TRACE METALS IN SOILS AND SEVERAL BRASSICACEAE PLANT SPECIES FROM SERPENTINE SITES OF SERBIA

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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 carniiolicum, 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 carnolicum* 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
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.

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          | Aethionema saxatile             |
|                                   |              |               |                     | SP11         | Erysimum linariifolium         |
| Mt Zlatibor (Prdavac stream)      | 43°44'10"    | 19°39'29"    | 900                 | SP16         | Rorippa lippizensis            |
| Mt Zlatibor (Trčinoga-Ravna Kosa) | 43°43'53"    | 19°38'12"    | 850                 | SP8          | Cardamine plumieri             |
| Mt Zlatibor (Krivi Potok stream)  | 43°41'11"    | 19°42'03"    | 1020                | SP13         | Erysimum carniolicum           |
| Mt Rogozna (Pasji stream-Vinogor) | 43°07'25"    | 20°37'48"    | 1120                | SP5          | Alyssum montanum               |
| Kraljevo (Gornja Lopatnica river) | 43°40'09"    | 20°26'30"    | 440                 | SP2          | Aethionema saxatile            |
|                                   | 43°40'48"    | 20°28'05"    | 450                 | SP7          | Alyssum repens                 |
|                                   | 43°40'48"    | 20°28'05"    | 450                 | SP10         | Cardamine plumieri             |
| Čačak (Vrnčanska river gorge)     | 43°55'05"    | 20°08'11"    | 410                 | SP9          | Cardamine plumieri             |
|                                   |              |               |                     | SP14         | Isatis tinctoria               |
| Gornji Milanovac (Brđani gorge)   | 43°59'27"    | 20°25'15"    | 330                 | SP3          | Aethionema saxatile            |
|                                   |              |               |                     | SP12         | Erysimum linariifolium         |
| Raška (Trnavska river gorge)      | 43°17'14"    | 20°36'07"    | 420                 | SP6          | Alyssum montanum               |
| Ibar river valley (Maglič ruins)  | 43°36'93"    | 20°33'04"    | 300                 | SP4          | Alyssum montanum               |
|                                   |              |               |                     | SP15         | Isatis tinctoria               |
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 Vrnjačka 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)              | % coarse sand | % fine sand | % silt | % clay |
|              | 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 ± standard deviations)

| Sample point | pH soil in H₂O | 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±0.2           | 0.0049±0.0000 | 0.0257±0.0001 | 0.58±0.01     | 2.19±0.08      | 1.48±0.08      | 1.48  |
| SP2           | 7.39           | 6.30               | 0.9±0.4           | 0.0186±0.0006 | 0.0857±0.0041 | 0.27±0.01     | 0.70±0.05      | 4.60±0.23      | 0.20  |
| SP3           | 6.56           | 5.66               | 8.9±0.6           | 0.0081±0.0006 | 0.219±0.0010  | 0.15±0.01     | 2.14±0.16      | 3.87±0.09      | 0.55  |
| SP4           | 7.42           | 6.48               | 3.7±0.3           | 0.0068±0.0013 | 0.0594±0.0013 | 0.59±0.01     | 1.14±0.05      | 2.39±0.10      | 0.48  |
| SP5           | 7.76           | 6.95               | 12.1±1.5          | <0.0001      | 0.141±0.0003  | 0.13±0.01     | 0.97±0.01      | 2.49±0.06      | 0.39  |
| SP6           | 7.67           | 6.93               | 4.2±0.5           | 0.0315±0.0047 | 0.156±0.006  | 0.49±0.01     | 0.75±0.03      | 1.10±0.03      | 0.68  |
| SP7           | 7.10           | 6.02               | 2.6±0.5           | 0.0049±0.0000 | 0.0499±0.0011 | 0.50±0.01     | 1.30±0.04      | 2.91±0.43      | 0.45  |
| SP8           | 7.27           | 6.32               | 7.1±0.8           | 0.0053±0.0006 | 0.0528±0.0013 | 0.38±0.01     | 2.01±0.05      | 3.32±0.12      | 0.61  |
| SP9           | 7.10           | 6.03               | 4.6±0.2           | 0.0063±0.0007 | 0.0875±0.0010 | 0.15±0.01     | 2.35±0.00      | 3.53±0.07      | 0.67  |
| SP10          | 7.10           | 6.02               | 2.6±0.5           | 0.0049±0.0000 | 0.0499±0.0011 | 0.50±0.01     | 1.30±0.04      | 2.91±0.43      | 0.45  |
| SP11          | 7.17           | 6.21               | 1.8±0.2           | 0.0049±0.0000 | 0.0257±0.0001 | 0.58±0.01     | 2.19±0.08      | 1.48±0.08      | 1.48  |
| SP12          | 6.56           | 5.66               | 8.9±0.6           | 0.0081±0.0006 | 0.219±0.0010  | 0.15±0.01     | 2.14±0.16      | 3.87±0.09      | 0.55  |
| SP13          | 6.77           | 5.87               | 8.0±1.4           | 0.0070±0.0002 | 0.110±0.0005  | 0.16±0.01     | 5.10±0.28      | 2.54±0.10      | 2.01  |
| SP14          | 7.01           | 5.82               | 10.1±0.4          | 0.0053±0.0006 | 0.0253±0.0010 | 0.67±0.02     | 0.31±0.00      | 3.80±0.12      | 0.08  |
| SP15          | 7.13           | 6.13               | 2.9±0.8           | 0.0053±0.0006 | 0.149±0.0002  | 0.17±0.01     | 2.56±0.05      | 2.65±0.14      | 0.97  |
| SP16          | 7.00           | 5.97               | 5.0±1.2           | 0.0049±0.0000 | 0.0358±0.0008 | 0.33±0.01     | 1.30±0.16      | 3.51±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          |
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 ± standard deviations)

