

TRACE METALS IN SOILS AND SEVERAL BRASSICACEAE PLANT
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Abstract: Serpentine soils from 16 sample points in Serbia as well as the roots and shoots of eight Brassicaceae family species: *Aethionema saxatile*, *Alyssum montanum*, *Alyssum repens*, *Cardamine plumieri*, *Erysimum linariifolium*, *Erysimum carniolicum*, *Isatis tinctoria*, *Rorippa lippizensis*, were analyzed with regard to their concentrations of P, K, Fe, Ca, Mg, Ni, Zn, Mn, Cu, Cr, Cd, and Pb. Most of the soil samples were typical of ultramafic sites with low concentrations of P, K and Ca and high concentrations of Mg, Fe, Ni and Zn. Ca/Mg ratio was <1 in most soil samples and Brassicaceae plants. Only in *A. montanum*, *A. repens*, *E. linariifolium* and *R. lippizensis* was the Ca/Mg ratio >1. The levels of P, K, Fe and Zn were high, Mn and Cu occurred in low amounts, whereas Cr, Cd, Co and Pb were only traceable. In the roots and shoots of *A. montanum* and *A. repens* the measured concentrations of Ni were 657 mg kg⁻¹ and 676 mg kg⁻¹ respectively, which is the first instance that such high concentrations of Ni were detected in these two species.

INTRODUCTION

Ultramafics ('ultrabasic' or 'serpentine') represent magmatic or metamorphic rocks which are characterized by high concentrations of Mg, Fe, Ni, Cr and Co and low concentrations of Ca, P, and K and contain less than 45% silica (SiO₂) [32]. In the strictly mineralogical sense, the 'serpentine' include not only one but a group of different minerals which are commonly hydrothermally altered products of olivine (MgFe)₂SiO₄. Their approximate formula is H₄(MgFe)₃Si₂O₉. In petrology, 'serpentine' is frequently used for 'serpentinite' to denote rock consisting primarily of many associated minerals (hydrated magnesium rich silicates, e.g. tremolite, or residual unaltered minerals, e.g. chromite) [33].

Ultramafics are distributed all over the world viz., the United States of America, Canada, Cuba, Brazil, Europe (Wales and Scotland, Sweden, Norway, Finland, Switzerland, Austria, Czech Republic, Poland, France, Spain, Portugal, Italy, Bosnia and Herzegovina, Albania, Serbia, Bulgaria, FYR Macedonia and Greece), Turkey, Iran, New

Caledonia, south east-Asia, Philippines, Japan, Zimbabwe, South Africa, New Zealand and greenstone belts of Australia [15, 26, 45].

Serpentine substrates commonly support vegetation and flora that is sparser and more stunted than that of other nearby soils. Many plant species are incapable of growing on serpentine substrates because they are extremely poor in essential nutrients and have a low Ca/Mg ratio [20]. The distinctive vegetations of soils derived from serpentine and related rocks sharply separate such areas from adjacent non-serpentine terrain in many regions of the world. The plant life of these serpentine areas varies greatly with topography, depth of soil, location, and other factors, but is usually sparse or stunted. It is characterized by the presence of many species which seem to be restricted to the serpentine habitat. Many of the species present on adjacent non-serpentine soils are not able to grow on the serpentine; others grow on the serpentine but are stunted, while only a few plants grow equally well on either type of soil. Some native species occur both on and off serpentine. Within such species, populations both well adapted and poorly adapted to serpentine may be encountered [26].

There are several morphological features that are characteristic of serpentine tolerant plants (species, subspecies or varieties) [7]. First, these plants typically possess xeromorphic foliage (including sclerophylls and reduced leaf size). Second, the stature of serpentine tolerant species (or varieties) is significantly reduced relative to the counterparts on nonserpentine soils. Finally, root systems of plants growing on and off serpentine sites are often more developed on serpentine soils than on neighboring soils.

On the basis of heavy metal uptake, plants which grow on ultramafic soils can be divided into 'normal' and metal hyperaccumulators. Most plants of ultramafic soils are of the former type and show only slightly elevated Ni concentrations in the shoot dry matter (about 5–100 mg kg⁻¹) in comparison to those from other soil types (0.5–10 mg kg⁻¹) [35]. While most serpentine plants are able to grow on these soils without excessive uptake of elements, hyperaccumulators take up more than 1000 mg kg⁻¹ Ni and accumulate it into their leaf dry matter [4]. To date, about 360 hyperaccumulators of Ni from ultramafic soils have been identified [38]. They are mostly found in subtropical and tropical areas, while some species (mostly from the Brassicaceae family) are encountered in temperate areas (especially Mediterranean and Turkey) [36].

By far the greatest number of nickel-accumulating species within any genus is found in *Alyssum* L. After a comprehensive screening of 168 *Alyssum* species for their nickel content, it was concluded that hyperaccumulation of nickel is restricted entirely to the section *Odontarrhena* and that 70% of the species assigned to this section are identified as accumulators [8]. Besides the *Alyssum* species, nickel hyperaccumulation was also discovered in species that belong to other genera of the Brassicaceae family of Europe and Turkey, such as *Leptoplax emarginata* (Boiss.) O.E. Schulz (syn. *Peltaria emarginata* (Boiss.) Hausskn.) [41], several species of *Bornmuellera* Hausskn. [21, 37, 40], two species of *Pseudosempervivum* (Boiss.) Grossh. (*Cochlearia* L.), one species of *Aethionema* (*A. spicatum* Post) in Turkey and a considerable number of plant taxa of the genus *Thlaspi* L. s.l. [34, 36]. In the Balkan Peninsula, hyperaccumulation by many species in the above genera has been reported in several publications [5, 6, 10, 43]. So far only the data about Ni accumulation in *A. murale* s.l. from the central part of Serbia (Mt Goč) (under the name *A. markgrafii*) have been published [29, 48], but there has been no information about the hyperaccumulative potential of the species from the ultramafic

areas of Serbia that belong to some other genera (*Isatis*, *Cardamine*, *Rorippa*, *Erysimum*) of the Brassicaceae family.

Hence, it is necessary to obtain more information about metal accumulation capacities of the plants from the Brassicaceae family growing on serpentine soils to evaluate their potential for phytoremediation including metal extraction (phytoextraction). Therefore, in this work we wished to: 1) determine concentrations of macro and micronutrients such as P, K, Ca, Mg, Fe, Ni, Mn, Zn, Cu, Cr, Pb, Co and Cd in several serpentine soils from Serbia; 2) examine trace metal accumulation in various Brassicaceae plants growing on the serpentine soils of Serbia; 3) assess the ability of the species that belong to the genera *Isatis*, *Cardamine*, *Rorippa*, *Erysimum*, *Aethionema* and *Alyssum* to tolerate or accumulate these elements in their tissues in order to identify new potential trace metal accumulating taxa.

MATERIALS AND METHODS

Sample collection

Plant and soil samples were collected in May 2010 and May 2011 from 16 sampling points (SP), SP1-SP16 (Table 1). Soil samples were chosen by eye based on the presence of the sampling plants. At each study site, a variable number of soil samples (5–10 replicates) were taken from the rhizosphere of several plants specimens. These samples (ca. 500 g per sample) were transported in polyethylene bags to the laboratory and afterward dried at 40°C for 3 days.

Plant species were sampled in 5–20 replicates from each of the investigated populations, according to their abundance and biomass. All the plant samples were collected, separated into roots and shoots, and washed carefully with bi-distilled water to remove soil particles. Dry weights were obtained after drying at 40°C for 3 days. Plants have been identified with the help of local floras [23]. These species are: *Aethionema saxatile* (L.) R. Br., *Alyssum montanum* L., *Alyssum repens* Baumg., *Cardamine plumieri* Vill., *Erysimum linariifolium* Tausch, *Erysimum carniolicum* Dolliner, *Isatis tinctoria* L., *Rorippa lippizensis* (Wulfen) Reichenb. Voucher specimens of the plants collected are deposited in the Collections of the Natural History Museum (BEO) and the Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade (BEOU).

Soil analysis

Particle size distribution was determined by sieving and sedimentation after samples were soaked in the dispersant sodium hexametaphosphate in 1:2 soil-water suspensions and shaken end-over-end for 16 h. The sand (>50 µm) was fractionated by dry sieving. The pipette method was used for the determination of clay (<2 µm) and silt (<50 µm) fractions [46]. The USDA classification was applied to determine soil textural classes [42].

