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RESEARCH ARTICLE

Biogeochemical survey of the Allchar (North Macedonia) arsenic-thallium ore body: a focus on hyperaccumulator plants

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Abstract
Background and aims The Allchar site in North Macedonia has a unique geology exceptionally enriched in arsenic and thallium, making the local soils extremely toxic to plant life. Surprisingly, the mineralized soils at Allchar host a diverse flora, with unknown metal(loid) accumulation potential for most of these plant species. The main aim of this study was to determine the elemental profiles ('elementomes') of plant species growing naturally in the Allchar area and to assess their elemental accumulation in relationship to concentrations in the soil in which the plants grow.
Methods Samples of in total 23 plant species (with at least 4 replicates per species) and their associated rhizospheric soils were collected in the field at the Allchar site in North Macedonia and analysed with monochromatic X-ray fluorescence analysis for total and DTPA-extractable metal and metalloid concentrations.
Results High foliar concentrations of thallium were found in some plant species, being the most extreme in *Silene latifolia*, at 79,200 µg g^{-1} thallium, whilst arsenic concentrations are generally low in most of the plant species analysed. Thallium hyperaccumulation (> 100 µg g^{-1}) was found in the families Violaceae, Lamiaceae and Caryophyllaceae. Particularly high foliar thallium concentrations were found in *Viola arsenica* and *V. tricolor* subsp. *macedonica*, reaching up to 31,600 and 11,700 µg g^{-1} thallium, respectively. The elemental concentrations in soil and plant samples reflect that of the local mineralogy of the three different areas investigated at the Allchar site, with the highest mean values for thallium and arsenic in the Crven Dol area, and 249 and 3970 µg g^{-1}, respectively, in the plants that were analysed.
Conclusion The present study led to the discovery of several new thallium hyperaccumulating plant species, such as *Clinopodium alpinum*, *Anthyllis vulneraria* and *Linum hirsutum*, whereas the

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thallium concentrations found in *Silene latifolia* are the highest thus far recorded in nature highlighting the potential of this species for thallium phytomining applications.

Keywords Bioindicator · Excluder · Hyperaccumulator · Metallophyte · *Silene latifolia*

Introduction

The extraction and processing of metal ores generates large quantities of waste, contaminated in metals and metalloids, and are usually released on site, altering the environmental properties and contaminating the soil over a long period of time (Hudson-Edwards et al. 2011). These properties can vary greatly over a small area, both in elemental composition and concentrations, which is further emphasised by the mechanical properties of the substrate. Plant response to metalliferous substrate varies, from exlcusion to accumulation, with hyperaccumulation being the most extreme response to high elemental concentrations in the soil (Baker 1981; Baker et al. 2010). To date, hyperaccumulation has been reported in just over 700 species of plants, with those hyperaccumulating Ni the most numerous (observed in 523 taxa), whereas the other elements have been found to accumulate above the hyperaccumulation thresholds in much lower numbers (Reeves et al. 2018). The potential of these species has been recognised for their use in phytoremediation and phytomining (Corzo Remigio et al. 2020), although excluders may be especially important in stabilising contaminated soils and mine wastes, preventing further spread into the surrounding areas (Craw et al. 2007; Lottermoser 2010).

A particular challenge for plant adaptation were active and closed mines with high metal concentrations in the soils, which in extreme cases can cause evolutionary changes in just one generation (Bradshaw 1984). The abandoned Allchar mine site is one of the largest deposits of thallium; a metal extremely toxic for plants, animals and humans, that can occur in two oxidation states –Tl^{+} and the less stable and thousands of times more toxic Tl^{3+} (Sadowska et al. 2016). The Allchar site comprises of a 2 km × 0.3−0.5 km area in the southern part of North Macedonia and is a volcanogenic hydrothermal Tl-As-Sb-Au deposit (Janković 1993; Janković and Jelenković 1994). Similar to other large Tl deposits, including those in France, Switzerland, the USA, and China (Liu et al. 2019), it is characterised by association with As, whose reserves have been estimated at *ca*. 15 kilotons (Boev et al. 2002), whereas those of Tl, with 500 tons are the largest in the world (Ivanov 1986). The hotspot of Tl reserves is located in the Crven Dol area in the northern part of the Allchar deposit, which is richest in lorándite (thallium-arsenic sulphosalt or $TlAsS_2$), the best-known Tl-bearing mineral with an almost stoichiometric composition (Jovanovski et al. 2018). Other Tl minerals are vrbaite ($Tl_4Hg_3Sb_2As_8S_{20}$), raguinite ($TlFeS_2$), picotpaulite ($TlFe_2S_3$), and parapierrotite ($TlSb_5S_8$) discovered at Allchar, most of which occur exclusively in this area (Krenner 1897; Boev et al. 2002; Jovanovski et al. 2018). The most abundant elements (As, Tl, Sb, Au) are unevenly distributed at Allchar in three distinct zones: (i) Crven Dol in the northern part with a dominance of As and Tl and Sb, Hg and Au as accompanying elements, (ii) Central Deposit with Sb and Au as dominant elements associated with Tl, As, Hg and Ba, and (iii) South Deposit with a dominance of Au minerals and varying proportions of As and Sb (Janković and Jelenković 1994).

The mineralogical composition of the Allchar area has led to a specific flora characterised by a high diversity despite the toxic concentrations of Tl and As in the soil (up to 18 and 142 g kg^{-1}, respectively; Đorđević et al. 2021), but at the same time has contributed to a significant participation of endemic species highly tolerant to high metal concentrations (Bačeva Andonovska et al. 2021; Jakovljević et al. 2023, 2024). *Viola arsenica*, a steno-endemic species of Allchar, contained foliar concentrations of up to 58,900 µg g^{-1} Tl (when growing in soils with pseudo-total Tl concentration of up to 4430 µg g^{-1}; Jakovljević et al. 2023), even higher than some of the strongest hyperaccumulators of this element – *Biscutella laevigata*, *Silene latifolia* and *Iberis linifolia* subsp. *intermedia*, with 32,700, 1500 and 400 µg g^{-1} Tl, respectively (Corzo Remigio et al. 2022a, b). Extremely high Tl concentrations were also found in *V. allchariensis*, another steno-endemic species, with up to 23,100 µg g^{-1} Tl in the leaves (Jakovljević

