Pol. J. Environ. Stud. Vol. 21, No. 6 (2012), 1855-1866

Original Research

Comparative Study of Hyperaccumulation of Nickel by *Alyssum murale* s.l. Populations from the Ultramafics of Serbia

Ahmed F. Tumi[1], Nevena Mihailović[2], Boško A. Gajić[3],
Marjan Niketić[4], Gordana Tomović[1]*

[1]Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade,
Takovska 43, 11000 Belgrade, Serbia
[2]Institute for the Application of Nuclear Energy – INEP, University of Belgrade,
Banatska 31b, 11080 Belgrade, Serbia
[3]Laboratory of Soil Physics, Institute of Land Management, Faculty of Agriculture, University of Belgrade,
Nemanjina 6, 11081 Belgrade, Serbia
[4]Natural History Museum, Njegoševa 51, 11000 Belgrade, Serbia

Received: 4 February 2012
Accepted: 13 June 2012

Abstract

Ultramafic areas exist in large blocks or as small outcrops separated from other geological substrates in Serbia and host a certain number of facultative serpentinophytes. Among them is *Alyssum murale* Waldst & Kit. s.l., widespread species distributed in SE Europe and SW Asia and well known example of Ni hyperaccumulators. The aims of the present study were to investigate the level of concentration of Ni (and some elements such as Fe, Mn, Zn, Cu, Ni, Cr, Pb, Co, and Cd) at several serpentine soils in Serbia and to determine the level of accumulation of these elements in roots, shoots, and leaves, with a focus on Ni. The maximum available concentrations of metals in soil dry matter (DM) were 950 mg·kg^{-1} Fe, 3,110 mg·kg^{-1} Ca, 4,600 mg·kg^{-1} Mg, 443 mg·kg^{-1} Ni, 803 mg·kg^{-1} Mn, 26 mg·kg^{-1} Zn, 4 mg·kg^{-1} Cu, 43 mg·kg^{-1} Cr, 82 mg·kg^{-1} Co, 1 mg·kg^{-1} Cd, and 17 mg·kg^{-1} Pb. The Ca/Mg ratio in serpentine soils varied from 0.08 to 6.22. In *A. murale* plants, the maximum concentrations of Ni were up to 2,926 mg·kg^{-1} in roots, 6,793 mg·kg^{-1} in shoots, and 13,160 mg·kg^{-1} in leaves. Ca/Mg ratio in plant tissues were up to 2.25 (roots), 9.25 (shoots), and 15.23 (leaves). Cu content in the roots and shoots was high (up to 1,044 mg·kg^{-1} Cu and 849 mg·kg^{-1} Cu). This survey suggests that some *A. murale* populations from serpentine soils of Serbia emerge as strong Ni hyperaccumulators and can be used for phytoextraction purposes.

Keywords: ultramafics, serpentine soils, *Alyssum murale*, nickel hyperaccumulator, Serbia

Introduction

Ultramafics ('ultrabasic') represent a group of igneous (magmatic) or meta-igneous (metamorphic) rocks that consist of less than 45% silica (SiO_2) and have high concentrations of Mg, Fe, Cr, Co, and Ni, and low concentrations of P, K, and Ca [1]. Ultramafics are divided into intrusive, volcanic, ultrapotassic, and metamorphic rocks, and among the intrusive ultramafic rocks the most common are harzburgite, dunite, and lherzolite. Serpentinites are rocks that form as a result of metamorphism or metasomatism of primary magnesium-iron silicate minerals. This entails the replace-

---

*e-mail: gtomovic@bio.bg.ac.rs

ment of the primary silicate minerals by magnesium silicate serpentine minerals and the concentration of excess iron in magnetite [2]. Serpentine soils are magnesium rich, calcium, potassium and phosphorus poor soils that develop on the layer of loose, heterogeneous material covering solid ultramafic rocks.

Ultramafic rocks are distributed all over the world, including in Europe: Sweden, Norway, Finland, Helgoland, Great Britain, Austria, Czech Republic, Switzerland, Balkan Peninsula, Italy, Spain, Portugal, and France. Serpentine vegetation is reported also from the Ural mountains, Southern Rhodesia, Japan, Karakelang in Indonesia, New Caledonia, New Zealand, Cuba, Puerto Rico, Newfoundland, Quebec, British Columbia, and the United States [3].

According to Stevanović et al. [4], the largest serpentine areas in Europe are in the Balkans and they exist in large blocks or as small outcrops separated from other geological substrates in central Bosnia, western and central Serbia, and central and southeastern Albania, ending at the serpentine formations of Epirus and Thessaly in Greece. Small portions of serpentine bedrock are distributed also in southwestern and central Bulgaria (eastern and central Rhodope Mountains). Some quite isolated serpentine 'islands' occur in the northern part of FYR Macedonia, southern and northeastern Serbia, and Sterea Ellas.

Areas of the soil-rock systems that may be grouped together as 'serpentines' are well known for their remarkable and often unique plant species. These widely scattered serpentine areas have many features in common. In almost all cases they:

(1) are sterile and unproductive, either as farm lands or timber lands
(2) possess unusual floras characterized by narrowly endemic species of great interest to the plant taxonomist and geneticist
(3) support vegetation in striking physiognomic contrast with that on other soils [3, 5-7]

Many plant taxa from the Brassicaceae family are quite well adapted to serpentine soils, the so-called hyperaccumulators, and have the capacity to accumulate nickel to such a degree that dry leaf concentrations of this element exceed 1,000 $\mu g \cdot g^{-1}$ (0.1%). Prasad and Freitas [8] listed c. 11 genera and 87 species of this family. By far the greatest number of nickel-accumulating species within any genus is found in *Alyssum* L. *Alyssum bertolonii* Desv. was the first nickel hyperaccumulator to have been discovered and documented in any genus. After this species, *Alyssum pinto-dasilvae* Dudley and *Alyssum murale* Waldst. & Kit. were also recognized as nickel hyperaccumulators. In addition to the 14 European hyperaccumulator species, a further 31 hyperaccumulors of the genus *Alyssum* (all in section *Odontarrhena*) were presented [9]. After a comprehensive analysis of the 168 *Alyssum* species for their nickel content, these authors concluded that hyperaccumulation of nickel is restricted entirely to the sect. *Odontarrhena,* and that 70% of the species assigned to this section are identified as accumulators. Within this section more than 50 hyperaccumulators are found in southern Europe and SW Asia [10].

However, in the most recent publication [11], on the basis of balance between molecular data, morphology, distribution and ecology, only 11 European Ni hyperaccumulator species of genus *Alyssum* were recognized. Cecchi et al. [11] stated that molecular and morphological evidence from native populations indicated that several species (e.g. *A. bertolonii* Desv. subsp. *scutarinum* E.I. Nyárády, *A. chlorocarpum* Hausskn., *A. janchenii* E. I. Nyárády, *A. markgrafii* O. E. Schulz, and *A. chalcidicum* Janka) previously regarded as endemic hyperaccumulators can hardly be recognized as distinct from *A. murale*, and treated all these taxa as synonyms of the species *A. murale* s.l. Cecchi et al. [11] accepted the opinion of Hartvig [12], who considered that the slight differentiation in trichome density and inflorescence morphology of these populations may support, at most, their recognition at the varietal rank. Therefore, these authors considered that literature reports of hyperaccumulation in *A. murale* [13, 14] are likely to be referred to this plant or to closely related taxa from Anatolian serpentine areas.

A. murale Waldst & Kit. is a species distributed in southeastern Europe (the Balkan Peninsula, Romania), Russia, Asia Minor, and southwestern Asia. It occurs on different types of geological substrata (limestone, andezite, granite), but it also is very common on peridotite and serpentinite [15]. This plant is among several *Alyssum* taxa that have been enormously successful, because it occurs on serpentine outcrops as extensive and nearly pure populations with an almost total absence of any other competing species [9]. Besides this publication, there are several additional papers about nickel accumulation of this species in different parts of the eastern Mediterranean (e.g. the Balkans, Turkey, and Iran) [13, 16-27].

