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Exploring Ni-accumulation in serpentinophytic taxa of Brassicaceae from Albania and Greece

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ABSTRACT

The family Brassicaceae includes the highest proportion of Ni-hyperaccumulating plants in western Eurasia. Though increasingly relevant for scientific research and biotechnological applications, data about metal concentrations in native populations are still incomplete. The Balkan peninsula is a major diversity centre for Ni-hyperaccumulators due to the wide distribution of ultramafic soils across the area. Using Atomic Absorption Spectrophotometry and material from our field collections, we determined Ni concentration in 31 accessions of 22 taxa from nine tribes: Aethionemeae, Alysseae, Arabideae, Cardamineae, Coluteocarpeae, Erysimeae, Hesperideae, Iberideae and Isatideae. Results confirm that Ni-hyperaccumulation ability is phylogenetically restricted to Alysseae and Coluteocarpeae. Highest Ni-concentrations and bioaccumulation factors were found in *Bornmuellera*, *Odontarrhena* and *Noccaea*. Plants of the facultative serpentinophyte *O. chalcidica* from the type locality displayed shoot Ni levels $> 2000 \mu\text{g g}^{-1}$ DW despite growing on non-ultramafic soils with low Ni. This resulted in a remarkably high bioaccumulation factor (91.7). High and low shoot Ni concentrations were instead detected in *O. muralis* accessions from serpentine and non-serpentine sites, respectively. Hyperaccumulation was confirmed in the endemic *O. euboica*, for which previous reports were contrasting. Shoot Ni concentration was negatively related to soil Ni concentration in non-accumulating taxa, suggesting efficient exclusion mechanisms on Ni-rich soils.

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Introduction

Hyperaccumulation is the ability of some plants to accumulate trace metals in the leaves to concentrations that are hundreds or thousands of times higher than those in the soil (Reeves et al. 2018). This ability is found in about 720 angiosperms and was shaped by evolution under multiple selection pressures, including herbivore and pathogen attack, interspecific competition, and drought (Bhatia et al. 2005; Revell et al. 2008; El Mehdawi et al. 2011; Goolsby and Mason 2015). Hyperaccumulators are a remarkable resource for investigating the physiology, genetic control and molecular mechanisms of metal accumulation, as well as the evolution and adaptation of plants to extreme environments (Peer et al. 2005; Bech et al. 2014). Moreover, their unique ability can be exploited in field applications, such as phytoremediation and agromining (Marques et al. 2009; van der Ent et al. 2015), where they are used to extract trace metals from mineralised soils and processed to obtain high-grade bio-ores (Chaney et al. 2007; Losfeld et al. 2015; van der Ent et al. 2015; Kidd et al. 2018).

Nickel (Ni) is the most frequently accumulated metal, with as many as 532 angiosperm species in 53 families and 130 genera worldwide that are specialized for it (Reeves et al. 2018; see also Van der Pas and Ingle 2019). According to

Reeves et al. (2018), Ni accumulation ability is characteristic to those plants that contain the metal at concentrations $> 1000 \mu\text{g g}^{-1}$ dry weight (DW) in their aerial tissues. Here, concentrations are 2–3 orders of magnitude greater than in most plants growing on comparable soils, which are known as “excluders” (van der Ent et al. 2013). Such plants are often obligate endemics of metalliferous soils derived from ultramafic rocks that are naturally enriched in Ni and other trace metals like chromium and cobalt (Baker and Brooks 1989; Reeves 2003). However, some Ni-hyperaccumulating species are facultative and can grow either on or off ultramafic soils (Pollard et al. 2014).

Although Ni-hyperaccumulators are becoming increasingly important both for scientific research (Peer et al. 2005; Bech et al. 2014), and biotechnological applications (van der Ent et al. 2015; Kidd et al. 2018; Nkrumah et al. 2018; Bani et al. 2021), information on metal concentrations in native plant populations with respect to soil metal composition is still incomplete. This also applies to Euro-Mediterranean hyperaccumulators, the majority of which belongs to the Brassicaceae family. At present, 80 species or nearly 12% of all known hyperaccumulators are included in this group (Reeves et al. 2018; Hopewell et al. 2021). The Mediterranean Region includes several centres of distribution for Ni-hyperaccumulating plants, the most diverse of which are

found in the Balkan peninsula. This region is characterized by the widespread occurrence of ultramafic soils along broad latitudinal and altitudinal gradients, from Serbia to southern Greece and from sea level to over 2500 meters in altitude (Brooks 1987; Stevanovic et al. 2003). Brassicaceae are a prominent component of the serpentine vegetation of these areas, with several herbaceous or frutescent species often forming large populations especially on exposed rocky or gravelly outcrops (Hopewell et al. 2021).

Previous studies on some groups of Ni-hyperaccumulators in this region showed a large variability in accumulation capacity by obligate and facultative serpentinophytes of Brassicaceae from different soil types (Bani et al. 2009; 2010; 2013). Recent investigations focused on the genus *Odontarrhena* C.A.Mey. ex Ledeb. from Albania, with the aim of bringing clarity on the complicated systematics of this group (Cecchi et al. 2018) and establishing unequivocal correspondence between taxonomic units and Ni-accumulation with respect to soil conditions (Bettarini et al. 2019; 2021). Further studies have been dedicated to single taxa in this genus with peculiar metal accumulation behaviour, such as *O. sibirica* (Willd.) Španiel, Al-Shehbaz, D.A.German & Marhold (Bettarini et al. 2020) or to newly described taxa such as the Greek endemic *O. stridii* Cecchi, Španiel & Selvi (Cecchi et al. 2020) or the Italian endemic *O. bertolonii* (Desv.) Jord. & Fourr. subsp. *cesalpina* Selvi (Selvi and Vivona 2021). New data about metal accumulation in Brassicaceae from the Balkans and the Aegean region have been provided by other recent investigations (Paçarizi et al. 2020; Reeves et al. 2022), showing the relevance of this topic and the need to advance knowledge about the geographic and systematic distribution of this ability in the family for further research and applications.

During recent field excursions across different ultramafic areas of Albania and Greece, plant material of obligate or facultative serpentine taxa of Brassicaceae belonging to nine clades was collected, namely the tribes Aethionemeae, Alyseae, Arabideae, Cardamineae, Coluteocarpeae, Erysimeae, Hesperideae, Iberideae and Isatideae, as defined by Koch et al. (2018; see also Francis et al. 2021). By applying Atomic Absorption Spectrophotometry (AAS), it was possible to assess the levels of Ni in root and shoot tissues and to evaluate the accumulation capacity of taxa and accessions in relation to concentrations of Ni in the corresponding native soil. Results of this survey brought new evidence about Ni accumulation across Brassicaceae and in still incompletely known species of *Odontarrhena* such as *O. muralis*, *O. chalcidica* and *O. euboea*, thus potentially contributing to the recently instituted Global Hyperaccumulator Database (Reeves et al. 2018).

Accordingly, the major aims of this article are: i) to increase the current body of knowledge about Ni-hyperaccumulating plants in the Euro-Mediterranean region; ii) to contribute new data about the phylogenetic and geographic distribution of Ni-accumulation across Brassicaceae, and iii) to provide further insights into the relationship between soil and plant Ni concentration, based on a representative sample of excluding and accumulating taxa from a broad range of Albanian and Greek serpentine sites.