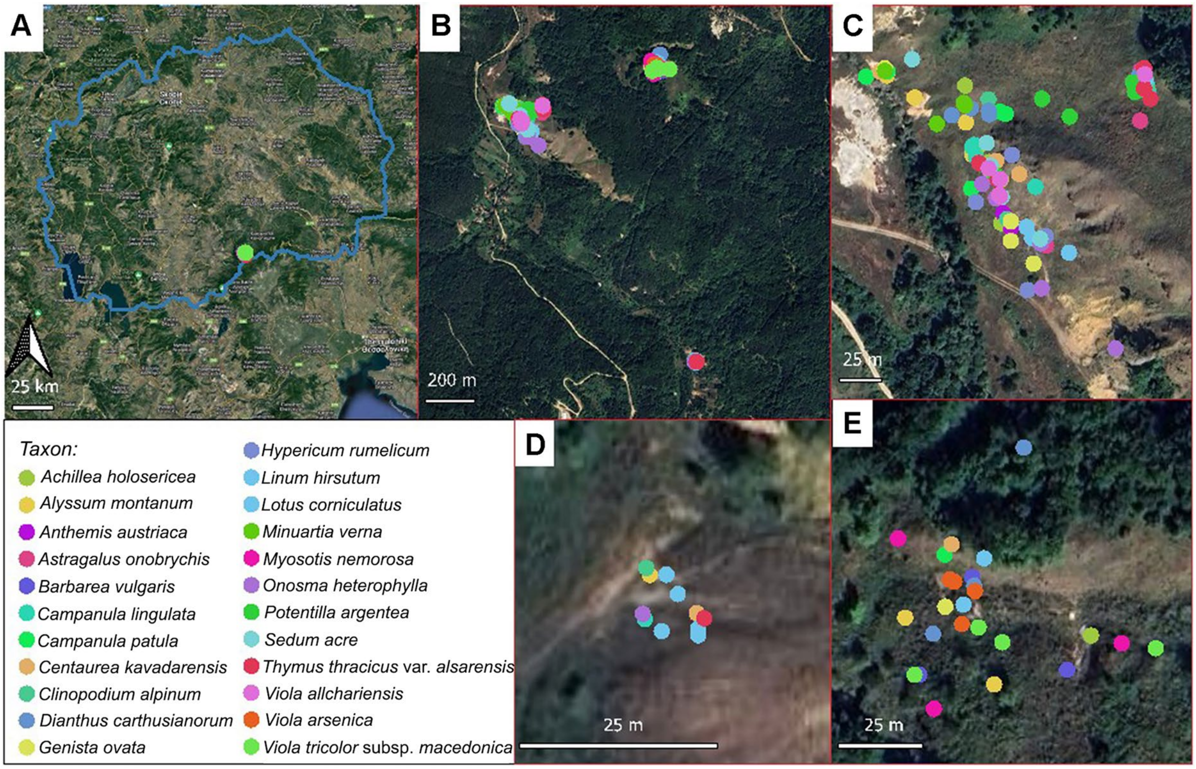


Fig. 1 Sampling points at the Allchar site showing the location of the site in North Macedonia near the Greek border (**A**), an overview of the Allchar site (**B**) and the Central Deposit (**C**), Crven Dol (**D**) and the South Deposit (**E**). Colour coding for different species sampled in this study

et al. 2023), while the threshold for hyperaccumulation of Tl (100 μg g^{-1}; van der Ent et al. 2013) was also exceeded in *Minuartia verna* (up to 3980 μg g^{-1}), *Silene vulgaris* (up to 1720 μg g^{-1}), *Alyssum kavadarcensis* (up to 127 μg g^{-1}), *Knautia caroli-rechingeri* (up to 109 μg g^{-1}) and *Centaurea leucomala* (up to 238 μg g^{-1}) (Bačeva Andonovska et al. 2021; Jakovljević et al. 2024). In *A. kavadarcensis*, the threshold for hyperaccumulation was also exceeded for As, with foliar concentrations reaching up to 1330 μg g^{-1} (Jakovljević et al. 2024).

The unique geochemistry of Allchar provides the opportunity to investigate the foliar accumulation of unusual elements in plant species occuring on the site. Therefore, the aim of this study to determine the elemental profiles of a wide range of plant species and their relationship to the soil in which they grow. Additionally, the data collected in this study complements the results previously obtained on other common species from this site.

Materials and methods

Collection of soil and plant samples

The fieldwork was conducted at the Allchar site, located on Mt. Kožuf in North Macedonia (N 41.158135°, E 21.94494°) (Figs. 1, 2, and 3). In total, 23 different plant species were collected across the site (Fig. 4) in at least four replicates. Flowering plants were randomly collected in each of the three deposits (Central Deposit, Crven Dol and the Southern Deposit, Fig. 1). The 'rhizospheric' soil near the roots of each collected plants (~5–10 cm deep) was also sampled to enable soil to plant relation assessments.

Elemental analysis of soil and plant samples

After collection, the soil samples were first dried in a ventilated oven at 40 °C for 48 h, with the

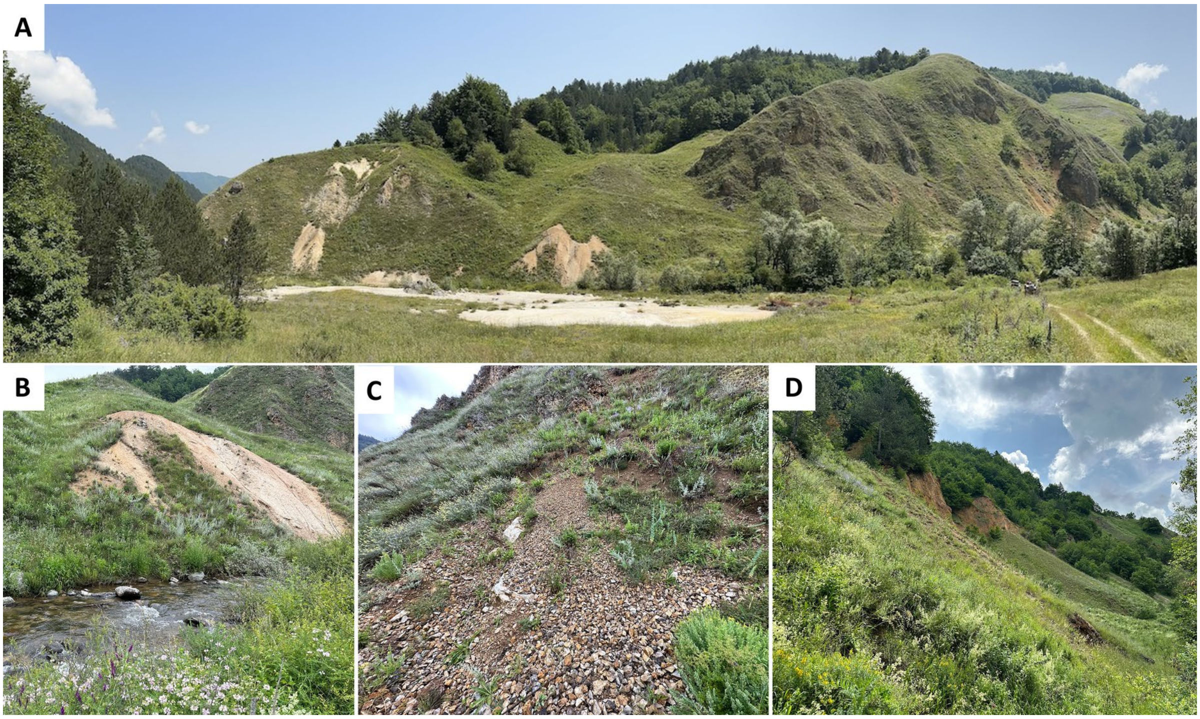


Fig. 2 The Allchar site in North Macedonia: (**A**) panoramic view of the site; (**B**) outcropping As-enriched mineralisation at the valley floor in the Central Deposit, (**C**) mineralised soils in the higher parts of the Central Deposit, (**D**) mine spoils under the audits of the South Deposit enriched in Sb

Fig. 3 Arsenic-thallium mineralisation at the Allchar site: outcropping gossan rich in lorándite (thallium arsenic sulfosalt) and realgar (arsenic sulfide) (**A**), strongly mineralised soil (**B**), soil sample with elemental sulphur and various sulphosalts (**C**)