So far only data about Ni accumulation in *A. murale* s.l. were published from the northern central part of Serbia (Mt Goč) [28, 29], under the name *A. markgrafii*. Although there are a lot of records about the presence of *A. markgrafii* and *A. janchenii* in the territory of Serbia [15, 30-32] etc., in light of the most recent publications [11, 12] it is most likely that all these data actually refer to the species *A. murale* s.l. In our opinion these taxa could be reduced to infraspecific rank within *A. murale*. Glabrous siliqules have been noted as their main differential features, but it seems that the morphological specificity of the serpentine forms in Serbia is actually reflected in the vegetative characters – short-lived perennials with a few ephemeral sterile shoots. In fact, populations with completely glabrous fruits only occur on serpentines of north-central Serbia, where *A. janchenii* was described.

The aims of the present study were:

(1) to investigate the level of concentration of Ni (and some other elements such as P_2O_5, K_2O, Ca, Mg, Fe, Mn, Zn, Cu, Cr, Pb, Co, and Cd) at several serpentine sites in western, southwestern, and central Serbia, where populations of *A. murale* grow
(2) to determine the level of accumulation of these elements in plant tissues (roots, shoots, and leaves), with a focus on Ni

Table 1. Latitude, longitude, and altitude of the sampling points of *Alyssum murale* s.l. populations from Serbia.

Species	Location	Sample point	Latitude (N)	Longitude (E)	Altitude (m. a. s. l.)	Vegetation alliance of the studied location	Type of ultra-mafic rocks
A. murale	Mt Zlatibor, Čelićko hill – Okolište	SP1	43º 37′57″	19º 44′14″	1090	*Centaureo-Bromion fibrosi*	Metamorphic (serpentinite)
A. murale	Čačak, Tučkovo village (Vrnčanska river gorge)	SP2	43º 55′05″	20º 08′11″	410	*Quercion frainetto* *Ostryo-Carpinion orientalis*	Metamorphic (serpentinite)
A. murale	Gornji Milanovac, Brđani gorge	SP3	43º 59′27″	20º 25′15″	330	*Chrysopogoni-Danthonion alpinae*	Metamorphic (serpentinite)
A. murale	Kraljevo, Bogutovac village (Gornja Lopatnica river gorge)	SP4	43º 40′49″	20º 27′46″	430	*Quercion frainetto* *Orno-Ostryon*	Intrusive (harzburgite)
A. murale	Raška (Trnavska river gorge)	SP5	43º 17′14″	20º 36′07″	420	*Centaureo-Bromion fibrosi*	Intrusive (harzburgite)
A. murale	Mt Kopaonik, Paljevštica (Brzećka river gorge)	SP6	43º 20′20″	20º 56′32″	680	*Centaureo-Bromion fibrosi*	Intrusive (harzburgite)
A. murale	Mt Rogozna (Izbice – Negotinac)	SP7	43º 06′46″	20º 36′22″	730	*Centaureo-Bromion fibrosi*	Intrusive (harzburgite)
A. murale	Ušće (Studenica river gorge)	SP8	43º 27′40″	20º 35′53″	400	*Orno-Ericion*	Intrusive (harzburgite)
A. murale	Mt Maljen, Divčibare (Golubac)	SP9	44º 06′39″	19º 58′30″	950	*Orno-Ericion*	Intrusive (harzburgite)

(3) to assess the potential of Ni hyperaccumulation in relation to the soil characteristics of the environment. This would be of special interest for potential use in phytoextraction or phytostabilization.

Research Areas

The distribution of ultramafic bedrocks occurrence, locations (latitude and longitude), and habitat characteristics (altitude, vegetation alliances, type of ultramafic rocks) of the studied *A. murale* populations are shown in Fig. 1 and Table 1. The selection was based on the presence at each site of large populations of *A. murale*. Plant and soil samples were collected in May 2010 and 2011 from nine sampling points: SP1-SP9 (Table 1). Soil samples were chosen by eye based on the presence of the *A. murale* 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 dried at 40ºC for 3 days.

Plant species were sampled according to their abundance and biomass, 5-10 replicates of each investigated population. All the plant samples were collected, separated into roots, shoots, and leaves, and washed carefully with distilled water to remove soil particles. Dry weights were obtained after drying at 40ºC for 3 days. Plants have been identified with the help of local flora [15]. 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).

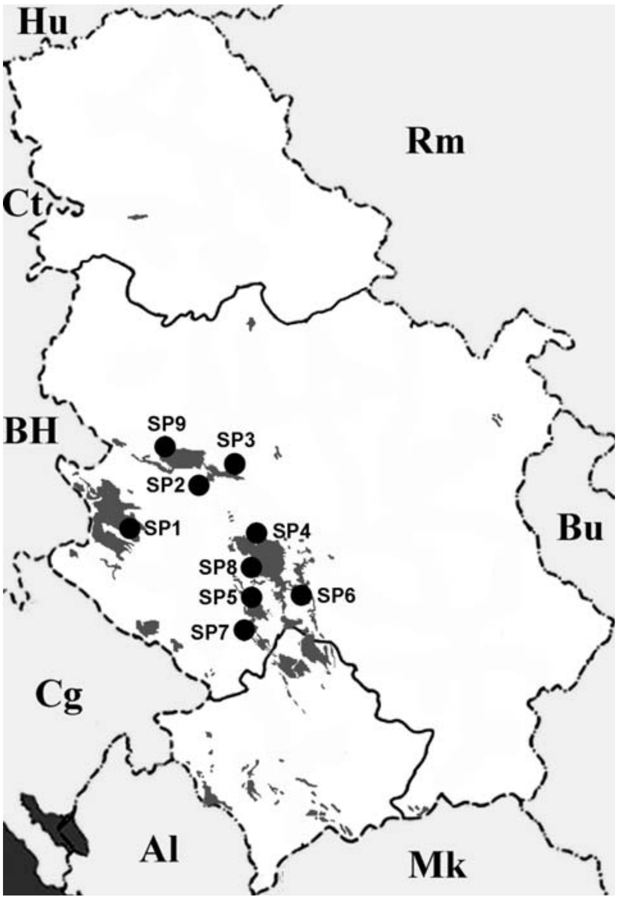

Fig. 1. Map of Serbia showing ultramafic bedrock occurence (in gray) and locations (SP1-SP9) of the studied *Alyssum murale* populations (black circles).

Experimental Procedures

Soil Analysis

Particle size distribution was determined after soil dispersion by sodium hexametaphosphate, using the pipette method for the silt and clay fractions and dry-sieving for the sand fraction [33]. The sand is separated from clay and silt with a 50 µm sieve. The USDA classification was applied to determine soil textural classes [34].

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} [35]. Available P_2O_5 and K_2O were measured in AL solution (0.1 M ammonium lactate and 0.4 M acetic acid) extract (S/L 1:20) [36]. 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. Available Ca and Mg were determined in 1 M ammonium acetate extract (S/L 1:50) [37] using AAS (atomic absorption spectrophotometry) (Pye Unicam SP 192). Organic matter concentration was determined by dichromate digestion based on FAO [38] procedure. Extraction of available (potentially leachable) metals in soil was performed by 0.1N HCl (S/L 1:10) according to the procedure recommended by Garcia et al. [39]. Total metal extraction was done by HCl and HNO_3 digestion (ISO 11466 1995). Metal concentrations in both extracts were determined using atomic absorption spectrophotometry (ISO 11047 1998) (Pye Unicam SP 192).

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 molybdenum blue method described by Chen et al. [40]. Potassium concentration was determined using FES (flame emission spectrophotometry) using a Pye Unicam SP 192 atomic absorption spectrophotometer. 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.