Materials and methods

Plant and soil material

Plant material was collected in 17 sites, five of which in central and southern Albania and 12 in continental Greece, in the summer of 2017, 2018 and 2019 (Figure 1). Plants were mainly growing on ultramafic outcrops, but we also included accessions from non-serpentine soil in the case of a few facultative serpentinophytes in the genera *Odontarrhena*, *Erysimum* L. and *Rorippa* L. At each site, three to five plants were collected for chemical analyses, plus a complete voucher specimen to be used for identification. This was performed by means of *Flora Hellenica* (Strid 1983) and *Flora Europaea* 2nd edition (Tutin et al. 1993), also checking data in the Web Flora of Greece (<http://portal.cybertaxonomy.org/flora-greece/intro>). In total, we analysed 31 accessions belonging to 22 specific or sub-specific taxa in 12 genera and 9 tribes: Aethionemeae Al-Shehbaz, Beilstein & E.A.Kellogg (*Aethionema* R. Br.), Alyseae DC. (*Alyssum* L., *Bornmuellera* Hausskn., *Odontarrhena* C.A.Mey.), Arabideae DC. (*Draba* L.), Cardamineae Dumort. (*Cardamine* L., *Rorippa* Scop.), Coluteocarpeae V.I.Dorof. (*Noccaea* Moench), Erysimeae Dumort. (*Erysimum* L.), Hesperideae Prantl (*Hesperis* L.), Iberideae Webb & Berthel. (*Iberis* L.) and Isatideae DC. (*Isatis* L.). Vouchers are deposited in the *Herbarium Centrale Italicum* at the University of Florence (FI). In addition, we analysed shoot tissue samples of herbarium specimens from Greece of *Bornmuellera baldaccii* (Degen) Heywood subsp. *rechingeri* Greuter and *Odontarrhena euboea* (Halácsy) Španiel, Al-Shehbaz, D.A.German & Marhold. Finally, a collection of *B. cappadocica* (DC.) Cullen & T.R.Dudley from east Turkey (Van, 2700 m; *Rechinger*, 53873, B) was also included in the study, as this species was never assessed before for Ni accumulation. Complete information about examined taxa and accessions from Greece and Albania is given in Table 1.

Soil samples were also collected in 15 of the localities explored (Table 2). At these localities, five aliquots of soil of ca. 40 g each were randomly collected within the plant population area, at 1–10 cm of depth; these were then pooled together to obtain a single bulk soil sample of ca. 200 g for subsequent analyses.

Determination of nickel concentration

Dry plant samples were carefully washed for ten minutes with milliQ-water and then dried at 50 °C for 48 h (Selvi et al. 2017). Samples (0.1 g) were then mineralized by a microwave-assisted digestion (Mars 6, CEM, USA) with 10 mL of concentrated HNO₃ (69%, for analysis, ACS, ISO) at 200 °C for 10 min, and finally diluted to 25 mL with milliQ-water. Each of the five samples was replicated three times, to obtain a mean value. Certified reference materials (LGC No 7162) were used to verify the accuracy and precision of the methods, whose values were <10% RDS.

Soil samples were sieved with a 2 mm mesh stainless steel sieve and placed in an oven at 50 °C for 7 days (Bettarini et al. 2019). From each sample, five subsamples of about 0.5 g were digested using 10 mL of 69% HNO₃ in a microwave digestion system, with the same procedure described above.



Figure 1. Geographical location of plant and soil collection sites; plant accession codes as in Table 1; corresponding soil codes are given in Table 3.

After digestion, Ni concentration in plant and soil samples was determined by means of an atomic absorption spectrophotometer (PinAAcle 500, Perkin Elmer, Waltham, Massachusetts, USA) with a detection limit of 6 ng mL^{-1} . To obtain the calibration line, standard solutions were prepared (0.5, 1, and 2 ppm Ni) by diluting available commercial Ni stock solution (PerkinElmer).

To assess the metal translocation ability of the plant and its efficiency in accumulating Ni compared to the metal concentration in the soil, the translocation factor (TF) and the bioaccumulation factor (BAF) were finally calculated for each sample, as follows (Masarovičová et al. 2010):

$$\text{TF} = [\text{Ni}]_{\text{shoot}} / [\text{Ni}]_{\text{root}}$$

$$\text{BAF} = [\text{Ni}]_{\text{shoot}} / [\text{Ni}]_{\text{soil}}$$

Mean TF and BAF values were obtained as average of shoot/root ratio and shoot/soil Ni concentration ratio of each sample, respectively.

Data analyses

Mean values of shoot, root and soil Ni concentration were compared by one-way ANOVA followed by Tukey *post-hoc* test. The Shapiro-Wilk test was used to check data normality.

A hierarchical cluster analysis was performed for classifying the specimens based on Ni accumulation capacity (Yang et al. 2014). The classification was based on shoot metal concentration, BAF, and TF values, therefore only specimens for which Ni concentrations were available for roots, shoots, and soil of origin were included in the analysis (19 specimens out of 31, 6 tribes out of 9, Table 3). UPGMA clustering was performed with Past version 4.03 (Hammer et al. 2001) using the Bray-Curtis similarity index.

Table 1. List of the examined specimens with country of origin (Albania or Greece), collection locality, accession code, collectors and vouchers (Herbarium codes follow *Index Herbariorum*).