Soil actual and exchangeable pH was determined in distilled water and in 1 M KCl solution respectively, in a solid-liquid (S/L) ratio of 1:2,5 ml g⁻¹ [28]. Available P and K were measured in AL solution (0.1 M ammonium lactate and 0.4 M acetic acid) extract (S/L 1:20) [13]. Phosphate concentration was determined by molybdenum blue method and potassium concentration was determined using FES (flame emission spectrophotometry) by Pye Unicam SP 192 atomic absorption spectrophotometer. Detection limit of the

Table 1. Characterization of the sampling points of different Brassicaceae species from the ultramafic areas of Serbia

Locality	Latitude (N)	Longitude (E)	Altitude (m.a.s.l.)	Sample point	Species
Mt Zlatibor (Skakavac river)	43°44'34"	19°36'42"	840	SP1	<i>Aethionema saxatile</i>
				SP11	<i>Erysimum linariifolium</i>
Mt Zlatibor (Prdavac stream)	43°44'10"	19°39'29"	900	SP16	<i>Rorippa lippizensis</i>
Mt Zlatibor (Trčinoga-Ravna Kosa)	43°43'53"	19°38'12"	850	SP8	<i>Cardamine plumieri</i>
Mt Zlatibor (Krivi Potok stream)	43°41'11"	19°42'03"	1020	SP13	<i>Erysimum carniolicum</i>
Mt Rogozna (Pasji stream-Vinogor)	43°07'25"	20°37'48"	1120	SP5	<i>Alyssum montanum</i>
Kraljevo (Gornja Lopatnica river)	43°40'09" 43°40'48" 43°40'48"	20°26'30" 20°28'05' 20°28'05'	440 450 450	SP2	<i>Aethionema saxatile</i>
				SP7	<i>Alyssum repens</i>
				SP10	<i>Cardamine plumieri</i>
Čačak (Vrnčanska river gorge)	43°55'05"	20°08'11"	410	SP9	<i>Cardamine plumieri</i>
				SP14	<i>Isatis tinctoria</i>
Gornji Milanovac (Brdani gorge)	43°59'27"	20°25'15"	330	SP3 SP12	<i>Aethionema saxatile</i> <i>Erysimum linariifolium</i>
Raška (Trnavska river gorge)	43°17'14"	20°36'07"	420	SP6	<i>Alyssum montanum</i>
Ibar river valley (Maglič ruins)	43°36'93"	20°33'04"	300	SP4 SP15	<i>Alyssum montanum</i> <i>Isatis tinctoria</i>

applied procedure is 0.0001 g·kg⁻¹ for both P and K. Exchangeable Ca and Mg were determined in 1 M ammonium acetate extract (S/L 1:50) [47] by AAS (atomic absorption spectrophotometry) (Pye Unicam SP 192) with the detection limits of 0.06 g·kg⁻¹ for Ca and 0.01 g·kg⁻¹ for Mg. Organic matter concentration was determined by dichromate digestion based on the FAO procedure [14]. Detection limit of the procedure is 0.1%. Extraction of available (potentially leachable) metals in soil was performed by 0.1 M HCl (S/L 1:10) according to the procedure recommended by Garcia *et al.* [16]. Total metal extraction was done by HCl and HNO₃ digestion (ISO 11466, 1995). Metal concentrations in both extracts were determined using atomic absorption spectrophotometry (ISO 11047, 1998) (Pye Unicam SP 192). The detection limit for these measurements is 0.1 mg·kg⁻¹ for most elements. For the evaluation of the analytical procedure for trace elements and heavy metals we used standard soil sample NCS DC87104.

Each analysis of the soil material was performed with three replicates. In addition, each of the chemical analyses was run also with three replicates. Arithmetic means and standard deviations are shown in tables.

Plant analysis

Dried and ground plant material was digested by slightly modified wet procedure described by ISO 6636/2, 1981, using a boiling mixture of nitric and sulphuric acids. Phosphorus concentration was determined by modified the molybdenum blue method described by Chen *et al.* [11]. The detection limit of the procedure was 0.1 g·kg⁻¹. Potassium concentration was determined using FES (flame emission spectrophotometry) by Pye Unicam SP 192 atomic absorption spectrophotometer with the detection limit of also 0.1 g·kg⁻¹. Metal concentrations were determined using atomic absorption spectrophotometry (Pye Unicam SP 192). The series of standard solutions for metals were made from 1 g l⁻¹ solutions purchased from Carlo Erba, Italy. The reliable detection limit was 1.0 mg·kg⁻¹. For the evaluation of the analytical procedure for plant material elemental analysis we used standard sample NIST 1515 (Apple leaves).

Each analysis of the plant material was performed with three replicates. In addition, each of the chemical analyses was also run with three replicates. Arithmetic means and standard deviations are shown in tables.

SITE DESCRIPTION

The distribution of the occurrence and locations of ultramafic bedrocks of the studied plant populations is shown in Table 1 and Figure 1. At each site, the selection was based on the presence of large populations of the investigated species. In Mt Zlatibor the investigated sites (SP1, SP11) with the vegetation alliance *Centaureo-Bromion fibrosi* were covered with xerophile pastures and rocky grounds, while three other sample points (SP8, SP13, SP16) were characterized by the presence of the secondary screes vegetation from the alliance *Achnatherion calamagrostis*. In the surroundings of Čačak town (the Vrnčanska river gorge) (SP9, SP14), soil and plants were collected at the edges of thermophilous deciduous forests which belong to the alliances *Quercion frainetto* and *Ostryo-Carpinion orientalis*. In the Brđani gorge (a vicinity of Gornji Milanovac town) (SP3, SP12) the investigated sites were characterized by the presence of pastures and rocky grounds with vegetation alliance *Chrysopogoni-Danthonion alpinae*. In these localities soils derived from ultramafic metamorphic rocks – serpentinites. In the surroundings of Kraljevo city (the Gornja Lopatnica river gorge) (SP2, SP7, SP10), soils and plants were collected at rocky grounds near thermophilous deciduous forests which belong to the alliances *Quercion frainetto* and *Orno-Ostryon*. Collecting sites in the Trnavska River gorge near the city of Raška (SP6), the Ibar River valley (SP4, SP15) and in Mt Rogozna (SP5) were covered with xerophile pastures and rocky grounds with the vegetation alliance *Centaureo-Bromion fibrosi*. At these seven sample points soils derived from intrusive ultramafic rocks – harzburgite.

RESULTS AND DISCUSSION

Soil Characteristics

Physical characteristics of 16 soil samples from ultramafic areas of Serbia are given in Table 2. The textural classes of the studied samples range from silty loam to sandy loam, but two soil samples belong to the loam class. Textures of serpentine soils vary from heavy clays to light loams [49], but silt and clay contents are generally minimal [7].



Fig. 1. Map of Serbia showing ultramafic bedrocks occurrence (in gray) and locations (SP1–SP16) of the studied soils and Brassicaceae species (black circles)

Table 2. Texture of soil samples

Sample point	Particle size distribution				USDA textural classes
	sand (total)		% silt	% clay	
	% coarse sand	% fine sand			
	2.00–0.2 mm	0.2–0.05 mm	0.05–0.002 mm	<0.002 mm	
SP1	45.7	17.9	24.9	11.5	sandy loam
SP2	46.7	14.2	30.7	8.4	sandy loam
SP3	10.6	6.8	67.5	15.1	silty loam
SP4	46.8	16.7	25.0	11.5	sandy loam
SP5	43.3	13.1	26.8	16.8	sandy loam
SP6	25.9	23.3	39.2	11.6	loam
SP7	7.9	14.1	65.5	12.5	silty loam
SP8	42.1	14.8	36.8	6.3	sandy loam
SP9	17.9	14.4	57.0	10.7	silty loam
SP10	7.9	14.1	65.5	12.5	silty loam
SP11	45.7	17.9	24.9	11.5	sandy loam
SP12	10.6	6.8	67.5	15.1	silty loam
SP13	22.2	11.3	57.1	9.4	silty loam
SP14	66.7	10.6	14.6	8.1	sandy loam
SP15	30.3	16.3	36.1	17.3	loam
SP16	23.0	12.6	56.1	8.3	silty loam

Chemical soil properties (pH in H₂O, pH in 1 M KCl, percentage of organic matter, and concentrations of P, K) as well as major elements (Fe, Ca, Mg) are shown in Table 3. The pH in H₂O of the soil samples varied from moderately acidic (6.56) to moderately alkaline (7.76), while pH in 1 M KCl was acidic (5.66) to almost neutral (6.95). The percentage of organic matter differed significantly among the sampling points and was within the range from 0.9% to 12.1%. The contents of P varied from <0.0001 g kg⁻¹ to 0.0315 g kg⁻¹ and K from 0.0253 g kg⁻¹ to 0.219 g kg⁻¹; these values are quite low and characteristic of soils developed on ultramafics [27]. As for the concentrations of major elements in soil samples, the soils had very low to high available Ca concentration (0.31–5.10 g kg⁻¹) and moderate to high concentration of available Mg (1.10–4.60 g kg⁻¹). The available Ca/Mg ratios in 13 soil samples are relatively low (0.20–0.97); this is in accordance with some other serpentine soils of the Apennine Peninsula, where the concentrations of available Ca tend to be much lower than those of Mg [27]. Magnesium is the predominant cation in neutral or moderately acid serpentine soils of the temperate zone [48]. In three serpentine soil samples (SP1, SP11, SP13) higher values of bioavailable Ca compared to Mg (Ca/Mg ratios 1.48 and 2.01) would indicate lower Ca deficiency stress for the plants, which was also noticed for the ultramafic soils in Iran [17]. The content of the available Fe varied from 0.13 g kg⁻¹ to 0.67 g kg⁻¹ and it was high in the loamy and alkaline soils [24].