Data Analysis

Each analysis of the soil and plant material was performed with three replicates. In addition, each of the chemical analyses also was run with three replicates. Arithmetic means and standard deviations are shown in tables. All the data were subjected to a statistical analysis, but since the data were not normally distributed, we used nonparametric statistics. Correlation matrices were produced to examine the inter-relationships between the investigated metal concentrations in the soils, roots, and shoots, as well as in the leaves of the investigated samples. Correlations were evaluated using the bi-variation method, with two-tailed significance and Spearman R correlation coefficients. Correlations were done only for the elements, the concentrations of which in the samples were >0.1 mg·kg^{-1}. Interpopulation variability of Ni concentration in plants was checked by nonparametrics ANOVA (Kruskal-Wallis test). Data analysis was performed using STATISTICA for Windows 5.1. work package.

Results

Soil Characteristics

The textural classes of the studied soil samples range from loam, silty loam to sandy clay, but most of the samples fall into the sandy loam class.

Chemical soil properties such as pH, organic matter, P_2O_5, K_2O, available concentrations of major elements (Fe, Ca, Mg), and total and available concentrations of trace elements (Ni, Mn, Zn, Cu, Cr, Co, Cd, Pb) are shown in Table 2. The pH of the soil samples varied from acidic to moderately alkaline and the percentage of organic matter differed significantly among the sampling points. Concerning the concentration of major elements in soil samples, the soils were more or less of typical ultramafic composition, with the moderate to high concentration of available Mg and low to moderate available Ca content. Total and available concentrations of trace elements such as Ni, Mn, Co and Cr are elevated and also typical for serpentinite sites, while most of the total and available concentrations of Cu, Zn, Cd and Pb in nine soil samples fall within the ranges for normal soils.

Chemical Composition of the Plant Material

Concentrations of P_2O_5, K_2O, and major elements (Fe, Ca, Mg) in the roots, shoots, and leaves of nine *A. murale* populations are presented in Table 3. Despite relatively low available Ca content in the soil samples, the concentrations of Ca in the roots, shoots, and leaves of *A. murale* populations were several times higher. On the contrary, concentration of available Mg in the soil samples was followed by only slightly higher Mg content in all plants samples. Concentrations of Fe in almost all plant samples were generally below 1000 mg·kg^{-1}.

Trace element concentrations (Ni, Mn, Zn, Cu, Cr, Co, Cd, Pb) in *A. murale* plants are shown in Table 4. The lowest concentrations of Ni and Mn were in the roots and the highest levels were in the leaves. The opposite trend was noticed for Zn content, since the concentration of Zn was the highest in the roots, while both in the shoots and in the leaves it was much lower. Contents of Cu in the roots and shoots were variable and in some cases even very high, but in the leaves of almost all the samples this element was found only in traces. Contents of Cr and Co in all the investigated plant parts were low, and only in the roots and shoots of four plant samples were small amounts of Cr found. In the case of Cd, concentrations in the roots and

Table 2. pH, organic matter, P_2O_5, K_2O, major elements (Fe, Ca, Mg), and trace elements (Ni, Mn, Zn, Cu, Cr, Co, Cd, Pb) concentrations (mg·kg^{-1}) in soils of sites in which *Alyssum murale* s.l. populations are present. Concentrations are expressed as means ± standard deviations.

Sample point	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9
pH soil in H_2O	6.2	7	6.5	7.3	7.6	7.6	7.6	7.8	7.6
pH soil in 1 N KCl	5.1	5.8	5.6	6.3	7	7	7.1	7.1	7
Organic matter %	17.9±0.7	10.1±0.4	8.9±0.6	0.9±0.4	0.7±0.1	3.3±0.4	2.4±0.1	5.1±0.7	0.7±0.1
P_2O_5 (available)	6.8±0.0	5.3±0.6	8.1±0.6	18.6±0.6	24.6±4.2	17.6±2.7	15.1±0.7	275±22	1.7±0.3
K_2O (available)	155±1	25±1	219±1	85±4	64±4	59±1	135±4	242±6	14±1
Fe (available)	120±10	670±20	150±10	270±10	950±50	110±20	540±20	120±10	610±10
Ca (available)	1360±170	310±0	2140±160	900±50	880±50	3110±70	1080±40	1760±60	1230±70
Mg (available)	4070±80	3800±120	3870±90	4600±230	1240±40	500±20	810±10	1750±40	2320±60
Ca/Mg	0.33	0.08	0.55	0.2	0.71	6.22	1.32	1.01	0.53
Ni (total)	1699±276	1843±43	1544±64	1158±61	2269±38	2125±8	2091±38	1669±31	1489±41
Ni (available)	181±12	75±2	171±6	67±6	155±1	155±5	181±2	140±5	443±80
Mn (total)	4972±383	1223±82	2717±153	1456±63	910±34	1186±14	1160±29	1070±15	2176±175
Mn (available)	304±10	84±2	241±2	98±3	371±22	460±15	538±7	473±14	803±19
Zn (total)	124±6	38±0.5	126±4	92±6	34±1	37±0.7	35±1	43±2	119±12
Zn (available)	11.2±0.4	0.4±0.1	12.2±0.6	1.8±0.1	9.2±0.2	2.9±0.3	6.4±0.5	10.2±0.5	26.5±6.6
Cu (total)	18.2±2.5	0.8±0.6	14.5±0.6	31.1±0.8	9.7±0.6	8.9±0.2	12.3±0.3	12.5±0.5	14.8±0.4
Cu (available)	<0.1	<0.1	<0.1	1.6±0.1	0.1±0.1	<0.1	0.1±0.1	<0.1	4.7±0.6
Cr (total)	1580±73	174±3	919±28	270±12	341±19	608±16	789±31	317±8	366±12
Cr (available)	<0.1	<0.1	<0.1	<0.1	20.5±0.1	19.6±0.7	43.5±0.1	13.6±0.1	14.4±0.9
Co (total)	241±36	120±7	155±3	92±2	90±9	108±2	102±2	81±6	<0.1
Co (available)	<0.1	<0.1	<0.1	<0.1	36.1±1.2	52.0±1.3	82.0±2.7	41.1±0.7	<0.1
Cd (total)	2.4±0.3	2.3±0.1	2.3±0.1	2.6±0.1	4.2±0.3	4.0±0.1	3.4±0.1	2.6±0.1	4.1±0.1
Cd (available)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.3±0.1
Pb (total)	75.2±6.4	11.5±1.3	66.8±0.8	15.9±3.3	43.8±2.9	26.6±1.6	33.9±0.9	28.1±1.7	95.4±8.1
Pb (available)	<0.1	5.3±0.6	<0.1	<0.1	15.6±0.9	0.50±0.1	5.1±0.5	2.5±0.5	17.6±0.9

shoots were low and only in the leaves of four samples was the content of this element slightly higher. Pb contents in all the plant samples were below 0.1 mg·kg^{-1}.

Interpopulation Variability in Ni Concentration

Results of Kruskal-Wallis ANOVA tests showed significant interpopulation variability in Ni concentration among analyzed populations in all plant tissues – roots, shoots, and leaves (Table 5).

Relationships between Metal Concentrations

In soils, the correlation analysis found most significant positive mutual relationships between Ni and Zn, Mn, while most significant negative correlations were between Mn and Mg and between Fe and Ca. In the roots of *A. murale*, most significant positive correlations were between Ni and Fe and between Mg and Fe, Zn, while significant negative correlations were between Ca and Fe (Table 6).

In the shoots of *A. murale,* most significant positive correlations were between Mn and Ca, Mg, while most significant negative correlations were between Ni and Ca, Mn. In the leaves of *A. murale*, most positive correlation was between Zn and Ni, while most negative correlations were between Ca and Ni, Zn (Table 7).

A comparison of Ni-accumulating capacity of *A. murale* leaves (Ni-concentration in leaves vs. available Ni concentration in soil) in analyzed populations revealed great variations (Fig. 2). Analyses of correlation of this capacity with the soil parameters and element contents in soil, roots, and shoots show that this capacity may be affected by several factors (Table 8).