	Tribe/Taxon	Collection locality	Lat - Long	Altitude (m)	Code	Collectors and voucher
	Aethionemeae Al-Shehbaz, Beilstein & E.A.Kellogg					
1	<i>Aethionema saxatile</i> R.Br. subsp. <i>graecum</i> (Boiss. & Spruner) Hayek	Greece, Euboea, Limni, serpentine rocks	38.7589 N, 23.3254 E	10	<i>Asg</i>	Selvi & Bettarini (FI056355)
	Alysseae DC.					
2	<i>Alyssum densistellatum</i> T.R.Dudley.	Greece, Euboea, Limni, serpentine rocks	38.7589 N, 23.3254 E	10	<i>Ad</i>	Selvi & Bettarini (FI056352)
3	<i>Bornmuellera baldaccii</i> (Degen) Heywood subsp. <i>baldaccii</i>	Albania, Lurë mountains, towards the summit peak	41.7669 N, 20.1834 E	1970	<i>Bb1</i>	Cecchi & Selvi (FI058352)
4	<i>Bornmuellera baldaccii</i> (Degen) Heywood subsp. <i>baldaccii</i>	Greece, Grevena, Mt. Vasilitsas, serpentine slopes	40.0544 N, 21.0801 E	1800	<i>Bb2</i>	Selvi & Bettarini (FI056365)
5	<i>Bornmuellera baldaccii</i> (Degen) Heywood subsp. <i>rechingeri</i> Greuter	Greece, Grevena, W of Smixi	40.0566 N, 21.0930 E	1625	<i>Bbr</i>	Willing 247.194 (B)
6	<i>Bornmuellera emarginata</i> (Boiss.) Rešetnik	Greece, Metsovo near Katara Pass	39.7949 N, 21.2028 E	1600	<i>Be1</i>	Selvi & Bettarini (FI055789)
7	<i>Bornmuellera emarginata</i> (Boiss.) Rešetnik	Greece, south of Kedhros, Sofaditis valley, serpentine	39.1809 N, 22.0443 E	260	<i>Be2</i>	Selvi & Bettarini s.no.
8	<i>Bornmuellera tymphaea</i> Hausskn.	Greece, Metsovo, near Katara pass, serpentine slopes	39.7949 N, 21.2028 E	1610	<i>Bt</i>	Selvi & Bettarini (FI055791)
9	<i>Odontarrhena chalcidica</i> (Janka) Španiel, Al-Shehbaz, D.A.German & Marhold.	Greece, Chalkidiki Gomati, schistous slopes	40.3918 N, 23.8049 E	156	<i>Oc1</i>	Selvi & Bettarini (FI055805)
10	<i>O. chalcidica</i> (Janka) Španiel, Al-Shehbaz, D.A.German & Marhold.	Greece, Thrace, Dadia, serpentine soil	41.1609 N, 26.2140 E	240	<i>Oc2</i>	Selvi & Bettarini (FI055810)
11	<i>Odontarrhena euboea</i> (Halácsy) Španiel, Al-Shehbaz, D.A.German & Marhold.	Greece, Euboea, Limni, serpentine rocks	38.7589 N, 23.3251 E	11	<i>Oe1</i>	Selvi & Bettarini (FI056354)
12	<i>O. euboea</i> (Halácsy) Španiel, Al-Shehbaz, D.A.German & Marhold.	Greece, Euboea, Papades, serpentine rocks	–	–	<i>Oe2</i>	Rechinger 19311 (B)
13	<i>O. euboea</i> (Halácsy) Španiel, Al-Shehbaz, D.A.German & Marhold.	Greece, Euboea Mt. Kandili, serpentine soil	–	550	<i>Oe3</i>	Rechinger 16416 (B)
14	<i>Odontarrhena muralis</i> (Waldst. & Kit.) Endl.	Greece, Loutraki, west foot of Gerania Ori, serpentine soil	37.9758 N, 23.0318 E	250	<i>Om1</i>	Selvi & Bettarini (FI056347)
15	<i>Odontarrhena muralis</i> (Waldst. & Kit.) Endl.	Greece, East Macedonia, north of Paranesti, granite rocks	41.3665 N, 24.4458 E	260	<i>Om2</i>	Selvi & Bettarini (FI055807)
	Arabideae DC.					
16	<i>Draba lasiocarpa</i> Adams	Albania, near Voskopojë, serpentine stony ground	40.5988 N, 20.5957 E	1430	<i>DI</i>	Cecchi & Selvi (FI051787)
	Cardamineae Dumort.					
17	<i>Cardamine glauca</i> Sprengel ex DC.	Albania, Bulqizë to Krastë, serpentine rocks	41.1753 N, 20.1747 E	1030	<i>Cg</i>	Cecchi & Selvi (FI051786)
18	<i>Cardamine glauca</i> Sprengel ex DC.	Greece, Grevena, Mt. Vasilitsas, serpentine rocks	40.0539 N, 21.0828 E	1800	<i>Cg2</i>	Selvi & Bettarini (FI056368)
19	<i>Cardamine plumieri</i> Vill.	Albania, Korçë, Mt. Moravë serpentine rocks	40.5772 N, 20.7955 E	1020	<i>Cp</i>	Cecchi & Selvi (FI051785)
20	<i>Rorippa pyrenaica</i> (All.) Rchb.	Albania, Korçë, Voskopojë, serpentine stony ground	40.6003 N, 20.5926 E	1430	<i>Rp1</i>	Cecchi & Selvi (FI051788)
21	<i>Rorippa pyrenaica</i> (All.) Rchb.	Greece, Grevena, Mt. Vasilitsas, serpentine slopes	40.0539 N, 21.0828 E	1800	<i>Rp2</i>	Selvi & Bettarini (FI055795)
22	<i>Rorippa sylvestris</i> (L.) Besser.	Albania, Korçë, Drenovë, humid serpentine soil	40.5835 N, 20.7937 E	960	<i>Rs</i>	Cecchi & Selvi (FI051798)
	Coluteocarpeae V.I. Dorof.					
23	<i>Noccaea ochroleuca</i> (Boiss. & Heldr.) F.K.Mey.	Greece, Konitsa, Aaos river valley, serpentine rocks	40.0241 N, 20.9847 E	80	<i>No</i>	Selvi & Bettarini (FI056371)
24	<i>Noccaea tymphaea</i> (Hausskn.) F.K.Mey.	Greece, Metsovo, Katara pass, serpentine soil	39.7908 N, 21.2291 E	1705	<i>Nt</i>	Selvi & Bettarini (FI055794)
	ErysimeCae Dumort.					
25	<i>Erysimum crassistylum</i> C.Presl	Greece, Kalambaka to Kastania, serpentine rocks	39.7388 N, 21.4961 E	330	<i>Ec1</i>	Cecchi & Selvi (FI056358)
26	<i>Erysimum crassistylum</i> C.Presl	Greece, Grevena, Mt. Vasilitsas, serpentine soil	40.0539 N, 21.0828 E	1800	<i>Ec2</i>	Selvi & Bettarini (FI056369)
27	<i>Erysimum pusillum</i> Bory & Chaub. subsp. <i>microstylum</i> (Hausskn.) Hayek	Albania, Shkumbin valley between Elbasan and Librazhd, serpentine soil	41.1819 N, 20.2736 E	220	<i>Epm</i>	Cecchi & Selvi (FI051792)
	Hesperideae Prantl					
28	<i>Hesperis laciniata</i> All. subsp. <i>laciniata</i>	Greece, Kalambaka to Kastania, serpentine hill	39.7388 N, 21.4939 E	330	<i>Hll</i>	Selvi & Bettarini (FI056361)

(Continued)

Table 1. (Continued)

	Tribe/Taxon	Collection locality	Lat - Long	Altitude (m)	Code	Collectors and voucher
Iberideae Webb & Berthel.						
29	<i>Iberis carnosa</i> Willd.	Greece, between Korinthos and Kato Alepochori, serpentine gravels	38.0560 N, 23.0837 E	10	<i>lc</i>	Selvi & Bettarini (FI056349)
30	<i>Iberis sempervirens</i> L.	Albania, Mts. Lurë, serpentine rocks	41.7780 N, 20.1935 E	1740	<i>ls</i>	Cecchi, Selvi & Bettarini (FI058315)
Isatideae DC.						
31	<i>Isatis tinctoria</i> L. subsp. <i>tinctoria</i>	Albania, Shkumbin valley between Elbasan and Librazhd, serpentine rocks	41.1820 N, 20.2760 E	220	<i>ltt</i>	Cecchi & Selvi (FI051791)

Table 2. List of examined soils with collection locality, soil code and Ni concentration ($\mu\text{g g}^{-1}$ DW), values are mean of n samples \pm standard deviation.

Soil code	Locality	Lat - Long	Altitude m (a.s.l.)	[Ni]soil
S.1	Albania, Bulqizë, Krastë	41.1753 N, 20.1747 E	1303	5193 \pm 295 (n=5) a
S.2	Greece, Epirus, Metsovo, towards Katara pass	39.7949 N, 21.2028 E	1610	4277 \pm 157.11 (n=3) b
S.3	Albania, Shkumbin valley between Elbasan and Librazhd	41.1819 N, 20.2736 E	220	3460 \pm 75 (n=5) c
S.4	Albania, Dibër, Lurë mountains	41.7669 N, 20.1834 E	1970	3336 \pm 100.28 (n=6) c
S.5	Greece, Epirus, Metsovo, Katara pass	39.7962 N, 21.2295 E	1705	2494 \pm 109.7 (n=5) d
S.6	Albania, Korçë, Drenovë	40.5772 N, 20.7955 E	960	2451 \pm 287 (n=5) e
S.7	Greece, Thrace, north of Dadia	41.1609 N, 26.2140 E	240	2443 \pm 97.49 (n=4) e
S.8	Greece, Loutraki, western foot of Gerania Ori	37.9745 N, 23.0322 E	250	2155 \pm 57.78 (n=6) e,f
S.9	Greece, Epirus, Mount Vasilitsas	40.0544 N, 21.0801 E	1800	2087 \pm 105.3 (n=4) e,f
S.10	Greece, Thessaly, Kedhros	39.1809 N, 22.0443 E	260	1960 \pm 40.2 (n=5) f
S.11	Greece, Euboea, Limni	38.7589 N, 23.3254 E	10	1938.5 \pm 10.62 (n=5) f
S.12	Greece, Epirus, Metsovo, towards Katara pass	39.7931 N, 21.2033 E	1600	1876 \pm 62 (n=5) f
S.13	Albania, Korçë, Voskopojë	40.5988 N, 20.5957 E	1430	1447 \pm 21 (n=5) g
S.14	Greece, Chalkidiki, close to Gomati	40.3918 N, 23.8049 E	156	69 \pm 3.03 (n=10) h
S.15	Greece, Macedonia, north of Paranesti	41.3665 N, 24.4458 E	260	46 \pm 2.5 (n=5) i

Letters indicate significant differences between concentrations at p -value < 0.05.

Linear regression was used to fit the relationship between soil Ni concentrations and plant Ni concentrations in the samples from the localities explored; significance level was set at p -value < 0.05. Statistical analyses and plot drawing were conducted using GraphPad Prism 7 for Windows .

