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RESEARCH ARTICLE

Testing thallium phytoextraction using *Silene latifolia* and *Biscutella laevigata* on natural thallium-enriched and thallium-spiked substrates

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Abstract

Background and aims Thallium is a non-essential trace element occurring as a contaminant from industrial pollution, but it is also economically valuable. Phytoextraction for thallium using hyperaccumulator plants offers a potential opportunity to remediate thallium-contaminated soil while obtaining a valuable product (then called 'phytomining'). *Silene latifolia* is among the most promising candidates for thallium phytomining, but its performance in this context remains untested. It was compared here with the better studied thallium hyperaccumulator *Biscutella laevigata*.

Methods *Silene latifolia* seeds were collected from Allchar (North Macedonia) and Ganges (France), while for *B. laevigata* the Ganges accession was used. Plants were cultivated under controlled conditions on substrate from the Allchar site in North Macedonia with natural high thallium enrichment and on artificially thallium-spiked substrate to obtain key parameters required for future upscaling of thallium phytomining. Furthermore, the possible effects of Spirulina as a biostimulant on thallium phytoextraction yield were evaluated.

Results Cultivated on thallium-enriched substrate for one month, *S. latifolia* (Allchar accession) attained up to 12,600 µg Tl g^{-1} DW in the shoot, compared to 3920 µg Tl g^{-1} DW when grown on thallium-spiked substrate. In the Ganges accession of *S. latifolia*, and particularly also in *B. laevigata*, notably lower shoot thallium concentrations were found. While biostimulants promoted plant biomass in all accessions analyzed, thallium yield in the shoots increased only in *B. laevigata.*

Conclusions Thallium phytomining has yet to be tested at field scale, but experimental results suggest high potential for the *S. latifolia* (Allchar accession). Substantial variation exists in phytoextraction potential between *S. latifolia* accessions, which can be exploited for plant selection, while a positive response

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to the biostimulant in thallium yield was observed only in *B. laevigata*.

Keywords Biostimulant · Hyperaccumulator · Phytomining · Thallium

Introduction

Thallium (Tl) is a non-essential element, with a lethal dose for adults of only 8–10 mg Tl per kg of body weight (Al Hammouri et al. 2011; Riyaz et al. 2013; ATSDR 2015). It is more dangerous to humans than arsenic (As), mercury (Hg), lead (Pb) or cadmium (Cd), earning it the notorious epithet 'the poisoner's poison' (Lennartson 2015). Thallium occurs in two oxidation states (mono- and trivalent) in the natural environment, both hazardous to living organisms, with the toxicity of Tl^{3+} being thousands of times higher than that of Tl^{+} (Rickwood et al. 2015). However, the bioavailable concentration of the former is much lower, as Tl^{+} is stable and soluble in water as free ions, which are easily absorbed by humans, animals, and plants, primarily because of the chemical similarity to potassium ions (K^{+}) (Peter and Viraraghavan 2005).

Globally, soil Tl concentrations are low, in uncontaminated soil typically below 1 μg g^{-1} (Fujihara and Nishimoto 2024), with Tl mainly present in sulfide ores of zinc (Zn), Pb, and Cd (Galván-Arzate and Santamaría 1998; Peter and Viraraghavan 2005). Anomalous soil Tl concentrations can result from human activities, such as mining, smelting, refining, coal burning, and other industrial processes involving Tl-rich ores (Kazantzis 2000), with up to 5000 tons of Tl released into the environment each year (Karbowska 2016). The annual worldwide production of Tl is estimated at 10 tons, mainly as a by-product from processing Tl-bearing sulfide Zn-Pb ores (Kazantzis 2000; Migaszewski and Gałuszka 2021; US Geological Survey 2024). About 17 million kilograms of Tl resources are present in Zn ores in Canada, the United States and Europe, whereas 630 million kilograms occur in coal mines worldwide (US Geological Survey 2024). The end uses of this technology-critical element are mainly advanced materials for gamma radiation detection, infrared optical materials, low-melting glass, photoelectric cells, high-temperature superconductors, and radioisotopes, and it had a market price of USD 9300 per kilogram in 2025 (US Geological Survey 2026).

Along with excess Tl in the environment and the resulting direct exposure, numerous studies have reported that Tl can substantially bioaccumulate in some food crops, posing a potential risk to human health (Tremel et al. 1997; LaCoste et al. 2001; Pavlickova et al. 2006; Corzo-Remigio et al. 2024; Xiao et al. 2024). Thallium accumulation in crops has been shown to occur more readily than that of other potentially toxic elements, such as Hg, Pb, Zn and Cd (Xiao et al. 2004), reaching concentrations of more than 20 μg g^{-1} dry weight (DW) in the edible parts of vegetables from contaminated areas (Xiao et al. 2024). Under controlled conditions, these concentrations reached up to 8300 μg Tl g^{-1} (Corzo-Remigio et al. 2024), with precipitation in solid Tl-crystalline deposits in *Brassica oleracea* (Corzo-Remigio et al. 2026). The threat posed by Tl to human life and ecosystems has raised significant public concern (Peter and Viraraghavan 2005), prompting call for improved legislative guidelines on Tl discharge into the environment, as well as development of novel, environmentally sustainable remediation technologies.

Nature-based solutions for metal pollution rely on phytoextraction/phytomining, which harnesses a hyperaccumulator plants' natural ability to extract metal(loid)s from soil and recover them from metal(loid)-rich biomass, particularly for economically valuable elements (van der Ent et al. 2015, 2026; Corzo Remigio et al. 2020; Rylott and van der Ent 2025). This technology specifically exploits hyperaccumulator plants, which have the remarkable ability to accumulate metal(loid)s in their shoots at concentrations far greater than those in the soil and beyond any physiological requirement (van der Ent et al. 2013). Although Tl phytomining is considered economically feasible, with values more than 600 times higher than those for nickel (Ni) in 2025 (US Geological Survey 2026), research on its practical application has been limited (Kidd et al. 2018; Robinson and Anderson 2018). Therefore, initial research steps towards realizing Tl phytoextraction should focus on selecting the most suitable hyperaccumulator plant species, with high shoot target metal concentration and high biomass production as essential traits for the success of this technology (Vangronsveld et al. 2009). For Tl, the threshold for hyperaccumulation has been identified as 100 μg Tl g^{-1} DW in the leaves (van der Ent et al. 2013; Reeves et al. 2018), a value exceeded in several

facultative hyperaccumulator species from Zn-Pb mines (Ganges) in Southern France and Northern Italy (Cave del Predil) (Escarré et al. 2011; Fellet et al. 2012), and the Tl-As outcrop at Allchar in North Macedonia (Jakovljević et al. 2024, 2025).