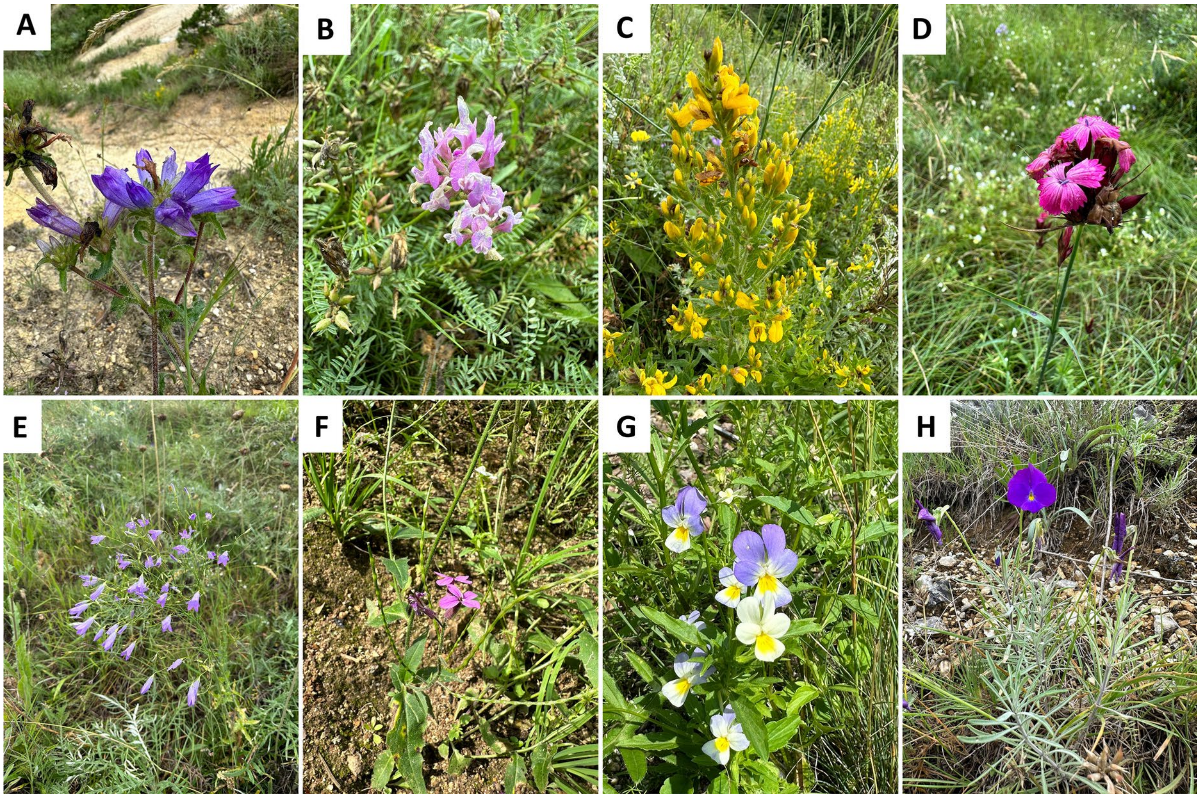
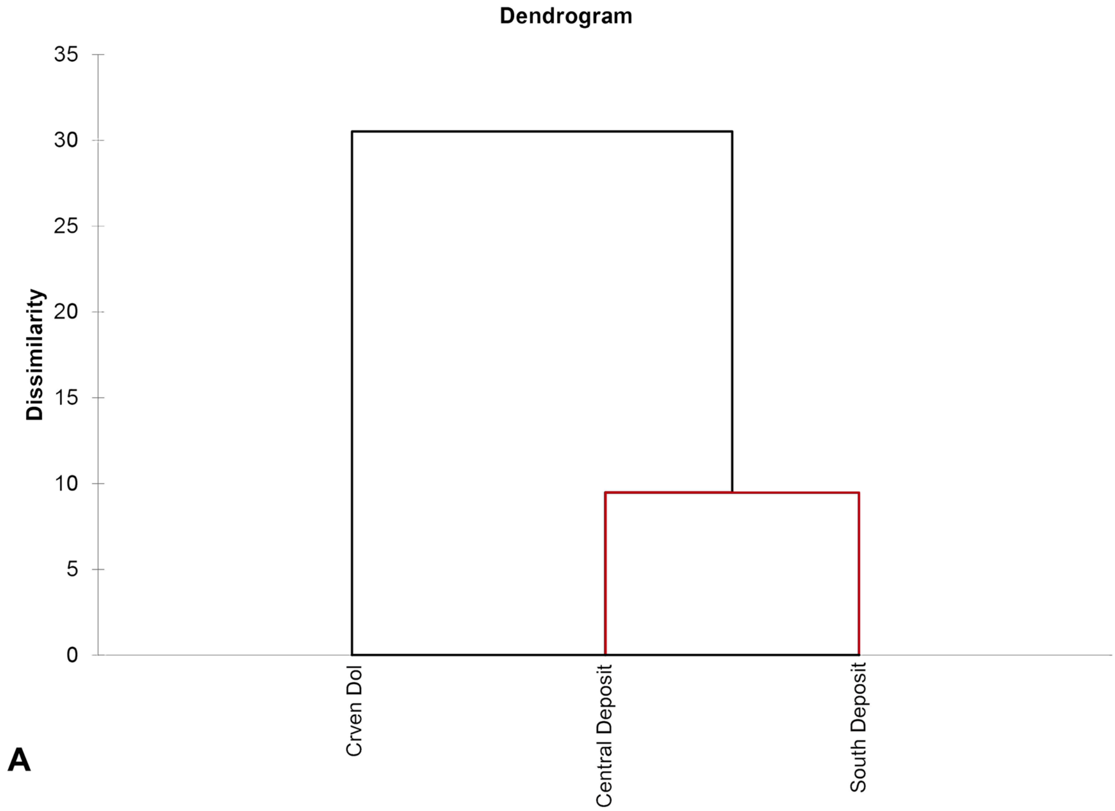


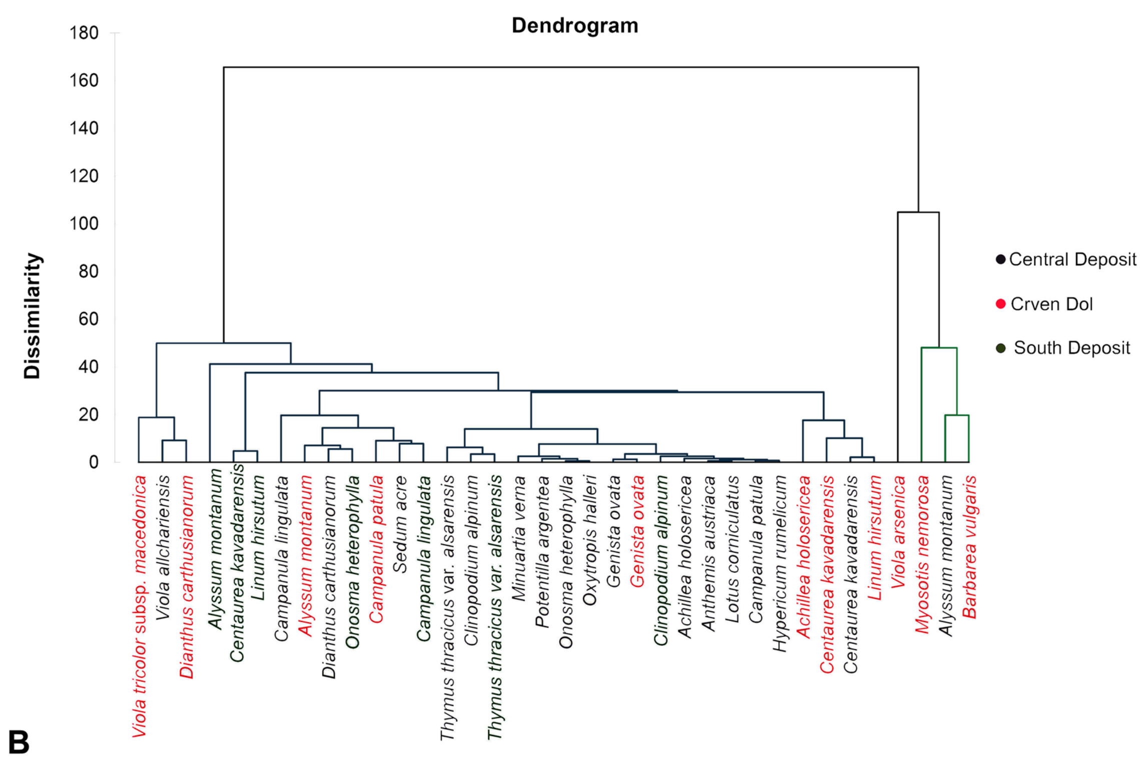
Fig. 4 Some of the plant species sampled at the Allchar site: *Campanula lingulata* (**A**), *Oxytropis halleri* (**B**), *Genista ovata* (**C**), *Dianthus carthusianorum* (**D**), *Campanula patula* (**E**), *Hesperis matronalis* (**F**), *Viola tricolor* subsp. *macedonica* (**G**), *Viola allchariensis* (**H**)

