UNIVERSITY OF BELGRADE

FACULTY OF BIOLOGY

Ahmed Farag Tumi

BIOACCUMULATION POTENTIAL OF SELECTED PLANT SPECIES OF THE FAMILY BRASSICACEAE FROM SERPENTINE HABITATS IN SERBIA

Doctoral Dissertation

Belgrade, 2013

UNIVERZITET U BEOGRADU

BIOLOŠKI FAKULTET

Ahmed Farag Tumi

BIOAKUMULACIONI POTENCIJAL ODABRANIH BILJNIH VRSTA IZ PORODICE BRASSICACEAE SA SERPENTINITSKIH STANIŠTA U SRBIJI

DOKTORSKA DISERTACIJA

Beograd, 2013

Data on the mentors and commission members:

Mentors:

PhD Gordana Tomović
Assistant Professor, University of Belgrade, Faculty of Biology

PhD Marjan Niketić
Senior Research Associate, Natural History Museum Belgrade

Commission members:

PhD Jasmina Šinžar-Sekulić
Assistant Professor, University of Belgrade, Faculty of Biology

PhD Nevena Mihailović
Senior Research Associate, University of Belgrade, Institute for the Application of Nuclear Energy

PhD Ana Čučulović
Research Associate, University of Belgrade, Institute for the Application of Nuclear Energy

Date of public defence:

Podaci o mentoru i članovima komisije:

Mentori:

dr Gordana Tomović
docent, Univerzitet u Beogradu, Biološki fakultet

dr Marjan Niketić
viši naučni saradnik, Prirodnjački muzej u Beogradu

Članovi komisije:

dr Jasmina Šinžar-Sekulić
docent, Univerzitet u Beogradu, Biološki fakultet

dr Nevena Mihailović
viši naučni saradnik, Univerzitet u Beogradu, Institut za primenu nuklearne energije

dr Ana Čučulović
naučni saradnik, Univerzitet u Beogradu, Institut za primenu nuklearne energije

Datum odbrane:

Bioaccumulation potential of selected plant species of the family Brassicaceae from serpentine habitats in Serbia

ABSTRACT

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 (SiO_2). 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. On the basis of heavy metal uptake, plants which grow on ultramafic soils can be divided into 'normal' and metal hyperaccumulators. 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. To date, about 360 hyperaccumulators of Ni from ultramafic soils have been identified. They are mostly found in Brassicaceae family in temperate areas (especially Mediterranean and Turkey). By far the greatest number of nickel-accumulating species within any genus is found in *Alyssum* L. Besides the *Alyssum* species, nickel hyperaccumulation was also discovered in species that belong to other genera of the Brassicaceae family of Europe and Turkey. So far only the data about Ni accumulation in *A. murale* from the central Serbia was published, 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*, *Aethionema*, *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, the aims of this thesis were to: 1) determine the basic habitat characteristics of the selected taxa from the Brassicaceae family, including information on the type of vegetation and/or community, altitude, slope, geological and pedological substrate and climate; 2) identify the basic physical (texture) and chemical (pH in H_2O, pH in 1N KCl, percent of organic matter, contents of P_2O_5 and K_2O) features of serpentine soil of the study sites; 3) determine the concentrations of the macro (Ca, Mg, Fe) as well as trace elements (Ni, Mn, Zn, Cu, Cr, Pb,

Co, Cd) in soils that are developed over serpentinite substrates at several locations in the northwestern, western, southwestern and central Serbia; 4) examine the concentrations of the macro and trace elements in underground and aboveground parts (stems and/or leaves) of plants of the family Brassicaceae that inhabit serpentinite habitats in Serbia; 5) based on the obtained results, the assessment of the capacity of some plant species of the family Brassicaceae for tolerance and/or bioaccumulation of certain trace elements (with special attention to nickel and zinc); 6) identificationn and discovery of new potential hyperaccumulator species in the family Brassicaceae from Serbia.

Soil and Brassicaceae plant samples were collected in 2010 and 2011 from 30 sampling points within 9 wider areas of the NW, W, SW and C Serbia. The following 11 plants were analyzed: *Erysimum linariifolium* Tausch, *Erysimum carniolicum* Dolliner, *Thlaspi kovatsii* Heuffel, *Thlaspi praecox* Wulfen, *Alyssum murale* Waldst. & Kit., *Alyssum montanum* L., *Alyssum repens* Baumg., *Cardamine plumieri* Vill., *Rorippa lippizensis* (Wulfen) Reichenb., *Aethionema saxatile* (L.) R. Br., *Isatis tinctoria* L. 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. The sand is fractionated by dry sieving. The pipette method was used for the determination of clay and silt fractions. 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. Available P and K were measured in AL solution extract (S/L 1:20). Phosphate concentration was determined by molybdenum blue method and potassium concentration was determined using flame emission spectrophotometry atomic absorption spectrophotometer. Exchangeable Ca and Mg were determined in 1 M ammonium acetate extract by AAS. Organic matter concentration was determined by dichromate digestion based on the FAO procedure. Extraction of available metals in soil was performed by 0.1 M HCl (S/L 1:10). Total metal extraction was done by HCl and HNO_3 digestion. Metal concentrations in both extracts were determined using atomic absorption spectrophotometry. Plant analysis – dried and ground plant material was digested by slightly modified wet procedure described by ISO 6636/2, using a boiling mixture of nitric and sulphuric acids. Phosphorus concentration was determined by modified the molybdenum blue method. Potassium concentration was determined using flame emission spectrophotometry. Metal concentrations were determined by using atomic absorption spectrophotometry.

On the basis of the analyses of physical soil characteristics that were done on 30 different ultramafic sites (within nine locations) in NW, W, SW and C Serbia it can be concluded that textural classes of the studied samples range from silty loam to sandy loam. Silt and clay contents in serpentine soils are generally minimal but in our study almost one third of the soil samples were of silty loam classes. The pH in H_2O of the serpentine soil samples varied from moderately acidic to moderately alkaline, while percent of organic matter in soils was low to moderate which indicate a relatively good humidization. Soils collected at Serbian sampling sites contained low level of P_2O_5, K_2O and Ca and high Fe and Mg contents which is also general characteristics of ultramafic soils. The serpentine soils of 30 Serbian sites were characterized by generally elevated total concentrations of trace elements such as Ni, Mn, Co and Cr, while most of the total Cu, Zn, Cd and Pb concentrations in analyzed serpentine soil samples fall within the ranges for normal soils. Concerning the available concentration of major elements, the soils were more or less of typical ultramafic composition, with the moderate to high concentration of available Mg and low to high available Ca content. The Ca/Mg quotients for the available fraction in 23 samples were relatively low; the content of the available Fe was high for the loamy and alkaline soils. Available concentrations of Ni, Zn and Mn were high in soil samples. Available Cr contents were low which confirms low mobility of Cr in surface soil layer and its limited availability to plants.

Generally speaking, the content of Ca was higher than Mg content in all plant tissues and the highest Ca/Mg ratio was noticed in *A. murale* leaves. In the roots and shoots of *A. montanum*, *A. repens*, *E. linariifolium* and only in shoots of *R. lippizensis* and *T. praecox* the Ca/Mg ratios > 1 were noticed. In the plant tissues of the rest of investigated Brassicaceae species, the contents of Ca were lower than Mg. Contents of Fe in the most of Brassicaceae plant tissues were elevated in relation to the natural Fe content of fodder plants, and it has been concluded that high Fe content of plants is may probably be applicable to all the serpentine flora of the Balkans. The total quantity of Ni in tissues of all nine *A. murale* samples studied was high to extremely high and in some cases, the ratio between concentration of Ni in leaves and in the corresponding soil was 67:1. The Ni concentrations in the shoots of *Thlaspi* species were high to extremely high, while in *A. montanum* and *A. repens* Ni contents were high and very unusual for the species that belong to section *Alyssum*. Actually, this is the first time that such high Ni content was measured within any species that belongs to this section. All the rest of Brassicaceae species studied here had Ni concentrations in their roots and shoots lower than 100 mg kg^{-1}, confirming that no nickel hyperaccumulating but tolerant species were recorded among these plants. Comparing to low Zn content in soil samples, in the roots of all *A. murale* samples Zn concentrations were the highest. Also, certain *Thlaspi* species studied here was noteworthy for containing Zn at concentrations 1000 mg kg^{-1}. Although Mn content in soil samples was relatively high, its concentration in plant tissues was noticeably lower; it is assumed that species growing in serpentine soils generally act as excluders of Mn and can restrict transport of this element to the shoots. In plant tissues of almost all the Brassicaceae plants Cu, Cr and Co were found only in traces.

Correlation of Ni accumulating capacity with soil properties lead to a 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 hyperaccumulators are defined as those plants which contain in their tissue more than 1000 mg kg^{-1} dry weight of Ni or more than 10000 mg kg^{-1} dry weight of Zn or Mn, only *A. murale*, *T. kovatsii* and *T. praecox* belong to the group of Ni hyperaccumulators. Strong accumulators can accumulate 100–999 mg kg^{-1} Ni in leaves and based on their accumulation properties, some populations of *A. montanum*, *A. repens* and *I. tinctoria* can be classified as Ni strong accumulators.

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. If we apply this criterion for the selection of accumulator Brassicaceae species from Serbian serpentine sites, some populations of *A. montanum*, *E. linariifolium*, *T. kovatsii* and *T. praecox* can be identified as Zn accumulators. Therefore, most Brassicaceae species studied here belong to the group of species tolerant to serpentine soils, and can be considered either as excluders or as indicators.

Alyssum and *Thlaspi* species should be useful for soil stabilization on ultramafic substrate, and can be used to establish a vegetative cover at serpentine sites or where vegetation is scarce due to high levels of metal contamination. This applies particularly to sites of former mining activity. Some populations of *A. murale*, *T. praecox* and *T. kovatsii*

display the best efficiency of Ni uptake, in terms of both the Ni concentrations attained and the biomass produced. Despite the fact that populations of *I. tinctoria* showed only low to moderate capacity to uptake, translocate or accumulate Ni and Zn in its roots and shoots, this biennial plant could be very good candidate for phytoextraction practice due to its well developed and deep roots, fast growth rate and high biomass producing. 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 heavy metals) could be a very convenient approach to phytoremediation and phytoextraction practices.

Key words: Ultramafics; Serpentine soils; Brassicaceae, Trace metals, Nickel hyperaccumulators; Serbia

Scientific field: Biology
Field of scientific specialization: Ecology, biogeography and environmental protection
UDC number: 504.5:582.683.2]:549.623.7(497.11)(043.3)

Bioakumulacioni potencijal odabranih biljnih vrsta iz porodice Brassicaceae sa serpentinitskih staništa u Srbiji

REZIME

Ultramafiti (serpentiniti) predstavljaju grupu magmatskih ili metamorfnih stena, koje se karakterišu visokim koncentracijama Mg, Fe, Ni, Cr i Co, kao i niskim koncentracijama Ca, P i K sadrže manje od 45% silicijuma (SiO_2). Flora i vegetacija koje naseljavaju serpentinitske supstrate obično su značajno oskudnije i siromašnije u odnosu na okolna staništa. Mnoge biljne vrste nisu sposobne da rastu na serpentinitski staništima, zbog njihovog ekstremno niskog sadržaja esencijalnih elemenata (nitrijenata), kao i nepovoljnog odnosa Ca/Mg u zemljištu. Na osnovu sposobnosti usvajanja teških metala, biljke koje rastu na ultramafitskim zemljištima mogu se podeliti u dve grupe: tzv. „normalne“ i metal hiperakumulatorske vrste. Dok je većina serpentinitskih biljaka sposobna da raste na ovakvim zemljištima, bez usvajanja velikih koncentracija teških metala, hiperakumulatorske vrste mogu akumulirati i do 1000 mg kg^{-1} Ni u suvoj masi svojih nadzemnih delova (najčešće u listovima). Do danas je identifikovano oko 360 vrsta koje su hiperakumulatori nikla, od kojih najveći broj pripada porodici Brassicaceae. Daleko najveći broj hiperakumulatora zabeležen je u okviru roda *Alyssum* L. Osim vrsta iz ovog roda, hiperakumulatorske vrste mogu pripadati i sledećim vrstama i/ili rodovima koje naseljavaju mediteranske delove Evrope i Male Azije: *Leptoplax emarginata* (Boiss.) O.E. Schulz, nekoliko vrsta iz roda *Bornmuellera* Hausskn., dve vrste iz roda *Pseudosempervivum* (Boiss.) Grossh. (Cochlearia L.), jedna vrsta iz roda *Aethionema*, kao i značajan broj vrsta iz roda *Thlaspi* L. Na području Srbije je do danas utvrđeno da jedino vrsta *Alyssum murale* sa planine Goč ima sposobnost hiperakumulacije nikla, dok za vrste iz nekih drugih rodova kao što su *Isatis*, *Cardamine*, *Rorippa*, *Erysimum* ne postoje informacije o hiperakumulativnim potencijalima.

Prema tome, bilo je neophodno sakupiti više informacija o akumulativnim kapacitetima biljaka iz porodice Brassicaceae sa serpentinitskih staništa u Srbiji, ali i proceniti njihov potencijal za fitoremedijaciju, pa su na osnovu ovih činjenica definisani ciljevi ove disertacije: 1) utvrđivanje osnovnih karakteristika staništa ispitivanih

taksona, uključujući podatke o tipu vegetacije i/ili zajednice, nadmorskoj visini, nagibu terena, geološkoj podlozi i klimi; 2) utvđivanje osnovnih fizičkih (tekstura, tj. mehanički sastav) i hemijskih (pH u H_2O, pH u 1 N KCl, procenat organske materije, koncentracija P_2O_5 i K_2O) svojstava serpentinitskih zemljišta na istraživanim lokalitetima; 3) utvrđivanje koncentracije makroelemenata (Ca, Mg, Fe) i teških metala (Ni, Mn, Zn, Cu, Cr, Pb, Co, Cd) u zemljištima koja se razvijaju iznad serpentinitske podloge na više lokaliteta u Srbiji; 4) ispitivanje koncentracije makroelemenata i teških metala u podzemnim i nadzemnim delovima biljaka iz porodice Brassicaceae koje naseljavaju serpentinitska staništa u Srbiji; 5) na osnovu dobijenih rezultata, proceniti kapacitet pojedinih biljnih vrsta iz porodice Brassicaceae za toleranciju ili bioakumulaciju određenih mikroelemenata (pre svega teških metala); 6) otkriti nove nikl i/ili cink akumulatorske ili hiperakumulatorske vrste iz porodice Brassicaceae.