Pioneering Tl phytomining experiments used *Iberis linifolia* L. (synonym *I. intermedia* Guers.), a Tl hyperaccumulator that can attain up to 13,430 µg Tl g^{-1} DW on Tl-spiked soil with 20 µg Tl g^{-1} (Scheckel et al. 2004). It has an estimated yield of 10 t ha^{-1} and with plants containing approximately 800 µg Tl g^{-1} DW, this species would yield about 8 kg of Tl per hectare per harvest (Anderson et al. 1999; Leblanc et al. 1999). However, this species has a rather low biomass and slow growth rate. Another Tl hyperaccumulator, *Biscutella laevigata* L., achieves higher biomass with greater Tl accumulation, reaching up to 32,000 µg Tl g^{-1} DW on soils with a mean of 427 µg Tl g^{-1} (Pošćić et al. 2015) and up to 16,100 µg Tl g^{-1} DW (Corzo Remigio et al. 2022a) or 24,130 µg Tl g^{-1} DW in hydroponics (Salinitro et al. 2024). Current knowledge of hyperaccumulation of Tl in different species, substrates and conditions is summarized in Table S1. *Silene latifolia* Poir. is another Tl hyperaccumulator, with the highest Tl concentration ever reported in a field-collected plant—up to 80,000 µg g^{-1} DW in samples from Allchar (Jakovljević et al. 2025), a site with exceptionally high soil Tl concentrations reaching up to 18,000 µg g^{-1} Tl (Đorđević et al. 2021). *Silene latifolia* is common across Europe, Western Asia, and Northern Africa (POWO 2025) and is a facultative Tl hyperaccumulator (Regini et al. 2024). To date, two metallicolous Tl hyperaccumulating populations have been identified: (i) one near Ganges/St Laurent le Minier (France), with up to 1489 µg Tl g^{-1} DW in nature (Escarré et al. 2011) and up to 35,700 µg Tl g^{-1} DW in young leaves of hydroponically grown plants (Corzo Remigio et al. 2022b), and (ii) the aforementioned population from Allchar (Jakovljević et al. 2025). Previous studies (Regini et al. 2024, 2025) have revealed Tl accumulation- and photosynthesis-related traits in this species, with Tl mainly localized in the root cortex and leaf epidermis, and enriched at the base of the midrib and in the veins of leaf surfaces (Corzo Remigio et al. 2022a; Regini et al. 2026). Due to its exceptionally high tissue Tl concentrations, rapid growth rate, and relatively high biomass production, *S. latifolia* is considered a good potential candidate for Tl phytoextraction (Corzo Remigio et al. 2020, 2022b). This is particularly relevant given that the commercial feasibility of phytomining is often limited by the slow growth and low biomass production of many hyperaccumulators (Kidd et al. 2015); thus improving these factors is crucial to the process (Chaney et al. 2007; Nkrumah et al. 2016; Cerdeira-Pérez et al. 2019). Biostimulants may promote these characteristics, as they include diverse bioactive natural substances that enhance plant nutrient use efficiency, quality traits and stress tolerance (Yakhin et al. 2017; Du Jardin et al. 2025). Among these, the cyanobacterium *Limnospira platensis*, commonly known as Spirulina, is one of the most investigated for agricultural applications, being a rich and renewable source of micro- and macronutrients, phytohormones, polysaccharides and antioxidants critical for plant development (Shedeed et al. 2022; Arahou et al. 2023). Beneficial effects have been reported on germination, growth, stress tolerance and defence elicitation in numerous food crops (Attia et al. 2024; Touzout et al. 2025; Essid et al. 2026). To date, however, the use of Spirulina to improve the yield of metal hyperaccumulators remains unexplored, opening new possibilities for sustainable biostimulant-aided phytoextraction.

Since the pioneering Tl phytomining experiments undertaken by Leblanc et al. (1999) very little experimental work has been done to assess Tl hyperaccumulator species for utility in phytomining. Given the favourable characteristics of *S. latifolia,* and the better studied Tl hyperaccumulator *B. laevigata*, for Tl phytomining, this study aimed to test their Tl extraction capacity on both naturally Tl-enriched and Tl-spiked substrates to obtain key parameters for future upscaling of this technology. Here, we tested the impact of natural biostimulant as an eco-efficient strategy to improve these factors and thus Tl recovery.

Materials and methods

Plant culture conditions and thallium phytomining experiment

Seeds of *S. latifolia* were collected at Allchar (N 41.157658°, E 21.944728°) and Ganges (N 43.9314000°, E 3.6644611°) and *B. laevigata* seeds were collected at Ganges by hand picking from capsules and rapidly air-dried in cellophane bags. The

seeds were cold stratified (6 days at 4 °C) and then germinated on a substrate composed by 50% peat and 50% sand v/v. The seedlings were transferred to the experimental treatments (Table 1) 10 days after germination, at the emergence of the second true leaf. The pots, measuring 6×6×8 cm (W×L×D), were filled with 400 g of substrate and one plant per pot was transplanted, to obtain six replicates per treatment (Fig. 1). For the phytoextraction test, control, naturally enriched Tl-substrate and Tl-spiked substrate were prepared as follows: the control substrate was based on sandy soil collected onsite at Wageningen University (The Netherlands), sieved at 2 mm and then amended with 5 wt% peat-based potting mix (Unifarm, Wageningen University), to increase its water holding capacity. The Tl-spiked substrate was prepared by adding a thallium (I) nitrate ($TlNO_3$) solution to control substrate, to achieve a final concentration of 10 µg Tl g^{-1} DW. The substrate was first water saturated, then the Tl-solution was added, followed by homogenization by manual mixing. The substrate was air-dried again before use. The Allchar substrate (Tl-enriched substrate) was a naturally Tl-rich soil with 10,170 ± 540 µg Tl g^{-1} and 3.62 ± 0.12 µg Tl g^{-1} DW as the total and DTPA-extractable metal concentrations, respectively, determined by monochromatic X-ray fluorescence analysis (MXRF) as described below. This soil was collected from the Crven Dol area (Allchar, Republic of North Macedonia) from naturally outcropping Tl-As mineralization (Jakovljević et al. 2025), air-dried and sieved at 2 mm. To lower the Tl concentrations and ensure the growth of Ganges accessions (taken from the soil with 245 µg Tl g^{-1} DW, Escarré et al. 2011), this soil was mixed with control substrate at a 1:1 w:w ratio. At the beginning of the experiment, concentrations of Tl in control, spiked and Allchar substrates were 0.6 ± 0.2, 11.3 ± 1.6, and 4021 ± 275 µg g^{-1} as the total, and 0.01 ± 0.01, 11.3 ± 1.6, and 2.02 ± 0.17 µg Tl g^{-1} DW as the available concentrations, respectively. This was determined by DTPA-extract using Z-Spec MXRF, as described below. The experiment took place in a growth chamber with a 12/12 h light/dark cycle; illumination provided by high-intensity LED lights (VYPRx PLUS with PhysioSpec Indoor spectrum, Fluence Bioengineering, Austin, TX, USA), with a photosynthetic photon flux density of 550 µmol m^{-2} s^{-1} (measured with an Apogee MQ-500 instrument); and a day/night temperature regime of 25°C/20°C. Half of the plants from each substrate condition were treated with Spirulina once a week. Dry, finely ground Spirulina (Spirulina Farm, Orbetello, Italy, concentrations of ~20,000, 840, and 1200 µg g^{-1} DW for K, Ca and Fe, respectively, determined as for plant samples) was administered through irrigation in a quantity corresponding to 0.1% w/w of the substrate dry weight according to the manufacturer's instructions. The composition of macro- and micronutrients in the Spirulina dry biomass and in the control substrate (determined as for plant and soil samples) are reported in Table S2.