Table 3. P_2O_5 and K_2O and major element (Fe, Ca, Mg) concentrations (mg·kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations.

Sample point	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9
P_2O_5 root	550±70	705±80	500±140	960±70	2550±50	1090±140	1910±110	2340±190	2080±20
P_2O_5 shoot	1190±30	610±210	820±40	1000±40	2020±50	1110±50	1400±30	1560±80	3240±780
K_2O root	8710±410	12990±630	9730±1090	10390±170	15220±540	9660±130	12110±220	15950±470	1310±90
K_2O shoot	8890±190	12090±480	9550±380	13380±70	14680±190	7470±240	15350±270	17440±120	1230±50
Fe root	930±140	340±60	500±120	410±30	70±40	710±50	320±50	110±10	800±240
Fe shoot	720±150	640±130	250±20	610±40	410±30	520±20	600±40	160±20	200±70
Fe leaves	1010±190	490±30	270±20	660±70	200±30	540±20	450±40	320±10	460±30
Ca root	5610±70	7920±480	6460±640	4810±270	6180±250	5980±50	7670±170	6860±40	3400±610
Ca shoot	8140±250	18250±650	10650±930	14210±490	36920±890	34590±1550	33990±500	35700±1070	8130±1450
Ca leaves	19540±1900	35350±2320	17190±7880	19360±880	64900±5240	73250±6110	61040±5400	64900±2570	17280±7930
Mg root	5420±230	3940±120	3950±300	3740±180	3050±160	5040±50	3750±40	3050±160	2750±1090
Mg shoot	4370±80	8330±740	4650±360	9260±130	3990±120	4970±60	8110±20	6870±130	2220±880
Mg leaves	6480±840	9340±510	5150±830	11800±790	4260±1130	5710±2050	9650±2490	7750±390	6390±810
Ca/Mg root	1.04	2.01	1.64	1.29	2.03	1.19	2.05	2.25	1.24
Ca/Mg shoot	1.86	2.19	2.29	1.53	9.25	6.96	4.19	5.2	3.66
Ca/Mg leaves	3.02	3.78	3.34	1.64	15.23	12.83	6.33	8.37	2.7

Discussion of Results

The serpentine soils of nine Serbian locations were characterized by elevated total concentrations of trace elements such as Ni, Mn, Co, and Cr that are typical of such sites. Total Ni content of serpentine soils are generally in the range 500-8,000 μg·g^{-1} [41], while total Mn content of worldwide soils vary from 411 to 550 mg·kg^{-1}, total Co in ultramafic soils is from 35 to 200 mg·kg^{-1}, and soils developed from serpentines have especially elevated total Cr content (170-3,400 mg·kg^{-1}, sometimes to above 100,000 mg·kg^{-1}) [42]. In our analyses, contents of the first three elements were more or less within the range of the previously recorded values for different ultramafic soil samples from Serbian and other Balkan peninsula areas, Turkish, and Iranian serpentinites [10, 19, 25, 26, 41, 43, 44]. Still, total Mn content in some other serpentine soils of Serbia was much higher, while total Cr value was significantly higher in several other Balkan peninsula soils [19, 25, 44] than in soil samples presented in this paper. Most of the total Cu, Zn, Cd, and Pb concentrations in nine soil samples fall within the ranges for normal soils, since in ultramafic rocks total Cu content ranges from 10 to 40 mg·kg^{-1}, while total Zn content lies in the range 40 to 60 mg·kg^{-1}. The average contents of Cd in soils are between 0.2 and 1.1 mg·kg^{-1}, and the overall mean value of total Pb for different soils is estimated at 27 mg·kg^{-1} [42]. Our results correspond to other three serpentine soils from western (Mt. Zlatibor), northwestern (Mt. Divčibare), and central Serbia (Goč Mountain) [44].

It is generally accepted that total soil metal concentration does not determine plant tissue concentrations, and that "plant available" concentrations (extractable fraction) generally correlate closely with plant uptake [45]. Concerning the available concentration of major elements in soil samples, the soils were more or less of typical ultramafic composition, with the moderate-to-high concentration of available Mg (500-4,600 mg·kg^{-1}) and low-to-moderate available Ca content (310-3,110 mg·kg^{-1}). The Mg/Ca quotients for the available fraction in seven soil samples are relatively high; this is in accordance with some other serpentine soils of the Apennine, where the concentrations of available Mg can be much higher than those of Ca [46]. In two serpentine soil samples (SP6, SP7) higher values of bioavailable Ca compared to Mg would indicate less Ca deficiency stress for the plants, which was also indicated by Ghaderian et al. [41] for the ultramafic soils in Iran. The content of the available Fe was high for the loamy and alkaline soils [42], while available concentrations of Ni and Mn were high in all soil samples and much higher than those presented by Ghaderian et al. [41] for ultramafic soils from Iran.

Despite relatively low available Ca content (310-3110 mg·kg^{-1}) in the soil samples, the concentrations of Ca in the roots, shoots, and leaves of *A. murale* populations were several times higher. Comparing to Ca content, concentrations of available Mg in the soil samples were followed by slightly higher Mg content in all plant samples. Generally, the content of Ca was higher than Mg content in all plant tissues and the highest Ca/Mg ratio (1.6-15.2) was noticed

Table 4. Trace element (Ni, Mn, Zn, Cu, Cr, Co, Cd, Pb) concentrations (mg·kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations.

Sample point	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9
Ni root	1712±107	1643±142	1813±248	777±23	508±48	877±14	862±22	241±19	2926±472
Ni shoot	2177±32	2198±85	2422±40	1680±38	687±39	1820±14	1184±51	549±27	6793±1201
Ni leaves	7664±713	5026±495	6478±1162	2031±277	943±47	4459±171	1616±114	733±32	13160±1652
Mn root	29.0±2.5	14.6±2.6	7.9±2.6	4.4±1.1	11.8±1.8	41.8±3.1	22.0±1.8	22.7±1.8	11.8±9.5
Mn shoot	37.6±4.3	50.6±3.3	15.8±1.3	46.8±1.3	31.8±2.7	77.8±0.8	69.1±3.7	107.2±5.9	5.0±1.7
Mn leaves	93.8±3.7	30.0±3.9	19.7±8.1	15.7±7.3	18.5±1.9	87.4±6.1	68.2±4.2	126.1±2.9	39.2±4.9
Zn root	466±50	564±82	101±21	248±27	68.4±1.7	221±10	31.7±6.2	44.0±4.4	50.9±6.2
Zn shoot	223±16	287±32	277±27	113±9	54.5±2.5	86.8±5.9	22.6±6.6	43.3±6.1	46.8±4.3
Zn leaves	115±18	21.6±6.6	75.9±6.4	19.7±4.9	11.8±1.1	21.3±2.8	20.4±2.2	26.1±0.4	33.3±7.6
Cu root	923±58	1044±60	159±19	236±18	<1.0	1023±60	84±7	89±7	3.6±0.6
Cu shoot	573±13	849±30	1127±49	440±31	<1.0	354±7	78±2	18.3±1.7	3.9±0.6
Cu leaves	<1.0	<1.0	<1.0	<1.0	2.5±0.6	7.3±0.6	5.0±1.0	7.6±1.0	3.2±0.7
Cr root	<1.0	<1.0	<1.0	<1.0	13.4±0.2	13.7±0.2	27.5±0.2	27.5±0.4	<1.0
Cr shoot	<1.0	<1.0	<1.0	<1.0	27.2±0.4	27.5±0.4	27.2±0.4	27.5±0.4	<1.0
Cr leaves	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Co root	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Co shoot	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	30.2±1.1	10.6±2.8	<1.0
Co leaves	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Cd root	5.4±1.7	6.4±1.4	3.5±0.1	<1.0	<1.0	4.5±0.7	<1.0	12.2±0.2	<1.0
Cd shoot	<1.0	7.2±1.0	<1.0	<1.0	6.9±1.8	<1.0	4.0±0.6	4.8±1.2	<1.0
Cd leaves	16.5±1.3	19.3±0.9	16.0±0.5	16.0±0.1	<1.0	<1.0	<1.0	<1.0	<1.0
Pb root	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Pb shoot	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Pb leaves	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0

in leaves. This is because Ca content in *A. murale* leaves is remarkably high, which is a consequence of the unusual ability of this plant to accumulate high Ca concentrations, even from soils with high Mg/Ca quotients that are characteristic of serpentines. The similar results were presented for different plants (including *Alyssum* species) from Greece (Lesbos Island), Albania, and Italy [10, 26, 46]. According to Pandolfini and Pancaro [47], serpentine plants must posses a mechanism to limit uptake of Mg and/or a high absorption capacity for selective Ca uptake.