Results

Plant Ni concentration

Nickel concentrations in shoots and roots of each investigated accession are reported in Table 3. Among tribes, most specimens of Alyseae showed shoot and root Ni values largely over the accumulation threshold of $1000 \mu\text{g g}^{-1}$ DW. Samples belonging to the genus *Bornmuellera* reached the highest Ni concentrations, ranging approximately from a minimum of $11000 \mu\text{g g}^{-1}$ DW in *B. tymphaea* (Bt) to a maximum of about

$24000 \mu\text{g g}^{-1}$ DW in *B. baldaccii* (Bb1) in shoots, and from around $2800 \mu\text{g g}^{-1}$ DW in *B. baldaccii* (Bb2) to $8700 \mu\text{g g}^{-1}$ DW in *B. emarginata* (Be1) in roots. Nickel values reached by the *Odontarrhena* samples were also elevated, ranging from a minimum of $4000 \mu\text{g g}^{-1}$ DW for *O. chalcidica* (Oc2) to a maximum of $14000 \mu\text{g g}^{-1}$ DW for *O. euboea* (Oe2) in shoots, and from around 20 in *O. muralis* (Om2) to $800 \mu\text{g g}^{-1}$ DW in *O. chalcidica* (Oc1) in roots. The only exception was found for the specimen *O. muralis* (Om2), which showed Ni shoot values significantly lower compared to the values found in the other samples of Alyseae analysed here (ca. $200 \mu\text{g g}^{-1}$ DW). *Alyssum densistellatum* (Ad), the only specimen of this genus analysed in this study, showed significantly lower values than the others of the same tribe, reaching only $40 \mu\text{g g}^{-1}$ DW of Ni in shoots and around $300 \mu\text{g g}^{-1}$ DW in roots. In tribe Coluteocapeae, both accessions of genus of *Noccea* showed elevated Ni levels reaching over $5000 \mu\text{g g}^{-1}$ DW in *Noccea ochroleuca* (No) and

Table 3. List of examined specimens (Plant code), with Ni-concentration in roots and shoots ($\mu\text{g g}^{-1}$ DW) of each specimen (means \pm standard deviation), code of soil samples from sites approximately corresponding to the collection sites of plant specimens; type of soil (U=ultramafic, NU=non ultramafic), translocation factor (TF: shoot/root ratio) and bioaccumulation factor (BAF: shoot/soil ratio).

Plant code	[Ni]root	[Ni]shoot	Soil code	Soil type	TF	BAF
Aethionemeae						
<i>Asg</i>	166 \pm 5.2 ij	102 \pm 2.6 j	S.11	U	0.6 \pm 0.02 a	0.05 \pm 0.001 ab
Alysseae						
<i>Ad</i>	334 \pm 3.6 hi	39 \pm 21.2 j	S.11	U	0.1 \pm 0.06 a	0.02 \pm 0.01 b
<i>Bb1</i>	3466 \pm 130 b	24939 \pm 4842 a	S.4	U	7.2 \pm 1.4 bc	7.5 \pm 1.4 e
<i>Bb2</i>	2847 \pm 521 bc	12610 \pm 2774 cd	S.9	U	4.7 \pm 1.2 b	6 \pm 1.3 e
<i>Bbr</i>	–	15779 \pm 1.52 abc	–	–	–	–
<i>Be1</i>	8770 \pm 4925 a	19707 \pm 5244 ab	S.10	U	2.1 \pm 1.2 a	10.5 \pm 2.6 d
<i>Be2</i>	3368 \pm 726 bc	13332 \pm 2123 bc	S.12	U	4.8 \pm 1.3 b	7.6 \pm 1.1 e
<i>Bt</i>	3245 \pm 272 b	11126 \pm 2469 cde	S.2	U	3.4 \pm 0.8 a	2.6 \pm 0.5 d
<i>Oc1</i>	830 \pm 364 ef	6152 \pm 1439 fg	S.14	NU	8.7 \pm 5.6 c	91.7 \pm 20.8 f
<i>Oc2</i>	585 \pm 192 fg	4145 \pm 1889 h	S.7	U	8.04 \pm 3.1 c	1.7 \pm 0.7 c
<i>Oe1</i>	158 \pm 34.7 j	5512 \pm 993 gh	S.11	U	36.6 \pm 12.1 e	2.84 \pm 0.5 d
<i>Oe2</i>	–	14707 \pm 59.5 abc	–	–	–	–
<i>Oe3</i>	–	7431 \pm 27.9 ef	–	U	–	–
<i>Om1</i>	1981 \pm 1098 cd	4383 \pm 1430 h	S.8	U	4 \pm 3.6 b	2.03 \pm 0.5 c
<i>Om2</i>	20 \pm 13.8 k	215 \pm 47.7 i	S.15	NU	31.4 \pm 34 de	4.66 \pm 1 e
Arabideae						
<i>Dl</i>	–	105 \pm 0.19 j	S.13	U	–	0.07 \pm 0.0001 ab
Cardamineae						
<i>Cg</i>	122 \pm 3 j	37.7 \pm 0.2 j	S.1	U	0.3 \pm 0.005 a	0.007 \pm 0.0001 a
<i>Cg2</i>	–	202 \pm 1 ij	S.9	U	–	0.09 \pm 0.0004 ab
<i>Cp</i>	–	68 \pm 38 j	S.6	U	–	0.02 \pm 0.01 a
<i>Rp1</i>	192 \pm 3.21 ij	129 \pm 1.18 j	S.13	U	0.7 \pm 0.01 a	0.08 \pm 0.0008 b
<i>Rp2</i>	370 \pm 5.1 gh	72 \pm 27.4 j	S.9	U	0.2 \pm 0.07 a	0.03 \pm 0.01 a
<i>Rs</i>	–	128.6 \pm 1.5 j	S.6	U	–	0.05 \pm 0.0006 ab
Coluteocarpeae						
<i>No</i>	–	9558 \pm 4600 de	–	U	–	–
<i>Nt</i>	1038 \pm 473 de	5138 \pm 1337 h	S.5	U	6.3 \pm 3.2 b	2.06 \pm 0.5 c
Erysimeae						
<i>Ec1</i>	260 \pm 1.52 i	143 \pm 2.8 j	–	U	0.5 \pm 0.01 a	–
<i>Ec2</i>	133 \pm 2.6 j	130 \pm 4.3 j	S.9	U	1 \pm 0.05 a	0.06 \pm 0.002 b
<i>Epm</i>	80 \pm 38 j	86 \pm 1 j	S.3	U	1 \pm 0.4 a	0.02 \pm 0.01 b
Hesperideae						
<i>Hl</i>	–	59.6 \pm 63 j	–	U	–	–
Iberideae						
<i>lc</i>	–	72 \pm 8 j	–	U	–	–
<i>ls</i>	73 \pm 60 jk	54.6 \pm 13 j	S.4	U	0.6 \pm 0.6 a	0.01 \pm 0.01 a
Isatideae						
<i>lts</i>	–	153 \pm 7.5 ij	S.3	U	–	0.04 \pm 0.001 ab

N. tymphaea (*Nt*) shoots and over 1000 $\mu\text{g g}^{-1}$ DW in *N. tymphaea* (*Nt*) roots. All specimens of the other tribes analysed (Aethionemeae, Arabideae, Cardamineae, Erysimeae, Iberideae, Isatideae) reached significantly lower Ni concentrations in both shoots and roots.

Translocation factors (TFs) could be determined when Ni concentrations in roots and shoots were both available for the same collection (Table 3). Wide variation was detected in *Bornmuellera*, where TFs ranged from 2.1 in *B. emarginata* (*Be1*) to 7.2 in *B. baldaccii* subsp. *baldaccii* (*Bb1*), and even more in *Odontarrhena*, ranging from 4 in *O. muralis* (*Om1*) to 36.6 in *O. euboica* (*Oe1*); the latter accession showed the highest TF value among all the examined samples. Specimens of Aethionemeae, Cardamineae, Erysimeae, Iberideae showed TFs < 1. Summarizing TF values at the tribe level showed to what extent these were higher in Alysseae and Coluteocarpeae than in the other ones investigated here (Figure 2).