Uzorci zemljišta i biljaka iz porodice Brassicaceae sakupljeni su tokom 2010. i 2011. godine sa 30 lokacija u okviru 9 širih područja severozapadne, zapadne, jugozapadne i centralne Srbije. Analizirane su sledećih 11 vrsta: *Erysimum linariifolium* Tausch, *Erysimum carniolicum* Dolliner, *Thlaspi kovatsii* Heuffel, *Thlaspi praecox* Wulfen, *Alyssum murale* Waldst. & Kit., *Alyssum montanum* L., *Alyssum repens* Baumg., *Cardamine plumieri* Vill., *Rorippa lippizensis* (Wulfen) Reichenb., *Aethionema saxatile* (L.) R. Br., *Isatis tinctoria* L. Metode za analize zemljišta – distribucija veličine čestica određivana je prosejavanjem i taloženjem. Uzorci su bili potpoljeni u disperzant natrijum heksametafosfat u zemljišno-vodenoj suspenziji 1:2 i mućkani u šejkeru 16h. Čestice peska su frakcionisane suvim prosejavanjem, dok je za određivanje frakcija gline i praha korištena pipet-metoda. Za određivanje teksturalnih klasa zemljišta primenjivana je USDA klasifikacija. Stvarna i izmenljiva pH vrednost zemljišta u destilovanoj vodi i 1 M rastvoru KCl očitavana je na pH-metru. Koncentracija pristupačnog P_2O_5, utvrđena je spektrofotometrijski, dok je koncentracija dostupnog K_2O određena korišćenjem metode plameno emisione spektrofotometrije (FES) na atomskom apsorbcionom spektrofotometru. Koncentracije kalijuma u uzorcima određivane su korišćenjem programa Origin 7.0. Koncentracije izmenljivih Ca i Mg u uzorku očitavane su zajedno sa standardnim rastvorima na atomskom apsorbcionom spektrofotometru. Koncentracija organske materije u zemljištu određivana je digestijom u dihromatu zasnovanog na FAO proceduri. Ekstrakcija pristupačnih metala u zemljištu izvršena je digestijom (ISO 11466 1995). Za određivanje koncentracija ukupnih elemenata u zemljištu izvršena je mineralizacija prema neznatno modifikovanoj proceduri koja je opisana u standardima (ISO 11466 1995 i ISO 11047 1998). Metode za analize biljnih tkiva – koncentracije fosfora u mineralizovanim uzorcima određivana je modifikovanom molibden-plavom metodom. Koncentracija kalijuma određivana je plamenoemisionom spektrofotometrijom na atomskom apsorpcionom spektrofotometru. Koncentracija kalijuma određivana je korišćenjem programa Origin 7.0. Da bi se odredile koncentracije makroelemenata (Ca, Mg, Fe), kao i pojedinih teških metala (Ni, Mn, Zn, Cu, Cr, Pb, Co, Cd) obavljena je mineralizacija uzoraka neznatno modifikovanom procedurom u standardimu (ISO 6636/2 1981). Koncentracije metala su određivane metodom atomske apsorbcione spektrofotometrije, primenom istih standarda kao i za određivanje koncentracije istih elemenata u uzorcima zemljišta. Koncentracije su određivane su korišćenjem programa Origin 7.0. Svaka analiza uzoraka zemljišta i biljaka izvršena je u tri ponavljanja. Statistička obrada dobijenih podataka urađena je u statističkom paketu Statistica for Windows (Stat Soft, 1996). Statističke procedure koje su korištene su analiza varijanse (nonparametrics ANOVA, Kruskal-Wallis test) i korelacija (bi-variation metod i Spearman-ov R koeficijent korelacije).

Na osnovu analiza granulometrijskog sastava 30 uzoraka serpentinitskih zemljišta na području Srbije, utvrđeno je da većina uzoraka pripada klasi peskovito-glinovitih zemljišta. pH uzorkovanih serpentinitskih zemljišta bio je u opsegu od blago kiselih do blago alkalnih, dok se procenat organske materije u uzorcima kretao od niskog do umereno visokog. Uzorci zemljišta na serpentinitima Srbije većinom su se odlikovali niskim sadržajem P_2O_5, K_2O i Ca i visokim koncentracijama Fe i Mg što je generalna karakteristika ultramafitskih (serpentinitskih) zemljišta. Većina zemljišnih uzoraka imala je povećane koncentracije ukupnog Ni, Mn, Co i Cr i nizak sadržaj ukupnog Cu, Zn, Cd i Pb. Po pitanju koncentracije dostupnih makroelemenata, zemljišta su imala tipičan sastav serpentinitskih tala, sa srednjom do visokom koncentracijom dostupnog Mg i niskim do srednje visokim sadržajem Ca. Odnos Ca i Mg bio je relativno nizak kod 23 uzorka zemljišta, dok je sadržaj dostupnog Fe bio visok za glinovita i bazna tla. Koncentracije dostupnog Ni, Zn i Mn bile su dosta visoke, dok je sadržaj dostupnog Cr bio nizak, što je očekivano jer je ovaj elemenat slabo dostupan za biljke.

U svim biljnim tkivima sadržaj Ca bio je znatno veći u odnosu na Mg, a najpozitivniji odnos Ca i Mg je zabeležen u listovima vrste *A. murale*. U korenovima i nazemnim delovima kod vrsta *A. montanum*, *A. repens*, *E. linariifolium* i nadzemnim delovima kod *R. lippizensis* i *T. praecox* odnos Ca/Mg bio je > 1. U biljnim organima ostalih ispitivanih vrsta, odnos Ca i Mg bio je < 1. Kod većine biljaka iz porodice Brassicaceae, sadržaj Fe bio je veći u odnosu na biljke koje ne rastu na serpentinitima, pa se pretpostavlja da je to opšta karakteristika serpentinitske flore Balkana. Ukupan sadržaj nikla u svim uzorcima vrste *A. murale* bio je visok ili ekstremno visok, čak je odnos koncentracije nikla u listovima u odnosu na odgovarajuće zemljište išao do 67:1. Koncentracija istog elementa kod vrsta iz roda *Thlaspi* bila je izuzetno visoka, dok je kod vrsta *A. montanum* i *A. repens* sadržaj nikla bio takođe visok i dosta neuobičajen za ove dve vrste koje pripadaju sekciji *Alyssum*. Zapravo, tokom ovih istraživanja po prvi put je zabeležena tako visoka koncentracija ovog elementa u navedenim biljnim vrstama. U svim ostalim ispitivanim biljkama, sadržaj nikla bio je niži od 100 mg kg^{-1}, što ukazuje na činjenicu da ove vrste pripadaju grupi biljaka tolerantnih na visok sadržaj ovog elementa u svojim organima, a ne hiperakumulatorskim vrstama. U odnosu na sadržaj cinka u zemljištu, u korenovima vrsta *A. murale* i vrstama iz roda *Thlaspi* zabeležen je znatno veći sadržaj ovog elementa. Svi ostali elementi (Mn, Cu, Cr i Co) su u podzemnim i nadzemnim organima ispitivanih vrsta iz porodice Brassicaceae zabeleženi u veoma niskim koncentracijama ili samo u tragovima.

Analizom korelacije kapaciteta usvajanja nikla u odnosu na određene osobine zemljišta utvrđeno je da su blago kisela zemljišta, sa niskim sadržajem P i K, niskim odnosom Ca i Mg, kao i malim koncentracijama Mn, Cr i Co najpovoljnija za usvajanje velilikih koncentracija nikla pre svega od strane vrste *A. murale*.

Ukoliko se kao hiperakumulatori definišu one vrste koje u svojim tkivima akumuliraju koncentracije nikla veće od 1000 mg kg^{-1} suve mase, ili koncentracije cinka veće od 10000 mg kg^{-1}, jedino se *A. murale*, *T. kovatsii* i *T. praecox* mogu smatrati nikl hiperakumulatorskim vrstama. Snažni akumulatori su vrste koje u svojim tkivima akumuliraju koncentracije nikla u količinama od 100–999 mg kg^{-1} suve mase, pa bi se na osnovu ovog kriterijuma neke populacije vrsta *A. montanum*, *A. repens* i *I. tinctoria* mogle uvrstiti u grupu snažnih akumulatora nikla. Akumulatorskim vrstama se smatraju one biljke kod kojih je odnos koncentracija teških metala u nadzemnim i

podzemnim organima >1.0, pa bi se primenom ovog kriterijuma neke populacije vrsta *A. montanum*, *E. linariifolium*, *T. kovatsii* i *T. praecox* mogle okarakterisati kao cink akumulatorske biljke. Sve ostale ispitivane vrste iz porodice Brassicaceae pripadaju grupi biljaka tolerantnih na serpentinitska zemljišta i mogu se smatrati samo kao indikatori ovakvih staništa.

Zahvaljujući svojim potencijalima za hiperakumulaciju nikla i/ili cinka, kao i većoj produkciji biomase, neke vrste iz rodova *Alyssum* (*A. murale*) i *Thlaspi* (*T. praecox* i *T. kovatsii*) mogu se veoma uspešno koristiti kako za fitostabilizaciju serpentinitskih zemljišta, tako i za površine koje su pod intenzivnom eksploatacijom ruda. Uprkos činjenici da su populacije vrste *I. tinctoria* pokazale slab ili umeren kapacitet za usvajanje, translokaciju i akumulaciju nikla i cinka u svojim podzemnim i nadzemnim organima, ova dvogodišnja biljka bi se mogla smatrati dobrim kandidatom za proces fitoekstrakcije pre svega zbog dobro razvijenoog korenovog sistema, kao i zbog brzog rasta i razvoja veće količine biomase tokom vegetacione sezone.

Na osnovu istraživanja sprovedenih u ovoj disertaciji, može se zaključiti da se autohtone vrste iz porodice Brassicaceae mogu veoma uspešno koristiti u procesima fitostabilizacije i fitoekstrakcije nikla i cinka, kako na serpentinitskim staništima, tako i na područjima koja su pod intenzivnom eksploatacijom ruda ovih metala.

Ključne reči: ultramafiti; serpentinitska zemljišta; Brassicaceae, teški metali, hiperakumulatori nikla; Srbija

Naučna oblast: Biologija
Uža naučna oblast: Ekologija, biogeografija i zaštita životne sredine
UDK broj: 504.5:582.683.2]:549.623.7(497.11)(043.3)

CONTENT

1. INTRODUCTION

1. 1. Ultramafics - general characteristics

Ultramafics (‘ultrabasic’) represent group of igneous (magmatic) or meta-igneous (metamorphic) rocks which 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 (Proctor 2003). Ultramafics are divided into intrusive, volcanic, ultrapotassic and metamorphic rocks and among the intrusive ultramafic rocks the most common are harzburgite, dunite and lherzolite. Essentially ultramafic rocks are composed of the minerals olivine, clinopyroxene, orthopyroxene, amphibole, biotite and serpentine. Each of the ultramafic minerals is represented by a monomineralic ultramafic rock such as pyroxenite (pyroxene), biotitite (biotite), dunite (olivine) and hornblendite (amphibole), while peridotites are olivine-rich rocks containing pyroxene but little or no felspar. Ultramafic rocks are sometimes described as ophiolites, however it should be noted that the latter term also embraces non-ultramafic rock types within gabbroic, mafic sheeted and mafic volcanic complexes, and it must also be remembered that ultramafites encompass rocks other than ophiolites (Brooks 1987).

Ultramafic rocks are distributed all over the world: in Europe (Sweden, Norway, Finland, Great Britain, Austria, Czech Republic, Switzerland, Bosnia and Herzegovina, Serbia, Macedonia, Albania, Greece, Bulgaria, 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 (Whittaker 1954). According to Stevanović *et al.* (2003), the largest serpentine areas in Europe are in the Balkans and they exist in large blocks or as small outcrops separated from other geological substrate in central Bosnia, western and central Serbia, 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.

According to Brooks (1987), before beginning the study of the ecology of ultramafites and their floras, very important points must first be established. The term "serpentine" *sensu stricto* refers only to the serpentine group of minerals (including antigorite and chrysolite) which have the general formula ($Mg_3Si_2O_5(OH)_4$ and which are important constituents of weathered "ultramafic rocks". These minerals are derived from the "serpentinization" of ultramafic rocks. Rocks which are rich in serpentine minerals derived from alteration of previously existing olivines and pyroxenes are known as "serpentinites". Serpentinites are rocks that form as a result of metamorphism or metasomatism of primary magnesium–iron silicate minerals. This entails the replacement of the primary silicate minerals by magnesium silicate serpentine minerals and the concentration of excess iron in magnetite (Alexander *et al.* 2007).

More specific definition of serpentine rocks is afforded by the terms "ultramafic" or "ultrabasic". The first one implies >70% ferromagnesian minerals, whereas the letter refers to rocks containing <45% silica. However, there is long-standing tendency for many scientists to classify all ultramafic rocks under the general term of serpentine. Furthermore, many botanists always refer ultramafic floras as "serpentine" floras, whether the soils are derived from serpentinized rocks or not. Although the term "ultramafic flora" is more adequate, it is used mainly by specialist, and therefore the long-standing traditional term "serpentine flora" is generally accepted and used in botanical scientific publications (Brooks 1987).