Concentrations of Fe in all plant samples were generally below 1,000 mg·kg^{-1}, and only in the leaves of SP1 was it above this value which, according to Reeves et al. [48], often indicates contamination of leaf samples by serpentine soil or dust, not always easily removed by simple washing procedures. However, the Fe content in all plant tissues was significantly elevated in relation to normal values for plants – approximately 100, maximum of about 700 mg·kg^{-1} [49]. In our survey in six sample points the highest quantity of iron was recorded either in the stems or in the leaves, while in three plant samples iron is dominant in the roots. It had been found previously that the increase of Ni concentration increased Fe accumulation in plants, primarily in roots [50, 51], which had been ascribed to the activation of the common metal uptake channels. Uptake of large quantities of Fe together with Ni in *A. murale* is thus caused by the presence of very abundant and active channels for Ni uptake. Iron is harmful for plant metabolism more than other heavy metals because it directly causes oxidative stress and the excess has to be inactivated, mostly by binding to low molecular SH ligands or in the form of Fe-S aggregates. Hyperaccumulators produce increased concentrations of such ligands, as has been confirmed for *A. lesbiacum* at high Ni concentrations [52].

Table 5. Results of Kruskal-Wallis ANOVA tests for Ni concentration among nine analyzed *Alyssum murale* s.l. populations for different plant tissues.

Plant tissue	H	df	p level
Roots	24.878	8	0.0016
Shoots	25.503.	8	0.0013
Leaves	25.542	8	0.0013

Ni concentrations in *A. murale* samples from Serbia were variable since there was significant interpopulation variability in Ni concentrations among analyzed populations in all plant tissues – roots, shoots, and leaves. The total quantity of Ni in tissues of all nine plant samples studied was high to extremely high (up to 13,160 mg·kg^{-1}), and in *A. murale* leaves at SP9 was 30 times higher than those in corresponding soil, while in the case of SP2 the ratio between concentration of Ni in leaves and in the corresponding soil was 66:1. Actually, in all the samples studied the concentration of Ni was higher in the above-ground parts compared to the root, which can be attributed to easy translocation of nickel in the acropetal direction [53]. This is completely in accordance with the finding of McNear et al. [54] that *A. murale* transports histidine-bound Ni from roots to shoots, where it is further deposited bound to malate and other organic acids within plant leaf dermal tissue. Ni hyperaccumulation was, under natural conditions, associated with serpentine or other Ni-rich soils and some native plants from such locations accumulated leaf concentrations of over 6,000 mg Ni/kg [42].

Generally speaking, Ni content in Serbian *A. murale* plant samples are lower then those recorded by Bani et al. [19], since the concentrations of Ni in *A. murale* plants from several locations in the Balkans ranged from 4,730 mg·kg^{-1} up to 20,100 mg·kg^{-1}. On the ultramafic slopes of Goč Mountain (central Serbia), plants of *A. markgrafii* contained from 5,125 mg·kg^{-1} to 6,250 mg·kg^{-1} Ni [28]. Vinterhalter and Vinterhalter [29] also proved that under conditions of *in vitro* culture *A. markgrafii* (seed collected from the same location in Goč Mountain) is as highly efficient nickel hyperaccumulator as in nature. These authors concluded that the ability to hyperaccumulate nickel was not restricted only to organized structures but also in undifferentiated, non-organogenic callus, and that hyperaccumulation in callus represents interesting finding since it indicates that the tolerance to nickel is a specific feature of cells and tissues of certain species and not only the result of translocation.

Although Mn content in soil samples was relatively high, its concentration in plant tissues was noticeably lower; the lowest one was in the roots while in the leaves it was up to 126.1 mg·kg^{-1}. Broadhurst et al. [55] presented much higher concentrations of Mn in leaves of several ecotypes of *A. murale* species. The opposite trend was noticed for Zn concentration in plant tissues. Comparing to low Zn content in soil samples, in the roots of all samples Zn concentration was the highest. Concentrations of both Mn and Zn found in this study are in agreement with values reported for other Balkan populations of *A. murale* [19]. It is assumed that species growing in serpentine soils generally act as excluders of Zn and Mn, and they can restrict transport of such metals to the shoots and maintain relatively low concentration of metals in leaves – even at high concentrations in soils [10].

Contents of Cr and Co in all the investigated plant parts were very low, and only in four plant samples were small amounts of Cr found in the roots and shoots (up to 27.5 mg·kg^{-1}). Reeves et al. [48] reported that concentrations of Cr in leaves are generally below 20 µg·g^{-1}, and chromium uptake by plants is usually very slight [13]. This can be explained by its low solubility in the serpentine soil solution due to the relatively high pH values [56]. In the case of Co, only in the shoots of two plant samples did we record small concentrations of this element (up to 30.2 mg·kg^{-1}), while in the leaves of all the samples this element was found only in traces. This is in contrast to the results of investigations done by Brooks and Radford [13] on the leaves from herbarium materials of *A. murale* populations from Europe, where content of this element was between 4 and 34 µg·g^{-1} of dry leaf mass. These authors claimed that the normal Ni/Co ratio in ultrabasic rocks is usually of the order of 10:1, but the species of the genus *Alyssum* preferentially accumulate nickel relative to cobalt. It is concluded that Ni-accumulating processes in *Alyssum* are not accompanied by any significant ability to accumulate other trace elements, since the concentrations of Cr, Co, Mn, and Fe in plant tissues of two *Alyssum* species from ultramafic soils of Iran were generally low [41].

Table 6. Correlation coefficients (r) for soil and *Alyssum murale* s.l. The upper right part for soil, the lower left part for roots; significant correlation coefficients are in bold (*p<0.05, **p<0.01, ***p<0.001).

Metal	Ca	Mg	Fe	Zn	Mn	Ni
Ca	–	-0.26	**-0.79*** **	**0.48***	0.37	0.33
Mg	0.00	–	-0.03	0.07	**-0.64*** **	-0.19
Fe	**-0.53** **	**0.60*** **	–	-0.16	-0.07	-0.03
Zn	-0.11	**0.69*** **	**0.42***	–	**0.49** **	**0.75*** **
Mn	0.20	**0.41***	0.27	0.04	–	**0.63*** **
Ni	-0.23	**0.38***	**0.75*** **	0.29	-0.04	–

Table 7. Correlation coefficients (r) for *Alyssum murale* s.l. The upper right part for shoots, the lower left part for leaves; significant correlation coefficients in bold (*p<0.05, **p<0.01, ***p<0.001).