temperature kept purposely as low as possible (maximum encountered in the field) to avoid major chemical speciation changes that would affect the DTPA-extraction. The samples were then sieved at 1 mm using a plastic mesh and 0.5 g subsamples were placed in custom-made XRF sample holders and covered with a 6 µm thin polypropylene film (Chemplex Industries Inc.). The monochromatic X-ray fluorescence (XRF) analysis was carried out using a JP500 instrument (Z-Spec Inc., East Greenbush, NY) in 'Soil Mode'. Diethylenetriamine pentaacetate (DTPA)-extraction was used for the determination of potentially 'plant-available' metals, and it useful for a wide range of metal ions (e.g., Tl^{+}, Cu^{2+}, Ni^{2+}, Zn^{2+}), even if not developed specifically for this purpose but for diagnizing micronutrient deficiency. Here a miniaturized version was used to enable analysis with the XRF instrument. Briefly, 0.6 g of 1 mm sieved soil was extracted with 1.2 mL DTPA solution in 2 mL tubes with a solid–liquid ration of 1:2 following the basic formulation of Lindsay and Norvell (1978). The DTPA solution was freshly prepared mixing: 14.92 g L^{-1} triethanolamine (TEA), 1.47 g L^{-1} calcium dichloride dihydrate ($CaCl_2 \cdot 2H_2O$), 1.97 g L^{-1} DTPA in a flask and made up to 1 L volume with MilliQ water. The pH was adjusted to 7.3 with HCl. The tubes were shaken for 2 h using an overhead shaker (Trayster digital, IKA-Werke GmbH & Co. KG) and centrifuged at 10,000 rpm for 3 min, and then 0.5 ml of clean supernatant was recovered and brought to a volume of 2 mL in a new tube with MilliQ water. DTPA extracts were also tested using Z-spec JP500 in water mode.

The plant material (leaves) was first air-dried after collection for a total of 4 days until constant weight. The dried leaves samples were then subjected to a stringent cleaning procedure to remove any surficial dust contamination using hexane following the methods described in Reeves and Kruckeberg (2018); Paul et al. (2019). Briefly, dried



Dendrogram
Dissimilarity
0
5
10
15
20
25
30
35
Crven Dol
Central Deposit
South Deposit
A



Dendrogram
Dissimilarity
0
20
40
60
80
100
120
140
160
180
Central Deposit
Crven Dol
South Deposit
Viola tricolor subsp. macedonica
Viola allchariensis
Dianthus carthusianorum
Alyssum montanum
Centaurea kavadarensis
Linum hirsutum
Campanula lingulata
Alyssum montanum
Dianthus carthusianorum
Onosma heterophylla
Campanula patula
Sedum acre
Campanula lingulata
Thymus thracicus var. alsarensis
Clinopodium alpinum
Thymus thracicus var. alsarensis
Minuartia verna
Potentilla argentea
Onosma heterophylla
Oxytropis halleri
Genista ovata
Genista ovata
Clinopodium alpinum
Achillea holosericea
Anthemis austriaca
Lotus corniculatus
Campanula patula
Hypericum rumelicum
Achillea holosericea
Centaurea kavadarensis
Centaurea kavadarensis
Linum hirsutum
Viola arsenica
Myosotis nemorosa
Alyssum montanum
Barbarea vulgaris
B

◂**Fig. 5** Dendrograms of the three different mineralised areas in the Allchar (**A**) and associated plant species (**B**) based on plant elemental profiles

plant material samples (~ 1 g) were immersed in 20 mL of anhydrous hexane (HPLC-grade, ≥ 95%, Sigma-Aldrich) in 50 mL polyproplene tubes, then the tubes were placed in an ultrasonic bath for 60 s. The leaves were then carefully extracted from the tube and the hexane was left to evaporate from the plant material. The thus cleaned plant material was then dried in a ventilated oven at 60 °C for 48 h. Following on, the plant material was ground to a fine powder (< 200 µm) in an impact mill (TubeMill 100 Control, IKA-Werke GmbH & Co. KG) and 0.5 g subsamples were placed in custom-made XRF holders and analysed with the Z-spec JP500 instrument in 'Plant Mode', as described below.

The JP500 (Z-Spec Inc.) instrument uses monochromatic X-ray fluorescence excitation at 17.48 keV to analyse elements from $Z = 15$ (P) to $Z = 39$ (Y) using the K-lines and up to $Z = 92$ (U) using the L-lines with optimum sensitivity for elements Cu–Se and Hg-Tl-Pb with limits of detection the range of 0.009–0.025 µg g^{-1}. Samples were analysed for 30 s. in Soil, Plant or Liquid Mode as specified above. Two certified standards were included for the plant material analysis (NIST SRM 1570a Trace elements in spinach leaves and NIST SRM 1573a Tomato leaves), two certified standards were included for the soil analysis (NIST SRM 2702, marine sediments and NIST SRM 2709a San Joaquin soil for soils) and one certified standard was included for the liquid sample analysis (TraceCERT®, Multielement standard solution V for ICP (SUPELCO, St. Louis). The recovery was between the 95% and 105% for each of the elements tested.

Data processing and statistical analyses

The elemental concentrations in soil and plant samples are given as minimum, maximum and mean values. Significant differences between samples were determined using the Kruskal–Wallis H test, followed by Dunn's test for pairwise multiple comparisons ($p < 0.05$), and marked by different letters. Spearman correlation was performed for the total element concentrations in the soil samples and the corresponding concentrations in the plant samples. The agglomerative hierarchical clustering was carried out using Ward's method. Boxplots were created using R package ggplot2 v.3.4.3 (Wickham 2016). Correlogram was prepared in OriginPro 2023 software (software from OriginLab Corporation).

Results

Patterns in the mineralisation zones (Crven Dol, Central Deposit and South Deposit)

The geology of Allchar consists of three distinct zones (Fig. 5A) with the highest Tl and As enrichment in the northern part (Crven Dol), as indicated by their concentrations in the soil (Table 1). The mean value of Tl was 476 µg g^{-1} in the South Deposit up to 1720 µg g^{-1} Tl in the soils of Crven Dol, whilst the mean value of As ranged from 9220 µg g^{-1} in the South Deposit up to 24,200 µg g^{-1} As in the soils of the Central Deposit. The mean concentrations of As and Tl in plants were also significantly higher in the Crven Dol area (249 µg g^{-1} and 3970 µg g^{-1}, respectively) compared to the South Deposit (10.1 µg g^{-1} and 233 µg g^{-1}, respectively), including *S. latifolia* with almost 80,000 µg g^{-1} Tl in its leaves. Cluster analysis of the plant species based on elemental concentrations in the leaves indicate that *V. arsenica* from Crven Dol is the most distant (Fig. 5B), due to extreme Tl concentrations. The second cluster, based on increased As concentrations, includes *M. nemorosa* and *B. vulgaris* from Crven Dol and *A. montanum* from the Central Deposit, whereas the remaining species from Crven Dol, Central Deposit, and all the species from the South Deposit, form the third, most numerous cluster with less conspicuous differences between the taxa analysed. The elemental concentrations in plants and associated rhizospheric soil are provided in Tables S1-S4.