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

| Species              | Sample point | Ca root        | Ca shoot       | Mg root  | Mg shoot  | Ca/Mg root | Ca/Mg shoot |
|----------------------|--------------|----------------|----------------|----------|-----------|------------|-------------|
| Aethionema saxatile  | SP1          | 4.45±0.28      | 9.66±0.09      | 6.84±0.12| 10.64±0.15| 0.65       | 0.91        |
| Aethionema saxatile  | SP2          | 4.01±0.32      | 8.71±0.27      | 5.43±0.17| 12.76±0.30| 0.74       | 0.68        |
| Aethionema saxatile  | SP3          | 3.49±0.28      | 4.95±0.29      | 5.82±0.27| 9.46±0.29 | 0.60       | 0.52        |
| Alyssum montanum     | SP4          | 7.28±0.32      | 20.71±0.48     | 3.37±0.18| 11.28±0.49| 2.16       | 1.84        |
| Alyssum montanum     | SP5          | 6.58±0.11      | 34.89±1.48     | 9.92±0.20| 11.15±0.19| 0.66       | 3.13        |
| Alyssum montanum     | SP6          | 7.90±0.14      | 43.41±1.29     | 10.08±0.34| 7.16±0.03 | 0.78       | 6.06        |
| Alyssum repens       | SP7          | 8.59±0.41      | 14.31±0.39     | 5.02±0.20| 5.99±0.05 | 1.71       | 2.39        |
| Cardamine plumieri   | SP8          | 6.91±0.33      | 2.03±0.16      | 10.79±0.32| 12.17±0.32| 0.64       | 0.17        |
| Cardamine plumieri   | SP9          | 6.77±0.31      | 4.44±0.18      | 10.02±0.26| 14.08±0.09| 0.68       | 0.32        |
| Cardamine plumieri   | SP10         | 4.32±0.12      | 7.72±0.31      | 10.29±0.49| 13.29±0.20| 0.42       | 0.58        |
| Erysimum linariifolium| SP11        | 7.59±0.33      | 3.75±0.17      | 6.33±0.13| 7.54±0.24 | 1.20       | 0.50        |
| Erysimum linariifolium| SP12        | 7.59±0.34      | 12.16±0.36     | 4.10±0.17| 5.01±0.37 | 1.85       | 2.43        |
| Erysimum carniolicum | SP13         | 1.60±0.21      | 3.90±0.13      | 5.23±0.54| 4.86±0.17 | 0.31       | 0.80        |
| Isatis tinctoria     | SP14         | 4.24±0.31      | 8.73±0.46      | 10.67±0.12| 15.07±0.14| 0.40       | 0.58        |
| Isatis tinctoria     | SP15         | 4.19±0.15      | 4.11±0.27      | 8.07±0.08| 8.33±0.08 | 0.52       | 0.49        |
| Rorippa lippizensis  | SP16         | 6.80±0.27      | 8.65±0.27      | 9.95±0.29| 7.71±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].
| Species                  | Sample point | Ni root  | Ni shoot | Zn root  | Zn shoot | Mn root  | Mn shoot | Cu root  | Cu shoot |
|-------------------------|--------------|----------|----------|----------|----------|----------|----------|----------|----------|
| Aethionema saxatile     | 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  |
| Aethionema saxatile     | 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 |
| Aethionema saxatile     | 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  |
| Alyssum montanum       | 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 |
| Alyssum montanum       | 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  |
| Alyssum montanum       | 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  |
| Alyssum repens         | 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    |
| Cardamine plumieri      | 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     |
| Cardamine plumieri      | 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  |
| Cardamine plumieri      | 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  |
| Erysimum linariifolium  | 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 |
| Erysimum linariifolium  | 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  |
| Erysimum carniolicum    | 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 |
| Isatis tinctoria        | 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 |
| Isatis tinctoria        | 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 |
| Rorippa lippizensis     | 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 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 100–999 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 9. Concentrations of Cr, Cd, Co, Pb in plant tissues of different Brassicaceae species (mg kg⁻¹). (Means ± standard deviations)

| Species                | Sample point | Cr root | Cr shoot | Cd root | Cd shoot | Co root | Co shoot | Pb root | Pb shoot |
|------------------------|--------------|---------|----------|---------|----------|---------|----------|---------|----------|
| *Aethionema saxatile*  | SP1          | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Aethionema saxatile*  | SP2          | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Aethionema saxatile*  | SP3          | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Alyssum montanum*     | SP4          | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Alyssum montanum*     | SP5          | 72.0±7.8| 83.3±0   | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Alyssum montanum*     | SP6          | 46.3±8.0| 32.1±8.2 | 7.0±0.7 | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Alyssum repens*       | SP7          | <1.0    | <1.0     | 2.8±0.7 | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Cardamine plumieri*   | SP8          | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Cardamine plumieri*   | SP9          | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Cardamine plumieri*   | SP10         | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Erysimum linariifolium*| SP11        | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Erysimum linariifolium*| SP12        | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Erysimum carniolicum*| SP13         | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Isatis tinctoria*     | SP14         | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Isatis tinctoria*     | SP15         | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
| *Rorippa lippicensis*  | SP16         | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     | <1.0    | <1.0     |
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 ultramafic sites of Serbia are considered to be generally characteristic of the serpentine flora of the Balkans. With the exception of certain instances where it is inversed, the Ca/Mg ratio typical of serpentine soils (< 1) is maintained as such

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