The process of serpentinization of ultramafic rocks involves the conversion of a peridotite to serpentine mainly by hydration of primary igneous minerals. Conversion of peridotite to serpentine is a metasomatic process in which there is change of composition of the rocks involved and the serpentinization occurs at temperatures between 100-300^oC. Besides the introduction of water, another chemical change which occurs during serpentinization is the partial oxidation of iron to magnetite. Also, the Fe^{3+}/Fe^{2+} in serpentines reflects changes in the alteration of iron during serpentinization since the original FeO contents of harzburgites, dunites and lherzolites is more or less the same. During serpentinization, the partition of iron among different minerals depends on the availability of oxygen (Brooks 1987).

1. 2. Physical properties of serpentine soils

Depending upon their exact chemical composition, serpentine soils can be red, green, blue or black. Serpentine soil's physical conditions are inhospitable for most plants. They are frequently rocky with granular texture, easily weathered at the earth's surface (vulnerable to erosion), and characterized by a lack of organic material (Kazakou *et al.* 2010). Peridotite and serpentinite formations are usually intrusions of great depth and often of ancient geologic origin and therefore it might be anticipated that the soils would be deep. In addition, many serpentine sites are in rolling or mountainous terrain, and the soils in such locations are usually shallow, probably because the steepness of the slopes and the sparseness of the vegetation encourage continual erosion. In many cases, these soils are made up mostly of relatively unweathered rock particles, and may be called lithosols. In many other instances, the shallow soils contain considerable clay and organic material and might better be called azonal soils of a relatively stable nature (Walker 1954). Robinson *et al.* (1935) considered that some serpentine rocks are too low in alumina to form sufficient clay for the establishment of a normal soil covering, regardless of the topography. These authors concluded that serpentine soils possess no physical characteristic which would render them particularly unfavourable for plant growth, although in some cases the clay contents of the subsoils were high enough to impede drainage. The textures of the soils vary from heavy clays to light loams while silt and clay contents in serpentine soils are generally minimal. Undesirable physical characteristics, such as gravely texture, low clay content, shallowness of the soil mantle and steepness of topography which encourages erosion, have important effects on the vegetation of serpentine areas, since they result in reduced moisture and nutrient levels. On moister serpentine sites, (north slopes), the plant cover is more abundant, and often includes additional species which are absent in drier sites. This probably indicates that plants which are widespread on serpentine soils possess drought resistance as well as tolerance for serpentine soils (Walker, 1954).

1. 3. Chemical properties of serpentine soils - the serpentine factor

The serpentine factor may be defined as the causal factor or factors (physical or chemical) related to the infertility of serpentine soils and to the nature of the vegetation colonising them. The general infertility of serpentine soils may also be defined as the "serpentine problem" (Brooks 1987). Also, Jenny (1980) introduced the term "serpentine syndrome", which illustrates the fact that it is the cumulative effect of the chemical, physical, and biotic components of the edaphic factor to which a plant must adapt. Although the physical characteristics of the serpentine soil and the restricted water supply are very important factors in limiting plant growth, the peculiarities (infertility) of serpentine soils are usually explained in terms of their chemical composition (Kazakou *et al.* 2010). A number of hypotheses have been established which attempt to explain the peculiarities of serpentine soils on the basis of soil chemistry. The development of different viewpoints on the subject probably can be attributed to the fact that the soils studied came from different climatic regions and to the probability that two or more of the proposed factors may operate simultaneously in many of the soils. Brooks (1987) proposed five factors which may be connected with the serpentine problem: the toxic effects of nickel, chromium and cobalt; the toxicity of excess magnesium; infertility due to low calcium content of serpentine soils; problems arising from an adverse calcium/magnesium quotient in the substrate; infertility arising from low levels of plant nutrients in the soils. Kazakou *et al.* 2010 presented three of the most popular hypotheses to explain the edaphic factors which may control serpentine floras: 1) Low availability of calcium relative to magnesium; 2) Deficiency of essential macronutrients; 3) High levels of phytotoxic heavy metals (Ni, Cr, Co, Mn). In addition to these three main factors, Walker (1954) considered that alkaline reaction of serpentine soils and low available molybdenum content contributes to their infertility.

1) <u>Calcium and Magnesium as serpentine factors</u>

Many scientists considered that the most important component of the serpentine factor represents the role played by calcium and magnesium. According to (Brooks 1987) there are three ways in which calcium and/or magnesium levels in serpentine soils can affect the vegetation which is able to colonize this type of habitats: a) the toxicity of magnesium; b) deficiency of calcium; c) an unfavourable Ca/Mg quotient in the soil.

a) The toxicity of Magnesium

The high level of magnesium was considered to be one of the most likely negative causes of the serpentine problem. This subject has been presented in detail by Krause (1982) and has been known over past 150 years. Part of this toxicity comes from the very high magnesium concentration of some soils, which can up to 36% MgO, coupled with the relatively high plant-availability of magnesium minerals. If plants need to develop an adequate tolerance to magnesium (contrary to chromium which appears to be practically 100% non-available to plants), the magnesium must be clearly phytotoxic (Brooks 1987). The role of magnesium in controlling plant growth on ultramafic sites is presented by Brooks and Yang (1984) on some serpentine endemic plants from Zimbabwe. These authors found that in the plant tissues there was a highly significant inverse relationship between the concentrations of magnesium and some nutrients such as iron, manganese, phosphorus, boron and sodium. These results reflect to the great importance of antagonism to nutrient uptake caused by high magnesium contents in the soil. Brooks and Yang (1984) and Brooks (1987) considered that high magnesium concentrations in soils were one of the principal factors in the serpentine problem.

b) Deficiency of Calcium

The concentration of calcium in serpentine soils is extremely low and in some cases is <100 mg/kg. However, low calcium levels are not the primary cause of serpentine infertility, since the low Ca/Mg quotients is the main cause of the serpentine syndrome. If present in large enough concentrations, calcium has the ability to neutralize harmful effects of ions such as Mg^{2+}, Na^{+} and H^{+} which could otherwise damage vegetation. Deficiency of calcium therefore results in a lowered tolerance to other elements in serpentine soils (Brooks 1987). Experiments which were done by Proctor (1971) have proved that addition of calcium can largely reduce the incidence of nickel toxicity symptoms in vegetation.

There are many literature sources which point to the beneficial effects of calcium addition to serpentine soils. According to Walker (1954), calcium deficiency can be treated as being one of the principal causes of the serpentine problem. However, Brooks (1987) considered that this might not be the case, as it was likely that the physiological effects of low calcium contents are related to the ability of this element to prevent damage from other heavy metals rather than to calcium deficiency *per se*. This author claimed that despite to the many published references, the role of low calcium contents in serpentine soils remains unresolved.

c) Unfavourable Ca/Mg Quotient

Loew & May (1901) first reported that poor productivity of serpentine sites is due to the low Ca:Mg ratio present in serpentine soils. From their experiments, they concluded that the Ca:Mg ratio must be at least unity for optimal growth. Vlamis and Jenny (1948) proposed that the low calcium content present in serpentine soils is the principal component of the serpentine syndrome, and high magnesium concentrations further compound the problem by depressing the availability of calcium. This hypothesis is supported by a number of studies (Kruckeberg 1954; Walker *et al.* 1955) in which growth of non-serpentine plants in serpentine soil significantly increases with the addition of calcium to the soil. Kruckeberg (1954) found that nonserpentine races of *Phacelia californica* (Hydrophyllaceae) could survive on serpentine soil if the soil was supplemented with calcium; however, plants lived no better on serpentine soil fertilized with N, P, and K than they did on unfertilized serpentine soil. Walker (1954) also concluded that the poor productivity of serpentine soils is a result of the low Ca concentration and coexisting high concentration of Mg, and only those species tolerant of low Ca:Mg levels could survive on serpentine soils. The total Ca/Mg quotient as opposed to the exchangeable quotient in soils differs by factor of about 50-100 as a consequence of the much lower availability of magnesium compared to calcium. This is in sharp contrast with Ca/Mg quotient of about 1 and 1.5 for the total and exchangeable values for non-serpentine soils (Brooks 1987). In plant tissues Ca/Mg quotients reflects to some extent the same quotients (total and exchangeable). The data for Ca/Mg values in various plant families show that there is a tendency for families well represented on serpentine soils to have lower than average Ca/Mg quotients. For example *Caryophyllaceae* have a mean value of only 2.77 (Brooks 1987).

2) Deficiency of essential macronutrients

The second factor which could be connected with the serpentine problem is the scarcity of plant nutrients in serpentine soils. Level of phosphorus, potassium and nitrogen are particularly low in these soils though this is partially offset by their optimum availability at pH 6.8 which is typical for serpentine soils. Macronutrient deficiency has been diagnosed as limiting for plant growth on serpentine soils of several continents but the limiting nutrient varied. The low contents of available phosphorus in serpentine soils may possibly be connected to the high affinity of soluble phosphates to serpentine (Brooks 1987).

Several studies in California proved that plant communities in serpentine soils are nitrogen (N)-limited (Koide *et al.* 1988; O'Dell and Claassen 2006; Turitzin 1982). Still, other scientists concluded that serpentine plant communities in Europe are strongly P- rather than N-limited (Chiarucci *et al.* 1999; Nagy and Proctor 1997).

However, it is more likely that multiple soil macronutrient deficiencies, rather than any single nutrient deficiency, are responsible for globally poor plant productivity on serpentine soils (Nagy and Proctor 1997). Although a low nutrient status is a common feature of serpentine soils, it is not always universal. For example Robinson *et al.* (1935) reported that phosphorus, potassium and calcium status of most serpentine soils, though low, was no lower than many non-serpentine soils which were farmed successfully.

3) High level of toxic heavy metals as a serpentine factor

Robinson *et al.* (1935) and Brooks (1987) suggested that high levels of phytotoxic elements such as nickel, chromium and cobalt in serpentine soils were responsible for their infertility, since high concentrations of these elements were more or less common in the serpentine soils. Therefore, it is appropriate to consider each of these elements separately:

Nickel

In the Earth's crust, the mean Ni abundance has been estimated around 20 mg/kg, whereas in the ultramafic rocks Ni ranges from 1400 to 2000 mg/kg. Its concentrations decrease with increasing acidity of rocks, down to the range of 5–20 mg/kg in granites. Sedimentary rocks contain Ni in the range from 5 to 90 mg/kg, with the highest values being for argillaceous sediments. Nickel reveals both chalcophilic and siderophilic affinity and readily combines with metallic Fe. Therefore, Ni–Fe compounds are common in the Earth's core and meteorites. After weathering, most Ni is coprecipitated with Fe and Mn oxides, and becomes included in goethite, limonite, serpentinite, as well as in other Fe minerals. It is also associated with carbonates, phosphates, and silicates (Kabata-Pendias 2011).

The Ni status of soils is highly dependent on its contents in parent material, but the concentration of Ni in surface soils reflects the additional impact of both soil-forming processes and anthropogenic activities. Ni is quite abundant in all soil groups and its greater accumulation is observed in calcisols and cambisols. Throughout the world soils contain Ni in the very broad range, and its mean concentrations are within the range 13–37 mg/kg. The Ni distribution in soil profiles is related either to OM or to amorphous oxides and clay fractions, depending on soil types. The lowest Ni level (7.6 mg/kg) was in soil with 0.9% clay content, and the highest (53.8 mg/kg) in soil with 4.6% clay content. Ni levels are elevated in some organic-rich soils. Especially peaty serpentine soils are known for high Ni levels, existing in easily soluble organic complexes (Nielsen *et al.* 1977). Also, soils of arid and semiarid regions are likely to have a high Ni content. Nickel in soils is slightly mobile and occurs mainly in the residual fraction, in more than 50%. In surface soil horizons, Ni occurs mainly in bound organic forms, a part of which are relatively easily soluble chelates. Bonding of Ni to organic ligands may be very strong, and can highly affect its mobility. Forms of Ni in soils are diverse and range from highly mobile to ones that have no reactivity. Several soil properties, particularly clay fraction, SOM, and pH, control Ni behaviour and phytoavailability (Kabata-Pendias 2011).

There is no evidence of an essential role of Ni in plant metabolism. The Ni content of plants seems to be highly controlled by soil properties and origin, plant abilities to absorb Ni (e.g., accumulators and hyperaccumulators) also has a strong influence. Nickel is usually easily extracted from soils by plants and its contents of plants are simple functions of Ni forms in soils. Both plant and pedological factors affect these processes and the most pronounced factor is soil pH. The mechanism of Ni toxicity to plants is not fully understood, although the restricted growth of plants and injuries caused by an excess of this metal was observed for quite a long time. Biological effects of Ni are highly related to its species. Generally, the range of excessive or toxic amounts of Ni in most plant species varies from 10 to 100 mg/kg. The range of excessive or toxic amounts of Ni in most plant species varies from 10 to 1000 mg/kg. Ni toxicity is associated with serpentine or other Ni rich soils. Some native plants from such location accumulate leaf concentrations of over 6000 mg Ni/kg. Several plant species are known for their great tolerance and hyperaccumulation of Ni. Usually these species, mainly of the Boraginaceae, Cruciferae, Myrtaceae, Leguminosae and Caryophyllaceae families, are also Co accumulators. Native vegetation of serpentine soils was reported to contain up to 19,000 mg/kg (AW) Ni (Lyon *et al.* 1968). The mechanism of the Ni hyperaccumulation is not yet well-understood, but evidently it is associated with organo-metallic complexes. An association has been found between Ni concentration in the sap of several genotypes of hyperaccumulators and concentrations of some organic acids (polycarboxylate), especially of citric acid (Brooks 1998). These complexes react as transport ligands for Ni within plants which have accumulated the nickel. According to Asemaneh *et al.* (2006), the main ligands for Ni in hyperaccumulators are citrate and malate, involved in the metal transfer within plant tissues. Ni is likely to be concentrated in cells of leaf epidermis, but in most plants Ni is accumulated mainly in roots. The interaction between Ni and other trace metals, Fe in particular, is believed to be a common mechanism involved in the Ni toxicity. Ni excess is believed to cause an actual Fe deficiency by inhibiting the translocation of Fe from roots to tops. Both antagonistic and synergistic interactions are observed between Ni and several trace metals (Kabata-Pendias 2011).