Concentrations of the total and available trace elements (Ni, Zn, Mn, Cu) in the soils are presented in Table 4. The serpentine soils of investigated localities were characterized by elevated total concentrations of Ni, Zn and Mn that are typical of such sites. Total and available Ni concentrations varied from 1158 mg kg⁻¹ to 2272 mg kg⁻¹ and from 67 mg kg⁻¹ to 218 mg kg⁻¹ respectively. Total and available Mn concentrations were very high and varied from 1223 mg kg⁻¹ to 5650 mg kg⁻¹ and from 84 mg kg⁻¹ to 588 mg kg⁻¹ respectively. Total Ni and Mn concentrations in this research corresponded to the range of previously recorded values for different ultramafic soil samples from Serbia, other Balkan Peninsula countries, Turkey and Iran [6, 25, 30, 37, 43]. Concentrations of available Ni and Mn in all soils were much higher than those presented for ultramafic soils from Iran [17, 18]. Total concentrations of Zn in all sites were above 30 mg kg⁻¹, lying in the range between 35.3 and 201 mg kg⁻¹, while concentration of available Zn was between 1.2 mg kg⁻¹ and 84.7 mg kg⁻¹. Available Zn concentrations in the analyzed soils were much higher than those presented for serpentine soils from Italy [27]. The total Ni concentrations of serpentine soils were generally within the range 500–8000 µg g⁻¹ [17], while total Zn concentration of ultramafic soils was 40–60 mg kg⁻¹ and Mn concentration of worldwide soils was 411–550 mg kg⁻¹ [24]. The total Cu concentration was in the range 1.0–31.1 mg kg⁻¹ while available Cu concentrations were below <0.1 mg kg⁻¹ in 11 soil samples, and only in six cases were they higher (up to 1.7 mg kg⁻¹).

Total and available Cr, Cd, Co and Pb concentrations in soils are given in Table 5. The total Cr concentrations were high and varied from 174 mg kg⁻¹ to 1511 mg kg⁻¹, and were similar to the levels found in various Balkan serpentine soils [6]. Available Cr was below 0.1 mg kg⁻¹ at 14 sample points and in two cases it amounted to 19.6 mg kg⁻¹.

These low values confirm low mobility of Cr in surface soil layer and its limited availability to plants [27, 44]. Total and available concentrations of Cd at all but three sample points were below < 0.1 mg kg⁻¹. The average concentration of Cd in soils is between 0.2 and 1.1 mg kg⁻¹ [24]. In uncontaminated soils, its concentrations are highly determined by soil texture; they range from 0.01 to 0.3 mg kg⁻¹ in sandy soils and from

Table 3. pH, organic matter and P, K, Fe, Ca, Mg concentrations in soil samples (g kg^{-1}). (Means \pm standard deviations)

Sample point	pH soil in H_2O	pH soil in 1 M KCl	Organic matter %	P (available)	K (available)	Fe (available)	Ca (available)	Mg (available)	Ca/Mg
SP1	7.17	6.21	1.8 \pm 0.2	0.0049 \pm 0.0000	0.0257 \pm 0.0001	0.58 \pm 0.01	2.19 \pm 0.08	1.48 \pm 0.08	1.48
SP2	7.39	6.30	0.9 \pm 0.4	0.0186 \pm 0.0006	0.0857 \pm 0.0041	0.27 \pm 0.01	0.90 \pm 0.05	4.60 \pm 0.23	0.20
SP3	6.56	5.66	8.9 \pm 0.6	0.0081 \pm 0.0006	0.219 \pm 0.0010	0.15 \pm 0.01	2.14 \pm 0.16	3.87 \pm 0.09	0.55
SP4	7.42	6.48	3.7 \pm 0.3	0.0068 \pm 0.0013	0.0594 \pm 0.0013	0.59 \pm 0.01	1.14 \pm 0.05	2.39 \pm 0.10	0.48
SP5	7.76	6.95	12.1 \pm 1.5	<0.0001	0.141 \pm 0.003	0.13 \pm 0.01	0.97 \pm 0.01	2.49 \pm 0.06	0.39
SP6	7.67	6.93	4.2 \pm 0.5	0.0315 \pm 0.0047	0.156 \pm 0.006	0.49 \pm 0.01	0.75 \pm 0.03	1.10 \pm 0.03	0.68
SP7	7.10	6.02	2.6 \pm 0.5	0.0049 \pm 0.0000	0.0499 \pm 0.0011	0.50 \pm 0.01	1.30 \pm 0.04	2.91 \pm 0.43	0.45
SP8	7.27	6.32	7.1 \pm 0.8	0.0053 \pm 0.0006	0.0528 \pm 0.0013	0.38 \pm 0.01	2.01 \pm 0.05	3.32 \pm 0.12	0.61
SP9	7.10	6.03	4.6 \pm 0.2	0.0063 \pm 0.0007	0.0875 \pm 0.0010	0.15 \pm 0.01	2.35 \pm 0.00	3.53 \pm 0.07	0.67
SP10	7.10	6.02	2.6 \pm 0.5	0.0049 \pm 0.0000	0.0499 \pm 0.0011	0.50 \pm 0.01	1.30 \pm 0.04	2.91 \pm 0.43	0.45
SP11	7.17	6.21	1.8 \pm 0.2	0.0049 \pm 0.0000	0.0257 \pm 0.0001	0.58 \pm 0.01	2.19 \pm 0.08	1.48 \pm 0.08	1.48
SP12	6.56	5.66	8.9 \pm 0.6	0.0081 \pm 0.0006	0.219 \pm 0.001	0.15 \pm 0.01	2.14 \pm 0.16	3.87 \pm 0.09	0.55
SP13	6.77	5.87	8.0 \pm 1.4	0.0070 \pm 0.0002	0.110 \pm 0.005	0.16 \pm 0.01	5.10 \pm 0.28	2.54 \pm 0.10	2.01
SP14	7.01	5.82	10.1 \pm 0.4	0.0053 \pm 0.0006	0.0253 \pm 0.0010	0.67 \pm 0.02	0.31 \pm 0.00	3.80 \pm 0.12	0.08
SP15	7.13	6.13	2.9 \pm 0.8	0.0053 \pm 0.0006	0.149 \pm 0.002	0.17 \pm 0.01	2.56 \pm 0.05	2.65 \pm 0.14	0.97
SP16	7.00	5.97	5.0 \pm 1.2	0.0049 \pm 0.0000	0.0358 \pm 0.0008	0.33 \pm 0.01	1.30 \pm 0.16	3.51 \pm 0.03	0.37

Table 4. Concentrations of Ni, Zn, Mn, Cu in soil samples (mg kg⁻¹). (Means ± standard deviations)