Soil Ni concentration

Table 2 shows the list of the examined soils from Albania and Greece, in order of decreasing Ni concentration. Variability in soil Ni concentration from site to site in Albania was

significant, ranging from a minimum of 1447 $\mu\text{g g}^{-1}$ from around Voskopojë (S.13) to a maximum of 5193 $\mu\text{g g}^{-1}$ in the serpentine site near Bulqizë (S.1). Elevated Ni concentrations were also detected in the Greek serpentine soils, with a minimum of 1876 $\mu\text{g g}^{-1}$ (S.12) and a maximum of 4277 $\mu\text{g g}^{-1}$ both at Katara pass (S.2). Two non-serpentine soils from Greece showed the lowest Ni concentrations: 69 $\mu\text{g g}^{-1}$ and 46 $\mu\text{g g}^{-1}$ respectively at Gomati (S.14) and in a site with granite parent rock north of Paranesti in Macedonia (S.15).

Bioaccumulation factors (BAFs) of the examined accessions are given in Table 3, while mean values by tribe are shown in Figure 2. In Alysseae and Coluteocarpeae mean BAF values were > 4, whereas these were < 0.1 in the other examined tribes. However, variation within Alysseae was remarkable, ranging from 0.02 in *A. densistellatum* (*Ad*) to 91.7 in the accession of *O. chalcidica* from the type locality in Greece (*Oc1*). The latter remarkably elevated value resulted from the high Ni concentration in the shoot vs. the very low concentration of the metal in the non-serpentine soil of this locality. No evidence of accumulation was found in the accessions of *Cardamine* and *Aethionema*, that could be classified as Ni-excluders.

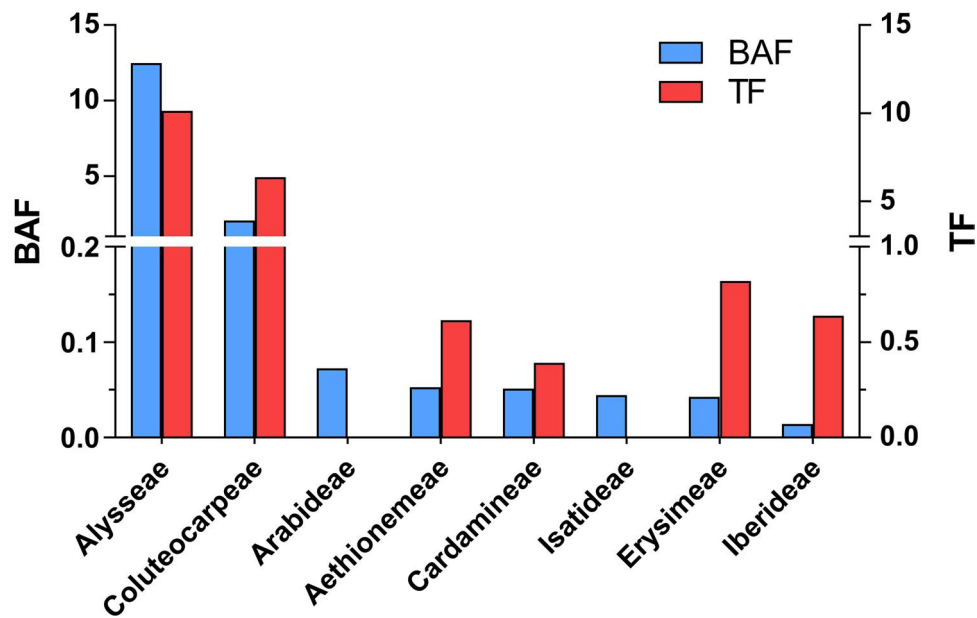


Figure 2. Mean values of the Bioconcentration Factor (BAF) and the Translocation Factor (TF) in the nine examined tribes.

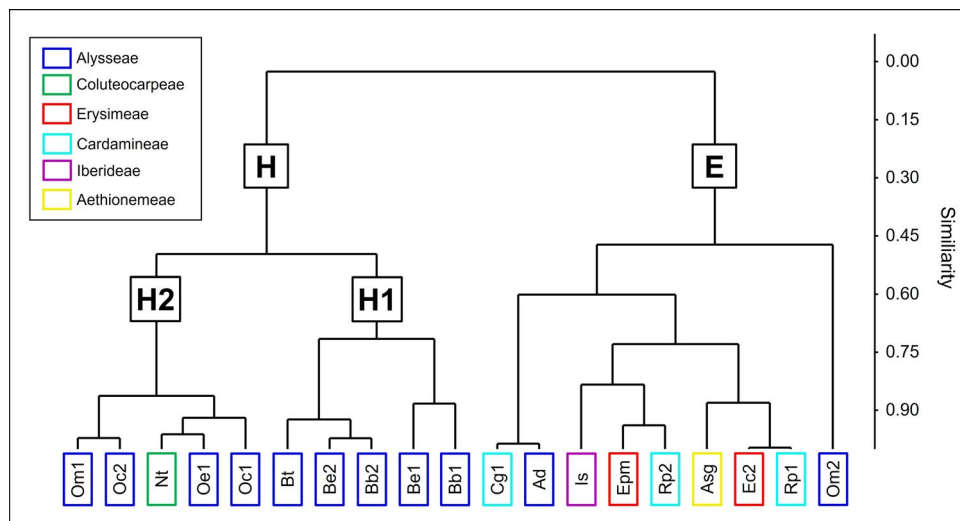


Figure 3. Classification of specimens for Ni accumulation capacity by Cluster analysis. Codes of accessions follow Table 1; different colours refer to the different tribes, as indicated in the legend box. H: Hyperaccumulator cluster; E: Excluder cluster.

The cluster analysis based on shoot Ni concentrations, BAFs and TFs (cophenetic correlation = 0.98) retrieved two main groups of specimens (Figure 3), one enclosing only hyperaccumulating specimens (cluster H, 10 accessions) and the other the excluder ones (cluster E, 9 accessions). All specimens of Alysseae and Coluteocarpeae were included in cluster H, except for *Om1* and *Ad* which confirmed to be classified as excluders. Moreover, the group of hyperaccumulators (H) consisted of two sub-clusters, one including the *Bornmuellera* specimens (H1) and one the *Odontarrhena* and *Noccaea* specimens (H2).

Relationships between Ni concentrations in shoots and soils were analysed only for accessions from ultramafic sites, separately for accumulating taxa with shoot Ni concentration > 1000 µg g⁻¹ and excluding taxa (< 1000 µg g⁻¹; Figure 4a,b). In the former group, shoot and soil Ni concentration tended to be positively related, though in a non-significant way

($r=0.225$, $p=0.142$). In the excluding taxa, shoot Ni concentration was negatively related to soil concentration ($r=0.343$, $p=0.005$), indicating increasing exclusion with increasing levels of the metal in the soil

Discussion

Although a large number of species in distant lineages of Brassicaceae are found on ultramafic soils across temperate regions, current knowledge supports that Ni-accumulation ability originated in only five out of the 52 tribes that are recognized in the family (Koch et al. 2018; Francis et al. 2021), namely Alysseae, Coluteocarpeae, Aethionemeae, Cardamineae in the Old World, and Schizopetaleae in the New World (Cecchi et al. 2010). This means that efficient mechanisms to limit metal uptake and translocation are likely working in the other serpentine taxa that belong to the