Plants appear to be remarkably tolerant to high nickel concentrations in serpentine soils and there is an appreciable amount of evidence that plants are readily able to develop tolerance to serpentinic environments. Among the tolerant plants are various *Alyssum* species; some of them had very high nickel content (ca. 10000 mg/kg) in its leaves and also tolerated much higher nickel content in the soil (up to 3000 mg/kg). Also, endemic species *Alyssum bertolonii* and *Alyssum argenteum* showed great tolerance to effects of nickel to germination, root elongation and protoplasmic resistance of epidermal cells (Brooks 1987). The most extreme example of nickel uptake and tolerance is recorded in New Caledonian tree *Sebertia acuminata* which contains a blue sap containing 11% nickel as the citrate complex (Jaffré *et al.* 1976). However, a survey of literature on nickel as an important contributor to the serpentine factor reveals that there is a great deal of contradictory evidence.

There are several factors that contribute to phytotoxicity of nickel. The first factor is pH, since it is well known that adding lime to serpentine soils decreases the mobility of nickel and decreases the toxicity of the soils (Halstead *et al.* 1969). The second factor is iron, since some workers (Mizuno 1968) found that the toxicity of nickel is due to iron deficiency caused by competition between these two elements. Still, according to Brooks (1987) even though serpentine soils contain a very high iron content, the proportion of plant-available iron is extremely low (of the order 0.001%), so that iron deficiency could eventually arise in the presence of high levels of nickel in serpentine soils. Therefore, because of greater availability of nickel, soil extract contain almost equal contents of iron and nickel even though the total iron amount of the soil is about 100 times greater. The third factor which contributes to the nickel content in soils is the presence of organic matter. The high content of organic matter can also reduce nickel toxicity by removing this metal as a chelate complex (Halstead *et al.* 1969). However, the organic content is relatively low in serpentine soils so that nickel is less likely to be complexed by organic matter. In addition, serpentine soils which are being less fertile, and also having low contents of nitrogen, potassium and magnesium than other soils, support vegetation to provide the organic matter, hence compounding nickel toxicity (Brooks 1987).

Chromium

The abundance of Cr in the Earth's upper crust averages 100 mg/kg. The distribution pattern of Cr in rocks shows the tendency to its concentration in mafic igneous rocks and in argillaceous sediments. In ultramafic rocks its content can be over 3000 mg/kg and in acid ones (e.g., granites) may be less than 50 mg/kg. The geochemistry of Cr is complex because of its easy conversion from one oxidation state to another (Kabata-Pendias 2011).

The world soil average content of Cr in soils has been considered as 60 mg/kg. Since soil Cr is inherited from parent rocks, higher contents are generally found in soil derived from mafic rocks and argillaceous sediments. Soils developed from serpentines are especially elevated in Cr, sometimes to above 100,000 mg/kg. Cr occurs in soils mainly (> 80% of total content) in the immobile residual fraction. A positive relationship between Cr and the fine granulometric fraction in soils has resulted in higher Cr content in silty and loamy soils than in sandy ones. Soils pH has an influence on the Cr speciation and solubility. In uncontaminated soils at neutral pH, Cr is slightly mobile and thus not easily available to plants which may have nutritional implication. In ultramafic soils, where a large part of Cr occurs mainly in insoluble phases, Cr was present in the soil solution, under natural vegetation, in the range of 2.7–10 µg|L, whereas under crop vegetation, due to effects of phosphorous fertilizers, its concentrations reached up to 700 µg|L (Becquer *et al.* 2003). The immobility of soil Cr may be responsible for an inadequate Cr supply to plants. Cr is of nutritional importance because it is a required element in human and animal nutrition (Kabata-Pendias 2011).

There is no evidence of an essential role of Cr in plant metabolism. Chromium is slightly available to plants and not easily translocated within plants, thus it is concentrated mainly in roots. The most available to plants is Cr^{6+}, which is the very unstable form under normal soil conditions and its availability depends on soils properties, and especially on soil texture and pH. Also Cr^{3+} and several complex Cr anions (e.g., CrO_4^{2-}) may be easily available to plants. The mechanism of absorption and translocation of Cr in plants is similar to those of Fe, and therefore the capability of roots to convert Cr^{3+} to CrO_4^{2-} is the key process in Cr absorption. There is a great difference in the accumulation of Cr by shoots and roots of various vegetable crops; the shoot/root ratio varies widely from 0.005 to 0.027. The highest concentration of Cr, supplied in two forms (Cr^{3+} and Cr^{6+}), was found in roots of plants of the Brassicaceae family, and the lowest in roots of *Allium* sp. (Kabata-Pendias 2011). According to Brooks (1987), the serpentine plants seldom, if ever, have a chromium content exceeding 100 mg/kg and this low level of uptake can be used to measure the degree of contamination of plant samples by soil.

Although stimulating effects of chromium on plants have been noticed by several authors, the phytotoxicity of this element has been often reported, especially in plants on contaminated soils or developed from ultramafic rocks. The toxicity of chromium depends on its oxidation state, but is also related to readily available forms of chromate. Symptoms of chromium toxicity appear as wilting of tops and root injury; also, chlorosis in young leaves, chlorotic bands on cereals, and brownish-red leaves are typical features (Kabata-Pendias 2011). Proctor (1971) proved that the chromic ion toxicity is reduced by the presence of calcium ions, which may explain the tolerance of some plants to serpentine soils. Brooks (1987) concluded that there was no real evidence that the chromium in serpentine soils is to any degree responsible for the toxicity of such substrates. This author did not believe that this trace and toxic element is part of the serpentine problem, particularly as the chromic ion concentration in equilibrium with the hydroxide at pH 6.8 is only 10 mg/kg. In addition, there are no reliable records of any floras specifically adapted to chromium rich soils anywhere on Earth.

Cobalt

Cobalt, in the Earth's crust, is highly concentrated in mafic rocks, up to 200 mg/kg, when compared to its content in acid igneous rocks, 1–15 mg/kg, and to its abundance in the upper continental crust, 10–12 mg/kg. The geochemical cycle of Co closely resembles that of Fe and Mn cycling, and is likely to be associated with several minerals of these metals. In soils, Co behaviour seems to be strongly influenced mainly by the Mn oxide phase formation. Relatively easy interactions of Co with all metals that are associated geochemically or biochemically with Fe, have a significant impact on its behaviour in soils and its phytoavailability (Kabata-Pendias 2011).

The worldwide mean value of Co in surface soils is considered as 10 mg/kg. Usually, higher levels of Co are in heavy loamy soils (cambisols) and sometimes in organic soil (histosols). The content and distribution of Co in soil profiles are dependent on soil-forming processes and therefore differ for soils of various climatic zones. Higher Co contents of surface soils are found in arid and semiarid regions. High Co contents are observed in soils over serpentine rocks, up to 520 mg/kg, and in soils around ore deposits, up to 85mg/kg. Soils over mafic rocks and soils derived from clay deposits contain the highest amounts of this metal. Cobalt speciation in soils depends on several factors, among which the Eh potential plays a significant role.

Contents of Co in plants are highly controlled by both soil factors and the ability of plants to absorb this metal. In higher plants, absorption of Co^{2+} by roots involves transport in the xylem, the metal is mainly transported by the transpiration flow. Co complexing with organic compounds controls its bioavailability. A low mobility of Co^{2+} in plants restricts its transport from stems to leaves. The Co uptake by plants is a function of content of its mobile fraction and of the Co concentration in solution during absorption; Co behaves like other trace metals (e.g. Fe, Mn) and is transported in forms bound to complexing organic compounds with a molecular weight in the range of 1000/5000. Co is easily taken up by leaves through the cuticle (Kabata-Pendias 2011).

The role of Co in the photosynthesis is controversial, although there are some evidences that it participates in chlorophyll formation. Low concentration of Co stimulates growth of both algae and higher plants. Positive effects of low Co concentrations on plant metabolism are not yet fully understood and probably several effects are cross-linked with interactions with other trace metals. The interaction of Co with other metals depends mainly on their concentrations. Co interacts with all metals that are associated biogeochemically with Fe. The most significant relationship has been observed between Co and Mn or Fe in plants. Both geochemical and biochemical antagonisms between these metals have arisen from their affinity to occupy the same sites in crystalline structures and from the similarity of their metallo organic compounds (Kabata-Pendias 2011).

The ability of plant species to absorb Co varies considerably. Legumes accumulate more Co than do grasses. Mean Co contents of clovers from various countries range from 100 to 570 μg/kg, whereas these values for grasses are between 60 and 270 μg/kg. Plants are known to accumulate large amounts of Co and to develop a mechanism of Co tolerance which is basically similar to that occurring in any metalliferous plant species. Several plant species, mainly of the families Cruciferae, Caryophyllaceae, Violaceae, Leguminosae, Boraginaceae, and Nyssaceae, are known for their high accumulation of Co and are also recommended as biogeochemical indicators (Kabata-Pendias 2011).

As the availability of cobalt and nickel to plants is similar, it is not surprising to find hyperaccumulators of cobalt (> 1000 mg/kg). However, among serpentinophytes no such accumulators are known. This is probably because of the relatively low, compared to nickel, concentrations of cobalt. Typical cobalt levels in serpentine plants are around 10 mg/kg; however, species capable of hyperaccumulating nickel are also capable of accumulating very high concentrations of cobalt (Brooks 1987).

As a general conclusion it can be said that the "serpentine syndrome is still not solved after nearly a century of effort, but some progress has been made in the past 30 years and there seems no doubt that some time, perhaps before the turn of the century, a firm consensus will emerge defining the true nature of serpentine problem and its solution. When that stage is reached, it will represent the unravelling of what is one of the most important problems in plant physiology today. The solution will depend above all on co-operation between scientists of many disciplines: analytical chemists, biochemists, plant physiologists, botanists, biosystematics and many others" (Brook 1987).

1. 4. Trace elements hyperaccumulation and tolerance in serpentine plants

Baker (1981) proposed four categories of plant-heavy metal relationships: excluders, accumulators, indicators, and hyperaccumulators.

Excluders are defined as 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. High metal accumulation may be found in the roots of excluder plants and their leaf/root metal concentration quotient is < 1.0 (Baker 1981; Baker and Brooks 1989). Another type of exclusion involves restricting metals from entering the plant (Tilstone and Macnair 1997) by the influence of root exudates, or the absence of an uptake mechanism.

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 leaf/root metal concentration quotient is >1.0 (Baker 1981; Baker and Brooks 1989; Tilstone and Macnair 1997).

Hyperaccumulators are plants that accumulate in excess of 100-fold more metal in their shoots than normal plants (Brooks *et al.* 1977; Brooks 1998). For Ni, Co, and

Cr, the concentration exceeds 1000 mg/kg and more than 10000 mg/kg dry weight for Zn or Mn (Baker and Brooks 1989). Hyperaccumulators can simply be viewed as accumulator plants that show extreme behaviour in metal uptake and translocation to the shoots. This distinctive feature of hyperaccumulators has attached great scientific interest, especially for their potential use in the remediation strategy of heavy metal contaminated soils and phytomining (Chaney *et al.* 2007). Many hyperaccumulators do not operate as good indicators of soil metal contents since them posses almost similar high levels of hyperaccumulation over a very wide range of soil metal levels.

Indicator plants show an intermediate response to high soil metal content with the metal concentration in the plants reflecting the soil concentration; the plant/soil metal concentration quotient in indicators is approximately 1.0 (Baker 1981). According to this author, effective biogeochemical indicator plants have an approximately linear relationship between elemental content in the plants and the concentration of the same element in the soil.

Serpentine soils have properties that are highly unfavourable for most plants. Among these, heavy metal tolerance, especially to Ni, is considered as a crucial factor for plant survival on certain serpentine soils (Kruckeberg 1954; Proctor and Woodell 1975). One strategy to cope with high levels of metals shown by serpentine plants is hyperaccumulation.

(1) Occurrence of Ni hyperaccumulation

According to Kazakou *et al.* (2008), the analysis of many thousands of herbarium and field specimens of serpentine plants has led to the identification of more than 360 species in over 90 genera and 40 families of almost exclusively dicotyledonous plants that display Ni concentrations above 1000 mg kg-1. A high percent of these species (85-90%) appear to be serpentine endemics. Others inhabit a variety types of substrata, but exhibit Ni hyperaccumulation on serpentine soils, for example *Alyssum bracteatum* (Iran, Caucasus), or *Thlaspi ochroleucum* (E. Europe, Turkey). Thorough lists of Ni hyperaccumulators are given by Brooks (1987), while supplementary recent additions can be found in Ghaderian *et al.* (2007) and Reeves *et al.* (2007). It should be underlined that globally Ni hyperaccumulation is actually quite rare, shown by some 2% of serpentine species worldwide (ranging from 0% in some areas to about 15% in some of those in Cuba). Physiological and biochemical attention in Ni hyperaccumulation and the potential applications of some of the hyperaccumulator species far outweigh this rarity (Kazakou *et al.* 2008).