Alkali and alkaline Earth metals (K, Ca, Mg, Ba, Sr, Rb) in soils and plants

Macro-elemental concentrations varied greatly between samples, with Ca and Mg being the highest in the soil sample of *Barbarea vulgaris*, whilst K concentrations were the highest in the rhizospheric soil associated with *Linum hirsutum* (Table S1). Concentrations of Ba were low in most samples, but with notable enrichment and concentrations > 1000 µg g^{-1}

Table 1 The range of elemental concentrations in different parts of the Allchar area (in µg g^{-1})

Site	Central Deposit		Central Deposit		Crven Dol		Crven Dol		South Deposit		South Deposit	
Sample	Soil		Plant		Soil		Plant		Soil		Plant	
	min−max	mean	min−max	mean	min−max	mean	min−max	mean	min−max	mean	min−max	mean
K	0−15,400 a	6250	2600−30,800 a	11,100	0−14,700 b	6330	5060−32,800 b	16,300	0−20,300 ab	8220	8430−23,600 ab	13,300
Ca	0−520,000 a	129,000	1640−77,000 a	15,100	0−343,000 a	139,000	2240−77,300 a	15,200	0−251,000 a	94,300	4050−26,100 a	13,400
Mg	0−251,000 a	37,300	235−6300 a	1240	0−219,000 b	70,300	435−7350 b	1650	0−248,000 ab	84,300	375−2990 ab	1150
Ba	0−1390 a	53.4	0−267 a	25.4	<LOD a	<LOD	0−116 a	12.8	<LOD a	<LOD	3.68−45.4 a	15.3
Sr	61.7−1200 a	262	1.5−88.4 a	9.31	59.4−500 b	134	1.56−124 b	41.8	60.4−92 ab	78.3	2.45−22.6 ab	9.31
Rb	2.89−254 a	50.3	5.62−208 a	45.3	12.8−219 b	80.5	26.2−431 b	129	11.3−202 b	125	26.6−172 b	80.1
Cl	0−299 a	25.6	32.5−9410 a	1580	0−300 a	23.2	325−13,600 a	2510	0−75.3 a	13.4	297−2740 a	1270
Br	0−79.1 a	11.7	0.273−18.4 a	2.97	0−58.5 b	11.6	0.709−47.6 b	14.3	3.1−81.4 a	35.3	0.674−5.64 a	2.65
Fe	17,800−104,000 a	50,100	12.3−1270 a	197	28,100−183,000 a	68,200	25.1−4150 a	655	42,200−78,700 a	63,400	50.3−400 a	149
Mn	113−9950 a	1870	3.11−366 a	53.7	285−18,300 b	4880	5.56−296 b	104	224−1750 a	993	6.86−53.9 a	30.2
Cr	17−245 a	62.7	0−12.1 a	0.703	26.9−150 a	63.9	0−5.41 a	0.942	19.9−124 a	71.4	0.432−1.29 a	0.692
Cu	0−63.1 ab	18.8	1.64−12 ab	5.01	0−52.7 a	4.92	0.254−11 a	3.98	0−20.1 b	7.7	2.05−12.1 b	7.44
Zn	30.8−463 a	164	9.11−232 a	39.1	43.3−569 b	169	16−142 b	58.5	47.2−104 ab	67.1	11.2−116 ab	46.8
Pb	2.14−109 a	45.1	0−15.9 a	1.55	9.23−157 b	47	0.107−241 b	29.5	2.74−47.2 a	25.3	0.136−12.8 a	1.61
Al	0−106,000 a	69,400	0−3440 a	264	0−95,000 a	62,900	0−5720 a	619	0−76,500 a	41,200	42.1−302 a	124
Tl	0−5230 a	598	0−2280 a	192	354−5750 b	1720	6.55−31,600 b	3970	18.1−1000 ab	476	0.233−1960 ab	233
As	289−95,700 a	11,600	0−1190 a	50.6	1760−105,000 a	24,200	0−2490 a	249	916−21,700 a	9220	0.922−29.5 a	10.1
P	0−3070 a	1460	163−3320 a	945	0−4320 ab	491	319−3040 ab	1210	0−1140 b	496	582−3010 b	1370
S	0−19,600 a	1530	241−5520 a	1250	0−6380 b	1830	446−14,500 b	3560	0−3380 b	1500	817−14,300 b	2920
Se	<LOD b	<LOD	0−0.136 b	0.02	0−2.66 a	0.102	0−0.056 a	0.002	<LOD c	<LOD	0−1.31 c	0.558

Significant differences between the samples according to the Kruskal–Wallis ANOVA and the subsequent Dunn's post-hoc test are labelled with different letters in soil samples associated with *Alyssum montanum* and *Sedum acre*. In plants these concentrations were relatively low (up to 267 μg g^{-1} Ba in *Campanula lingulata* (Campanulaceae)).

Halogens (Cl, Br) in soils and plants

The Allchar deposit is enriched in Cl and Br, which is reflected in the soils containing up to 300 μg g^{-1} Cl (mean of 115 μg g^{-1}) and 81 μg g^{-1} Br (mean of 48 μg g^{-1}), respectively. The highest concentrations were found in soils associated with *V. arsenica* (Violaceae) and *B. vulgaris* (Brassicaceae) with up to 47.6 μg g^{-1} Cl and 35.4 μg g^{-1}Br.

Transition metals (Fe, Mn, Cr, Cu, Zn) in soils and plants

None of the transition metals is notably enriched in the Allchar deposit and the soils showed unremarkable concentrations, especially DTPA-extractable, with the highest values of 15.8 and 30.9 μg g^{-1} for Zn and Mn, respectively. For Pb and Cr these concentrations were even lower, up to 2.69 and 3.09 μg g^{-1} (Tables S1 and S2). This is also reflected in the plants, none of which have unusual concentrations of Fe, Mn, or Cu, and none are (hyper)accumulators (Table S3). The highest Zn concentrations occur in *Viola allchariensis* (up to 232 μg g^{-1}), far below the hyperaccumulation threshold (3000 μg g^{-1}) of this element. Concentrations of Cr in plant samples from Allchar were < 13 μg g^{-1}, considerably lower than those found in the soil.

Post-transition metals (Tl, Pb, Al) in soils and plants

The soils at the Allchar site are exceptionally enriched with Tl, with concentrations reaching up to 5750 μg g^{-1} Tl (Fig. 6A). The DTPA-extractable concentrations were significantly lower, < 5 μg g^{-1} in most samples, and the highest concentration of 24.1 μg g^{-1} Tl found in the soil associated with *A. montanum* from the Central Part (Table S2). The highest Tl concentrations occur in the leaves of *S. latifolia* (Caryophyllaceae), *V. arsenica*, and *V. tricolor* subsp. *macedonica* (Violaceae) with concentrations of 79,200, 31,600 and 11,700 μg g^{-1} Tl, respectively (Fig. 7A). With Tl concentrations above the threshold (> 100 μg g^{-1} Tl), *Clinopodium alpinum* (Lamiaceae), *Hesperis matronalis* (Brassicaceae), *Anthyllis vulneraria* (Fabaceae) and *Linum hirsutum* (Linaceae) are also hyperaccumulators of this element, with 2280, 2190, 1220 and 254 μg g^{-1} Tl in the leaves, respectively (S2A; Table S4). Thallium hyperaccumulation was found in all analysed samples of the Violaceae, Lamiaceae and Caryophyllaceae families. Soil concentrations of Pb were low (up to 157 μg g^{-1}) and no plant species accumulates this element.