Metal	Ca	Mg	Fe	Zn	Mn	Ni
Ca	–	0.21	-0.13	**-0.41***	**0.62*****	**-0.81*****
Mg	-0.01	–	**0.50****	0.10	**0.61*****	-0.34
Fe	-0.16	**0.47***	–	0.29	0.17	0.01
Zn	**-0.59*****	-0.19	0.22	–	-0.28	**0.50****
Mn	0.36	0.02	0.26	0.37	–	**-0.67*****
Ni	**-0.66*****	-0.14	**0.40***	**0.63*****	-0.06	–

Despite the fact that available Cu content in soil samples was very low, in five plant samples very high concentrations of this element were found – both in the roots and in the shoots. The accumulation of Cu by higher plants is not so common a feature, and hyperaccumulation of copper is noticed in some terrestrial plants such as *Mimulus guttatus* DC. [57] or *Helichrysum candolleanum* as both Ni and Cu hyperaccumulator [58], as well as in aquatic macrophytes [59]. In the leaves of almost all the samples, this element was found only in traces that correspond to the results given by Bani et al. [19]. Cu has low mobility relative to other elements in plants and most of this metal appears to remain in roots [42]. Cd content in all the samples and in all the plant parts were relatively low (up to 19.30 mg·kg^{-1}), while in the case of Pb, concentrations of this element in plant tissues was below 0.1 mg·kg^{-1}.

Analyses of correlation of Ni TF (soil/leaves) in *A. murale* plants with soil parameters revealed that soils with lower pH, Ca/Mg ratio, P_2O_5 and Co, Cr, and Mn contents favoured the development of highly efficient Ni hyperaccumulators. Higher Ni accumulation at lower pH values in soil was to be expected, because acidic conditions increase the mobility and availability of Ni ions [60]. Inhibition of Ni accumulation by increased concentration of Co, Cr, and Mn would indicate an antagonism between these ions and Ni, probably due to the competition for the same binding sites of the root channels. Such antagonism was already found in another Ni hyperaccumulator, *Brassica juncea* [61].

Negative correlation of Ni uptake capacity with P_2O_5 contents in soil and plants that was found in this study is possibly a result of organic acid synthesis stimulation. Actually, it had been found that in several non-accumulating plant species, P-starvation lead to increased synthesis of organic acids for the purpose of root exudation to enable more efficient P-acquisition [62]. If constitutionally high organic acid synthesis in *A. murale* [54] would be increased by P-starvation, that would lead to additionally increased transport within the plant.

In *A. murale* shoots and leaves, concentrations of Ni were negatively correlated with concentrations of Ca, which correspond to the results for serpentine plants from Lesbos Island (Greece) [10]. However, Bani et al. [19] found no correlation between Ca and Ni concentrations in *Alyssum* leaves. Negative correlation of Ni uptake and translocation capacity with Ca concentration in leaves and Ca/Mg ratio in soil and shoots reflects a well-known finding that Ca alleviates toxic effects of some metals (including Ni) by substituting them and thus decreasing their uptake and transport [63]. The trait of *A. murale* to concen-

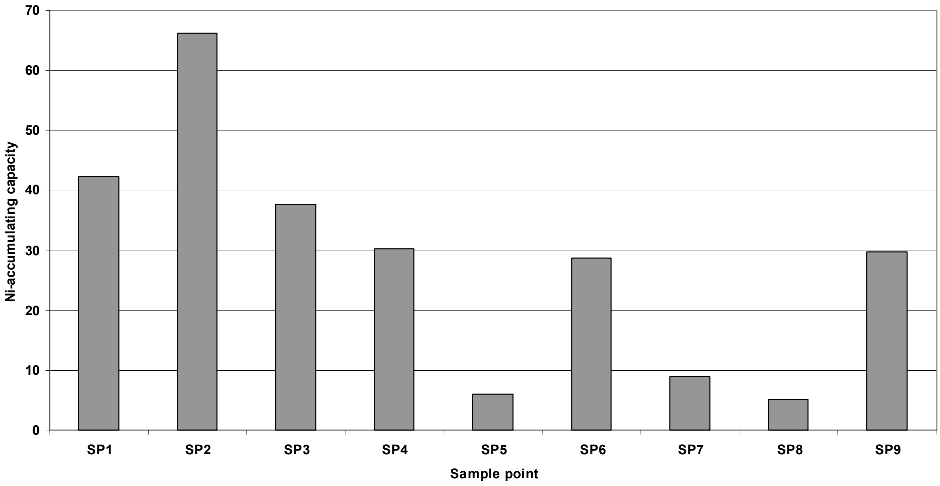

Fig. 2. Variations in Ni TF (Ni-concentrations in leaves vs. available Ni-concentrations in soil) in *Alyssum murale* s.l. plants from different sampling sites.

Table 8. Correlations between Ni TF in *Alyssum murale* s.l. leaves and soil parameters, element contents in soil, roots, and shoots (only significant correlation coefficients are presented ($p \leq 0.001$)).

Soil parameters	r	Element content in roots	r	Element content in shoots	r
pH	-0.67	P_2O_5	-0.81	P_2O_5	-0.43
P_2O_5	-0.50	Ni	0.57	K_2O	-0.60
Ca/Mg	-0.79	Zn	0.86	Cu	0.77
Available Mn	-0.51	Cu	0.70	Ca (leaves)	-0.60
Available Cr	-0.69	Cr	-0.77	Ca/Mg	-0.65
Available Co	-0.69				

trate Ca in spite of the low concentration of this element in soil indicates that this species may have two mechanisms for surpassing heavy metal toxicity: great affinity toward Ca that leads to lower uptake of some elements (including Ni), and production of organic ligands for Ni uptake, neutralization, transport, and safe storage. Possibly, in populations deprived of Ca, the second mechanism becomes more developed. Stronger negative correlation with Ca/Mg ratio than with Ca content itself indicates a positive correlation of Mg content with Ni hyperaccumulation. Küpper et al. [64] found the same compartmentalization of Mg and Ni (contrary to Ca and Ni) in leaves of Ni hyperaccumulators *A. bertolonii* and *A. lesbiacum*, from which they concluded that Mg accumulation is a result of active defensive mechanism against Ni and other metals aimed at preventing their incorporation in chlorophyll.

Contrary to Kaur et al. [61], who found antagonism of Ni with Cu and Zn in *B. juncea*, in *A. murale* plants analyzed in this study, correlation of Ni TF for leaves with Zn and Cu contents in plants was positive. It had been found in some non-accumulating plants like bean [65] or *Trigonella corniculata* [51] that Ni increased Cu concentration in roots, but no correlation was found with Ni content excluding synergism of these ions. One of the possible reasons for the positive correlation of Ni TF in leaves and Zn and Cu contents in roots and Cu content in stems may be a better developed antioxidant system in these plants, involving higher activities of Cu/Zn superoxide dismutase. Although it had been stated many times that the antioxidative system in hyperaccumulators was not the primary detoxifying mechanism, it showed high activity in these plants. For instance, superoxide dismutase activity in hairy roots of Ni hyperaccumulator *A. bertolonii* was found to be 2.5 times higher in the presence of Ni than in non-accumulating *Nicotiana tabacum*. This showed that the hyperaccumulator possessed constitutionally high capacity to fight off oxidative stress. Co-incidence of high antioxidative capacity with high accumulation in an *A. murale* population would indicate that more "vigorous" or better adapted genotypes developed both of these mechanisms to a higher degree. However, the connection of Cu and Zn accumulations with superoxide dismutase activity still remains to be investigated in the analyzed populations.

Conclusions

Soil samples collected at nine ultramafic sites in western, southwestern, and central Serbia contain elevated levels of Ni, Mn, Co, and Cr. The Mg/Ca quotients for the available fraction in seven soil samples are relatively high.

Despite relatively low available Ca content in the soil samples, the concentrations of Ca in *A. murale* tissues were several times higher. Generally, the content of Ca was higher than Mg content in all plant tissues and the highest Ca/Mg ratio (1.6-15.2) was noticed in the leaves. The total quantity of Ni in tissues of all nine plant samples studied was high to extremely high and in two cases it was 30 and 66 times higher in leaves than those in corresponding soil. Correlation of Ni accumulating capacity with soil properties lead to the conclusion that weakly acidic soils, poor in P and K, with low Ca/Mg ratio and with low contents of Mn, Cr and Co, are most favourable for the development of *A. murale* specimens with high hyperaccumulating capacity.