Table 1 Experimental design of this study including the species, number of biological replicates, accessions and treatments

Species	Substrate	Treatment	Number of plants
Biscutella laevigata (Ganges)	Control	Water	6
		Spirulina	6
	Tl-spiked (10 µg g^{-1})	Water	6
		Spirulina	6
	Allchar	Water	6
		Spirulina	6
Silene latifolia (Ganges)	Control	Water	6
		Spirulina	6
	Tl-spiked (10 µg g^{-1})	Water	6
		Spirulina	6
	Allchar	Water	6
		Spirulina	6
Silene latifolia (Allchar)	Control	Water	6
		Spirulina	6
	Tl-spiked (10 µg g^{-1})	Water	6
		Spirulina	6
	Allchar	Water	6
		Spirulina	6

Plant and substrate elemental analysis

Shoots were harvested after the 30-day experimental period, weighed, and rinsed with deionized water. All plant and substrate samples were dried in a ventilated oven at 60 °C for 48 h. The cleaned samples were then ground to a fine powder (< 200 µm) in an impact mill (IKA TubeMill 100 Control) and 0.5 g subsamples were placed into custom MXRF sample holders and covered with 12 µm polypropylene thin film (Z-Spec Inc.) for MXRF analysis. The MXRF analysis of

Fig. 1 *Silene latifolia* from Ganges accession in France (a, b) and *Biscutella laevigata* from Ganges accession in France (c, d) in their natural habitat and in the pot experiment in this study (e)

the plant powdered material was performed using a Z-Spec E-max instrument (Z-Spec Inc.) in 'Plant' or 'Soil' mode (Zhang et al. 2026). This instrumentation uses monochromatic X-ray fluorescence excitation to analyze elements from Z = 13 (Al) to Z = 53 (I) 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 and limits of detection (LODs) ranging from 0.009 to 0.025 μg g^{-1}. Three replicate measurements per sample were taken, each time rotating the sample cup to three different positions and averaged. Two certified standards were included for the plant material analysis (NIST SRM 1570a Trace elements in spinach leaves and NIST SRM 1573a tomato leaves), and two certified standards were included for the substrate analysis (NIST SRM 2702, marine sediments and NIST SRM 2709a San Joaquin soil for soils). The recovery was between 95% and 105% for each of the elements tested. Available metal concentrations were determined using the DTPA extraction method (Lindsay and Norvell 1978). Three grams of 2 mm-sieved substrate were extracted with 6 mL of DTPA solution in 15 mL tubes, shaken for 2 h, and centrifuged at 10,000 rpm for 3 min. Aliquots of 2 mL clear supernatant were collected for analysis. The DTPA solution contained 14.92 g L^{-1} triethanolamine (TEA), 1.47 g L^{-1} $CaCl_2 \cdot 2H_2O$, and 1.97 g L^{-1} DTPA, adjusted to pH 7.3 with HCl. Extracts were also analysed using the Z-Spec E-max after being transferred into special MXRF holders designed for liquids and available metal concentrations were expressed as μg g^{-1} DW of the extracted soil.

The substrate samples were also tested for total organic carbon (TOC) and total nitrogen (N) content at the beginning and end of the phytomining experiment. Substrates were ground in a MM400 ball mill (Retsch, Haan, Germany) at 30 rpm, for 1 min. Thirty milligrams of substrate powder were placed in small tin capsules and weighed on a precision scale. The tin capsules were then pressed to remove all air and placed in the instrument autosampler. Elemental carbon and nitrogen were quantified using a CHNS-O Flash 2000 Elemental Analyzer (Thermo-Fisher Scientific, Waltham, MA, USA).

Chlorophyll fluorescence analysis

The efficiency of the photosynthetic apparatus was monitored by weekly measurements of chlorophyll fluorescence parameters using a MultispeQ V 2.0 (PHOTOSYNQ INC. 325 E. Grand River Ave. Suite 225 East Lansing, MI 48823 USA), a device that combines a handheld fluorometer, a chlorophyll meter, and a benchtop spectrometer (Kuhlgert et al. 2016). Single measurements were taken on individual young, fully expanded leaves, lasting 30 to 60 s and were always conducted during the same period of the day (between 09:00 and 13:00). The photosynthesis-related parameters effective quantum yield of PSII (φPSII), non-photochemical quenching (φNPQ) and non-regulated energy dissipation (φNO) were calculated as follows: $\varphi PSII = (F_{m'} - F_s)/F_{m'}$, where $F_{m'}$ is maximal fluorescence yield under the steady-state illumination and F_s is steady-state fluorescence yield, $\varphi NPQ = 1 - (\varphi PSII + \varphi NO)$ and $\varphi NO = F_s/F_{m'}$.

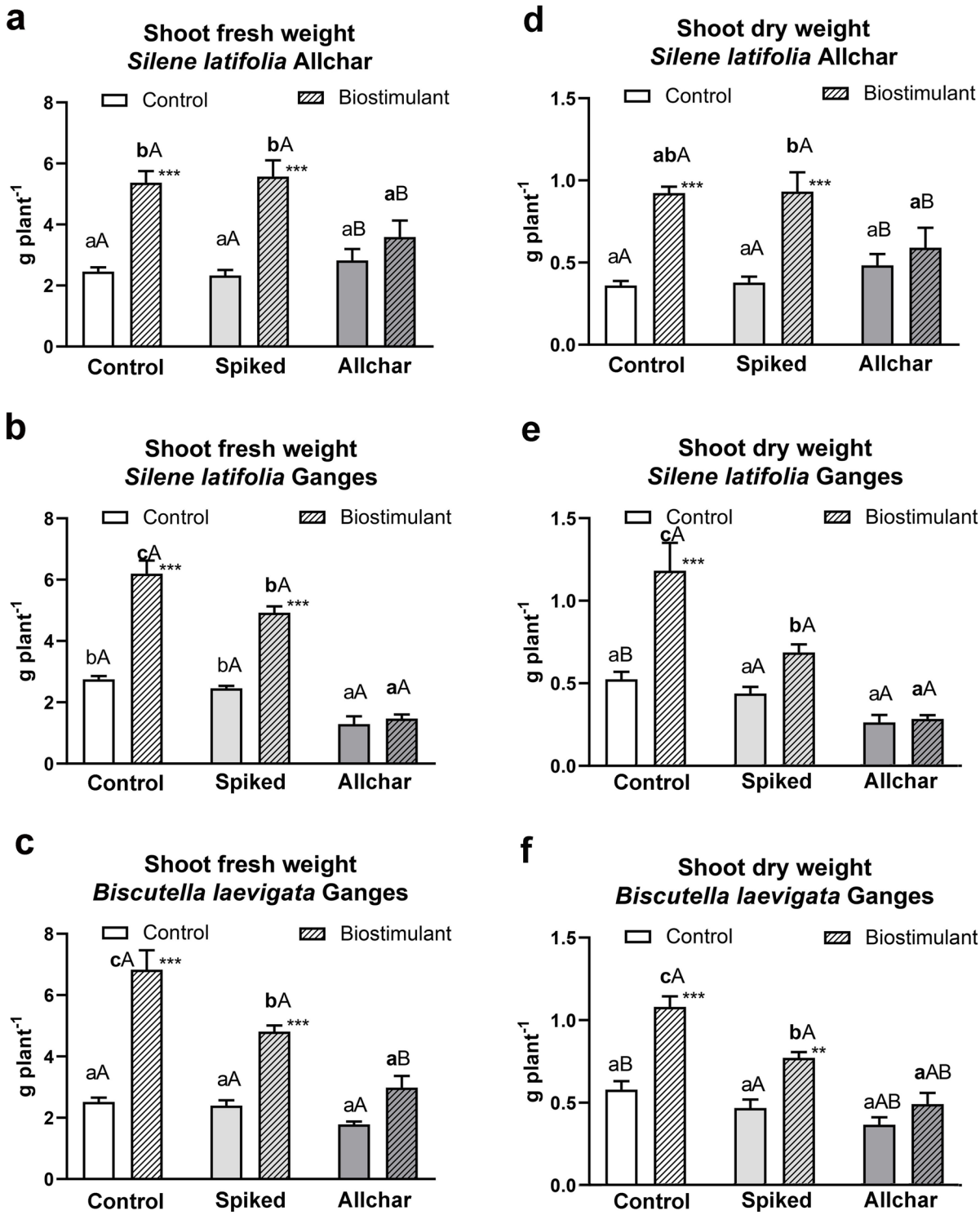
Data analysis

A two- way ANOVA (statistical significance at $p < 0.05$) was performed to evaluate the effects of substrate treatment and biostimulant on the three accessions and their interaction. Post hoc comparisons were conducted using Tukey HSD test in GraphPad Prism 7 (GraphPad Software). A one- way ANOVA ($p < 0.05$) was used to assess differences among the three accessions exposed to the same treatment. The Tl yield per hectare was calculated from the projected biomass, based on the weight of mature *S. latifolia* plants (50–200 g per plant) at a density of 4 plants per m^2, and whole biomass Tl concentrations (1000–5000 μg Tl g^{-1}) derived from values obtained from plants growing on moderately Tl-contaminated soil containing 10 μg Tl g^{-1}. For visualisation of phytoextraction efficiency RStudio software v. 4.2.0 (R Core Team 2020) was used.