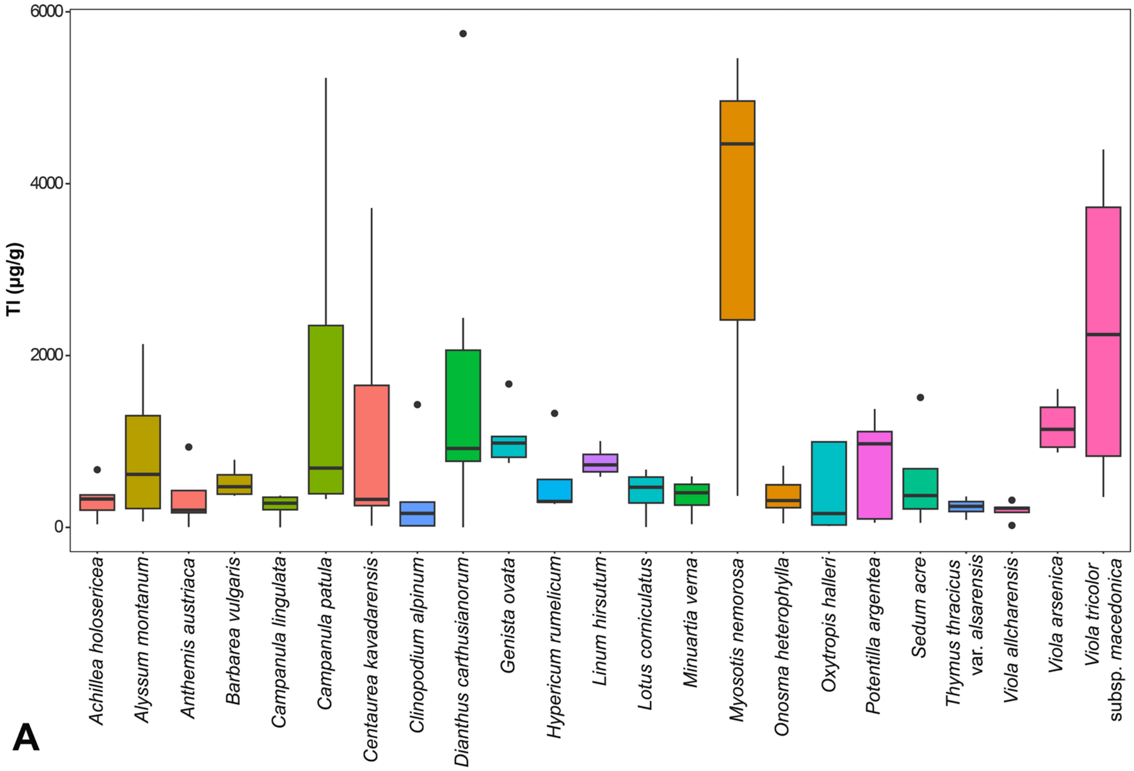
Nonmetals and metalloids (P, S, Se, As) in soils and plants

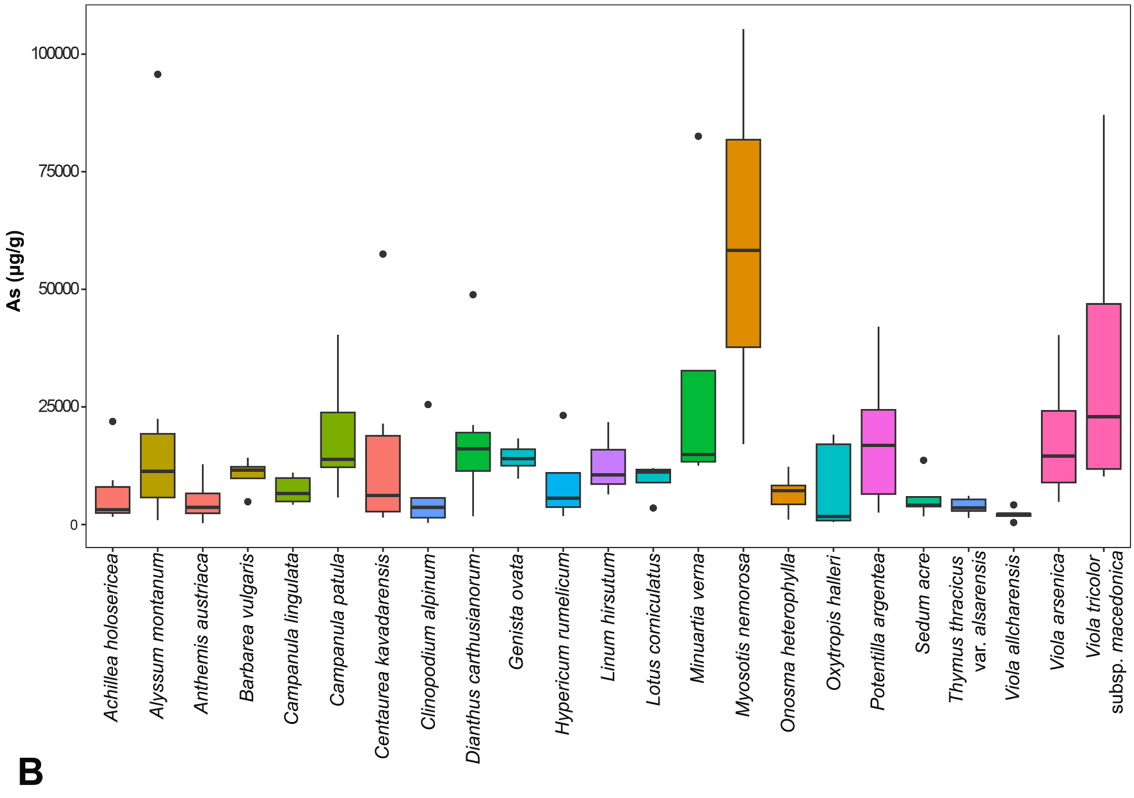
Prevailing soil concentrations of S are high to extremely high, up to 19,600 μg g−1 S as many ore minerals are sulphosalts. Although the total concentrations of As exceeded values of 100 g kg−1, as in the soil sample associated with Myosotis nemorosa from Crven Dol, the highest DTPA extractable concentration reached only 232 μg g−1 As (Tables S1 and S2; Fig. 6B). A particularly strong synergism was observed between the total concentrations of As and Tl, with a positive correlation also observed with the total concentration of Fe (Figure S1). Excessive concentrations of As were found in one sample each of A. montanum, B. vulgaris (Brassicaceae) and M. nemorosa (Boraginaceae), with 1190, 1280 and 2490 μg g−1 As of this element, respectively (Fig. 7B), but also in a sample of S. latifolia (Table S4).