(2) Hypotheses of the ecological function of hyperaccumulation

The most fundamental questions regarding hyperaccumulation is why some plants accumulate trace elements to concentrations that are toxic to most other organisms and what selective advantage has led to the evolution of hyperaccumulation? (Pollard 2000; Kazakou *et al.* 2008). The following hypotheses were suggested by Boyd and Martens (1992): *Hypothesis a*: drought resistance. Proctor and Woodell (1975) proposed that xeromorphism is frequent on serpentine soils and drought resistance as a function of hyperaccumulation was suggested by Severne (1974). Baker and Walker (1989) proposed that Ni might be hyperaccumulated as an osmoticum in plant cells to decrease the water potential of the plant. *Hypothesis b*: interference. Baker and Brooks (1989) suggested that hyperaccumulation of Ni could be a ‘‘survival or defence strategy against competition from other species’’. However, it was concluded (Zhang *et al.* 2007) that hyperaccumulators growing on serpentine soils are not likely to acquire any benefit from the allelopathic effect of high-Ni leaves falling onto the soil surface. *Hypothesis c*: inadvertent uptake. High concentrations of metals in hyperaccumulators may be an unintended consequence of other features such as a lack of mycorrhizae (Baker and Walker 1989) or the greater transpiration of plants in xeric habitats in serpentine. *Hypothesis d*: defense. That hyperaccumulation of Ni serves a defense function has been supported by surveys examining the levels of predation by insects or attack by fungal pathogens on hyperaccumulators grown on high-Ni and low-Ni media (Boyd 2007). This author concluded that there is some evidence for defense by hyperaccumulated Se As, Ni, Cd, and Zn, but that relatively few plant taxa and natural enemies have been investigated. There is evidence that other plants such as accumulators may use substances such as Cd, Co, Cr, Cu, Mn, Ni, Pd, and Zn in defense, so Boyd (2007) suggested that the evolution of hyperaccumulation from accumulation could be possible.

(3) Tolerance to serpenine soils

The subject of adaptation and tolerance of plant species to the environmental conditions of serpentine soils has been occupying researchers for many years.

Metal tolerance is a fine example of micro-evolution involving evolutionary change by natural selection within a species. Serpentine-tolerant populations may either belong to species of natural metal tolerance or be distinct races or ecotypes (plants that have developed tolerance not possessed by the remainder of the species) (Kazakou *et al.* 2008). Plants tolerate higher metal contents on serpentine soils either by a constitutive trait present in all members of the species growing either on non-serpentine or serpentine substrata or by an adaptive mechanism present only in tolerant ecotypes (Antonovics *et al.* 1971; Baker and Proctor 1990). If tolerance to serpentine soils is examined in plants which are found on both serpentine and non-serpentine soils, it is possible to differentiate the evolution of ecotypes (local adaptation) from constitutive tolerances (Kazakou *et al.* 2008).

Several such studies have been accomplished. In some cases, similar metal uptake features and performance of plants from serpentine and non-serpentine populations suggests a constitutional metal tolerance at the species level (Reeves and Baker 1984). Contrasting assumptions were gained in other studies demonstrating that serpentine tolerance is an adaptive rather than a constitutive trait (Proctor 1971; Boyd and Martens 1992).

According to Kazakou *et al.* (2008) the current list of Ni hyperaccumulators includes taxa widely distributed through the plant kingdom suggesting independent convergent evolution of this ability perhaps in response to a wide variety of selection pressures. It may therefore be unreasonable to search for either a single reason for the evolution of Ni hyperaccumulation, or a single common biochemical process for its uptake and sequestering. In fact, studies even between two genera (*Thlaspi* and *Alyssum*) within a single family (Brassicaceae) indicate differences in the chemical behaviour and Ni-binding ligands involved.

According to Kruckeberg (1954), a major criterion for serpentine tolerance must be the capacity of serpentine plants to grow on soils of low calcium levels. Walker (1954) suggested that serpentine endemics appear to be characterized by their ability to obtain sufficient calcium, even at the low concentrations characteristic of serpentine soils. Some native species occur both on and off serpentine, however, and studies of these may further clarify the problem. Therefore, it could be expected to find, within species of this latter type, some populations adapted to serpentine and others not so adapted.

However, the more recent studies (Kazakou *et al.* 2008), revealed the fact that the ability of a plant to tolerate a combination of heavy metals can be achieved either by co-tolerance or multiple metal tolerance. Co-tolerance is accomplished by pleiotropy, whereby the same gene(s) produces tolerance to more than one metal. Multiple metal tolerance results when genes for several tolerances spread independently within a serpentine population. According to Macnair (1993) the only way to distinguish between multiple tolerance and pleiotropy in plants is by a thorough genetic analysis investigating the independence of genes for tolerance to different metals. Some evidence indicated that heavy metal tolerance is mostly metal-specific since plants only tolerate the metals that occur at toxic contents in their environment (Verkleij *et al.* 1991).

There are many studies showing that heavy metals are antagonistic to the uptake of other elements. It has been found a significant inverse relationship between Mg in serpentine plants and the plant concentrations of B, Fe, Mn and P (Brooks and Yang 1984). The findings of these authors showed that these relationships were caused by high Mg levels in the soil; high Mg concentration is probably the main cause of infertility of serpentine soils and therefore of the development of specialized serpentine floras adapted to this unfavourable edaphic condition.

1. 5. Plant species in relation to serpentine soils

Serpentine plants exhibit adaptations to the low nutrient availability and high heavy metal concentrations of serpentine soils. It has been suggested that the physiological adaptations of serpentine plants may have some degree of morphological expression (Kruckeberg 1954). According to Clarkson and Hanson (1980), physiological traits of plants from nutrient-poor soils include: 1) slow growth rates; 2) high investment in anti-herbivore defences; 3) low saturation rates of nutrient uptake coupled with failure to respond to fertilizer application; 4) selectivity; 5) flexible allocation patterns (e.g. plasticity within the same root system); 6) storage of nutrients and efficient nutrient utilization; 7) high investment in mycorrhizal development; 8) efficient mechanisms of internal nutrient recycling to ensure minimal nutrient losses during leaf senescence.

Many serpentine species are xeromorphic, and water deficiency has been suggested as another stress factor of many serpentine soils (Proctor and Woodell 1975). Freitas and Mooney (1996) compared the water-stress tolerance of two different ecotypes of *Bromus hordeaceus* from sandstone and serpentine soils. They revealed that the serpentine ecotype showed greater physiological adaptation (decreased stomatal conductance resulting in lower transpiration rate and higher leaf water potentials) to water deficiency. Brooks (1987) commented that serpentine plants have a powerfully developed root system to help the uptake of water and nutrients which may be seen as an adaptation to poor and well-drained serpentine soils. Some serpentine plant populations display a strategy whereby the formation of lateral roots is down-regulated at high concentrations of Mg with more resources allocated to the longer and deep-growing roots important in dry conditions (O'Dell *et al.* 2006). Boyd and Martens (1992) analyzed mineral element concentrations of *Thlaspi montanum* var. *montanum* populations and concluded that plants from serpentine populations have a tendency to lower levels of several elements than plants from non-serpentine populations. They suggested that these findings may be explained by lower nutrient uptake abilities of populations from serpentine soils. Due to lower nutrient concentrations of serpentine soils (Proctor and Woodell 1975), uptake systems for these plants may be saturated at relatively low soil nutrient levels.

Brooks (1987) applied terms facultative and obligate serpentine plants; *facultative* taxa are plants which will grow quite well on serpentine soils without having a specific requirement for any of the edaphic or physical properties of the soil, while *obligate* taxa are presumed to grow on serpentine because of a specific nutritional or other requirement which only such soils can provide. However, Baker (1987) warned that great care must be taken in definition of the term *obligate*, since by his experience it can be noticed that all serpentine endemics will grow quite well on non-serpentine soils until they succumb to either fungal attack or competition from strong weed species. This author commented that it would seem that these taxa are *obligate* to serpentine only insofar as such soils offer a refuge from biotic factors existing in non-serpentine substrates. Additionally, Reeves *et al.* (1999) divided plant species found on serpentine soils into two groups: a) *serpentinicolous*, serpentine-endemic or serpentine-obligate plants, which grow exclusively on serpentine soils and are not found elsewhere and, b) *serpentine-tolerant* or serpentine-facultative plants, which are able to survive on serpentine soils but grow better elsewhere. More recently, Stevanović *et al.* (2003) additionally made classification of the Balkan endemics according to their preference for serpentine to the following three large groups: *1) endemics which are mainly growing on serpentine – obligate serpentine endemics*; *2) endemics often growing on serpentine but also inhabiting other kinds of substrate (limestone or silicate) – facultative serpentine endemics*; *3) endemics mainly growing on non-serpentine substrate (limestone or silicate) but some times (or rarely) on serpentine – accidental serpentine endemics*.

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) in comparison to those from other soil types (0.5–10 mg/kg) (Reeves 1992). 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 (Baker and Brooks 1989). To date, about 360 hyperaccumulators of Ni from ultramafic soils have been identified (Reeves and Baker 2000). They are mostly found in subtropical and tropical areas, while some species (mostly from the family Brassicaceae) are encountered in temperate areas (especially Mediterranean and Turkey) (Reeves and Adigüzel 2008).

Prasad and Freitas (2003) listed c. 11 genera and 87 species of the family Brassicaceae. 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 pintodasilvae* Dudley and *Alyssum murale* Waldst. & Kit. were also recognized as nickel hyperaccumulators. In addition to the 14 European hyperaccumulator species, further 31 hyperaccumulors of the genus *Alyssum* (all in section *Odontarrhena*) were presented (Brooks *et al.* 1979). 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 (Kazakou *et al.* 2010).

However, in the most recent publication (Cecchi *et al.* 2010), on the basis of balance between molecular data, morphology, distribution and ecology, only 11 European Ni hyperaccumulator species of genus *Alyssum* were recognized. These authors 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.* (2010) accepted opinion of Hartvig (2002) who considered that the slight differentiation in trichome density and inflorescence morphology of these populations may support, at most, their recognition at the varietals rank. Therefore these authors considered that literature reports of hyperaccumulation in *A. murale* (Brooks and Radford 1978; Reeves *et al.* 2001) are likely to be referred to this plant or to closely related taxa from Anatolian serpentine areas. 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) (Babalonas *et al.* 1984; Bani *et al.* 2007, 2009, 2010, 2010a; Çullaj *et al.* 2004; Hasko and Çullaj 2001; Reeves and Adigüzel 2008; Reeves *et al.* 1983, 2009; Shallari *et al.* 1998).

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.) (Reeves *et al.* 1980), several species of *Bornmuellera* Hausskn. (Hasko and Çullaj 2001; Reeves *et al.* 1983; Reeves *et al.* 2009), 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. (Reeves 1988; Reeves and Adigüzel 2008). In the Balkan Peninsula, hyperaccumulation by many species in the above genera has been reported in several publications (Bani *et al.* 2009; Bani *et al.* 2010; Chardot *et al.* 2005; Shallari *et al.* 1998).

1. 6. History of survey of the serpentine flora and vegetation in Serbia

Investigations concerning serpentine flora and vegetation in Serbia already began in the 19th century when numerous plants growing on serpentine were discovered and described by, among others, Boissier, Heldreich, Haussknecht, Halácsy, Pančić and Baldacci.

The first record of the serpentine flora came from Josif Pančić who commenced his studies of the plant world of Serbia in 1846, and already in 1859 published a paper the very first work “On Serpentine Flora of Central Serbia". He cited around 300 species, and for 39 of them he underlined that they were first found on serpentine in Serbia. For these plants he recorded several distinctive morphological features with respect to other conspecifc plants living on adequate substratum, such as a smaller number of flowers and fruits, more slender and less hard, often recumbent stalks, denser and longer hairs. Pancic was obviously speaking of serpentinomorphoses, as they were named later. His special contribution were the species he discovered in the serpentinite areas and described by himself (*Potentilla visianii*, *Eryngium serbicum*, *Potentilla mollis*, *Centaurea melanocephala*) or together with Roberto Visiani (*Scabiosa achaeta*, *Scabiosa fumarioides*, *Haplophyllum boissieranum* (Visiani and Pančić 1866) or by Visiani on the basis of the same Pančić's collections (*Euphorbia glabriflora*).

After Pančić, at the turn of this century, also Lujo Adamović (1909) studied the flora on serpentinite and described the plants related to this substratum in Moesia as: exclusive serpentinophytes, inclined to serpentinite, and possible on serpentinite. Of the 39 species cited by Pančić he considered only 14 related to serpentinite, at different degrees. *Thymus adamovicii* was described from Brđanska Klisura gorge by Velenovský (1906) who named it after Adamović in recognition of the material received. *Helleborus serbicus* was described by Adamović (1906), but not as serpentinophyte, even if it has so far been found only on such substratum.

Interest for these studies continued to increase during the last century and the trend shows no sign of abating as witnessed by the relatively more recent publications of Degen (1897, 1900), Janchen (1920), Jávorka (1926), Markgraf (1932), Novák (1926, 1927, 1928), Košanin (1939), Soška (1939) etc.

Nedeljko Košanin (1914) published a comprehensive study on *Forsythia europaea*, a Tertiary relict endemic to Balkan and its communities are classed as a special type of shiblak. He also discovered *Vola dukadjinica* on serpentinite in Albania. Lately, Stevanović and Niketić (1990) found it on Šar-Planina Mt., also on serpentinite substratum; the species was thought not occurring in Serbia.

The first detailed study of Serbian serpentinite flora was attracted by Czech botanist František Novák. Unfortunately, Novák (1926, 1928, 1929), following the system of plant classification, analyzed only 196 species ending with the representatives of the *Apiaceae* family. He considered serpentinomorphosis as polymorphous structures more or less worthy of taxonomic interpretation and described a series of infraspecific forms, subspecies, varieties and forms as the adaptive result to serpentinite conditions.

Kosta Urošević (1949) and Zagorka Pavlović (1950, 1951, 1953, 1955, 1962, 1962-1964, 1964, 1974) investigated flora and vegetation on serpentinite in W. Serbia, paying attention to the areas still not well investigated. She emphasized relationship between phytogeography and geological substratum and described several pasture associations: *Poeto molineri-Plantaginetum carinatae*, *Festuco-Potentilletum zlatiborensis*, *Koelerio-Danthonietum calycinae* etc. Intensive research in W. Serbia, has been followed by Tatić *et al.* (1981), Jovanović-Dunjić and Jovanović 1987, Jovanović *et al.* (1992), Lakušić and Karadžić (2010), Vićentijević-Marković (2004), Kabaš *et al.* (2013).