Results

Plant growth on Allchar and Tl-spiked substrates

Cultivation of *S. latifolia* from Allchar and *S. latifolia* and *B. laevigata* from Ganges under three treatments that varied considerably in total Tl concentrations resulted in variation in the shoot fresh and dry weights, which were further influenced by the presence of the biostimulant (Fig. 2, Tables S3 and S4). When grown in Allchar substrate, the Allchar accession of *S. latifolia* achieved the highest values (2.82 g and 0.483 g per plant for shoot fresh and dry weight,


a
Shoot fresh weight
Silene latifolia Allchar
Control
Biostimulant
g plant-1
Control
Spiked
Allchar
b
Shoot fresh weight
Silene latifolia Ganges
c
Shoot fresh weight
Biscutella laevigata Ganges
d
Shoot dry weight
Silene latifolia Allchar
e
Shoot dry weight
Silene latifolia Ganges
f
Shoot dry weight
Biscutella laevigata Ganges

◂**Fig. 2** Shoot fresh and dry weight of (a, d) *Silene latifolia* Allchar, (b, e) *S. latifolia* Ganges, and (c, f) *Biscutella laevigata* Ganges cultivated for 30 days on the three substrates, with and without biostimulant (Spirulina) incorporation. Values are means ± SE, $n = 6$. Letters indicate significant differences among samples according to Tukey's test ($p < 0.05$), lowercase letters indicate intra-treatment comparisons (same species in different substrates, in bold with biostimulant) and uppercase letters indicate inter-species comparisons within the same treatment. Asterisks indicate the effect of biostimulant compared to the corresponding treatment (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

respectively). In this substrate, the Ganges accession of *S. latifolia* and *B. laevigata* had the lowest values for both shoot fresh and dry weights, with significant differences compared with spiked and control substrates for *S. latifolia*. Under the biostimulant treatment, this increase was statistically significant in plants grown in control and spiked substrates, while the lowest effects were observed in Allchar substrate (with an increase of only 14% for the Ganges accession of *S. latifolia*). In the order *S. latifolia* Allchar, *S. latifolia* Ganges and *B. laevigata*, the Spirulina-induced shoot biomass increase for fresh weight was 119%, 125% and 172% on control substrate and 139%, 100% and 100% on spiked substrate, respectively. For dry weight, the increases were 155%, 125% and 87% on control substrate and 146%, 57% and 64% on spiked substrate. No significant effects were determined in Allchar substrate for any accession. Except for the increase induced by the biostimulant in *B. laevigata* grown on control substrate, no significant variations in water content among the different accessions were found (Figure S1, Table S5).

Chlorophyll fluorescence under different treatments

Temporal variation in photosynthetic parameters for the three accessions grown on different substrates is shown in Fig. 3, for all of which two-way ANOVA indicated a significant interaction between time and treatment (Table S6). Regarding the effective PSII quantum yield (φPSII), *S. latifolia* from Allchar achieved the highest photosynthetic efficiency on the Allchar substrate (0.616 at the end of the experiment), with significantly lower values on control and Tl-spiked substrates. In contrast, there were no differences across treatments in either Ganges accession, except for a significantly lower value on Allchar substrate (0.510) compared to spiked substrate (0.659) in *S. latifolia* from Ganges after 14 days. On control and spiked substrates, biostimulant application resulted in significantly higher values for all accessions compared to their respective controls without Spirulina, with only *S. latifolia* Ganges also reaching higher values on Allchar substrate at the end of the treatment.

The variation in non-photochemical quenching (φNPQ) displayed the opposite trend to φPSII, with accession- and treatment-specific differences. The Allchar accession of *S. latifolia* exhibited the lowest φNPQ levels when grown in Allchar substrate compared to control and Tl-spiked substrates, reaching a value of 0.25 at the end of the experiment. In contrast, both Ganges accessions showed the reverse pattern, with *B. laevigata* attaining significantly higher φNPQ values at the end of the treatment when cultivated in Allchar substrate. Biostimulant application significantly reduced φNPQ levels only on control and spiked substrates for all accessions, but only at shorter exposure times for *B. laevigata*, with 0.157 being the lowest value observed after the two-week experiment for *S. latifolia* Ganges on spiked substrate.

Regarding non-regulated energy dissipation (φNO), although changes over time and treatments were minimal (values ranged from 0.103 to 0.155 after 14 and 30 days), some significant differences were observed. *Silene latifolia* Ganges and *B. laevigata* exhibited lower values when grown on Allchar substrate, and biostimulant treatment affected control and spiked substrates, increasing values in *S. latifolia* Allchar and decreasing them in *B. laevigata*.

Thallium accumulation on Allchar and spiked substrates

Significantly different Tl concentrations were found in the shoots of the three accessions in response to varying metal amounts across substrates, with a significant biostimulant × substrate type interaction (Fig. 4, Table S7). The highest shoot Tl concentrations were found in *S. latifolia* from Allchar, grown in on Allchar substrate, averaging 12,600 μg Tl g^{-1} DW, with the maximum value of 18,500 μg g^{-1} DW. For plants from this accession, the values were significantly lower when grown in the Tl-spiked substrate (3920 μg Tl g^{-1} DW). A similar concentration was accumulated on spiked substrate by the *S. latifolia* accession from