Discussion

Elemental concentrations in Allchar plants and the associated soil samples

The flora of the Allchar area is unusually rich in plant species, despite that the soil elemental concentrations are considered toxic for most plant species, as for As and Tl, being well above the toxicity levels of 20 and 3 μg g^{-1}, respectively (Xiao et al. 2004; Garg and Singla 2011). The edaphic factor posed by the soil to survival of these plant species results in numerous plant adaptations, particularly in coping with extreme elemental concentrations, with response varying from the exclusion in the root to the accumulation of extremely high elemental concentrations in shoots. This pertains especially to the genus *Viola*, which is known not only for a strikingly high number of metallophytes, but also for true hyperaccumulators of a

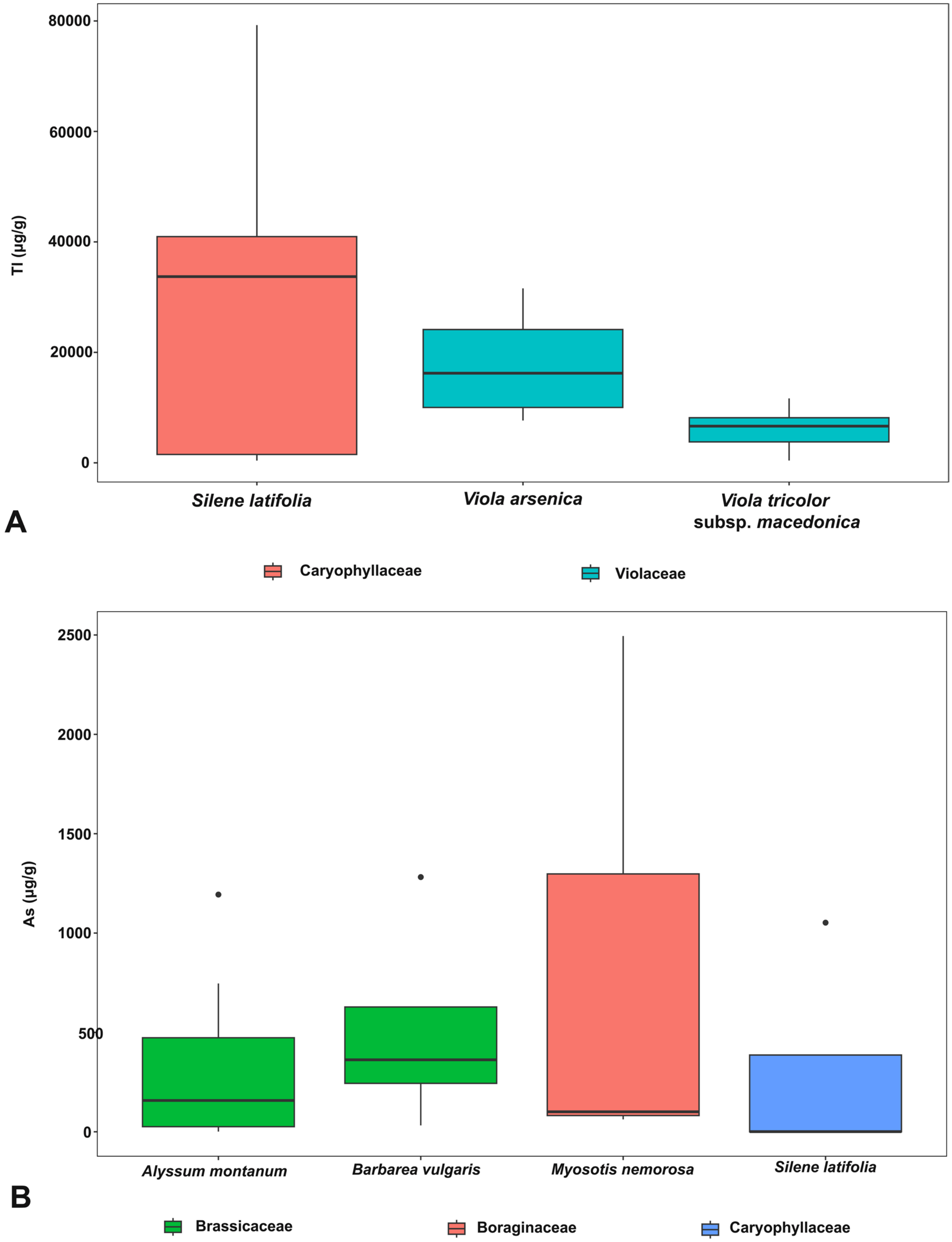

Tl (µg/g)
6000
4000
2000
0
Achillea holosericea
Alyssum montanum
Anthemis austriaca
Barbarea vulgaris
Campanula lingulata
Campanula patula
Centaurea kavadarensis
Clinopodium alpinum
Dianthus carthusianorum
Genista ovata
Hypericum rumelicum
Linum hirsutum
Lotus corniculatus
Minuartia verna
Myosotis nemorosa
Onosma heterophylla
Oxytropis halleri
Potentilla argentea
Sedum acre
Thymus thracicus var. alsarensis
Viola allcharensis
Viola arsenica
Viola tricolor subsp. macedonica
A


As (µg/g)
100000
75000
50000
25000
0
Achillea holosericea
Alyssum montanum
Anthemis austriaca
Barbarea vulgaris
Campanula lingulata
Campanula patula
Centaurea kavadarensis
Clinopodium alpinum
Dianthus carthusianorum
Genista ovata
Hypericum rumelicum
Linum hirsutum
Lotus corniculatus
Minuartia verna
Myosotis nemorosa
Onosma heterophylla
Oxytropis halleri
Potentilla argentea
Sedum acre
Thymus thracicus var. alsarensis
Viola allcharensis
Viola arsenica
Viola tricolor subsp. macedonica
B

◂**Fig. 6** Boxplots of minimum, maximum and mean total concentrations of Tl (**A**) and As (**B**) (in µg g^{-1}) in the soil samples associated with 23 plant taxa from Allchar