A very important centre for forming serpentinite endemics also lies in Kosovo and Metohija, whose flora and vegetation attracted many researchers: Blečić and Krasnići (1972); Blečić and Tatić (1960a; 1960b); Blečić *et al.* (1968, 1969), Martinovský (1971), Rexhepi (1979a, 1979b, 1982, 1984); Ranđelović and Ružić (1983), Tatić (1969), Tatić and Krivošej (1997), Mayer and Greuter (1985), Stevanović and Niketić (1990), Niketić and Diklić (1990), Niketić (1994), Krasniqi and Millaku (2007), Prodanović (2010), Prodanović *et al.* (2010; 2012), Millaku *et al.* (2011). That resulted by descriptions of several local endemics on serpentinites from Kosovo (*Aristolochia merxmuelleri*, *Cerastium neoscardicum*, *Stipa mayeri*, *Tulipa serbica* etc.) as well as by designations of several herbaceous communities.

There are also some reviews, biogeographic and synthetic studies on serpentinite areas in Serbia and Balkan Peninsula: Tatić and Veljović (1990), Vasić and Diklić 2001; Stevanović *et al.* (2003), Jakovljević *et al.* (2011).

Concerning the investigation of hyperaccumulative potential of plants from the territory of Serbia, 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 (Obratov *et al.* 1997; Vinterhalter and Vinterhalter 2005; Vinterhalter *et al.* 2008). Also, some additional surveys were conducted on several Balkan-endemic obligate and facultative serpentinophytes from differerent plant families (Radotić *et al.* 1992; Kadović and Knežević 2003; Obratov-Petković *et al.* 2006; Dudić *et al.* 2007; Licina *et al.* 2010). However there has been previous 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.

The most recent publications (Tumi *et al.* 2012; Tomović *et al.* 2013) provide new data about serpentine soil characteristics as well as Ni and Zn accumulation potential of *A. murale* s.l. and other selected Brassicaceae taxa from several ultramafic localities in Serbia.

1. 7. General characteristics of studied ultramaphite localities in Serbia

The climate characteristics of studied areas were defined by statistical data compiled by Republic Hidrometeorologic Service of Serbia, available at http://www.hidmet.gov.rs/index_eng.php. Data from the closest meteorological stations pertain to the period of last 30 or 12 years. In order to roughly estimate micro- and mesoclimate of the studied localities it was necessary to use maps of average temperatures and sums of precipitation, as available at the website of Republic Agency for Spatial Planning of the Republic of Serbia (http://www.rapp.gov.rs/index.php?jezik=e). (Figure 1 and Figure 2).

Table 1. The climate characteristics of studied ultramafic areas in Serbia

Locality	Sublocality	Latitude (N)	Longitude (E)	Altitude (m)	Temp.	Precip.	Meteorological stations	Temp. m.s.	Precip. m.s.
Mt Maljen	Divčibare	44.09611	19.99639	850	6-8 °C	900-1000 mm	Valjevo (176 m)	11.4°C	788 mm
		44.11083	19.97500	950					
		44.10333	19.98667	950					
Mt Zlatibor	Krivi Potok stream	43.68639	19.70083	1020	6-8 °C	900-1000 mm	Zlatibor (1028 m)	7.7°C	1019 mm
	Prdavac stream	43.73611	19.65806	900					
	Skakavac river	43.74278	19.61167	840					
		43.74278	19.61167	840					
	Trčinoga – Ravna Kosa	43.73139	19.63667	850					
	Čelićko hill – Okolište	43.63250	19.73722	1090					
		43.63250	19.73194	1090					
Požega, Tučkovo	Vrnčanska river gorge	43.91806	20.13639	410	8-10 °C	600-700 mm	Požega (310 m)	9.4 °C	726 mm
		43.91806	20.13639	410					
		43.91806	20.13639	410					
Gornji Milanovac	Brđani gorge	43.99083	20.42083	330	10-12 °C	800-900 mm	G. Milanovac (312 m)	10.0 °C	788 mm
		43.99083	20.42083	330					
		43.99083	20.42083	330					

Kraljevo, Bogutovac	Gornja Lopatnica river gorge	43.68028	20.46278	430	8-10 °C	900-1000 mm	Kaona (570 m)	12 °C	1014 mm
		43.66917	20.44167	440					
		43.68000	20.46806	450					
		43.68000	20.46806	450					
Ibar river valley	Maglič ruins	43.62583	20.55111	300	10-12 °C	800-900 mm	Kraljevo (215 m)	11.5 °C	740 mm
		43.62583	20.55111	300					
	Ušće (Studenica river gorge)	43.46111	20.59806	400	10-12 °C	600-700 mm	Još. Banja (555)	10.2 °C	729 mm
	Raška (Trnavska river gorge)	43.28722	20.60194	420	8-10 °C	600-700 mm	N. Pazar (545 m)	10.6 °C	659 mm
		43.28722	20.60194	420					
Mt Kopaonik	Šiljak	43.26222	20.78583	1550	4-6 °C	900-1000 mm	Kopaonik (1711)	3.6 °C	986 mm
Mt Kopaonik	Paljevštica (Brzećka river gorge)	43.33889	20.94222	680	6-8 °C	700-800 mm	Blaževo (800)	9.1 °C	841 mm
Mt Rogozna	Izbice – Negotinac	43.11278	20.60611	730	6-8 °C	700-800 mm	N. Pazar (545 m)	10.6 °C	659 mm
		43.11278	20.60611	730					
	Pasji stream – Vinogor	43.12361	20.63000	1120					

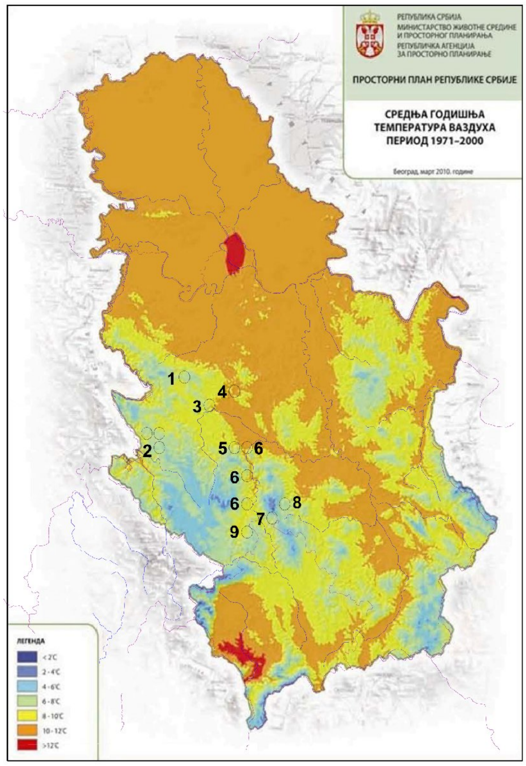

Figure 1. Map of Serbia showing mean annual temperatures and position of locations (1–9) of the studied Brassicaceae plant populations (red circles)

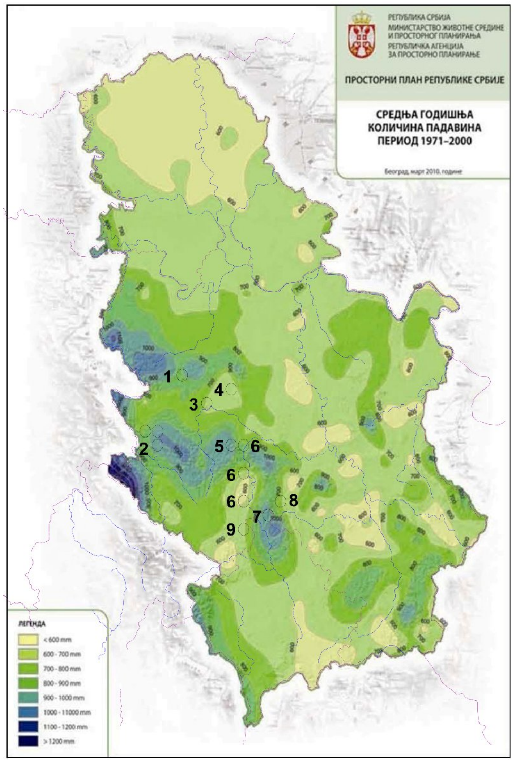

Figure 2. Map of Serbia showing mean annual precipitations and position of locations (1–9) of the studied Brassicaceae plant populations (red circles)

1. Mt Maljen, Divčibare

This locality is characterized by mountain climate with short cool summers and long cold winters. The climate at the foothills of the mountain has temperate-continental character. The mean annual temperature recorded at the closest meteorological station at Valjevo was 11.4 °C, while annual precipitation was 787.7 mm with 146 days with precipitation and 79 days with frost. The greatest amount of precipitation was recorded in July. There is usually a semiarid period in July and August. At the studied locality Divčibare the mean annual temperatures range from 6 to 8 °C, while annual sum of precipitation is 900-1000 mm so the climate is significantly more humid (Table 1).

Tectonically, this area belongs to Inner Dinarides, Ibar Zone and Ibar subzone of roots. The study area belongs to Maljen dislocation in NW-SE direction. It is characterized by a high amount of ophiolite rocks, while presence of Boranja granodiorite indicates continuation of its activity through the younger stages of Alpine orogenesis. Along the Maljen dislocation there was a movement of Triassic and Paleozoic masses of Jadar Zone that later overlaid the Ibar subzone of roots (Anđelković 1982). The serpentinized harzburgites recorded at the studied localities represent the W fringe of the large Bukovica-Maljen peridotite massif. The process of serpentinization is most pronounced at the edges and along the dislocations within the massif proper. The peridotite rocks are present along the whole area, in tectonic relationship with Upper Cretaceous, Triassic and Paleozoic formations. In area of Divčibare there are also some ultrabasic troclotites, reaching thickness of 2 m and penetrating through the harzburgites (Mojsilović *et al.* 1975; Filipović *et al.* 1978).

Following types of deciduous forests were cited as climatogeneous for Maljen area: *Fagetum montanum*, *Quercetum petraeae*, *Querco-Carpinetum*, *Quercetum cerris moesiacum* (Jovanović *et al.* 1986). The denuded serpentinite terrain is dominated by pine stands (*Erico-Pinetum gocensis*, *Seslerio serbicae-Pinetum gocensis*, *Pinetum sylvestris-nigrae*, *Erico-Pinetum sylvestris*) as well as by stands of Balkan pak (*Ostryo carpinifoliae-Quercetum dalechampii*) (Škorić and Vasić 2006). Following herbaceous associations appear in more humid areas: *Euphorbieto (cyparissias)-Brachypodietum pinnati*, *Nardetum strictae*, *Molinia caerulaea-Deschampsia caespitosa* and *Cynosuretum cristati.* Other phytocenoses appear in sunlit serpentinite limestones: *Koelerio-Danthonietum alpinae*, *Koelerietum montanae* and *Poa alpina-Plantago carinata* (Lakušić *et al.* 2005).

2. Mt Zlatibor

This locality (Appendix 1, Figure A) is characterized by a more or less humid mountain climate, with short cool summers and long cold winters, but there are also certain submediterranean influences, so the minimal recorded temperature of -19.8 °C is quite high for mountain areas. Climate at the foothills of this mountain has temperate-continental character. The mean annual temperature recorded at Zlatibor was 7.7 °C, with annual sum of precipitation of 1019 mm and 171 days with precipitation. The greatest amount of precipitation was recorded in June. There is generally no arid period in summer. At the studied localities the mean annual temperatures range from 6 to 8 °C, while annual sum of precipitation is 900-1019 mm (Table 1).

Tectonically, this area belongs to Inner Dinarides, Ibar Zone and so-called subzone Ibar meso-nappe, which became separated from the Ibar subzone of roots and overlaid far toward SW and W over the Drina and Lim Zones of Inner Dinarides as well as over the Central Dinarides, while in the SE part it is also overlaid over the Helenides and Outer Dinarides in Albania. The Ibar meso-nappe represents the most prominent nappe in Balkan Peninsula, from Karlovac in Croatia at the NW to the Mirdita zone in Albania at the SW. The study area is situated at Zlatibor nappe between rivers Đetina at the N and Lim at the S. There are two separate parts: the lower part consists of an igneous-sedimentary series (limestones and dolomites of Upper Triassic or metamorphous rocks of diabase-chert formation) while the upper part belonging to ophiolitic series. The ophiolites are overlaid over the igneous-sedimentary series, which is the lower nappe over the Paleozoic and Triassic layers of Drina and Lim zones (Anđelković 1982). The peridotite massif of Zlatibor mostly has harzburgite composition. The lherzolites occupy smaller areas while dunites are quite rare. The intensity of serpentinization may vary. Complete serpentinization was recorded only in the fringe areas of the massif, where harzburgite and dunite serpentinites appear in tectonized zones, as well as numerous lines of magnesite. Harzburgites were recorded in most studied localities, except at Čelićko hill – Okolište where serpentinites appear (Mojsilović *et al.* 1978).