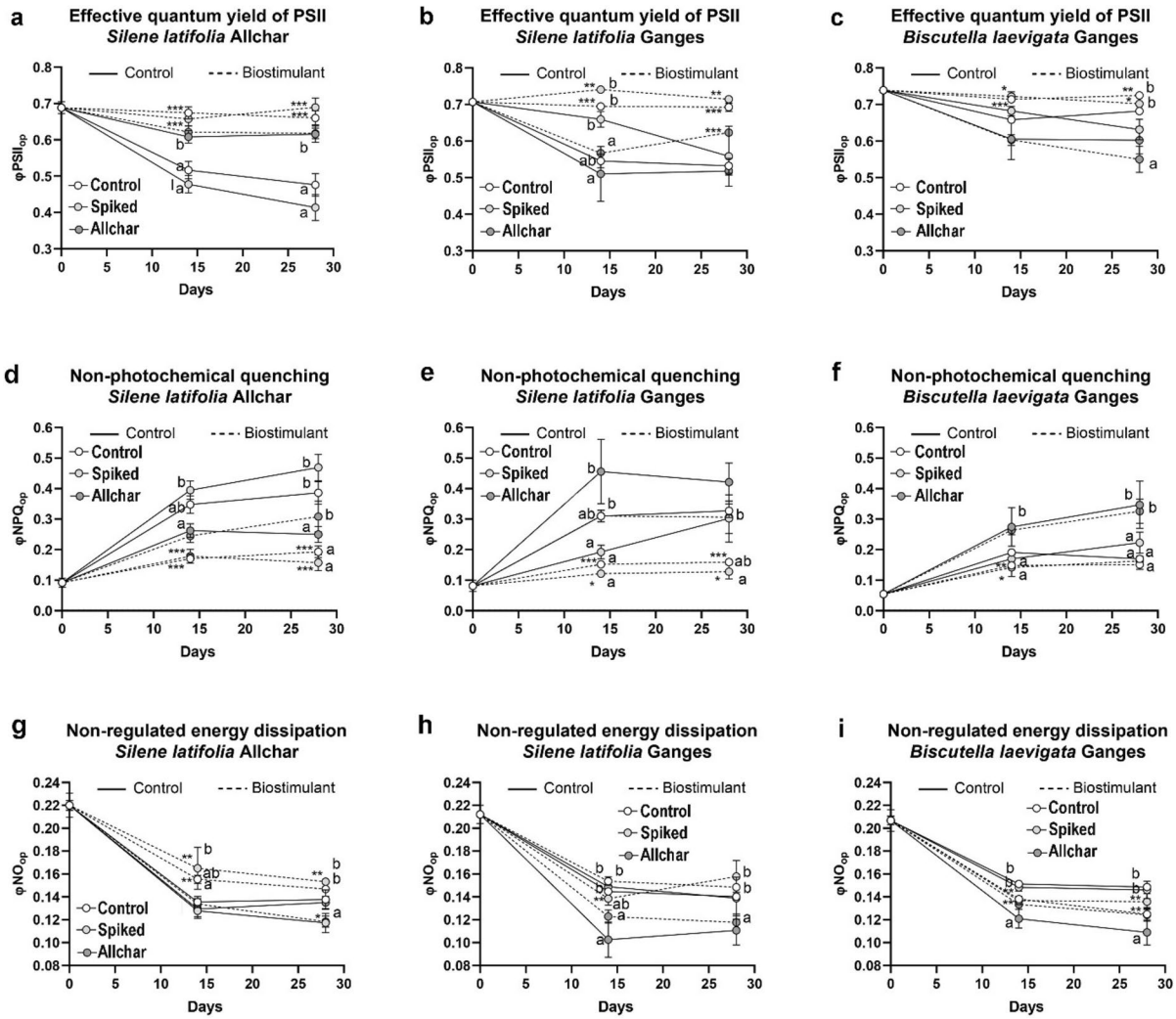


Fig. 3 Effective quantum yield of PSII (a, b, c), non-photochemical quenching (d, e, f) and non-regulated energy dissipation (g, h, i) of *Silene latifolia* Allchar, *S. latifolia* Ganges, and *Biscutella laevigata* Ganges cultivated for 30 days on the three substrates, with and without biostimulant (Spirulina) incorporation. Values are means ± SE, $n = 6$. Where present, letters indicate significant differences within each treatment group (to the left of the symbol for the no biostimulant group, to the right for the biostimulant group) at the same time point according to Tukey's test ($p < 0.05$). Asterisks indicate the effect of biostimulant within each treatment and each time point according to the t-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Ganges (4650 µg g^{-1} DW), while a contrasting pattern was observed in Allchar substrate, with considerably higher total, but lower DTPA-extractable Tl concentrations, where shoot Tl concentrations averaged only 459 µg Tl g^{-1} DW. In *B. laevigata* shoot concentrations were lower in plants grown in spiked substrate compared to those grown in Allchar substrate, mirroring soil Tl concentrations, but reaching much lower levels than in the other plants.

Biostimulant application reduced shoot Tl concentrations in both *S. latifolia* accessions when grown in Tl-rich substrates (spiked or naturally enriched), although the reduction was not significant for Ganges plants. In the Allchar *S. latifolia* accession, the biostimulant reduced shoot Tl concentrations by up to 50% in both treatments, with a mean concentration of 6510 µg Tl g^{-1} DW in plants grown in Allchar substrate; however, this value remained significantly

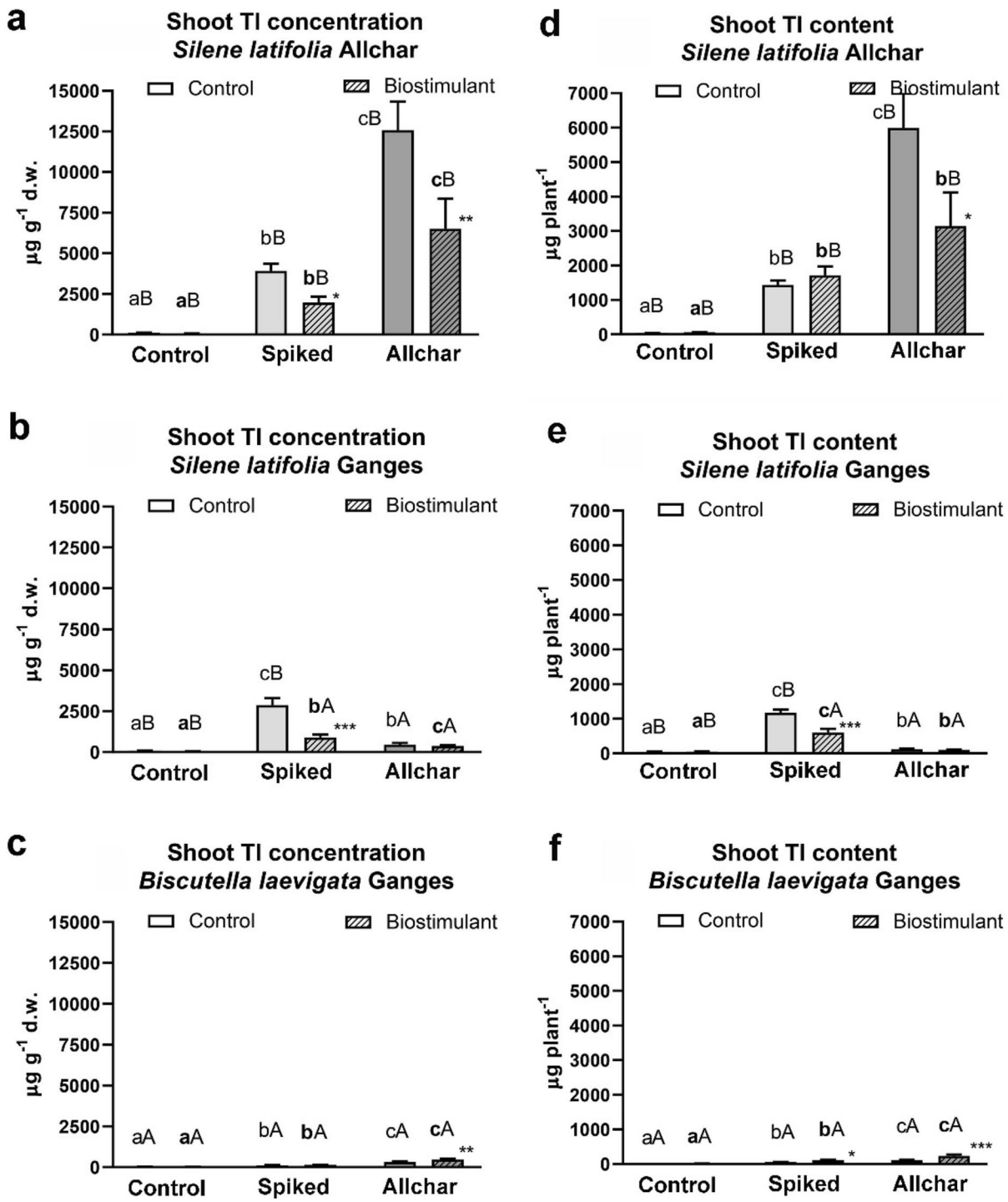


Fig. 4 Shoot concentrations and content of Tl in (a, d) *Silene latifolia* Allchar, (b, e) *S. latifolia* Ganges, and (c, f) *Biscutella laevigata* Ganges cultivated for 30 days on the three substrates, with and without biostimulant (Spirulina) incorporation. Values are means ± SE, $n = 6$. Letters indicate significant differences among samples according to Tukey's test ($p < 0.05$), lowercase letters for intra-treatment comparison (same species in different substrates, in bold with biostimulant) and uppercase letters for inter-species comparison within the same treatment. Asterisks indicate the effect of biostimulant compared to the corresponding treatment (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