If metal hyperaccumulators are defined as plants that have the capacity to accumulate Ni to such a degree that dry leaf concentrations of this element exceed 1,000 $mg \cdot kg^{-1}$, (0.1%) [9], most *A. murale* plant samples from Serbia emerge as strong Ni hyperaccumulators. Our results demonstrate that populations of this species from ultramafic soils of Serbia, due to high biomass production and high efficiency of Ni uptake, have the best potential for phytoextraction purposes.

Acknowledgements

The authors are grateful to Dr. Ljiljana Tomović for helping us with statistical data processing, and to Dr. Milica Tomašević for improvement of the draft version of the manuscript. The Ministry of Education, Science and Technological Development of the Republic of Serbia supported this research through Grant Nos. 173030 "Plant biodiversity of Serbia and the Balkans – assessment, sustainable use, and protection" (N. Mihailović, M. Niketić, and G. Tomović) and III43009 "New technologies for monitoring and environmental protection from hazard chemical substances and radiation burden" (B. Gajić).

References

1. PROCTOR J. Vegetation and soil and plant chemistry on ultramafic rocks in the tropical Far East. Persp. Plant. Ecol. Evol. Syst. **6**, (1-2), 105, **2003**.
2. ALEXANDER E.B., COLEMAN R.G., KEELER-WOLFE T. Serpentine Geoecology of Western North America: Geology, Soils, and Vegetation. Oxford University Press: Cary, NC. **2007**.
3. WHITTAKER R.H. (Ed.). The Ecology of Serpentine Soils. Ecology **35**, (2), 258, **1954**.
4. STEVANOVIĆ V., TAN K., IATROU G. Distribution of the endemic Balkan flora on serpentine I. – obligate serpentine endemics. Plant. Syst. Evol. **242**, (1-4), 149, **2003**.
5. BRADY K.U., KRUCKEBERG A.R., BRADSHAW H.D. Evolutionary ecology of plant adaptation to serpentine soils. Annu. Rev. Ecol. Evol. Syst. **36**, 243, **2005**.
6. KAZAKOU E., DIMITRAKOPOULOS P.G., BAKER A.J.M., REEVES R.D., TROUMBIS A.Y. Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. Biol. Rev. **83**, 495, **2008**.
7. HARRISON S.P., RAJAKARUNA N. (Eds.) Serpentine: Evolution and Ecology in a Model System. University of California Press: Berkeley. **2011**.
8. PRASAD M.N.V., FREITAS H. Metal hyperaccumulation in plants – Biodiversity prospecting for phytoremediation technology. Electron. J. Biotechnol. **6**, (3), 285, **2003**.
9. BROOKS R.R., MORRISON R.S., REEVES R.D., DUDLEY T.R., AKMAN Y. Hyperaccumulation of Nickel by *Alyssum Linnaeus* (Cruciferae). Proc. Royal. Soc. Lond. B **203**, (1153), 387, **1979**.
10. KAZAKOU E., ADAMIDIS G.C., BAKER A.J.M., REEVES R.D., GODINO M., DIMITRAKOPOULOS P.G. Species adaptation in serpentine soils in Lesbos Island (Greece): metal hyperaccumulation and tolerance. Plant Soil **332**, 369, **2010**.
11. CECCHI L., GABBRIELLI R., ARNETOLI M., GONNELLI C., HASKO A., SELVI F. Evolutionary lineages of nickel hyperaccumulation and systematics in European Alysseae (Brassicaceae): evidence from nrDNA sequence data. Ann. Botany **106**, (5), 751, **2010**.
12. HARTVIG P. *Alyssum* L., in: STRID A., TAN, K. (Eds.), Flora Hellenica 2. A. R. G. Gantner Verlag K. G.: Ruggell, pp 199-224, **2002**.
13. BROOKS R.R., RADFORD C.C. Nickel Accumulation by European Species of the Genus *Alyssum*. Proc. Royal. Soc. Lond. B **200**, (1139), 217, **1978**.
14. REEVES R.D., KRUCKEBERG A.R., ADIGÜZEL N., KRÄMER U. Studies on the flora of serpentine and other metalliferous areas of western Turkey. S. Afr. J. Sci. **97**, 513, **2001**.
15. DIKLIĆ N. *Alyssum* L., In: JOSIFOVIĆ M. (Ed.), Flore de la Serbie 3. Academie Serbe des Sciences et des Arts, Belgrade, pp. 286-311, **1973**.
16. BABALONAS D., KARATAGLIS S., KABASSAKALIS V. The Ecology of Plant Populations Growing on Serpentine Soils. Phyton **24**, (2), 225, **1984**.
17. BANI A., ECHEVARRIA G., SULÇE S., MULLAJ A., MOREL J.L. *In-situ* phytoextraction of nickel by native population of *A. murale* on ultramafic site in Albania. Plant Soil **293**, 79, **2007**.
18. BANI A., ECHEVARRIA G., MULLAJ A., REEVES R., MOREL J.L., SULÇE S. 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. Northeast. Nat. **16**, (sp5), 385, **2009**.
19. BANI A., PAVLOVA D., ECHEVARRIA G., MULLAJ A. Nickel hyperaccumulation by the species of *Alyssum* and *Thlaspi* (Brassicaceae) from the ultramafic soils of the Balkans. Bot. Serb. **34**, (1), 3, **2010**.
20. BANI A., THOMA F., SKURA E., ECHEVARRIA G., SULÇE S., MOREL J.L. Biodiversity in Metal Rich Soil. Proceedings of the 4th Conference on Water Observation and Information System for Decission Support BALWOIS, Ohrid, Republic of Macedonia, http://www.balwois.com/2010/. Accessed 22 December 2011. **2010**.
21. ÇULLAJ A., HASKO A., KONGOLI F. Investigation of the potential of several plants for phytoremediation of nickel contaminated soils and for nickel phytoextraction. Eur. J. Miner. Process. Environ. Protect. **4**, (2), 144, **2004**.
22. HASKO A., ÇULLAJ A. Nickel hyper-accumulating species and their potential use for the phyto-remediation of polluted areas. Options Méditerranéennes. Série A: Séminaires Méditerranéens **47**, 137, **2001**.
23. REEVES R.D., ADIGÜZEL N. The Nickel Hyperaccumulating Plants of the Serpentines of Turkey and Adjacent Areas: A Review with New Data. Turk. J. Bot. **32**, 143, **2008**.
24. REEVES R.D., BROOKS R.R., DUDLEY Th.R. Uptake of Nickel by Species of *Alyssum*, *Bornmuellera*, and Other Genera of Old World Tribus Alysseae. Taxon **32**, (2), 184, **1983**.
25. REEVES R.D., ADIGÜZEL N., BAKER A.J.M. Nickel Hyperaccumulation in *Bornmuellera kiyakii* Aytaç & Aksoy and Associated Plants of the Brassicaceae from Kızıldağ (Derebucak, Konya-Turkey). Turk. J. Bot. **33**, 33, **2009**.
26. SHALLARI S., SCHWARTZA C., HASKOB A., MORELA J.L. Heavy metals in soils and plants of serpentine and industrial sites of Albania. Sci. Total Environ. **209**, 133, **1998**.
27. VINTERHALTER B., SAVIĆ J., PLATIŠA J., RASPOR M., NINKOVIĆ S., MITIĆ N., VINTERHALTER D. Nickel tolerance and hyperaccumulation in shoot cultures regenerated from hairy root cultures of *Alyssum murale* Waldst et Kit. Plant Cell Tiss. Organ. Cult. **94**, (3), 299, **2008**.
28. OBRATOV D., KADOVIĆ R., MIHAJLOVIĆ N. *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, pp. 24-28, **1997**.
29. VINTERHALTER B., VINTERHALTER D. Nickel hyperccumulation in shoot cultures of *Alyssum markgrafii*. Biol. Plant. **49**, 121, **2005**.
30. NOVÁK F.A. Ad florae Serbiae cognitionem additamentum alterum. Preslia **5**, 65, **1927**.
31. PAVLOVIĆ Z. Charakteristische Elemente der serpentinischen Flora und Vegetation Serbiens. Glas. Prir. Muz. Beograd **B18**, 3, **1962**.
32. NIKOLIĆ V., SIGUNOV A., DIKLIĆ N. Dopuna flori SR Srbije novim podacima o rasprostranjenju biljnih vrsta., in: Sarić M., Diklić N. (Eds.), Flore de la Serbie 10. Academie Serbe des Sciences et des Arts: Belgrade, pp. 259-336, **1986**.
33. VAN REEUWIJK L.P. (Ed.) Procedures for Soil Analysis. 5th ed. Technical Paper//International Soil Reference and Information Centre: Wageningen. **1995**.