Sample point	Ni (total)	Ni (available)	Zn (total)	Zn (available)	Mn (total)	Mn (available)	Cu (total)	Cu (available)
SP1	1552±52	72.8±1.8	35.3±4.6	1.2±0.1	1716±84	131±3	4.0±0.3	<0.1
SP2	1158±61	67.0±6.7	92.9±6.5	1.8±0.1	1456±63	98±3	31.1±0.8	1.6±0.1
SP3	1544±64	171±6	126±4	12.2±0.6	2717±153	241±2	14.5±0.6	<0.1
SP4	1463±44	122±7	201±9	84.7±3.9	1671±98	126±5	11.3±0.6	1.0±0.1
SP5	1922±34	201±1	45.1±0.4	5.2±0.4	1674±27	588±21	18.1±0.4	<0.1
SP6	2272±75	218±1	45.4±2.1	12.7±0.1	1262±30	534±12	10.4±0.2	<0.1
SP7	1592±38	142±8	71.7±3.5	2.3±0.2	2857±78	192±7	18.9±0.8	1.7±0.1
SP8	1697±70	127±4	56.4±4.9	2.9±0.1	5650±372	264±11	18.2±0.5	<0.1
SP9	1574±60	130±6	54.0±4.0	2.7±0.1	2631±165	203±5	12.3±0.7	<0.1
SP10	1592±38	142±8	71.7±3.5	2.3±0.2	2857±78	192±7	18.9±0.8	1.7±0.1
SP11	1552±52	72.8±1.8	35.3±4.6	1.2±0.1	1716±84	131±3	4.0±0.3	<0.1
SP12	1544±64	171±6	126±4	12.2±0.6	2717±153	241±2	14.5±0.6	<0.1
SP13	1340±77	157±5	91.7±8.1	4.5±0.1	3199±114	220±6	20.4±0.2	<0.1
SP14	1843±43	75.8±2.6	38.6±0.4	1.4±0.1	1223±82	84±2	1.0±0.6	<0.1
SP15	1490±88	132±2	59.2±5.6	10.1±0.9	2236±112	211±2	12.6±0.5	0.9±0.1
SP16	1548±58	130±6	69.9±1.5	2.1±0.1	2682±90	152±12	15.5±0.6	<0.1

0.2 to 0.8 mg kg⁻¹ in loamy soils. The concentration of total Co was between 92 mg kg⁻¹ and 177 mg kg⁻¹. Available Co was also below 0.1 mg kg⁻¹ at 14 sample points and in two cases it was 55.4 mg kg⁻¹ and 58.5 mg kg⁻¹, respectively. Finally, total Pb concentrations varied from 11.5 mg kg⁻¹ to 120 mg kg⁻¹, while available Pb concentrations were in all but three samples below < 0.1 mg kg⁻¹. Total Co concentration in mafic soils was from 35 to 200 mg kg⁻¹, while the ranges of total Pb concentrations in ultramafic rocks and calcareous sediments were 0.1–8 mg kg⁻¹ and 3–10 mg kg⁻¹, respectively [24]. However, it had been reported that only about 0.005–0.13% of Pb in soil solution is available to plants [12].

Chemical composition of the plant material

Concentrations of P, K, Fe, Ca and Mg in the roots and shoots of investigated plant populations are presented in Tables 6 and 7. Concentrations of P varied from 0.43 g kg⁻¹ to 3.43 g kg⁻¹ in roots; in shoots they were quite similar and within the range of 0.6–3.4 g kg⁻¹. Concentrations of K were high both in roots and shoots of all populations; in roots they varied from 10.40 g kg⁻¹ to 32.46 g kg⁻¹ and in shoots from 10.82 g kg⁻¹ to 41.95 g kg⁻¹. Concentrations of Fe varied from 0.34 g kg⁻¹ (*A. saxatile* at SP3) to 1.86 g kg⁻¹ (*A. montanum* at SP5) in roots, and from 0.15 g kg⁻¹ (*E. linariifolium* at SP12) to 1.82 g kg⁻¹ (*A. montanum* at SP4) in shoots. Fe concentration in all plant tissues was similar to those observed in the North Apennines serpentine plants [27] and elevated in relation to the natural Fe content of fodder plants which ranges within 18–1000 mg kg⁻¹ [24]. It had been stated that Fe concentration above 1000 mg kg⁻¹ often indicated a contamination of samples by serpentine soil or dust, not always easily removed by simple washing procedures [39]. In our study, Fe content above this value was recorded in six root samples, as well as in six shoot samples. This cannot be a matter only of mistake or coincidence since all the plant samples were washed carefully with bi-distilled water to remove soil particles. In the soils with easily soluble Fe, plants may take up a very large amount of Fe, which was clearly shown in vegetation grown on soils derived from serpentine, where grass contained Fe within the range of 2127–3580 mg kg⁻¹ [22].

Babalonas *et al.* [2] detected Fe concentration up to 9070 mg kg⁻¹ in leaves of serpentine plants from Greece and concluded that high Fe concentration of the plants was not a unique characteristic of the serpentine flora in Bosnia or Greece and may well be applicable to all the serpentine flora of the Balkans.

Despite relatively low available Ca concentration (0.31–5.10 g kg⁻¹) in the soil samples, concentrations of Ca in roots and shoots of the investigated plant populations were several times higher and varied between 1.60 g kg⁻¹ (*E. carniolicum* at SP13) and 8.59 g kg⁻¹ (*A. repens* at SP7) in roots and from 2.03 g kg⁻¹ (*C. plumieri* at SP8) to 43.41 g kg⁻¹ (*A. montanum* at SP6) in shoots. Mg concentration varied from 3.37 g kg⁻¹ (*A. montanum* at SP4) to 10.79 g kg⁻¹ (*C. plumieri* at SP8) in roots and from 4.86 g kg⁻¹ (*E. carniolicum* at SP13) to 15.07 g kg⁻¹ (*I. tinctoria* at SP14) in shoots. Generally, the concentration of Ca was lower than that of Mg in plant tissues (Ca/Mg ratio <1) and it seems that all these plants were tolerant to low Ca/Mg level in the soils, as well as in their tissues. In roots and shoots of *A. montanum*, *A. repens*, *E. linariifolium* and *R. lippizensis* (shoots only) the Ca/Mg ratio > 1 (1.12–6.06) was noticed. High Ca contents in roots and shoots of these three species are the consequence of the unusual ability of these plants to accumulate high Ca concentrations in their tissues, even from the soils with low

Table 5. Concentrations of Cr, Cd, Co, Pb in soil samples (mg kg^{-1}). (Means \pm standard deviations)

Sample point	Cr (total)	Cr (available)	Cd (total)	Cd (available)	Co (total)	Co (available)	Pb (total)	Pb (available)
SP1	244 \pm 6	<0.1	<0.1	<0.1	133 \pm 2	<0.1	22.0 \pm 1.6	<0.1
SP2	270 \pm 12	<0.1	<0.1	<0.1	92 \pm 2	<0.1	15.9 \pm 3.3	<0.1
SP3	919 \pm 28	<0.1	<0.1	<0.1	155 \pm 3	<0.1	66.8 \pm 0.8	<0.1
SP4	868 \pm 26	<0.1	<0.1	<0.1	126 \pm 1	<0.1	49.2 \pm 4.8	<0.1
SP5	610 \pm 26	16.8 \pm 0.8	3.2 \pm 0.2	<0.1	115 \pm 7	55.4 \pm 0.7	75.5 \pm 2.4	3.7 \pm 0.5
SP6	349 \pm 11	19.6 \pm 0.8	3.4 \pm 0.1	<0.1	104 \pm 11	58.5 \pm 1.1	120 \pm 5	38.4 \pm 1.0
SP7	357 \pm 12	<0.1	2.4 \pm 0.1	<0.1	176 \pm 3	<0.1	29.5 \pm 3.7	<0.1
SP8	359 \pm 10	<0.1	<0.1	<0.1	157 \pm 2	<0.1	23.5 \pm 5.5	<0.1
SP9	310 \pm 5	<0.1	<0.1	<0.1	139 \pm 4	<0.1	26.7 \pm 4.4	<0.1
SP10	357 \pm 12	<0.1	<0.1	<0.1	176 \pm 3	<0.1	29.5 \pm 3.7	<0.1
SP11	244 \pm 6	<0.1	<0.1	<0.1	133 \pm 2	<0.1	22.0 \pm 1.6	<0.1
SP12	919 \pm 28	<0.1	<0.1	<0.1	155 \pm 3	<0.1	66.8 \pm 0.8	<0.1
SP13	1511 \pm 116	<0.1	<0.1	<0.1	177 \pm 3	<0.1	66.1 \pm 2.9	<0.1
SP14	174 \pm 3	<0.1	<0.1	<0.1	120 \pm 7	<0.1	11.5 \pm 1.3	5.3 \pm 0.6
SP15	407 \pm 18	<0.1	<0.1	<0.1	143 \pm 6	<0.1	54.1 \pm 2.1	<0.1
SP16	1042 \pm 80	<0.1	<0.1	<0.1	164 \pm 5	<0.1	30.7 \pm 3.7	<0.1