According to the map of potential vegetation, following forest phytocenoses are climatogeneous for Zlatibor: *Helleboro odori-Fagetum moesiacae*, *Luzulo albidae-Fagetum*, *Abieti-Fagetum moesiacae*, *Quercetum petraeae*, *Ostryo carpinifoliae-Quercetum* and *Oxali acetosellae-Piceetum abietis* (Jovanović *et al.* 1986). At the serpentinite massif of Zlatibor the greatest area is occupied by mixed forests of Black and Scots Pine (*Pinetum sylvestris-nigrae*), but there are also other types of pine forests (*Seslerio serbicae-Pinetum gocensis*, *Erico-Pinetum sylvestris*) and mixed forests of Balkan oak and Black Pine *Asplenio cuneifoliae-Quercetum dalechampii* subsp. subass. *pinetosum gocensis* (Škorić and Vasić 2006). The rocky pastures are dominated by grass phytocenoses (*Festuca fallax-Dianthus deltoides*, *Festuco sulcatae-Potentilletum zlatiborensis*, *Festuco rupicolae-Euphorbietum glabriflorae*), while highly eroded places are inhabited by *Poo molinieri-Plantaginetum holostei* and *Halascya sentneri-Potentilla mollis* (Lakušić *et al.* 2005).

3. Požega, Tučkovo, Vrnčanska River Gorge

This locality is characterized by temperate-continental to continental climate with warm summers and cold winters, with four well-pronounced seasons of the year. The closest meteorological station is Požega. The mean annual temperature recorded at Požega was 9.7 °C, with annual sum of precipitation of 726.4 mm and 148 days with precipitation. Due to presence of large amount of flowing water, Požeška Kotlina Valley is characterized by the greatest number of recorded fog days in Serbia. Due to high levels of fog and water Požega is one of the coldest lowland areas in Serbia in winter (after Sjenica), with the mean January temperature of -1.6 °C and minimal recorded temperature of -30.7 °C. On the other hand summer temperatures are quite high, and the recorded temperature maximum was 41 °C. The greatest amount of precipitation was recorded in July. There is generally a semiarid period in August, leading to the name of toponym Požega. Due to the orography, climate in the studied locality in the gorge of Vrnčanska river is significantly more temperate and humid. The mean annual temperature values range from 6 to 8 °C, while the annual sum of precipitation is 800-900 mm (Table 1).

Tectonically, this area belongs to Inner Dinarides, Ibar Zone and Ibar subzone of roots. The internal structure of this subzone is very complex and characterized by presence of deep cracks and numerous faults. In the northern part there is a characteristic nappe of ophiolitic-radiolarian complex over the Paleozoic and Triassic layers of Drina zone (and Jadar subzone). The studied area essentially represents the SE fringe of nappe of Maljen and Suvobor over the Triassic limestones of Ovčar and Kablar, belonging to an isolated klippe of the Jadar subzone (Anđelković 1982). At the studied localities it matches the harzburgite serpentinites with extremely rare occurrences of somewhat younger rocks or relicts of primary materials (olivine and rhombic pyroxene). The process of serpentinization is mostly intensive. Approximately 50% of the total amount of olivine was metamorphosed into serpentinite, so with the increase in intensity of this process the harzburgites were gradually connected to serpentinites (Filipović *et al.* 1978).

The climatogeneous forest of this area is *Quercetum frainetto-cerris* (Jovanović *et al.* 1986). On serpentinite there are some associations dominated by Downy Oak (*Quercus pubescens*) and Oriental Hornbeam (*Carpinus orientalis*), while associations of Smoke Tree (*Cotinus coggygria*) are common in more rocky areas.

4. Gornji Milanovac, Brđani River Gorge

This locality (Appendix 1, Figure D) is characterized by temperate-continental climate, with moderately warm summers and moderately cold winters. All four seasons of the year are well-developed. The closest meteorological station is Gornji Milanovac. The average recorded annual temperature for Gornji Milanovac was 10.0 °C while the annual sum of precipitation is 788 mm. July is the warmest month while January is coldest. Autumn is warmer than spring, in average by 1 °C. The absolute temperature maximum was 38.8 °C, while the absolute minimum was -30.5 °C. The highest levels of precipitation were recorded in period April-July, while lowest levels were recorded in period October-February. The month with the highest level of precipitation is June. There is usually a semiarid period in August. Winds are not prominent in this area. At the studied locality at Brđani Gorge climate is more temperate and warmer due to its orography. The mean annual temperature values range from 10 to 12 °C, while the annual sum of precipitation is 900-1000 mm (Table 1).

Tectonically, this area belongs to Inner Dinarides, Ibar Zone and Ibar subzone of roots, leading from Drina at NW through Sokolske Planine, Povlen, Maljen, Ovčar, Kablar, Dragačevo, valley of R. Ibar, to Uroševac, Kačanik and Veles in Macedonia, all the way to N Greece in S. The whole subzone has a W-SW direction, with a nappe over the Drina zone and partially over the Lim zone. The studied area belongs to the Maljen dislocation in NW-SE direction, characterized by a large mass of ophiolitic rocks. In the area southward from Gornji Milanovac there is nappe of serpentinite from this dislocation toward E, over the Upper Cretaceous flysch of Rudnik zone of Šumadides (Anđelković 1982). The geological substrate of the studied localities matches the harzburgite serpentinites with extremely rare occurrences of somewhat younger rocks or relicts of primary materials (olivine and rhombic pyroxene). They contain serpentine (partly as bastite) and accessory chromite, as well as iron oxide powder, a small amount of talcum and some secondary amphibole and thin lines of asbestos (Brković *et al.* 1968).

The climatogeneous forest of this area is *Quercetum frainetto-cerris* (Jovanović *et al.* 1986). However, on serpentinite there are some associations dominated by Downy Oak (*Quercus pubescens*) and Oriental Hornbeam (*Carpinus orientalis*), while associations of Smoke Tree (*Cotinus coggygria*) are common in more rocky areas. The dry stony pastures are characterized by association *Bromus fibrosus-Chrysopogon gryllus* (Lakušić *et al.* 2005). There are also smaller areas with a specific association where the edificatory species is endemic *Halacsya sendtneri*.

5. Kraljevo, Bogutovac, Gornja Lopatnica River Gorge

This locality (Appendix 1, Figure E) is characterized by temperate-continental climate, with moderately warm summers and moderately cold winters. All four seasons of the year are well-developed. The closest meteorological station is Kaona. The average recorded annual temperature for Kaona was 12 °C while the annual sum of precipitation is 1014.2 mm. July is the warmest month while January is coldest, although the average January temperature is 1.5 °C. The average precipitation levels exceed 20 mm in all months and they are highest in July (40 mm). Summers are humid and warm, mostly without any semiarid period. As winds are not prominent in this area, climate is of humid valley type. At the studied locality in gorge of Lopatnica climate is cooler and slightly less humid, due to orography and vicinity of Mt. Troglav. The mean annual temperature values range from 8 to 10 °C, while the annual sum of precipitation is 900-1000 mm (Table 1).

Tectonically, this area belongs to Inner Dinarides, Ibar Zone and subzone of Ibar sinclonitorium, leading along the Ibar Valley from Kraljevo at N to Trepča at S. The studied area belongs to nappe of Stolovi and Troglav and is situated at the foothills of Troglav. This nappe is mostly composed of ophiolitic-radiolarian complex which is overlaying the Paleozoic rocks of Radočelo anticline at SW, while both of them lay over the Drina Zone. At the W this nappe is overlaid over the Upper Cretaceous sediments, while at the S it continues into the W Kopaonik, laying over the Kopaonik ultramafite (Anđelković 1982). In the studied localities most of these rocks match the harzburgite type (about 75%), then lherzolite (about 15%) and dunite (about 10%), while pyroxenites were represented with up to 1% (Marković *et al.* 1963; Urošević *et al.* 1973).

The main type of forest vegetation includes *Quercetum frainetto-cerris* and *Asplenio cuneifoliae-Quercetum dalechampii* (=*Quercetum montanum*) (Jovanović *et al.* 1986). However, the bare stony slopes are dominated by stands of Black Pine and Balkan oak with Hop Hornbeam: *Potentillo heptaphyllae-Pinetum gocensis, Erico-Pinetum gocensis*, *Querco dalechampii-Pinetum gocensis*, *Ostryo carpinifoliae-Quercetum dalechampii* (Škorić and Vasić 2006). The sunlit stony slopes are dominated by associations of grass species (*Bromus fibrosus-Chrysopogon gryllus*, *Eryngio-Brometum fibrosi*, *Koelerio-Danthonietum alpinae*), while phytocenosis *Poo molinieri-Plantaginetum holostei* (Lakušić *et al.* 2005) is present in bare areas of loose rocky ground.

6. Ibar River Valley

This locality is characterized by temperate-continental climate, with moderately warm summers and moderately cold winters. All four seasons of the year are well-developed, but there are also some submediterranean influences. The valley has lateral gorges and spreads in N-S direction, including the three studied localities: Maglič Ruins, Ušće (Studenica River Gorge) and Raška (Trnavska River Gorge). The closest meteorological stations are Kraljevo, Jošanička Banja and Novi Pazar. The mean annual temperatures range from 10.2 °C to 11.5 °C, while average temperatures increase in direction of the river flow, from S toward N. The annual sum of precipitation varies from 659 mm in S to 740 mm in N. The warmest months are July and August, while January is the coldest month. The highest amount of precipitation was recorded in May and June, while in the northern part values for July are also significant. Winds are not prominent in this area, so the climate is of warm valley (župa) type. At the studied locality Maglič Ruins, the mean annual temperature values range from 10 to 12 °C, while the annual sum of precipitation is 800-900 mm. There is a semiarid period in August. At the studied locality Ušće (Studenica River Gorge), the mean annual temperature values range from 10 to 12 °C, while the annual sum of precipitation is 600-700 mm. There is a semiarid or arid period in August. At the studied locality Raška (Trnavska River Gorge), the mean annual temperature values range from 8 to 10 °C, while the annual sum of precipitation is 600-700 mm. There is a semiarid period in July and an arid period in August (Table 1).

Tectonically, this area belongs to Inner Dinarides, to the Ibar Zone which is the least stable part of Inner Dinarides. Most of this area belongs to the Ibar syncline that stretches westwards from Kraljevo, through Ibar Valley, across Jarandol and Lešak to Stari Trg. The Ibar syncline is composed of serpentinite-peridotite rocks, igneous-sedimentary series and Upper Jurassic limestones, overlaid by formations from Upper Cretaceous, Tertiary molasses and a thick series of tertiary vulcanite. The Ibar syncline is broken by faults. Only at the extreme S (Raška, Trnavska Reka) there is a nappe of Rogozna over the Upper Cretaceous formations, while in deeper parts it is also over the Paleozoic of Drina Zone (Anđelković 1982). Harzburgites from Jurassic were recorded in the studied localities. They belong to the ophiolite series of ophiolitic-radiolarian complex (Marković *et al.* 1963; Urošević *et al.* 1973; Urošević *et al.* 1973a).

The climatogeneous forest of this area is phytocenosis of Hungarian Oak-Turkey Oak forest, *Quercetum frainetto-cerris* (Jovanović *et al.* 1986). Due to the pronounced Mediterranean impact and eroded serpentinite terrain this area has stands of *Carpino orientalis-Quercetum frainetto-cerris* (in vicinity of Ušće), *Ostryo carpinifoliae-Quercetum.* In the stony areas there is also lilac scrub (‘shibljak’) *Cotino coggygriae-Syringetum vulgaris*. Vegetation of rocky ground is represented by the association *Koelerio-Danthonietum alpinae* (Lakušić *et al.* 2005).

7. Mt Kopaonik, Šiljak

This locality (Appendix 1, Figure B) is characterized by more or less humid mountain climate, with short cool summers and long cold winters. At the foothills climate is temperate-continental. The mean annual temperature recorded at Kopaonik was 3.6 °C while the annual sum of precipitation is 985.5 mm, with 174 days with precipitation. July and August are the warmest months while January is the coldest month. The highest amount of precipitation was recorded in May, followed by June. There is no arid period in the summer. At the studied locality Šiljak the mean annual temperature values range from 4 to 6 °C, while the annual sum of precipitation is 900-1000 mm (Table 1).

Tectonically this area belongs to Inner Dinarides, to the Ibar Zone which is the least stable part of Inner Dinarides. Most of this area belongs to the Kopaonik syncline that stretches westwards from Goč at the N through the central Kopaonik (Suvo Rudište) and further toward SE. It is composed of Paleozoic rocks, metamorphosed formations of Triassic, peridotites and serpentinites, volcanogenic-sedimentary series and Kopaonik granodiorite and andesite-dacite rocks with Upper Cretaceous formations. The Kopaonik anticline is continuous with the anticline of Željin and Goč at the N. The western arm of Kopaonik anticline forms the eastern arm of Ibar syncline, while the eastern arm is mostly deformed along the Kopaonik dislocation (Anđelković 1982). Harzburgites from Jurassic were recorded in the studied localities. They belong to the ophiolite series of ophiolitic-radiolarian complex (Urošević *et al.* 1973a).

In the subalpine zone of Mt. Kopaonik the dominant associations are forests of beech, fir and spruce: *Abieti-Fagetum*, *Piceetum excelsae*, *Helleboro odori-Fagenion moesiacae* and *Asplenio cuneifoliae-Quercetum dalechampii* (Jovanović *et al.* 1986). In vicinity of serpentinite peak Šiljak there are also some forests of subalpine beech, *Fagetum longipedunculatae* (=*Fagetum subalpinum serbicum*), as well as heaths with blueberries and juniper: *Vaccinio-Juniperetum sibiricae*, *Vaccinietum uliginosi* and *Vaccinietum myrtillii*. The massif of the peak Šiljak shows an extreme case of lack of vegetation, with following recorded associations of rocky and stony ground: *Luzulo-Seslerieteum rigidae*, *Sileneto serbicae-Stipetum pulcherrimae*, *Koelerietum montanae*, *Deschampsietum subalpinum*, *Brometum erecti* and *Carici humilis-Festucetum pancicianae* (Lakušić *et al.* 2005).