34. ROWELL D.L. Soil Science. Research methods and their applications. Springer: Berlin. **1997** [In German].
35. MCKEAGUE J.A. (Ed.) Manual on soil sampling and methods of analysis. Canadian Society of Soil Science, pp. 66-68, **1978**.
36. EGNER H., RIEHM H., DOMINGO W.R. Studies on the chemical soil analysis as a basis for the assessment of the ground nutrient status of the soil II Chemical Extraction methods to Phosphorus and Potassium determination. K. Lantbr. Hogsk. Annlr. W.R. **26**, 199, **1960** [In German].
37. VAN REEUWIJK L.P. (Ed.) Procedures for Soil Analysis. 6th ed. Technical Paper/International Soil Reference and Information Centre: Wageningen. **2002**.
38. FAO The Euphrates Pilot Irrigation Project. Methods of soil analzsis. Gadeb Soil Laboratory (A laboratory manual). Food and Agriculture Organization: Rome. **1974**.
39. GARCIA W.J., BLESSIN C.W., SANDFORD H.W., INGLETT G.E. Translation and accumulation of seven heavy metals in tissues of corn plants grown on sludge-treated strip-mined soil. J. Agric. Food Chem. **27**, (5), 1088, **1979**.
40. CHEN P.S., TORIBARA T.Y., WARNER H. Microdetermination of phosphorus. Anal. Chem. **28**, 1756, **1956**.
41. GHADERIAN S.M., MOHTADI A., RAHIMINEJAD M.R., BAKER A.J.M. Nickel and other metal uptake and accumulation by species of *Alyssum* (Brassicaceae) from the ultramafics of Iran. Environ. Pollut. **145**, 293, **2007**.
42. KABATA-PENDIAS A. Trace elements in soils and plants. 4th edn CRC Press. Taylor & Francis Group, Boca Raton: London, New York. **2011**.
43. GHADERIAN S.M., MOHTADI A., RAHIMINEJAD M.R., REEVES R.D., BAKER A.J.M. Hyperaccumulation of nickel by two *Alyssum* species from the serpentine soils of Iran. Plant Soil **293**, 91, **2007**.
44. OBRATOV-PETKOVIĆ D., BJEDOV I., BELANOVIĆ S. The content of heavy metals in the leaves of *Hypericum perforatum* L. on serpentinite soils in Serbia. Bull. Fac. For. **98**, 143, **2008**.
45. ADRIANO D.C. Trace elements in terrestrial environments Biogeochemistry, bioavailability, and risks of metals. Springer-Verlag, New York. **2001**.
46. LOMBINI A., DINELLI E., FERRARI C., SIMONI A. Plant-soil relationships in the serpentinite screes of Mt Prinzera (Northern Apennines, Italy). J. Geochem. Explor. **64**, 19, **1998**.
47. PANDOLFINI T., PANCARO L. Biogeochemical survey of some ophiolitic outcrops in Tuscany. Flora **187**, 341, **1992**.
48. REEVES R.D., BAKER A.J.M., BORHIDI A., BERAZAIN R. Nickel hyperaccumulation in the serpentine flora of Cuba. Ann. Bot. **83**, 29, **1999**.
49. LARCHER H. Physiological Plant Ecology. Springer-Verlag: Berlin. **1995**.
50. NARWAL R.P., SINGH M., SINGH J.P. Effect of Ni enriched sewage water on the accumulation of Ni and other heavy metals in corn. J. Ind. Soc. Soil. Sci. **39**, 123, **1991**.
51. PARIDA B.K., CHHIBBA I.M., NAYYAR V.K. Influence of nickel-contaminated soil on fenugreek (*Trigonella corniculata* L.) growth and mineral composition. Sci. Hort. **98**, 113, **2003**.
52. INGLE R.A., SMITH J.A.C., SWEETLOVE L.J. Responses to nickel in the proteome of the hyperaccumulator plant *Alyssum lesbiacum*. BioMetals **18**, 527, **2005**.
53. DUDIĆ B., RAKIĆ T., ŠINŽAR-SEKULIĆ J., ATANACKOVIĆ V., STEVANOVIĆ B. Differences of metal concentrations and morpho-anatomical adaptations between obligate and facultative serpentinophytes from Western Serbia. Arch. Biol. Sci., Belgrade **59**, (4), 341, **2007**.
54. MCNEAR D.H., CHANEY R.L., SPARKS D.L. The hypeaccumulator *Alyssum murale* uses complexation with nitrogen and oxygen donor ligands for Ni transport and storage. Phytochem. **71**, 188, **2010**.
55. BROADHURST C.L., CHANEY R.L., ANGLE J.S., MAUGEL T.K., ERBE E.F., MURPHY C.A. Simultaneous hyperaccumulation of nickel, manganese and calcium in *Alyssum* leaf trichomes. Environ. Sci. Technol. **38**, 5797, **2004**.
56. FREITAS H., PRASAD M.N.V., PRATAS J. Analysis of serpentinophytes from north-east of Portugal for trace metal accumulation–relevance to the management of mine environment. Chemosphere **54**, (11), 1625, **2004**.
57. ALLEN W.R., SHEPPARD P.M. Copper Tolerance in Some Californian Populations of the Monkey Flower, *Mimulus guttatus*. Proc. Royal. Soc. Lond. B **177**, (1047), 177, **1971**.
58. NKOANE B.B.M., SAWULA G.M., WIBETOE G., LUND W. Identification of Cu and Ni indicator plants from mineralised locations in Botswana. J. Geochem. Explor. **86**, 130, **2005**.
59. WELSH R.P.H., DENNY P. The uptake of lead and copper by submerged aquatic macrophytes in two English lakes. J. Ecol. **68**, 443, **1980**.
60. ROBINSON B.H., BROOKS R.R., KIRKMAN J.H., GREGG P.E.H., GREMIGNI P. Plant-available elements in soils and their influence on the vegetation over ultramafic ("serpentine") rocks in New Zealand. J. Royal Soc. N. Zealand **26**, 457, **1996**.
61. KAUR R., BHARDWAJ R., THUKRAL A.K. Growth and heavy metal uptake in *B. juncea* seedlings as affected by binary interactions between nickel and other heavy metals. Can. J. Pure. Appl. Sci. **4**, 1011, **2010**.
62. LOPEZ-BUCIO J., NIETO-JACOBO M.F., RAMIREZ-RODRIGUEZ V., HERREA-ESTRELLA L. Organic acid metabolism in plants: From adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Sci., **160**, 1, **2000**.
63. ROBINSON B.H., BROOKS R.R., CLOTHIER B.E. Soil amendments affecting nickel and cobalt uptake by *Berkheya codii*: potential use for phytomining and phytoremediation. Ann. Bot. **84**, 689, **1999**.
64. KÜPPER H., LOMBI E., ZHAO F.J., WIESHAMMER G., MCGRATH S.P. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. J. Exp. Bot. **52**, 2291, **2001**.
65. MIHAILOVIC N., DRAZIC G. Incomplete alleviation of nickel toxicity in bean by nitric oxide supplementation. Plant. Soil. Environ. **57**, 396, **2011**.