higher than the concentrations measured in the other two accessions in Tl-enriched substrates. In the Ganges *S. latifolia* accession, shoot Tl concentrations declined by up to 70% in Tl-enriched substrates, although the effect was less pronounced in Allchar substrate, reaching a minimum value of 897 µg Tl g^{-1} DW in spiked substrate. In contrast, *B. laevigata* showed the opposite response to biostimulant application, with an increase in shoot Tl concentrations, which was significant in Allchar substrate. Individual values reached a maximum of 600 µg Tl g^{-1}DW, with an average of 475 µg Tl g^{-1} DW.

For the other elements (Table S8), K concentration was significantly reduced only in the shoots of *S. latifolia* Allchar when grown in both spiked and Allchar substrates. In the biostimulant treatment, this reduction occurred in all three accessions but only on Allchar substrate, whereas *S. latifolia* Ganges grown on spiked substrate was the only sample with a biostimulant-induced increase in K levels. Across all three substrates, shoot K concentration was highest in *S. latifolia* Allchar and lowest in *B. laevigata*. In biostimulant-treated plants, *B. laevigata* consistently showed the lowest shoot K concentration, whereas the two *S. latifolia* accessions had similar values, except in Allchar substrate. Sulfur and As concentrations were highest in plants grown in Allchar substrate, across all three accessions analyzed (Table S8). Similar As values were recorded in both *S. latifolia* accessions, while the shoots of *B. laevigata* exhibited statistically different values, up to five times higher in the absence of biostimulation. On this same substrate, *B. laevigata* showed the highest shoot Cl concentrations, whereas in all treatments it displayed a higher shoot Ca amount and generally lower values for the other elements. When the effect was significant, the presence of Spirulina resulted in a general increase in Cl concentrations and, only in *B. laevigata* on Allchar substrate, a decrease in Mn, Fe, and Cu.

Thallium phytomining yields

The Tl content per shoot, representing the product of shoot Tl concentration and shoot DW biomass, varied among the samples, showing a significant interaction between biostimulant and substrate type (Fig. 4, Table S9). Specifically, the shoot concentration of 12,600 µg Tl g^{-1} DW in *S. latifolia* Allchar grown in Allchar substrate for 30 days resulted in a shoot Tl content of 6.08 mg per plant, based on a dry biomass of 0.483 g. In plants grown on spiked substrate, shoot Tl content was 1.48 mg, given the shoot concentration of 3900 µg Tl g^{-1} DW and a biomass of 0.378 g. In *S. latifolia* Ganges grown in spiked substrate, shoot Tl content was similar, with a shoot concentration of 2870 µg Tl g^{-1} DW translating to 1.25 mg Tl per plant, whereas *B. laevigata* had the lowest value. When grown in Allchar substrate, mean shoot Tl content in *S. latifolia* Ganges and *B. laevigata* reached 0.12 mg, which was significantly lower than that in *S. latifolia* Allchar. In biostimulant treated plants, shoot Tl content varied in a species-specific manner; there was a decrease in *S. latifolia* Ganges grown in spiked substrate (mean content 0.616 mg) and in Allchar plants grown in Allchar substrate (mean Tl content 3.84 mg). Shoot Tl content in *B. laevigata* increased in biostimulant-treated plants on both Tl-containing substrates, with the highest mean value at 0.233 mg in plants grown in Allchar substrate.

Compared to the start of the experiment, total soil Tl concentrations significantly decreased after cultivation with *S. latifolia* Allchar in all of the treatments, with reductions of approximately 45% in Tl-spiked and 17% in the Allchar substrate (Table 2). For the other accessions, the only significant decrease was observed in the Tl-spiked substrate cultivated with *S. latifolia* Ganges (around 55%). Two-way ANOVA did not reveal any significant differences among the samples (Table S10). For extractable Tl concentrations, significant decreases compared to the start of the experiment were found for all accessions and treatments (Table 3). Total and extractable concentrations of other elements and the percentage of substrate C and N are shown in Tables S11 and S12, respectively. Except for Cu and K, the Allchar substrate generally had higher total element concentrations than the control and spiked substrates, regardless of accession and biostimulant presence (Table S11), whereas intra-treatments comparisons, as well as the effect of Spirulina, were rarely significant. Similar results were found for the available fraction of metals in the substrates.

The current experiment did not assess the maximum attainable Tl yield, as plants were only grown for 30 days and remained hence small in size. However, assuming the main parameters affecting Tl yield at plant maturity, with plants weighing 50–200 g

Table 2 Total substrate Tl concentrations for *Silene latifolia* Allchar, *S. latifolia* Ganges and *Biscutella laevigata* Ganges cultivated for 30 days on three substrates, with and without biostimulant incorporation. Values are presented in μg Tl g^{-1} DW as means ± SE for $n = 6$

	Spiked substrate	Spiked substrate + biostimulant	Allchar substrate	Allchar substrate + biostimulant
S. latifolia Allchar	6.2 ± 2.8 **	5.3 ± 2.4***	3346 ± 448 *	3470 ± 532*
S. latifolia Ganges	5.10 ± 1.40***	8.1 ± 4.3	3584 ± 533	3959 ± 567
B. laevigata Ganges	11.2 ± 7.5	8.9 ± 5.8	3645 ± 351	3715 ± 362

Asterisks indicate significant differences from total substrate Tl concentration at the beginning of the experiment according to the t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Initial values were: 0.6 ± 0.2, 11.3 ± 1.6, and 4021 ± 275 μg Tl g^{-1} DW for control substrate, spiked and Allchar substrates, respectively. Values for control substrates at the end of the cultivation period were not reported, as they were always between 0 and 1 μg Tl g^{-1} DW

Table 3 Substrate available Tl concentration of *Silene latifolia* Allchar, *S. latifolia* Ganges and *Biscutella laevigata* Ganges cultivated for 30 days on three different substrates and treated with biostimulant. Values are presented in μg Tl g^{-1} DW as means ± SE of six replicates

μg Tl g^{-1} d.w	Spiked substrate	Spiked substrate + biostimulant	Allchar substrate	Allchar substrate + biostimulant
S. latifolia Allchar	0.068 ± 0.022***	0.098 ± 0.082***	0.843 ± 0.082***	1.332 ± 0.509aA*
S. latifolia Ganges	0.080 ± 0.027***	0.168 ± 0.098***	0.961 ± 0.269aA***	1.381 ± 0.205aA***
B. laevigata Ganges	0.226 ± 0.165***	0.314 ± 0.177***	0.969 ± 0.171aA***	1.191 ± 0.178aA***

Letters indicate significant differences among samples according to Tukey's test (at least $p < 0.05$), lower-case letters for intra-treatment comparison (same species in the same substrate, with or without biostimulant) and capital letters for inter-species comparison within the same treatment. Asterisks indicate significant differences from substrate available Tl concentration at the beginning of the experiment according to t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Initial values were: 0.01 ± 0.01, 11.3 ± 1.6, 2.02 ± 0.17 μg Tl g^{-1} DW for control substrate, spiked substrate and Allchar substrate respectively. Values for control substrate at the end of the cultivation period were not reported as always below detection limit

planted at a density of 4 plants per m^2, resulting in a biomass yield of 2000–8000 kg ha^{-1}, and shoot Tl concentrations of 1000 μg g^{-1}, 3000 μg g^{-1} and 5000 μg g^{-1}, the yield is expected to range from 2 to 40 kg per hectare per harvest (Fig. 5).