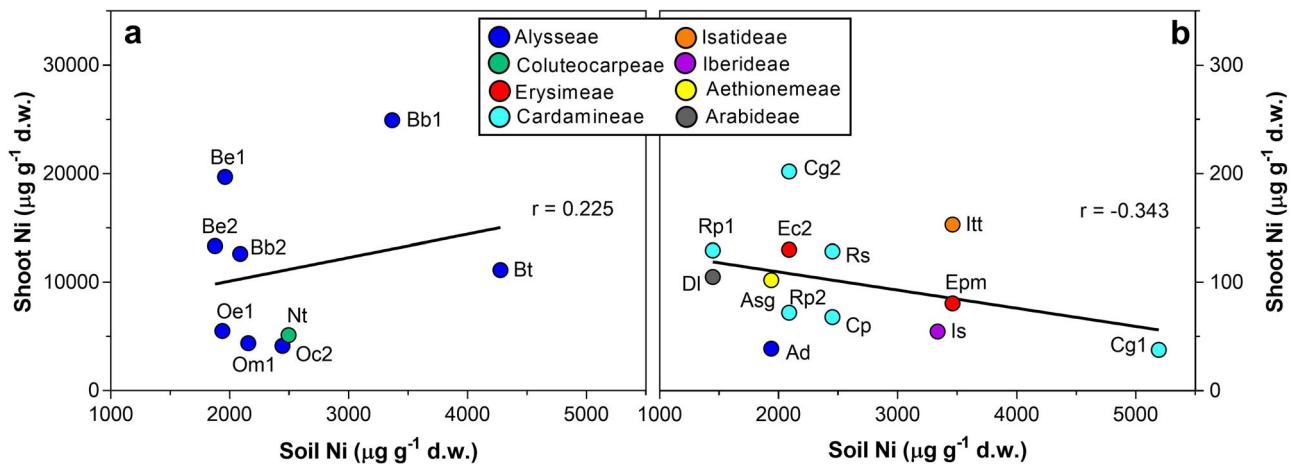


Figure 4. Relationship between Ni concentration in shoots and in relative soils of (a) Ni-hyperaccumulating and (b) Ni-excluding accessions from Albania and Greece.

remaining 47 tribes. Present findings, however, support that in western temperate Eurasia Ni-accumulation ability became established and widespread only in Alysseae and Coluteocarpeae, while we found no confirmation for Cardamineae and Aethionemeae. In the two latter tribes there is, respectively, only a single Ni accumulation report for *Cardamine resedifolia* L. from northern Italy (shoot Ni $1050 \mu\text{g g}^{-1}$; Vergnano Gambi & Gabbriell 1979; Reeves et al. 1983; Reeves et al. 2018) and *Aethionema spicatum* Post from Turkey ($764\text{--}1110 \mu\text{g g}^{-1}$; Reeves and Adigüzel 2008), suggesting that the ability may be quite sporadic and not fully developed.

Instead, present results support the notion that the small Balkan-Anatolian genus *Bornmuellera* is one of the evolutionary lineages in the family where metal tolerance and accumulation ability has reached maximum levels, with a mean bioaccumulation factor of 6.7. This is corroborated by several previous findings, reporting shoot Ni concentrations as high as $34400 \mu\text{g g}^{-1}$ in *B. emarginata* (formerly known as *Leptoplax e.*) and $31200 \mu\text{g g}^{-1}$ in *B. tymphaea* (Bani et al. 2010; see also Reeves et al. 1983; Bani et al. 2013). Recent evidence about *B. dieckii* Degen, an obligate serpentine endemic to a narrow mountain area in Kosovo, showed that this plant is able to accumulate up to $24295 \mu\text{g g}^{-1}$ in the leaves, with a bioaccumulation factor ranging from 2 to 12 (Paçarizi et al. 2020). In *B. baldaccii*, our findings suggest a higher accumulation capacity in Albanian plants of subsp. *baldaccii* than in Greek ones of both subsp. *baldaccii* and subsp. *rechingeri*, which is also reported from Albania (Meço et al. 2017). Previous reports about Turkish species also confirm the remarkable accumulation capacity in *Bornmuellera*, with values of up to 19240 and $12590 \mu\text{g g}^{-1}$ in, respectively, the serpentine endemics *B. glabrescens* (Boiss. & Bal.) Cullen & T.R. Dudley and *B. kiyakii* Aytaç & Aksoy (Reeves and Adigüzel 2008). On the other hand, our finding on *B. cappadocica* (DC) Cullen & T.R. Dudley from east Anatolia, the first report for this taxon, shows that not all the species of this genus are able to accumulate Ni, most likely depending on their edaphic preferences.

Odontarrhena is the other lineage of tribe Alysseae where Ni accumulation is widespread, being found in nearly all taxa that grow on ultramafic soils, either obligate or facultative (Bettarini et al. 2020). On the contrary, no Ni-hyperaccumulator

species are known to exist in the apparently similar but phylogenetically separate genus *Alyssum*, although several taxa in it are able to grow on ultramafic soils. In our study, such a sharply divergent behaviour is exemplified by the two species *O. euboica* and *A. densistellatum*, which were growing side by side at the same serpentine site on the western side of Euboea. While the former was found to accumulate $5500\text{--}14000 \mu\text{g g}^{-1}$ of Ni in the leaves (BAF 2.8), the latter resulted to be a typical Ni-excluder with a BAF of 0.02 and a TF of 0.1. Concerning *O. euboica*, our findings help to solve the existing doubts about Ni accumulation capacity in this species expressed by Reeves et al. (1983; see also Reeves et al. 2018). According to this author there is a need for detailed investigation into the causes of the wide variation in Ni accumulation reported for this species endemic to the serpentine soils of Euboea. Brooks and Radford (1978) analysed two herbarium specimens (Rechinger 16592 and 19311) and found 26 and $4550 \mu\text{g g}^{-1}$, respectively. Later, also Reeves et al. (1983) found contrasting values on two herbarium specimens, $38 \mu\text{g g}^{-1}$ for Rechinger 16598, collected on serpentine soil, and $8114 \mu\text{g g}^{-1}$ in another specimen of unrecorded origin. Two later analyses by Reeves (specimens Georgiadis 1978 and Reeves 2395) gave 14000 and $752 \mu\text{g g}^{-1}$, respectively. Our findings on plants from the northwest part of Euboea support that this species grows on Ni-rich serpentine soils and is fully able to accumulate the metal ($5512 \mu\text{g g}^{-1}$). Accumulation capacity is confirmed by results of our analysis of two herbarium specimens from serpentines of Mt. Kandilio (Rechinger no. 161416) and another site in the northern part of the island (Rechinger no. 19311, the same collection examined by Reeves et al. 1983), both of which had shoot Ni concentrations well above the $1000 \mu\text{g g}^{-1}$ threshold. Based on our direct field experience, we may suppose that the reasons for the very low Ni values reported above for *O. euboica* could be due to misidentification of the analysed plant samples. At the serpentine collection site along the coast south of Limni, in fact, this endemic grows side by side with two morphologically very similar species, *Alyssum densistellatum* and *O. sibirica*, both facultative serpentinophytes. In a recent study, Bettarini et al. (2020) showed that plants of *O. sibirica* from this locality (collection Selvi and Bettarini F1056353) also have very low shoot Ni ($52\text{--}110 \mu\text{g g}^{-1}$), as other plants of the same species from other Greek

localities, ultimately showing that this is the only species in *Odontarrhena* unable to accumulate Ni when growing on serpentine. Hence, checking the identity of the *O. euboica* herbarium specimens that gave low Ni values would be helpful to assess the reasons of the contrasting results discussed above.