range of different metals and metalloids (Tomović et al. 2021), with three taxa recognised as (hyper) accumulators of As and Tl in the Allchar area (Bačeva Andonovska et al. 2021). Concentration of most of the elements analysed varied to a different extent, being mainly related to the geological characteristics of the area (Stafilov et al. 2024). The concentrations of K did not vary greatly either in the soil or in the plant. The same applies to Rb, except in the leaves of *Viola tricolor* subsp. *macedonica*, where the Rb concentrations > 400 µg g^{-1} were considerably higher than those previously known in most plants, noting that a hyperaccumulation threshold for Rb has not yet been recognised (van der Ent et al. 2021). Rubidium is known to compete with K for uptake in plants (Nyholm and Tiler 2000) and these two elements showed a statistically strong positive correlation in samples from Allchar (Table S5; Figure S1). Thallium is another element that competes with K during uptake in plants, as these two chemical analogues are hypothesised to use the same K^+ channels for uptake, even though in hyperaccumulators such as *Biscutella laevigata* any putative Tl-specific transporters have not been identified yet (Corzo-Remigio et al. 2022b). Concentrations of Sr and Ba were well below the provisional hyperaccumulation thresholds set at 3000 µg g^{-1} for Sr and 1000 µg g^{-1} for Ba (van der Ent et al. 2021), despite the micro-areas of strong mineralisation, where Sr and Ba concentrations exceeded 1000 µg g^{-1}. A similar pattern of Ba enrichment was found in sulphide-mineralised areas in Greece (Reeves et al. 1986). For both Ba and Sr, no clear correlations were found between the plant concentrations and the total concentrations found in the soil ($\rho = 0.072$ and $\rho = 0.163$ for Ba and Sr, respectively), suggesting that other factors may be responsible for controlling accumulation, as in the case of Ba, whose uptake is promoted in acidic soils (Madejón 2013). Unusual concentrations were also not observed for any of the transition metals (Fe, Mn, Cr, Cu and Zn). This is particularly true for Cr due to its low bioavailability and plant uptake (Reeves et al. 2018), and high foliar Cr concentrations are strongly indicative of surficial contamination rather than genuine accumulation. On the contrary, post-transition metals, such as Al and Tl, accumulate in concentrations that clearly exceed the hyperaccumulation thresholds. This holds especially true for Tl, with the Allchar site having the greatest resource and highest concentrations globally (Boev et al. 2012) and Tl hyperaccumulation has been detected in nine plant taxa analysed from Allchar. The highest Tl concentrations were found in the leaves of *S. latifolia*, up to 79,200 µg g^{-1}, making it the strongest hyperaccumulator of Tl, as the highest foliar Tl concentration recorded in field growing plants was 1500 µg g^{-1} (Escarré et al. 2011), although this can reach up to 35,700 µg g^{-1} Tl in the leaves of hydroponically grown plants exposed to 30 µM Tl (Corzo Remigio et al. 2022b). This Tl concentration was all the more striking considering that the total Tl concentration in the soil was 862 µg g^{-1} Tl of which only 3.01 µg g^{-1} was DTPA extractable Tl. Note, however, that DTPA is not the best method for estimating Tl available Tl, refer to Jia et al. (2018). *Alyssum montanum*, *Clinopodium alpinum*, *Dianthus carthusianorum*, and *M. nemorosa* were not previously known to hyperaccumulate Tl, whereas in *Thymus thracicus* var. *alsarensis* and *Viola tricolor* subsp. *macedonica* Tl concentrations reported here significantly exceed the values observed in previous studies (Bačeva Andonovska et al. 2021; Jakovljević et al. 2023), but not in *V. arsenica* (58,900 µg g^{-1}) which grows in the Allchar area on soil significantly less enriched in this element (Jakovljević et al. 2023). The Violaceae is the family in which Tl hyperaccumulation was found in all taxa studied and with exceptionally high concentrations of this element. This feature led to its distinction from the other families, as shown by the cluster analysis (Figure S3). In most of the plants, Al concentrations were below 1000 µg g^{-1} (Kabata Pendias 2000) being the threshold for designation of Al accumulators (Metali et al. 2012) and hyperaccumulators (Jansen et al. 2002; van der Ent et al. 2021). Foliar Al concentrations were the highest in *B. vulgaris* (up to 5700 µg g^{-1}), whereas > 1000 µg g^{-1} Al was also found in the leaves of *A. montanum*, *C. lingulata* and *M. nemorosa*. However, these Al concentrations were still far from the extreme values found in tropical *Symplocos* spp., where 70,000 µg g^{-1} has been recorded (*e.g. S. spicata*; Faber 1925).

Besides Tl, As is the element most enriched in soil samples throughout the Allchar area (Đorđević et al. 2021). However, this did not result in unusually high foliar As concentrations in most plant species,


80000
60000
40000
20000
0
Tl (µg/g)
Silene latifolia
Viola arsenica
Viola tricolor subsp. macedonica
A
Caryophyllaceae
Violaceae
2500
2000
1500
1000
500
0
As (µg/g)
Alyssum montanum
Barbarea vulgaris
Myosotis nemorosa
Silene latifolia
B
Brassicaceae
Boraginaceae
Caryophyllaceae

◂**Fig. 7** Boxplots of minimum, maximum and mean concentrations of Tl (**A**) and As (**B**) (in µg g^{-1} DW) in the leaf samples from Allchar. The species with concentrations of Tl > 10,000 µg g^{-1} and As > 1000 µg g^{-1} were presented

suggesting exclusion as the main strategy for most plant to tolerate high As soils. The most important mechanism for As uptake includes the phosphate transporters, a very numerous group comprising more than 100 transporters within the phosphate transporter 1 family alone (Bucher 2007). Their suppression is one of the most important methods of reducing As uptake, together with the reduction of arsenate (As(V)) to arsenite (As(III)), the form that is not analogous to phosphate and is therefore easily re-exported to the soil (Meadows 2014). Hyperaccumulation concentrations levels of As were recorded in *A. montanum*, *B. vulgaris* and *M. nemorosa*, however only in one sample, and due to the erratic nature of As accumulation in these species it must be considered suspect and hence not classified as hyperaccumulation (> 1000 µg g^{-1} As). The anomalous As concentrations in these samples of *A. montanum* and *M. nemorosa* may be due to inadvertent uptake of extremely high As concentrations in the soil (even > 100,000 µg g^{-1}), whereas the total concentrations in *B. vulgaris* were considerably lower (4870 µg g^{-1}), and not much higher than those in the leaves. A relatively high As concentration (1050 µg g^{-1}), above the hyperaccumulation threshold (Reeves 2005), was also found in a sample of *S. latifolia*. Considering the Tl foliar concentrations of 33,700 µg g^{-1}, *S. latifolia* is a co-accumulator of these two elements, which is a rare phenomenon observed so far only in *V. arsenica* and *M. verna* (Jakovljević et al. 2023, 2024) from the same area, as well as in *Pteris vittata*, the known hyperaccumulator of As (Wei et al. 2020).

Patterns in the mineralisation zones (Crven Dol, Central Deposit and South Deposit)

The distribution of As and Tl in the Allchar are showed the similar pattern, with the highest values, both in the soil and plant, in Crven Dol, and lower concentrations in areas of Central and particularly of South Deposit. This is in line with area characteristics, and prevailing of As and Tl occurrence in Crven Dol (the northern part of deposit) with the mean mineralisation grade of 6 wt% As and 0.3 wt% Tl in the ore body (Boev et al. 2012). In addition to the As and Tl contents, Central Deposit is also characterised by strong Ba and Sr mineralisation, but is also enriched in Zn, Cu and Pb (Table 1; Amthauer et al. 2012). The decrease in temperature in the direction from south to north during ore formation most likely caused this variation in mineralisation (Amthauer et al. 2012).

However, the differences in elemental concentrations observed in the soil were far less noticeable in the plants growing on these soils. The greatest differences between species were found in the concentrations of As and Tl, with five species singled out as reaching the highest concentrations of these two elements (Tl in *S. latifolia* and *V. arsenica* and As in *M. nemorosa*, *B. vulgaris*, all from Crven Dol and *A. montanum* from the Central Deposit). Significantly lower elemental concentrations in the plant samples from South Deposit distinguish this area even more clearly from the other two areas at the Allchar site.

Conclusions

This study has revealed the peculiarities of the elemental profile ('elementome') of plant species from the Allchar area, with *Anthyllis vulneraria*, *Clinopodium alpinum* and *Linum hirsutum* representing newly discovered hyperaccumulators of Tl. In addition, the Tl concentrations at nearly 8 wt% in the leaves of *Silene latifolia* are the highest recorded thus far in nature, opening the possibilities for plant utilisation in phytomining of Tl on contaminated sites. There are few localities globally more extreme in terms of toxicity of the soils than Allchar, making it the perfect natural laboratory for studying soil–plant interactions and the adaptability of plant species to extreme trace element concentrations in situ. The study of areas with highly adverse geochemistry is of particular interest to better understanding plant adaptation and evolution, which is why additional analyses under control conditions will further complement the results obtained, focusing on elemental uptake, translocation to the aerial parts and localisation of predominant site of elemental accumulation.

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Author contributions K.J., T.M., K.B.A., M.S. and A.vd.E collected the samples in the field. T.M. and A.vd.E prepared the samples and conducted the XRF analysis. K.J. conducted the statistical analysis and prepared the figures. K.J. and A.vd.E wrote the first draft of the manuscript.

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

Declarations

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

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