Discussion

Plant growth on natural Tl-enriched and spiked substrates

The metal(loid)-rich substrates used in our test did not significantly reduce plant growth in any of the accessions tested, demonstrating that our experimental setup, although it cannot replicate the full range of soil and site conditions, provides a representative scenario to evaluate the feasibility of a phytomining trial. The differing trends in biomass production between *S. latifolia* Allchar and *S. latifolia* Ganges resulted in significant variations in shoot DW yield in Allchar substrate, which were already evident after one month of cultivation. The higher biomass achieved by *S. latifolia* Allchar likely results from evolutionary adaptation to its extreme environment, characterized by unique mineralogy with high prevailing Tl and As concentrations in the soil (Đorđević et al. 2021; Jakovljević et al. 2025). In both control and Tl-spiked substrates, Spirulina application generally increased plant biomass in all three accessions, probably acting as a biofertilizer, as has been extensively documented in crops (Attia et al. 2024; El-Shazoly et al. 2025; Touzout et al. 2025; Essid et al. 2026), even when exposed to metal stress (Rady et al. 2023; Shaari et al. 2023). The present findings in spiked substrate indicate that improved growth can also occur in certain types of wild plants, such as metal hyperaccumulators. However, the increase in plant biomass was never significant when cultivated in the Allchar substrate, likely due to its different

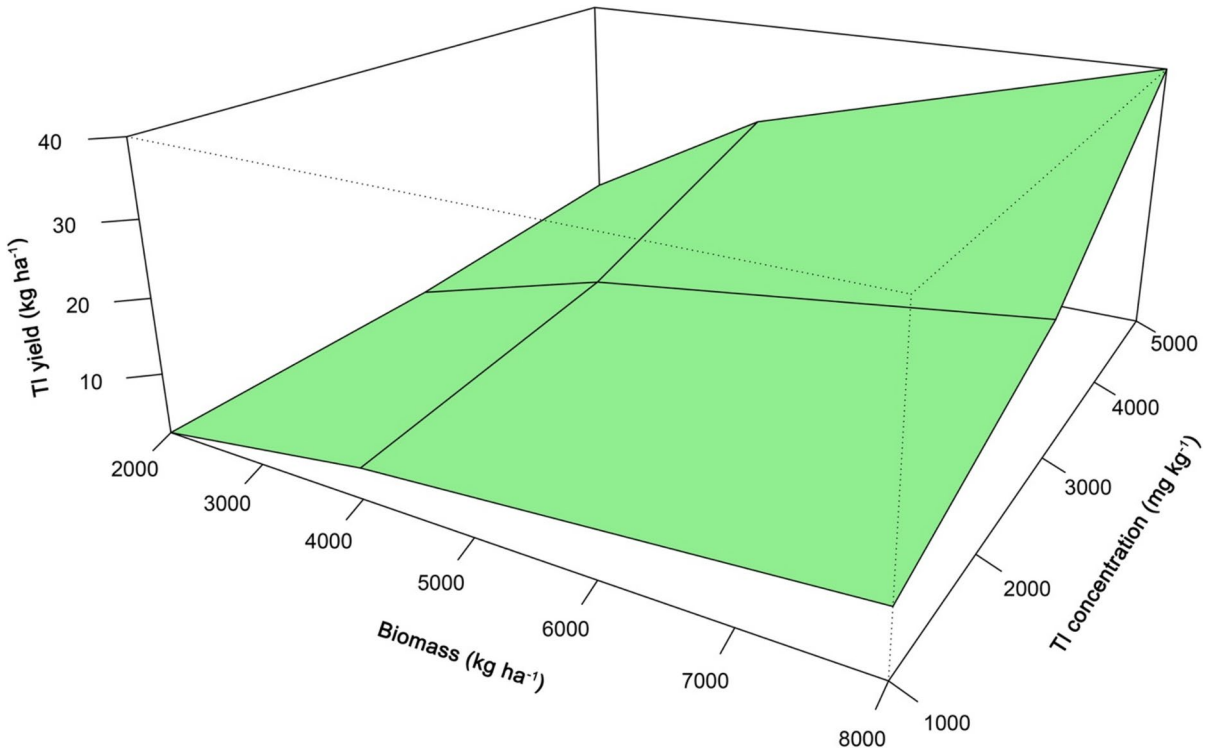


Fig. 5 Projected Tl yields per hectare per harvest using *Silene latifolia* (Allchar accession). The range of whole biomass per square meter is based on weights of mature plants (50–200 g per plant) with four plants per square meter and expressed per hectare. Whole shoot Tl concentrations (1000–5000 μg Tl g^{-1}) are based on values obtained from plants growing on moderately Tl- contaminated substrate with 10 μg Tl g^{-1}, concentrations suggested by Leblanc et al. (1999). Plants reach maximum size, before flowering, approximately three months after sowing at 20–25°C

physicochemical and biological characteristics, compared to the sandy substrate used in the other treatments.

Chlorophyll fluorescence analysis

In the present pot trial, PSII quantum yields indicated an ability to maintain photosynthetic activity in the presence of Tl in both Ganges accessions. In contrast, *S. latifolia* from Allchar showed an increase in this parameter when cultivated on Allchar substrate, coupled with the lowest levels of non-photochemical quenching and unchanged levels of non-regulated dissipation. This indicates that, in Allchar substrate, this accession was able to increase the percentage of light energy used for photochemistry, confirming its tolerance of this substrate. While further investigation is needed to elucidate the underlying mechanisms, the concurrent decline in φNPQ, indicating a reduced need to dissipate energy as heat, suggests that, when *S. latifolia* Allchar was grown on Allchar substrate, limitations on linear electron flow were alleviated, potentially reflecting increased Rubisco accumulation and enhanced CO_2 assimilation capacity (Johansson et al. 2004; Bielczynski et al. 2017). This would explain the increased shoot fresh and dry biomass compared to cultivation on control and spiked substrates. In contrast, in Ganges accessions high concentrations of Tl, but also higher phytoavailable concentrations of elements such as As, are likely associated with reduced values of photosynthetic parameters. Despite this, the extreme conditions of this substrate still seemed well tolerated, since the increase in φNPQ without a corresponding rise in non-regulated energy dissipation suggests that excess excitation energy was efficiently dissipated through regulated photoprotective mechanisms, preventing activation of the non-regulated pathway associated with the formation of reactive oxygen species. Treatment with Spirulina significantly increased photosystem efficiency in plants grown in both control

and spiked substrates, as well as in *S. latifolia* Ganges in Allchar substrate, which partly explains the higher biomass observed in these samples. Biostimulation of plant growth with microalgae such as Spirulina has already been associated with improved photosynthetic parameters in crop species (Parmar et al. 2023). In addition to the overall improvement in the chemical, physical, and biological characteristics of substrates amended with biostimulants, which results in enhanced plant photosynthetic traits, only a specific mechanism via possible activation of chlorophyll biosynthetic enzymes through Spirulina-improved Fe supply has been hypothesized (Rady et al. 2023). Enhanced photosynthetic activity, together with improved antioxidant capacity, has been reported to reduce metal toxicity in plants exposed to Spirulina (Rady et al. 2023), even when applied as a seed priming agent (Essid et al. 2026).