Additional findings of interest concern *O. chalcidica* and *O. muralis*, two closely related species that have been, and still are, often confused (Cecchi et al. 2018; Coppi et al. 2020). The former is a tetraploid facultative serpentinophyte that always accumulates high amounts of Ni when growing on Ni-rich substrates, either in natural (ultramafic soils) or experimental conditions (hydroponics; Bettarini et al. 2021). The present result on plants from the non-serpentine type locality in the Chalkidiki peninsula is remarkable as it shows this taxon to be able to express accumulation ability even when growing on soils with very low Ni content. To our best knowledge, this is the first report of Ni-accumulation for a plant growing on such “normal” soils, resulting in an exceptionally high bioaccumulation factor among Alyseae and even family Brassicaceae (91.7). Moreover, the experimental study by Bettarini et al. (2021) revealed a Ni-induced stimulation effect on the growth of these plants and shoot Ni concentrations above $1000 \mu\text{g g}^{-1}$, similar to other serpentine and hyperaccumulating populations of the same species from different Balkan sites. Also, in consideration of the tall plant size and large biomass, this “type” population of *O. chalcidica* could be tested as a candidate for agromining applications as those currently performed in Europe (Kidd et al. 2018). *Odontarrhena muralis* is instead a diploid species described from the volcanic site of the Deva citadel in Romania and is rarely found on ultramafic soils (Hartvig 2002). Although it is considered an important model taxon for studies on Ni-accumulation physiology and agromining applications, most accumulation reports from the Balkan ultramafics (Bani et al. 2010) should be referred to *O. chalcidica* or to the closely related serpentinophyte Ni-hyperaccumulator *O. decipiens* (Nyár.) L.Cecchi & Selvi (Coppi et al. 2020). To explore Ni-accumulation and tolerance in genuine *O. muralis*, Bettarini et al. (2021) experimentally tested two non-serpentine populations from Romania and Greece and found enhanced growth only in those plants exposed to low Ni concentrations, while maximum shoot Ni levels only approached the Ni hyperaccumulation threshold. Accordingly, the authors concluded that *O. muralis* cannot be considered a Ni-hyperaccumulator. In the light of this, the present finding of two contrasting Ni levels in the two examined accessions is relevant as it suggests the behaviour of facultative hyperaccumulator of *O. muralis*, unlike *O. chalcidica*. Although we confirm very low levels of Ni in non-serpentine plants of the accession *Om2* from northern Greece, these showed high BAF and TF values that are comparable to those of typical hyperaccumulators. Also, we demonstrated elevated shoot Ni levels in the plants from serpentine soils of Gerania Ori (*Om1* $>4000 \mu\text{g g}^{-1}$), which can be considered genuine hyperaccumulators. Further comparative studies of *O. muralis* populations would therefore be helpful to assess the existence of ecotypes with different accumulation ability possibly related to different soil types. Findings on *Noccaea* confirm accumulation in *N. tymphaea* and *N. ochroleuca*, which showed Ni levels even higher than

those previously reported for Greek and Turkish accessions of this species (Reeves et al. 1983; see also Reeves et al. 2018).

Examining the soil-plant Ni concentration relationship also provided hints into the metal tolerance mechanisms in the accessions and taxa examined. In fact, this relationship resulted of opposite sign between hyperaccumulating and excluding taxa (Figure 4). In the former group, shoot Ni tended to increase with soil Ni levels, though not significantly, in line with recent similar evidence on hyperaccumulating species of *Odontarrhena* from Albania (Bettarini et al. 2019). Hence present evidence suggests that this positive relationship might apply at a broader taxonomic scale across Brassicaceae. On the other hand, relation was here found to be negative for the 13 excluding taxa, with accessions from soils with the higher Ni concentrations showing the lower shoot element concentrations, regardless of species identity. In this case, the plant's need to limit Ni entrance when growing on the Ni-richest soils could have generated increasingly efficient exclusion mechanisms, thus resulting in metal shoot concentrations lower than those in plants from less mineralised soils.

Conclusions

Our results support that among the Eurasian lineages of Brassicaceae, Ni-accumulation ability originated only in tribes Alyseae and Coluteocarpeae. In Alyseae, this physiological specialization is strongly developed and widespread in the genera *Bornmuellera* and *Odontarrhena*, as indicated by the high shoot Ni-levels found in the previously “uncertain” *O. euboica* and the remarkably high BAF value (> 90) detected in the non-serpentine population of *O. chalcidica* from the type locality in Greece. The latter species has the unique ability to accumulate high shoot metal concentrations even when growing on Ni-poor soils and is thus likely to be an obligate Ni-hyperaccumulator. Instead, the closely related *O. muralis*, used as model species in many previous studies on Ni-accumulation, mostly consists of non-hyperaccumulator populations and a few hyperaccumulator populations, from non-serpentine and serpentine soils respectively, and should therefore be regarded as a facultative Ni-hyperaccumulator. Present findings confirm that Ni-accumulation ability did not originate in *Alyssum*, despite that several taxa in this genus are well adapted to grow on serpentine soils thanks to effective metal-exclusion mechanisms. Overall, present data can contribute to enrich the Global Hyperaccumulator Database. Further studies would be helpful to investigate, also from a molecular and physiological perspective, the accumulation ability especially in tribes Cardamineae and Aethionemae and to test the soil-plant Ni concentration relationship on a broader pool of taxa and accessions of Brassicaceae from Eurasian serpentine soils.