Table 6. Concentrations of P, K, Fe in plant tissues of different Brassicaceae species (g kg⁻¹). (Means ± standard deviations)

Species	Sample point	P root	P shoot	K root	K shoot	Fe root	Fe shoot
<i>Aethionema saxatile</i>	SP1	2.4±0.2	2.83±0.15	11.24±0.18	14.11±0.06	0.87±0.01	0.30±0.01
<i>Aethionema saxatile</i>	SP2	1.90±0.06	2.1±0.2	17.68±0.82	24.97±0.20	0.53±0.03	0.26±0.02
<i>Aethionema saxatile</i>	SP3	0.43±0.06	0.6±0.0	11.74±0.67	15.41±0.13	0.34±0.02	0.67±0.14
<i>Alyssum montanum</i>	SP4	1.2±0.3	0.74±0.03	10.61±0.93	12.24±0.39	0.70±0.07	1.82±0.02
<i>Alyssum montanum</i>	SP5	1.42±0.11	1.37±0.07	13.23±0.18	14.51±0.16	1.86±0.09	1.37±0.03
<i>Alyssum montanum</i>	SP6	1.52±0.05	1.09±0.06	14.67±0.24	13.45±0.21	1.31±0.05	0.45±0.02
<i>Alyssum repens</i>	SP7	0.66±0.10	0.81±0.06	10.40±0.48	10.82±0.25	0.47±0.04	0.57±0.03
<i>Cardamine plumieri</i>	SP8	2.80±0.26	1.90±0.17	16.87±0.48	18.91±0.20	1.49±0.08	1.0±0.1
<i>Cardamine plumieri</i>	SP9	1.6±0.1	2.00±0.17	28.79±0.73	38.72±0.86	1.40±0.09	1.33±0.03
<i>Cardamine plumieri</i>	SP10	1.2±0.1	1.2±0.3	19.85±0.48	27.46±0.88	1.60±0.09	1.32±0.04
<i>Erysimum linariifolium</i>	SP11	3.43±0.21	3.4±0.2	11.72±0.14	13.93±0.19	0.80±0.06	0.86±0.03
<i>Erysimum linariifolium</i>	SP12	1.2±0.3	2.0±0.2	12.02±0.55	21.95±1.18	0.45±0.10	0.15±0.05
<i>Erysimum carnioolicum</i>	SP13	1.73±0.11	1.99±0.23	16.34±0.06	18.43±0.61	0.96±0.05	1.07±0.08
<i>Isatis tinctoria</i>	SP14	1.13±0.10	2.0±0.0	29.95±0.15	41.95±0.62	0.74±0.08	0.76±0.03
<i>Isatis tinctoria</i>	SP15	1.9±0.1	2.1±0.2	32.46±0.29	35.79±3.80	0.80±0.07	0.73±0.05
<i>Rorippa lippizensis</i>	SP16	1.17±0.15	1.77±0.23	14.53±0.49	16.82±0.46	1.63±0.06	0.45±0.02

Table 7. Concentrations of Ca, Mg in plant tissues of different Brassicaceae species (g kg^{-1}). (Means \pm standard deviations)

Species	Sample point	Ca root	Ca shoot	Mg root	Mg shoot	Ca/Mg root	Ca/Mg shoot
<i>Aethionema saxatile</i>	SP1	4.45 \pm 0.28	9.66 \pm 0.09	6.84 \pm 0.12	10.64 \pm 0.15	0.65	0.91
<i>Aethionema saxatile</i>	SP2	4.01 \pm 0.32	8.71 \pm 0.27	5.43 \pm 0.17	12.76 \pm 0.30	0.74	0.68
<i>Aethionema saxatile</i>	SP3	3.49 \pm 0.28	4.95 \pm 0.29	5.82 \pm 0.27	9.46 \pm 0.29	0.60	0.52
<i>Alyssum montanum</i>	SP4	7.28 \pm 0.32	20.71 \pm 0.48	3.37 \pm 0.18	11.28 \pm 0.49	2.16	1.84
<i>Alyssum montanum</i>	SP5	6.58 \pm 0.11	34.89 \pm 1.48	9.92 \pm 0.20	11.15 \pm 0.19	0.66	3.13
<i>Alyssum montanum</i>	SP6	7.90 \pm 0.14	43.41 \pm 1.29	10.08 \pm 0.34	7.16 \pm 0.03	0.78	6.06
<i>Alyssum repens</i>	SP7	8.59 \pm 0.41	14.31 \pm 0.39	5.02 \pm 0.20	5.99 \pm 0.05	1.71	2.39
<i>Cardamine plumieri</i>	SP8	6.91 \pm 0.33	2.03 \pm 0.16	10.79 \pm 0.32	12.17 \pm 0.32	0.64	0.17
<i>Cardamine plumieri</i>	SP9	6.77 \pm 0.31	4.44 \pm 0.18	10.02 \pm 0.26	14.08 \pm 0.09	0.68	0.32
<i>Cardamine plumieri</i>	SP10	4.32 \pm 0.12	7.72 \pm 0.31	10.29 \pm 0.49	13.29 \pm 0.20	0.42	0.58
<i>Erysimum linariifolium</i>	SP11	7.59 \pm 0.33	3.75 \pm 0.17	6.33 \pm 0.13	7.54 \pm 0.24	1.20	0.50
<i>Erysimum linariifolium</i>	SP12	7.59 \pm 0.34	12.16 \pm 0.36	4.10 \pm 0.17	5.01 \pm 0.37	1.85	2.43
<i>Erysimum carnioiticum</i>	SP13	1.60 \pm 0.21	3.90 \pm 0.13	5.23 \pm 0.54	4.86 \pm 0.17	0.31	0.80
<i>Isatis tinctoria</i>	SP14	4.24 \pm 0.31	8.73 \pm 0.46	10.67 \pm 0.12	15.07 \pm 0.14	0.40	0.58
<i>Isatis tinctoria</i>	SP15	4.19 \pm 0.15	4.11 \pm 0.27	8.07 \pm 0.08	8.33 \pm 0.08	0.52	0.49
<i>Rorippa lippizensis</i>	SP16	6.80 \pm 0.27	8.65 \pm 0.27	9.95 \pm 0.29	7.71 \pm 0.29	0.68	1.12

Ca/Mg ratios that are a characteristic of serpentines. Similar results were presented for various plants from Italy, Albania and Greece (Lesbos Island) respectively [25, 27, 43]. Therefore, some of the serpentine plants must possess a mechanism to limit uptake of Mg and/or a high absorption capacity for selective Ca uptake [31].

A. montanum, *A. repens*, *E. linariifolium* and *R. lippizensis* occur both on and off serpentine soils [23] and the use of serpentine and non-serpentine populations of such species may be especially effective in the study of serpentine adaptation, since the populations being compared should be closely similar in every respect except in adaptation to serpentine [26].

Trace elements concentrations (Ni, Zn, Mn, Cu) in roots and shoots of eight Brassicaceae species are shown in Table 8. On ultramafic soils containing more than 1000 mg Ni kg⁻¹ most of the plants that survive there show Ni concentrations about 10 times higher than elsewhere, i.e. from about 2 to 80 mg kg⁻¹ [36], while the uptake of this element by Brassicaceae species on these soils generally falls in the range 20–150 mg kg⁻¹ [34]. In our present research, most of the plants accumulated Ni in the range < 1.0–100 mg kg⁻¹, while in the roots and shoots of *I. tinctoria* at SP15, the detected Ni content was slightly above this value (113 mg kg⁻¹ and 152 mg kg⁻¹ respectively). In roots (up to 657 mg kg⁻¹) and shoots (up to 676 mg kg⁻¹) of *A. montanum* at SP4 and *A. repens* at SP7 Ni concentrations were high and this is the first instance that such values were measured within these two species. Reeves *et al.* [40] measured only 22 mg kg⁻¹ Ni in the leaves of *A. montanum* from herbarium material and concluded that *Alyssum* species from the section *Alyssum* consistently show unremarkable Ni levels even serpentine endemics in this section. Brooks *et al.* [8] considered that Ni hyperaccumulation is almost entirely restricted to the section *Odontarrhena* of the genus *Alyssum*, while in other four sections (*Meniocus*, *Psilonema*, *Gamosepalum*, *Tetradenia*) no value higher than 152 mg kg⁻¹ was found. These authors found that Ni contents in *A. montanum* and *A. repens* leaves were up to 5 mg kg⁻¹ and 6 mg kg⁻¹ respectively. But, based on their accumulation properties, some populations of *A. montanum*, *A. repens* and *I. tinctoria* can be classified as Ni strong accumulators, since such plants can accumulate 100–999 mg kg⁻¹ Ni in leaves [8].