Thallium accumulation on natural Tl-enriched and spiked substrates

Although both *S. latifolia* and *B. laevigata* are genuine Tl hyperaccumulators (Salinitro et al. 2024), they exhibited accession-specific differences in Tl accumulation. When grown in substrate naturally enriched in Tl, shoot Tl concentrations in *S. latifolia* Allchar exceeded 12,000 μg Tl g^{-1} DW, far above the threshold for hyperaccumulation of 100 μg g^{-1} (van der Ent et al. 2013, 2021). Compared to *Silene latifolia*, has an exceptionally high capacity for Tl accumulation with foliar concentrations in plants at Allchar having nearly 80,000 μg Tl g^{-1} DW, but field-collected plants from can always be contaminated and Tl could be present in stomatal chambers and epidermal wax (Jakovljević et al. 2025), Tl concentrations in *B. laevigata*. Differences were also observed between *S. latifolia* accessions, with shoot Tl concentrations in *S. latifolia* Allchar nearly 30 times higher than those in Ganges plants, further suggesting the role of anomalously high metal(loid) concentrations in Allchar substrate in the evolution of plant hyperaccumulation. Therefore, in addition to genetic differences in plants with distinct edaphic origins (Pošćić et al. 2015; Babst-Kostecka et al. 2016), variations can also be presumed between plants from Tl-enriched substrates, as found between the Allchar and Ganges *S. latifolia* accessions when cultivated at high Tl dose levels. At lower Tl concentrations in the Tl-spiked substrate, these different accumulation trends were less apparent, likely because the less extreme substrate allowed greater expression of the hyperaccumulation ability of the Ganges accession, while the Tl concentrations were not sufficient for the Allchar accession to reach its typical Tl accumulation levels.

The biostimulant effect was species-specific, with a general decrease in Tl concentration in both *S. latifolia* accessions, particularly in the Allchar one, and an increase in *B. laevigata*. Since Spirulina biomass was especially enriched in K, this opposite behaviour could result from possible Tl-K competition for a common uptake pathway in *S. latifolia*, while a more independent pathway may exist in *B. laevigata*. In crop species, a reduction in trace metal accumulation is generally reported under Spirulina treatment and attributed to an overall improvement in plant antioxidative traits, including membrane protection and thus preserved functioning of exclusion mechanisms (Rady et al. 2023). Conversely, in a hyperaccumulator such as *B. laevigata*, such Spirulina-driven improvement in plant functioning could explain the observed increase in shoot Tl concentration on Allchar substrate. In addition, this accession was the only one to show a biostimulant-mediated increase in Tl content per plant, resulting from increased biomass on spiked substrate and the aforementioned increase in shoot concentration on Allchar substrate.

Regarding the other elements, all three accessions showed an increase in sulfur (S) concentrations when grown on Allchar substrate, which may, among other factors, be attributed to a metal-induced rise in glutathione levels to counteract the oxidative effects of accumulated metals, as reported in many hyperaccumulators (Na and Salt 2011), as well as higher uptake of SO_4^{2-}, which represents the beginning of the sulphate pathway. Additionally, the involvement of S-based ligands in Tl detoxification or hyperaccumulation could also be considered, since Tl complexation with phytochelatins has been reported in species such as *Coix lacryma-jobi* (Zhan et al. 2024). Differences in element concentrations among the plants were also observed for K, a chemical analogue of Tl, with strong competition in accumulation observed in a number of species (Chang et al. 2024). *Silene latifolia* Allchar was the only accession to show a Tl-induced decrease in K concentration and, at the same time, the highest concentrations of this element, even when plants were cultivated on the control substrate. The highest concentrations of As, another non-essential element for living organisms (Ganie et al. 2024), present geogenically in

anomalous concentrations in Allchar soil (Đorđević et al. 2021), were found in *B. laevigata*. In this study, growing in this substrate with over 23,000 µg g^{-1} of As, which is markedly higher than the 3300 µg g^{-1} of Tl, both accessions of *S. latifolia* accumulatedonly very little As (mean 120 µg As g^{-1} DW), in contrast to *B. laevigata*, where As averaged 631 µg g^{-1}, reaching a maximum of 1250 µg g^{-1}. Another interesting association was *B. laevigata* samples with the Cl, with generally higher amount of this element compared to *S. latifolia* accessions, probably due to Tl detoxification/hyperaccumulation mechanisms involving the formation of TlCl crystals (Salinitro et al. 2024).

Thallium phytomining yields

The exceptionally high extraction capacity of *S. latifolia* Allchar, which led to anomalous Tl concentrations in shoots, resulted in a significant reduction in substrate Tl concentrations by the end of the experiment, with the removal of 16.8% and 58% of the total and extractable Tl concentrations, respectively. The efficiency was even higher in spiked substrate, and more pronounced in *S. latifolia* accessions, with 54.9% and 45.1% of the total substrate Tl concentrations removed in accessions from Ganges and Allchar, respectively, however, with a significantly lower reduction of the element in absolute terms. Assuming a long-term field trial on a substrate 20 cm deep with a density of 1.3, for those from Allchar, this would amount to about 5.25 kg of available Tl per hectare. With a projected yield of the Allchar accession of *S. latifolia* of 2–40 kg per hectare, the minimum efficiency of removal of the phytoavailable fraction from the substrate is estimated at 38% per harvest, representing up to 0.4% of the total Tl in the substrate. However, as these calculations are based on projected estimated values rather than actual experiments, the values should be treated with caution, until fully verified under realistic conditions in the field.

Conclusions

The 30-day cultivation of *S. latifolia* accession from the Allchar site, and *S. latifolia* and *B. laevigata* accessions from the Ganges site, with accession-dependent variations, Tl concentrations in the substrate, and the presence of biostimulants, demonstrated a preference for utilizing the Allchar accession over those from Ganges for Tl phytomining purposes. Growing on its natural Tl-rich substrate, *S. latifolia* from Allchar developed the highest shoot biomass, along with a significant increase in photosynthetic activity and a markedly higher shoot Tl accumulation, identifying this accession as a promising candidate for Tl phytomining. In addition to testing a known Tl-hyperaccumulator species for its phytomining potential, this study also provides a possible experimental framework for assessing the feasibility of using prospective species for phytomining. Considering the multi-element-rich substrate used in this study; to distinguish more clearly the effects of Tl exposure, future research should focus on soils with lower concentrations of non-target elements. Moreover, given the variation in Tl availability when applied in different chemical forms (Rodríguez-Mercado and Altamirano-Lozano 2013), Tl-compounds, other than Tl-nitrate, should be tested for differences in Tl accumulation in hyperaccumlator plants in future studies.

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Author contributions GR, CG, MS, and AvdE conceived and planned the study. GR, MD, PCV, IC and NB conducted the experiment. GR, CG, KJ and AvdE evaluated and interpreted the data. CG, KJ and AvdE designed the figures. KJ, CG and AvdE drafted the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Data availability The data that support this study will be shared upon reasonable request to the corresponding author.

Declarations

Conflict of interest The authors declare no conflicts of interest relevant to the content of this manuscript.

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