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References

- Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. *Biorecovery* 1:81–126.
- Bani A, Echevarria G, Mullaj A, Reeves RD, Morel JL, Sulce S. 2009. Ni hyperaccumulation by Brassicaceae in serpentine soils of Albania and NW Greece. *Northeast Nat.* 16(sp5):385–404.
- Bani A, Imeri A, Echevarria G, Pavlova D, Reeves RD, Morel JL, Sulce S. 2013. Nickel hyperaccumulation in the serpentine flora of Albania. *Fresenius Environ. Bull.* 22:1792–1801.
- Bani A, Pavlova D, Echevarria G, Mullaj A, Reeves RD, Morel JL, Sulce S. 2010. Nickel hyperaccumulation by species of *Alyssum* and *Thlaspi* (Brassicaceae) from ultramafic soils of the Balkans. *Bot. Serb.* 34:3–14.
- Bech J, Abreu MM, Chon HT, Roca N. 2014. Remediation of potentially toxic elements in contaminated soils. In Bini C and Bech J. eds., PHEs, environment and human health. Springer, Dordrecht, Heidelberg, New York, London, p. 253–308.
- Bani A, Pavlova D, Rodríguez-Garrido B, Kidd P, Konstantinou M, Kyrkas D, Morel J-L, Prieto-Fernandez A, Puschenreiter M, Echevarria G. 2021. Element case studies in the temperate/mediterranean regions of Europe: Nickel. In: Van der Ent A, Echevarria G, Baker AJM, Morel JL, editors. *Agromining: farming for metals; mineral resource reviews*. Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-030-58904-2_16.
- Bettarini I, Colzi I, Coppi A, Cecchi L, Falsini S, Echevarria G, Pazzagli L, Selvi F, Gonnelli C. 2019. Unravelling soil and plant metal relationships in Albanian Ni-hyperaccumulators of genus *Odontarrhena* (syn. *Alyssum* sect. *Odontarrhena*, Brassicaceae). *Plant Soil.* 440(1–2):135–149. <https://doi.org/10.1007/s11104-019-04077-y>.
- Bettarini I, Colzi I, Gonnelli C, Pazzagli L, Reeves RD, Selvi F. 2020. Inability to accumulate Ni in a genus of hyperaccumulators: the paradox of *Odontarrhena sibirica* (Brassicaceae). *Planta.* 252(6):1–12. <https://doi.org/10.1007/s00425-020-03507-x>.
- Bettarini I, Gonnelli C, Selvi F, Coppi A, Pazzagli L, Colzi I. 2021. Diversity of Ni growth response and accumulation in Central-Eastern Mediterranean *Odontarrhena* (Brassicaceae) populations on and off serpentine sites. *Env. Exp. Bot.* 186:104455.
- Bhatia NP, Baker AJM, Walsh KB, Midmore DJ. 2005. A role for nickel in osmotic adjustment in drought-stressed plants of the nickel hyperaccumulator *Stackhousia tryonii* Bailey. *Planta.* 223(1):134–e139.
- Brooks RR. 1987. Serpentine and its vegetation: a multidisciplinary approach. Dioscorides Press, Portland, OR.
- Brooks RR, Radford CC. 1978. Ni accumulation in European species of *Alyssum*. *Proc. R. Soc. Lond. B.* 200:217–224. <https://doi.org/10.1098/rspb.1978.0016>.
- Cecchi L, Bettarini I, Colzi I, Coppi A, Echevarria G, Pazzagli L, Bani A, Gonnelli C, Selvi F. 2018. The genus *Odontarrhena* (Brassicaceae) in Albania: Taxonomy and nickel accumulation in a critical group of metallophytes from a major serpentine hot spot. *Phytotaxa.* 351(1):1–28. <https://doi.org/10.11646/phytotaxa.351.1.1>.
- Cecchi L, Gabbriellini R, Arnetoli M, Gonnelli C, Hasko A, Selvi F. 2010. Evolutionary lineages of nickel hyperaccumulation and systematics in European Alyseae (Brassicaceae): evidence from nrDNA sequence data. *Ann Bot.* 106(5):751–767. <https://doi.org/10.1093/aob/mcq162>.
- Cecchi L, Španiel S, Bianchi E, Coppi A, GC, Selvi F. 2020. *Odontarrhena stridii* (Brassicaceae), a new Nickel-hyperaccumulating species from mainland Greece. *Plant Syst. Evol.* 306(4):1–14. <https://doi.org/10.1007/s00606-020-01687-3>.
- Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL. 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. *J Environ Qual.* 36(5):1429–1443.
- Coppi A, Baker AJM, Bettarini I, Colzi I, Echevarria G, Pazzagli L, Gonnelli C, Selvi F. 2020. Population genetics of diploid-polyploid taxa of *Odontarrhena* (Brassicaceae) from Albania: the effects of anthropic habitat disturbance, soil and altitude on a Ni-hyperaccumulator plant group from a major serpentine hotspot. *Plants.* 9(12):1686. <https://doi.org/10.3390/plants9121686>.
- El Mehdawi AF, Quinn CF, Pilon-Smits EAH. 2011. Effects of selenium hyperaccumulation on plant-plant interactions: evidence for elemental allelopathy. *New Phytol.* 191(1):120–e131. <https://doi.org/10.1111/j.1469-8137.2011.03670.x>.
- Francis A, Lujan-Toro BE, Warwick SI, Macklin JA, Martin SL. 2021. Update on the Brassicaceae species checklist. *Biodivers Data J.* 9:e58773. <https://doi.org/10.3897/BDJ.9.e58773>.
- Goolsby EW, Mason CM. 2015. Toward a more physiologically and evolutionarily relevant definition of metal hyperaccumulation in plants. *Front. Plant Sci.* 6:33.
- Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica.* 4(1):9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.
- Hartvig P. 2002. *Alyssum*. In: Strid A, Tan K, editors, *Flora Hellenica 2*. Gantner Verlag, Ruggell, p. 199–224.
- Hopewell T, Selvi F, Ensikat HJ, Weigend M. 2021. Trichome biomineralization and soil chemistry in Brassicaceae from Mediterranean ultramafic and calcareous soils. *Plants.* 10(2):377.
- Kidd PS, Bani A, Benizri E, Gonnelli C, Hazotte C, Kissler J, Echevarria G. 2018. Developing sustainable agromining systems in agricultural ultramafic soils for nickel recovery. *Fron. Env. Sc.* 6:44.
- Koch MA, German DA, Kiefer M, Franzke A. 2018. Database taxonomics as key to modern plant biology. *Trends Plant Sci.* 23(1):4–6.
- Losfeld G, L’Huillier L, Fogliani B, Mc Coy S, Grison C, Jaffré T. 2015. Leafage and soil-plant relationships: key factors for reporting trace-elements hyperaccumulation by plants and design applications. *Environ Sci Pollut Res Int.* 22(8):5620–5632.
- Marques APGC, Rangel AOSS, Castro PML. 2009. Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. *Crit. Rev. Environ. Sci. Technol.* 39(8):622e654–622e654.
- Masarovičová E, Kráľová K, Kummerová M. 2010. Principles of classification of medicinal plants as hyperaccumulators or excluders. *Acta Physiol Plant.* 32(5):823–829.
- Meço M, Pavlova D, Mahmutaj E, Bani A, Mullaj A. 2017. Conservation status of some rare plant species on the watershed of the middle section of river Devoll (South Central Albania) and their distribution in Albania. *Phyt. Balc.* 23(2):199–205.
- Nkrumah PN, Echevarria G, Erskine PD, van der Ent A. 2018. Nickel hyperaccumulation in *Antidesma montis-silam*: from herbarium discovery to collection in the native habitat. *Ecol Res.* 33(3):675–e685.
- Paçarizi M, Krasniqi E, ZM, Ukaj S. 2020. Hyper-accumulation of nickel by *Bornmuellera dieckii* Degen as an endemic plant species in Kosovo. T2P International Scientific Conference Theory to Practice as a Cognitive, Educational and Social Challenge 17–18th September 2020. Mitrovica, Kosovo.
- Peer WA, Baxter IR, Richards EL, Freeman JL, Murphy AS. 2005. Phytoremediation and hyperaccumulator plants. In: Tamas MJ, Martinoia E, editors, *Molecular biology of metal homeostasis and*

- detoxification. Topics in current genetics, vol. 14. Springer, Berlin, Heidelberg, p. 299–340.
- Pollard AJ, Reeves RD, Baker AJ. 2014. Facultative hyperaccumulation of heavy metals and metalloids. *Plant Sci.* 217-218:8–17.
- Reeves RD. 2003. Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant Soil.* 249(1):57–65. <https://doi.org/10.1023/A:1022572517197>.
- Reeves RD, Adıgüzel N. 2008. The nickel hyperaccumulating plants of the serpentines of Turkey and adjacent areas: a review with new data. *Turk J Biol.* 32:143–153.
- Reeves RD, Aloupi M, Daftsis EI, Stratis JA, Mastoras P, Dimitrakopoulos PG. 2022. Biogeochemical aspects of the serpentines of Rhodes (Greece) and Cyprus. *Plant Soil.* 472(1-2):491–508.
- Reeves RD, Baker AJM, Jaffré T, Erskine PD, Echevarria G, Ent A. 2018. A global database for hyperaccumulator plants of metal and metalloid trace elements. *New Phytol.* 218(2):407–411.
- Reeves RD, Brooks RR, Dudley TR. 1983. Uptake of nickel by species of *Alyssum*, *Bommuellera*, and other genera of Old World tribus Alyseae. *Taxon.* 32(2):184–192.
- Reeves RD, van der Ent A, Baker AJM. 2018. Global Distribution and ecology of Hyperaccumulator plants. In: van der Ent A., Echevarria G, Baker AJM, Morel JL, editors. *Agromining: farming for metals, extracting unconventional resources using plants*. New York: Springer International Publishing; p. 75–92.
- Revell L, Harmon L, Collar D. 2008. Phylogenetic signal, evolutionary process, and rate. *Syst Biol.* 57(4):591–e601.
- Selvi F, Carrari E, Colzi I, Coppi A, Gonnelli C. 2017. Responses of serpentine plants to pine invasion: vegetation diversity and nickel accumulation in species with contrasting adaptive strategies. *Sci. Total Environ.* 595:72–80.
- Selvi F, Vivona L. 2021. Polyploidy in *Odontarrhena bertolonii* (Brassicaceae) in relation to seed germination performance and plant phenotype, with taxonomic implications. *Plant Biosyst.* 1–10. <http://doi.org/10.1080/11263504.2021.1985001>.
- Stevanovic V, Tan K, Iatrou G. 2003. Distribution of the endemic Balkan flora on serpentine I. – obligate serpentine endemics. *Plant Syst. Evol.* 242(1–4):149–170.
- Strid A. 1983. IOPB chromosome number reports LXXVIII. *Taxon.* 32:138–140.
- Tutin TG, Burges NA, Chaters AO, Edmondson JR, Moore TM, Valentine DH, Walters SM, Webb DA, editors. 1993. *Flora Europaea 1*, 2nd Ediz. Cambridge, England: Cambridge University Press.
- van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H. 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant Soil.* 362(1–2):319–334.
- van der Ent A, Repin R, Sugau J, Wong KM. 2015. Plant diversity and ecology of ultramafic outcrops in Sabah (Malaysia). *Aust J Bot.* 63(4):204–212.
- Van der Pas L, Ingle RA. 2019. Towards an understanding of the molecular basis of nickel hyperaccumulation in plants. *Plants.* 8(1):11.
- Vergnano Gambi O, Gabbriell R. 1979. Ecophysiological and geochemical aspects of nickel chromium and cobalt accumulation in the vegetation of some Italian ophiolitic outcrops. *Ofoliti.* 4:199–208.
- Yang W, Li H, Zhang T, Sen L, Ni W. 2014. Classification and identification of metal-accumulating plant species by cluster analysis. *Environ Sci Pollut Res Int.* 21(18):10626–10637.