In comparison with low Zn concentration in soil samples, Zn concentration was much higher in roots and shoots of all plant samples. Concentrations of Zn lay in the range of 12.3–107 mg kg⁻¹ in roots and 7.3–111 mg kg⁻¹ in shoots. The opposite trend was noticed for Mn concentration in plant tissues. Although Mn content in soil samples was relatively high, its concentration in plant tissues was several times lower; in roots Mn content was in the range from 7.6 mg kg⁻¹ (*A. repens* at SP7) to 96.6 mg kg⁻¹ (*C. plumieri* at SP9) and in shoots it varied from 13.2 mg kg⁻¹ (*A. repens* at SP7) to 120 mg kg⁻¹ (*C. plumieri* at SP8). It is assumed that species growing in serpentine soils can restrict transport of Mn to the shoots and maintain a relatively low concentration of metals in aboveground parts even at high concentrations in soils [25]. Mn concentrations in plants lower than those in corresponding soils can be attributed to soil pH (mean value 7), since at this pH value Mn forms insoluble oxides resulting in reduced availability [2]. Concentrations of Cu were generally low; in roots and shoots they were from < 1.0 mg kg⁻¹ to 78.6 mg kg⁻¹ and from < 1.0 mg kg⁻¹ to 28.8 mg kg⁻¹ respectively. Only in the roots and shoots of *A. repens* were much higher concentrations (178 mg kg⁻¹ and 143 mg kg⁻¹) of this element recorded. Similar results were presented for different *Alyssum* and *Thlaspi* plants from the ultramafic soils of the Balkans [6].

Table 8. Concentrations of Ni, Zn, Mn, Cu in plant tissues of different Brassicaceae species (mg kg⁻¹). (Means ± standard deviations)

Species	Sample point	Ni		Zn		Mn		Cu	
		root	shoot	root	shoot	root	shoot	root	shoot
<i>Aethionema saxatile</i>	SP1	20.4±4.2	<1.0	44.8±3.9	52.9±2.5	25.7±2.9	16.2±1.6	<1.0	3.7±0.4
<i>Aethionema saxatile</i>	SP2	84.3±4.6	41.8±13.6	12.7±1.5	11.2±2.7	25.4±0.8	35.2±0.1	2.1±0.1	3.2±0.01
<i>Aethionema saxatile</i>	SP3	<1.0	<1.0	12.3±1.8	7.3±0.8	16.8±3.1	20.0±3.9	5.7±0.3	4.6±0.1
<i>Alyssum montanum</i>	SP4	296±7	350±13	19.9±3.0	63.7±2.5	10.1±1.7	49.0±5.0	1.2±0.1	28.8±4.9
<i>Alyssum montanum</i>	SP5	31.6±17.9	11.6±4.1	22.8±1.8	32.9±3.0	83.0±6.6	72.6±5.7	3.8±0.6	6.5±1.1
<i>Alyssum montanum</i>	SP6	96.4±17.5	69.4±9.3	30.7±3.3	20.2±2.0	46.7±3.9	25.2±1.0	14.3±0.6	8.7±0.5
<i>Alyssum repens</i>	SP7	657±78	676±8	62.3±0.4	50.9±2.5	7.6±2.3	13.2±1.4	178±12	143±9
<i>Cardamine plumieri</i>	SP8	76.5±7.6	30.2±0.3	80.1±2.3	87.2±3.4	125±3	120±6	<1.0	<1.0
<i>Cardamine plumieri</i>	SP9	111±9	99.8±16.2	33.3±1.9	30.5±2.2	96.6±6.6	100±2	6.5±0.0	5.4±0.1
<i>Cardamine plumieri</i>	SP10	84.1±12.9	72.6±16.1	57.9±2.6	44.8±1.0	76.4±5.0	90.0±5.4	3.2±0.1	3.2±0.1
<i>Erysimum linariifolium</i>	SP11	22.7±7.1	42.6±4.3	47.9±7.1	111±20	15.2±3.4	33.9±2.8	<1.0	16.4±7.5
<i>Erysimum linariifolium</i>	SP12	91.2±9.2	41.8±8.3	25.9±4.0	31.7±1.9	34.4±2.6	26.4±1.5	3.2±0.1	3.2±0.1
<i>Erysimum carnioiticum</i>	SP13	10.5±8.4	12.9±4.1	47.0±6.2	67.2±4.3	23.5±5.1	23.9±3.4	10.5±2.9	19.1±2.8
<i>Isatis tinctoria</i>	SP14	81.6±7.2	89.6±31.0	29.5±7.7	18.8±3.7	40.6±3.7	43.5±1.6	17.7±1.9	16.0±2.0
<i>Isatis tinctoria</i>	SP15	113±7	152±1	62.0±4.8	22.6±2.4	31.5±2.0	31.5±1.8	3.2±0.1	21.0±1.8
<i>Rorippa lippizensis</i>	SP16	104±10	<1.0	107±9	77.6±7.5	63.5±9.8	17.4±0.8	78.6±7.1	19.8±2.2

Concentrations of all the remaining trace elements (Cr, Cd, Co, Pb) in the roots and shoots are presented in Table 9. Concentrations of Cr and Cd in all the investigated plants were below 1.0 mg kg^{-1} . In only four plant samples was Cr detected in the roots and shoots (up to 83.3 mg kg^{-1}), as well as in the case of Cd, where this element was detected only in traces (up to 7.0 mg kg^{-1}) and only in two root samples. Chromium uptake by plants is usually slight [9] and this was explained by the fact that much of the Cr in ultramafic rocks and the soils derived from them is likely to be in the form of relatively insoluble minerals such as chromite [25].

Concentrations of Co and Pb in all plant samples were below 1.0 mg kg^{-1} . Lead is usually not highly available to plants because it occurs in water-insoluble chemical forms in the soil [24]. In addition, the transport of this metal to the shoot is commonly very limited, with up to about 90% of the trace element taken up by a plant remaining in the roots [1].

Four categories of plant-heavy metal relationships were suggested [3]: excluders, indicators, accumulators and hyperaccumulators. In order to decide within which of the four above-presented plant-heavy metal relationships the investigated Brassicaceae plants can be classified, we calculated their accumulation potential for Ni and Zn (Table 10).

Accumulation and transport characteristics for Ni and Zn of the Brassicaceae species were estimated from the ratio between trace elements concentrations in soil, roots, and shoots. The transport factor (TF) was calculated as the shoot-to-root ratio of Ni or Zn concentrations. The accumulation factor (AF) was calculated as the ratio between Ni or Zn concentration in the shoot and the bioavailable concentration in the corresponding soil. In the case of Ni, only the roots of *A. montanum* (SP4) and *A. repens* accumulated Ni from the corresponding soil by a factor distinctly > 1 (2.43 and 4.63 respectively), while in the roots of all the remaining plant samples accumulation of Ni was not found (Ni concentrations in soil and roots were almost equal or distinctly < 1). TF and AF were low (< 1) for most of the plant samples (from 0.06 to 0.90), while only in the case of *E. linariifolium* (SP11) and *E. carniolicum* TF were values > 1 (1.88 and 1.23 respectively). Both TF and AF values > 1 were recorded in *A. montanum* (SP4), *A. repens* and *I. tinctoria*. In the case of Zn roots, almost all Brassicaceae species accumulated Zn from the corresponding soil by a factor distinctly > 1 (up to 50.95 in *R. lippizensis*). TF was low (< 1 or slightly above this value) for the majority of plant samples (from 0.36 to 1.44), and only in the case of *A. montanum* (SP4) and *E. linariifolium* (SP11) TF were values > 2 (3.20 and 2.32 respectively). AF values > 1 were recorded in 14 plant samples (up to 92.50 in *E. linariifolium* at SP11) and only in the case of *A. saxatile* (SP3) and *A. montanum* (SP4) AF were values < 1 (0.60 and 0.75 respectively).

If hyperaccumulators are defined as those plants which contain more than 1000 mg kg^{-1} dry weight of Ni, Co, Cu, Cr or Pb, or more than $10\,000 \text{ mg kg}^{-1}$ dry weight of Zn or Mn in their tissue [4], none of the investigated Brassicaceae species belongs to the group of hyperaccumulators. Based on their accumulation properties, some populations of *A. montanum*, *A. repens* and *I. tinctoria*, can be classified as Ni strong accumulators or Zn accumulators. Strong accumulators can accumulate $100\text{--}999 \text{ mg kg}^{-1}$ Ni in leaves [8], while accumulators show a tendency or ability to translocate and accumulate high metal levels in above-ground plant parts from both low and high soil metal concentrations without toxicity symptoms; their shoot/root metal concentration quotient is > 1.0 . However, most Brassicaceae species studied here belong to the group of species tolerant

Table 10. Accumulation potential for Ni and Zn of different Brassicaceae species

Species	Sample point	Ni root/soil	Ni TF	Ni AF	Zn root/soil	Zn TF	Zn AF
<i>Aethionema saxatile</i>	SP1	0.28	–	–	37.33	1.18	44.08
<i>Aethionema saxatile</i>	SP2	1.26	0.50	0.62	7.06	0.88	6.22
<i>Aethionema saxatile</i>	SP3	–	–	–	1.01	0.59	0.60
<i>Alyssum montanum</i>	SP4	2.43	1.18	2.87	0.23	3.20	0.75
<i>Alyssum montanum</i>	SP5	0.16	0.37	0.06	4.38	1.44	6.33
<i>Alyssum montanum</i>	SP6	0.44	0.72	0.32	2.42	0.66	1.59
<i>Alyssum repens</i>	SP7	4.63	1.03	4.76	27.09	0.82	22.13
<i>Cardamine plumieri</i>	SP8	0.60	0.39	0.24	27.62	1.09	30.07
<i>Cardamine plumieri</i>	SP9	0.85	0.90	0.77	12.33	0.92	11.30
<i>Cardamine plumieri</i>	SP10	0.59	0.86	0.51	25.17	0.77	19.48
<i>Erysimum linariifolium</i>	SP11	0.31	1.88	0.59	39.92	2.32	92.50
<i>Erysimum linariifolium</i>	SP12	0.53	0.46	0.24	2.12	1.22	2.60
<i>Erysimum carniolicum</i>	SP13	0.07	1.23	0.08	10.44	1.43	14.93
<i>Isatis tinctoria</i>	SP14	1.08	1.10	1.18	21.07	0.64	13.43
<i>Isatis tinctoria</i>	SP15	0.86	1.35	1.15	6.14	0.36	2.24
<i>Rorippa lippizensis</i>	SP16	0.80	–	–	50.95	0.73	36.95

to serpentine soils, and can be considered either as excluders or as indicators. Excluders are the plants that restrict transport of metals to the shoot, and maintain relatively low metal concentrations in the shoot over a wide range of soil metal concentrations; their leaf/root metal concentration quotient is < 1.0 ; indicators are plants that show an intermediate response to high soil metal concentrations with the metal concentration in the plants reflecting the soil concentration; the plant/soil metal concentration quotient in indicators is approximately 1.0 [3, 4].

CONCLUSIONS

Soils collected as 16 soil samples from 10 serpentine locations in Serbia contained low levels of P, K and Ca and high Fe and Mg ratios. The available Ca/Mg ratios in 13 soil samples were low (< 1). Soil samples were characterized by elevated total and available concentrations of trace elements such as Ni, Zn and Mn that are typical of ultramafic sites.

Fe and Zn were found in plants at higher levels than in the soil. High Fe quantities within Brassicaceae plants from ultramafics of Serbia are considered to be generally characteristic of the serpentine flora of the Balkans. With the exception of certain instances where it is inverted, the Ca/Mg ratio typical of serpentine soils (< 1) is maintained as such

even within the plants. Mn, Cu, Cr, Cd, Co and Pb, although existing in small amounts in the soil, were found either in small concentrations within the plants (Mn, Cu) or in traces (Cr, Cd, Co, Pb).

Storage of the highly toxic Ni seems to be a characteristic of only certain plant species. *Alyssum repens* and some populations of *Alyssum montanum* behaved differently from the other six Brassicaceae plant species examined in this study. The Ni concentrations of some specimens of *Alyssum montanum* and *Alyssum repens* were high even for plants growing in a nickel-rich environment and very unusual for the species that belong to the section *Alyssum*. Actually, this is the first time that such high Ni concentration was measured within any species that belongs to this section. These values may indicate a stage of evolution proceeding towards hyperaccumulation of nickel as a mechanism of tolerance [40]. Besides the fact that they accumulated several times more Ni than those in corresponding soil, these two species and *Erysimum linariifolium* also shared a somewhat peculiar behavior as regards the Ca/Mg ratio. They had a higher value of this ratio (both in roots and shoots) than most of the species, due to the unusual ability of these plants to accumulate high Ca concentrations in their tissues, even from the soils with low Ca/Mg ratios that are characteristic of serpentines.

Despite the fact that the populations of *Isatis tinctoria* showed only low to moderate capacity to uptake, translocate or accumulate trace elements such as Ni and Zn in its roots and shoots, this biennial plant could be a very good candidate for phytoextraction practice due to its well developed and deep roots, fast growth rate and high biomass production. For the commercial application of phytoremediation of soil trace metals, fast growing plants with high biomass and good metal uptake ability are needed [19]. Further exploration and sampling of *I. tinctoria* and soil over the full serpentine range of this species may reveal even higher Ni contents in these plants, raising the possibility that they could be used as indicators of Ni concentrations of the soil.

The use of indigenous plants from the Brassicaceae family with high tolerance and accumulation capacity for Ni (and some other trace metals) could be a very convenient approach to phytoremediation and phytoextraction practices.

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REFERENCES

- [1] Antosiewicz, D.M. (2004). Study of calcium-dependent lead tolerance on plants differing in their level of Ca-deficiency tolerance, *Environmental Pollution*, 134, 23–34.
- [2] Babalonas, D., Karataglis, S., & Kabassakalis, V. (1984). The Ecology of Plant Populations Growing on Serpentine Soils, *Phyton*, 24 (2), 225–238.
- [3] Baker, A.J.M. (1981). Accumulators and excluders – strategies in the response of plants to heavy metals, *Journal of Plant Nutrition*, 3, 643–654.

- [4] Baker, A.J.M., & Brooks, R.R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry, *Biorecovery*, 1, 81–126.
- [5] Bani, A., Echevarria, G., Mullaj, A., Reeves, R., Morel, J.L., & Sulçe, S. (2009). Nickel Hyperaccumulation by Brassicaceae in Serpentine Soils of Albania and Northwestern Greece, In: Soil and Biota of Serpentine: A World View, Proceedings of the Sixth International Conference on Serpentine Ecology, *Northeastern Naturalist*, 16 (sp5), 385–404.
- [6] Bani, A., Pavlova, D., Echevarria, G., & Mullaj, A. (2010). Nickel hyperaccumulation by the species of Alyssum and Thlaspi (Brassicaceae) from the ultramafic soils of the Balkans, *Botanica Serbica*, 34 (1), 3–14.
- [7] Brady, K.U., Kruckeberg, A.R., & Bradshaw, H.D. (2005). Evolutionary Ecology of Plant Adaptation to Serpentine Soils, *Annual Review of Ecology Evolution and Systematics*, 36, 243–266.
- [8] Brooks, R.R., Morrison, R.S., Reeves, R.D., Dudley, T.R., & Akman, Y. (1979). Hyperaccumulation of Nickel by Alyssum Linnaeus (Cruciferae), *Proceedings of the Royal Society of London B*, 203, 1153, 387–403.
- [9] Brooks, R.R., & Radford, C.C. (1978). Nickel Accumulation by European Species of the Genus Alyssum, *Proceedings of the Royal Society of London B*, 200, 1139, 217–224.
- [10] Chardot, V., Massoura, S.T., & Echevarria, G. (2005). Phytoextraction Potential of the Nickel Hyperaccumulators *Leptoplax emarginata* and *Bornmuellera tymphaea*, *International Journal of Phytoremediation*, 7, 3, 323–335.
- [11] Chen, P.S., Toribara, T.Y., & Warner, H. (1956). Microdetermination of phosphorus, *Analytical Chemistry*, 28, 1756–1758.
- [12] Davies, B.E. (1995). Lead. In B.J. Alloway (Ed.), *Heavy metals in soils* (pp. 206–223). Blackie Academic, London, 1995.
- [13] Egnér, H., Riehm, H., & Domingo, W.R. (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Boden II. Chemische Extraktionmethoden zu Phosphor- und Kaliumbestimmung, *Kunlinga Landboukshogskolans Annaler*, 26, 199–215.
- [14] FAO: *The Euphrates Pilot Irrigation Project. Methods of soil analysis. Gadeb Soil Laboratory (A laboratory manual)*, Food and Agriculture Organization, Rome, 1974.
- [15] Freitas, H., Prasad, M.N.V., & Pratas, J. (2004). Analysis of serpentinophytes from north-east of Portugal for trace metal accumulation-relevance to the management of mine environment, *Chemosphere*, 54, 11, 1625–1642.
- [16] Garcia, W.J., Blessin, C.W., Sandford, H.W., & Inglett, G.E. (1979). Translation and accumulation of seven heavy metals in tissues of corn plants grown on sludge-treated strip-mined soil, *Journal of Agricultural and Food Chemistry*, 27, 5, 1088–1094.
- [17] Ghaderian, S.M., Mohtadi, A., Rahiminejad, M.R., & Baker, A.J.M. (2007). Nickel and other metal uptake and accumulation by species of Alyssum (Brassicaceae) from the ultramafics of Iran, *Environmental Pollution*, 145, 293–298.
- [18] Ghaderian, S.M., Mohtadi, A., Rahiminejad, M.R., Reeves, R.D., & Baker, A.J.M. (2007^a) Hyperaccumulation of nickel by two Alyssum species from the serpentine soils of Iran, *Plant and Soil*, 293, 91–97.
- [19] Ghosh, M., & Singh, S.P. (2005). A Review on Phytoremediation of Heavy Metals and Utilization of its Byproducts, *Applied Ecology and Environmental Research*, 3, 1, 1–18.
- [20] Harrison, S., Viers, J.H., & Quinn, J.F. (2000). Climatic and spatial patterns of diversity in the serpentine plants of California, *Diversity and Distributions*, 6, 153–161.
- [21] Hasko, A., & Çullaj, A. (2001). Nickel hyper-accumulating species and their potential use for the phyto-remediation of polluted areas, *Options Méditerranéennes. Série A: Séminaires Méditerranéens*, 47, 137–150.
- [22] Johnston, W. R., & Proctor, J. (1977). Metal concentrations in plants and soils from two British serpentine sites, *Plant and Soil*, 46, 275–278.
- [23] Jovanović-Dunjić, R., Diklić, N., & Nikolić, V. (1973). Brassicaceae L. In M. Josifović (Ed.), *Flore de la Serbie*, Académie Serbe des Sciences et des Arts, 177–394, Belgrade, 1973.
- [24] Kabata-Pendias, A. (2011). *Trace elements in soils and plants* (4th ed.), CRC Press, Taylor & Francis Group, Boca Raton, London, New York, 2011.
- [25] Kazakou, E., Adamidis, G.C., Baker, A.J.M., Reeves, R.D., Godino, M., & Dimitrakopoulos, P.G. (2010). Species adaptation in serpentine soils in Lesbos Island (Greece): metal hyperaccumulation and tolerance, *Plant and Soil*, 332, 1–2, 369–385.

- [26] Kruckeberg, A.R. (1954). Plant species in relation to serpentine soils, In: Whittaker, R.H. (Ed.). *The Ecology of Serpentine Soils*, *Ecology*, 35, 2, 267–274.
- [27] Lombini, A., Dinelli, E., Ferrari, C., & Simoni, A. (1998). Plant-soil relationships in the serpentinite screes of Mt Prinzerza (Northern Apennines, Italy), *Journal of Geochemical Exploration*, 64, 1–3, 19–33.
- [28] McKeague, J.A. (1978). (Ed.), *Manual on soil sampling and methods of analysis*, Canadian Society of Soil Science, 1978, 66–68.
- [29] Obratov, D., Kadović, R., & Mihajlović, N. (1997). Alyssum markgrafii (Brassicaceae, Capparales) as nickel hiperaccumulator on Goč and Kopaonik serpentinites, Proceedings of the 3rd International Conference on the Development of Forestry & Wood Science/Technology, ICFWST '97 volume II, Belgrade & Mt. Goč, 1997, 24–28.
- [30] Obratov-Petković, D., Bjedov, I., & Belanović, S. (2008). The content of heavy metals in the leaves of Hypericum perforatum L. on serpentinite soils in Serbia, *Bulletin of the Faculty of Forestry – University of Belgrade*, 98, 143–154.
- [31] Pandolfini, T., & Pancaro, L. (1992). Biogeochemical survey of some ophiolitic outcrops in Tuscany, *Flora*, 187, 341–351.
- [32] Proctor, J. (2003). Vegetation and soil and plant chemistry on ultramafic rocks in the tropical Far East, *Perspectives in Plant Ecology Evolution & Systematics*, 6, 1–2, 105–124.
- [33] Proctor, J., & Woodell, S.R.J. (1971). The Plant Ecology of Serpentine: I. Serpentine Vegetation of England and Scotland, *Journal of Ecology*, 59, 2, 375–395.
- [34] Reeves, R.D. (1988). Nickel and Zinc Accumulation by Species of Thlaspi L., Cochlearia L., and Other Genera of the Brassicaceae, *Taxon*, 37, 2, 308–318.
- [35] Reeves, R.D. (1992). The hyperaccumulation of nickel by serpentine plants, In A.J.M. Baker, J., Proctor, R.D. Reeves (Eds.), *The Vegetation of Ultramafic (Serpentine) Soils* Intercept Ltd, Andover, 1992, 253–278.
- [36] Reeves, R.D., & Adigüzel, N. (2008). The Nickel Hyperaccumulating Plants of the Serpentines of Turkey and Adjacent Areas: A Review with New Data, *Turkish Journal of Biology*, 32, 3, 143–153.
- [37] Reeves, R.D., Adigüzel, N., & Baker, A.J.M. (2009). Nickel Hyperaccumulation in Bornmuellera kiyakii Aytaç & Aksoy and Associated Plants of the Brassicaceae from Kızıldağ (Derebucak, Konya-Turkey), *Turkish Journal of Botany*, 33, 33–40.
- [38] Reeves, R.D., & Baker, A.J.M. (2000). Metal-accumulating plants. In I. Raskin & B.D. Ensley (Eds.), *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*, 193–229 John Wiley & Sons, Inc., New York, 2000.
- [39] Reeves, R.D., Baker, A.J.M., Borhidi, A., & Berazain, R. (1999). Nickel hyperaccumulation in the serpentine flora of Cuba, *Annals of Botany*, 83, 29–38.
- [40] Reeves, R.D., Brooks, R.R., & Dudley, Th.R. (1983). Uptake of Nickel by Species of Alyssum, Bornmuellera, and Other Genera of Old World Tribus Alysseae, *Taxon*, 32, 2, 184–192.
- [41] Reeves, R.D., Brooks, R.R., & Preiss, J.R. (1980). Nickel accumulation by species of Peltaria Jacq. (Cruciferae), *Taxon*, 29, 5–6, 629–633.
- [42] Rowell, D.L. (1997). *Bodenkunde. Untersuchungsmethoden und ihre Anwendungen*, Springer, Berlin, 1997.
- [43] Shallari, S., Schwartz, C., Haskob, A., & Morela, J.L. (1998). Heavy metals in soils and plants of serpentine and industrial sites of Albania, *The Science of The Total Environment*, 209, 133–142.
- [44] Słowik, M., Młynarczyk, Z., & Sobczyński, T. (2011). Mobility of Chromium and Lead Originating from Weaving Industry: Implications for Relative Dating of Lowland River Floodplain Deposits (The Obra River, Poland), *Archives of Environmental Protection*, 37, 2, 131–150.
- [45] Stevanović, V., Tan Kit & Iatrou, G. (2003). Distribution of the endemic Balkan flora on serpentine I. – obligate serpentine endemics, *Plant Systematics and Evolution*, 242, 1–4, 149–170.
- [46] Van Reeuwijk, L.P. (Ed.) (1995). Procedures for Soil Analysis (5th ed.), Technical Paper/International Soil Reference and Information Centre, Wageningen, 1995.
- [47] Van Reeuwijk, L.P. (Ed.) (2002). Procedures for Soil Analysis (6th ed.), Technical Paper/International Soil Reference and Information Centre, Wageningen, 2002.
- [48] Vinterhalter, B., & Vinterhalter, D. (2005). Nickel hyperaccumulation in shoot cultures of Alyssum markgrafii, *Biologia Plantarum*, 49, 121–124.
- [49] Walker, R.B. (1954). Factors affecting plant growth on serpentine soils. In R.H. Whittaker, (Ed.), *The Ecology of Serpentine Soils*, *Ecology*, 35, 2, 259–266.