8. Mt Kopaonik, Paljevštica (Brzećka River Gorge)

This locality is characterized by temperate-continental climate, with mild summers and mild winters. All four seasons are well-pronounced. The closest meteorological station is Blaževo. The mean annual temperature recorded at Blaževo was 9.1 °C while the annual sum of precipitation is 840.8 mm. July is the warmest month while January is the coldest month. The highest amount of precipitation was recorded in May, followed by June. There is usually a semiarid period in August. At the studied locality Paljevštica the climate is somewhat cooler and drier. The mean annual temperature values range from 6 to 8 °C, while the annual sum of precipitation is 700-800 mm (Table 1).

Tectonically, this area belongs to Šumadides, including the terrains of SW Romania, C and S Serbia, C Macedonia and Greece. Within this tectonic unit, the studied locality is situated in the zone of Kopaonik block. This area is situated between the Zapadna Morava at N and Kosovo basin at S. The W boundary is the Kopaonik dislocation, stretching along the eastern slopes of Kopaonik and separating the Inner Dinarides from the Šumadides. The E boundary is the Morava-Šumadija dislocation which separates this block from the Moravides at the E. Along this block there are rocks belonging to ophiolitic-radiolarian complex with a nappe in direction of NE (Anđelković 1982). Harzburgites from Jurassic were recorded in the studied localities. They belong to the ophiolite series of ophiolitic-radiolarian complex (Urošević *et al.* 1973).

The primary associations at foothills of Kopaonik are forests of Hungarian and Turkey Oaks, *Quercetum frainetto-cerris*, as well as forests of Sessile and Turkey Oaks, *Quercetum petraeae-cerridis* (Jovanović *et al.* 1986). At the serpentinites there are also some associations with Black Pine, *Erico-Pinetum gocensis*, while deciduous forests also include *Ostryo-Fagetum moesiacae (=Seslerio rigidae-Fagetum)* and *Luzulo-Fagetum moesiacae*. At the denuded serpentinite rocky ground there is an association of Black Pine and spurge *Erico-Pinetum gocensis* (= *Euphorbio glabriflorae-Pinetum*) (Lakušić *et al.* 2005).

9. Mt Rogozna

This locality (Appendix 1, Figure C) is characterized by temperate-continental and mountain climate, with mild summers and mild winters. All four seasons are well-pronounced and there is a partial submediterranean influence. The closest meteorological station is Novi Pazar. The mean annual temperature recorded at Novi Pazar was 10.6 °C while the annual sum of precipitation is 659.1 mm. July and August are the warmest months while January is the coldest month. The highest amount of precipitation was recorded in May and June. There is a semiarid period in July and an arid period in August. The winds are not prominent at this locality. At the studied locality Mt Rogozna the climate is cooler and more humid due to orography. The mean annual temperature values range from 6 to 8 °C, while the annual sum of precipitation is 700-800 mm, representing a very low value for a mountain area in Serbia (Table 1).

Tectonically this area belongs to Inner Dinarides, to the Ibar Zone that represents the least stable part of Inner Dinarides. The Ibar Zone is composed by formations of lower and upper Paleozoic, Triassic, Jurassic, Upper and Lower Cretaceous and Paleogene. Southward from Raška, the anticline of Radočelo continues into the anticline of Berberište which has NW-SE direction. Nappe of Rogozna stretches from Raška at N to Kosovska Mitrovica at S. The formations of nappe of Rogozna are overlaid over the Upper Cretaceous formations throughout the area, while in deeper parts they also cover the Paleozoic of Drina Zone (Anđelković 1982). In the immediate vicinity there are recorded amphibole peridotites composed of serpentine, amphibole, olivine, accessory monoclinal pyroxene and chromite (Urošević *et al.* 1973a).

The main type of forest vegetation in Rogozna area includes deciduous and mixed forests: *Abieti-Fagetum moesiacae*, *Helleboro odori-Fagetum moesiacae* (= *Fagetum montanum*) and *Quercetum petraeae-cerridis* (Jovanović *et al.* 1986). Pines are also present at higher altitudes. Following associations were recorded at eroded serpentinite rocky ground: *Eryngio-Brometum fibrosi*, *Sedo-Dianthetum serbici*, *Potentillo-Fumanetum bonaparti* and *Halascya sentneri-Potentilla mollis* (Lakušić *et al.* 2005).

1. 8. Characteristics of the studied taxa

Brassicaceae Burnett (**Cruciferae**)

Herbs annual, biennial, or perennial, sometimes subshrubs or shrubs, with a pungent, watery juice. Eglandular trichomes unicellular, simple, stalked or sessile, 2- to many forked, stellate, dendritic, or malpighiaceous (medifixed, bifid, appressed), rarely peltate and scalelike; glandular trichomes multicellular, with uniseriate or multiseriate stalk. Stems erect, ascending, or prostrate, sometimes absent. Leaves exstipulate, simple, entire or variously pinnately dissected, rarely trifoliolate or pinnately, palmately, or bipinnately compound; basal leaf rosette present or absent; cauline leaves almost always alternate, rarely opposite or whorled, petiolate or sessile, sometimes absent. Inflorescence bracteate or ebracteate racemes, corymbs, or panicles, sometimes flowers solitary on long pedicels originating from axils of rosette leaves. Flowers hypogynous, mostly actinomorphic. Sepals 4, in 2 decussate pairs, free or rarely united, not saccate or lateral (inner) pair saccate. Petals 4, alternate with sepals, arranged in the form of a cross (cruciform; hence the earlier family name Cruciferae), rarely rudimentary or absent. Stamens 6, in 2 whorls, tetradynamous (lateral (outer) pair shorter than median (inner) 2 pairs), rarely equal or in 3 pairs of unequal length, sometimes stamens 2 or 4, very rarely 8-24; filaments slender, winged, or appendaged, median pairs free or rarely united; anthers dithecal, dehiscing by longitudinal slits. Pollen grains 3-colpate, trinucleate. Nectar glands receptacular, highly diversified in number, shape, size, and disposition around base of filaments, always present opposite bases of lateral filaments, median glands present or absent. Pistil 2-carpelled; ovary superior, sessile or borne on a distinct gynophore, mostly 2-locular and with a false septum connecting 2 placentae; placentation parietal, rarely apical; ovules anatropous or campylotropous, bitegmic, 1 to many per locule. Fruit typically a 2-valved capsule, generally termed silique (siliqua) when length 3 × or more than width, or silicle (silicula) when length less than 3 × width, dehiscent or indehiscent, sometimes schizocarpic, nutletlike, lomentaceous, or samaroid, segmented or not, terete, angled, or flattened parallel to septum (latiseptate) or at a right angle to septum (angustiseptate); valves 2(or 3-6); replum (persistent placenta) rounded, rarely flattened or winged; septum complete, perforated, reduced to a rim, or lacking; style 1, distinct, obsolete, or absent; stigma capitate or conical, entire or 2-lobed, sometimes lobes decurrent and free or connate. Seeds without endosperm, uniseriately or biseriately arranged in each locule, aseriate when 1, winged or wingless, mucilaginous or not when wetted; cotyledons incumbent (embryo notorrhizal: radicle lying along back of 1 cotyledon), accumbent (embryo pleurorrhizal: radicle applied to margins of both cotyledons), or conduplicate (embryo orthoplocal: cotyledons folded longitudinally around radicle), rarely spirally coiled (embryo spirolobal). Germination epigeal.

About 330 genera and 3500 species: all continents except Antarctica, mainly in temperate areas, with highest diversity in Irano-Turanian, Mediterranean, and W North American regions; 102 genera (eight endemic) and 412 species (115 endemic) in China.

The Brassicaceae include many important crop plants that are grown as vegetables (*Brassica, Nasturtium, Raphanus*) and sources of vegetable oils (*Brassica*) and condiments (*Armoracia, Brassica, Eutrema, Sinapis*). Oils of *Brassica* probably rank first in terms of tonnage of the world's production of edible oils. The family includes many ornamentals in the genera *Erysimum, Iberis* Linnaeus, *Lobularia, Malcolmia*, and *Matthiola*. The family also includes more than 120 species of weeds.

The delimitation of genera in the Brassicaceae is often difficult because of the frequent independent evolution of what appear to be similar character states, the variability of a given character in one genus and its fixture in another, and the inadequate sampling of material by most authors. Fruit characters are essential in the identification of genera. However, a key emphasizing flowering material is given in addition to the one emphasizing fruit. The most reliable determination of genera can be achieved when the material has both fruit and flowers and when both keys are successfully used to reach the same genus.

Erysimum L., Sp. Pl. 2: 660. 1753.

Herbs annual, biennial, or perennial, rarely subshrubs or shrubs. Trichomes sessile, medifixed, appressed, malpighiaceous or 3-5(-8)-rayed stellate. Stems simple or branched basally and/or apically. Basal leaves petiolate, rosulate, simple, entire or dentate, rarely pinnatifid or pinnatisect. Cauline leaves petiolate or sessile, cuneate or attenuate at base, rarely auriculate, entire or dentate. Racemes ebracteate or basally bracteate, rarely bracteate throughout, corymbose, elongated or not in fruit. Fruiting pedicels slender or thickened and nearly as wide as fruit, erect, ascending, divaricate, or reflexed. Sepals oblong or linear, erect, pubescent, base of lateral pair saccate or not. Petals yellow or orange, rarely white, pink, purple, or violet; blade suborbicular, obovate, spatulate, or oblong, apex rounded or emarginate; claw differentiated from blade, subequaling or longer than sepals. Stamens 6, erect, tetradynamous; anthers oblong or linear. Nectar glands 1, 2, or 4, distinct or confluent and subtending bases of all stamens; median glands present or absent. Ovules 15-100 per ovary. Fruit dehiscent siliques or rarely silicles, linear or rarely oblong, terete, 4-angled, latiseptate, or angustiseptate, sessile or rarely shortly stipitate; valves with an obscure to prominent midvein, pubescent on outside, rarely also on inside, keeled or not, smooth or torulose; replum rounded; septum complete, membranous, translucent or opaque, veinless; style obsolete or short, rarely half as long as or subequaling fruit, often pubescent; stigma capitate, entire or 2-lobed. Seeds uniseriate or rarely biseriate, winged, margined, or wingless, oblong, plump or flattened; seed coat minutely reticulate, mucilaginous when wetted; cotyledons incumbent or rarely accumbent. About 150 species: N hemisphere, primarily in Asia and Europe, with 14 species in Central and North America and eight species in N Africa and Macaronesia (Taiyan *et al.* 2001).

Erysimum linariifolium Tausch, *Flora* (*Regensb.*) **14**: 212 (1831) (*E. linearifolium* auct., non Moench). Perennial with branched, woody stock bearing long, procumbent leafy shoots; stems 10-40 cm in flower, up to 45 cm in fruit; hairs 2(3)-fid. Leaves 20-60 × (0·5-)2·5-5 mm, linear to oblanceolate, entire to sinuate-dentate. Pedicels 2-4 mm in flower, up to 7 mm in fruit. Sepals 6-9 mm; petals 12-18 × 4-5 mm, yellow, glabrous or sparsely pubescent on the back. Siliqua 40-80 × 1-1·5 mm, erecto-patent (30-50°), grey, sometimes with glabrescent angles; style 0·5-2 mm. Seeds 1·5-2 mm. $2n = 14$. Not always clearly distinct from *E. pusillum* Bory & Chaub**.** in the southern part of its range. *Limestone slopes, cliffs and rocks. Mountains of W. part of Balkan peninsula.* Al Ju (BH, Cg, Ct, Gr, Sr) (Ball 1993) (Appendix 2, Figure A).

Erysimum carniolicum Dolliner, *Flora* (*Regensb.*) **10:** 254 (1827) (*E. pannonicum* var. *sinuatum* (Neilr.) Janchen). Biennial; stems 10-60 cm; hairs bi- or trifid. Leaves 20-50 × 4-12 mm, linear-oblong to oblong, pectinate-pinnatifid to sinuate-dentate, the basal usually persistent, the lower and middle leaves sessile or very shortly petiolate. Pedicels 3-8 mm in flower, up to 11 mm in fruit. Sepals 8·5-12 mm; petals 16-24 × 5-10 mm, yellow, pubescent on the back. Siliqua 60-110 × c. 1·5 mm, erecto-patent (20-30°), sparsely pubescent; style 0·5-1 mm. 2n = 32. W. *Jugoslavia, S. E. Austria.* Au Ju (BH, Cg, Ct, Sl. Sr) (Ball 1993).

Thlaspi L., Sp. Pl. 2: 645. 1753.

Herbs annual, biennial, or perennial, often glabrous and glaucous. Trichomes absent or simple. Stems prostrate or erect, simple or branched. Basal leaves petiolate or subsessile, rosulate or not, simple, entire or dentate. Cauline leaves sessile, often auriculate, sagittate, or amplexicaul at base, entire or rarely dentate. Racemes ebracteate, elongated or not in fruit. Fruiting pedicels slender, divaricate or ascending, rarely reflexed. Sepals ovate or oblong, erect or ascending, base of lateral pair not saccate, margin membranous. Petals white, rarely purple or yellow; blade obovate, oblong, or spatulate, apex obtuse or emarginate; claw differentiated or not from blade. Stamens 6, tetradynamous; filaments dilated or not at base; anthers ovate or oblong, obtuse at apex. Nectar glands 2 or 4, lateral, often 1 on each side of lateral stamen; median glands absent. Ovules 4-24 per ovary. Fruit dehiscent siliques or silicles, linear, oblong, obovate, obcordate, elliptic, lanceolate, or suborbicular, often apically notched, strongly angustiseptate, sessile; valves keeled, winged or wingless; replum rounded; septum complete, membranous, translucent, veinless; style obsolete or prominent, exserted or included in apical notch of fruit; stigma capitate, entire or slightly 2-lobed. Seeds uniseriate, wingless, oblong, ovoid, or ellipsoid, plump; seed coat smooth, reticulate, rugose, or striate, mucilaginous or not when wetted; cotyledons accumbent or rarely incumbent.About 75 species: temperate Eurasia, especially SW Asia and C and S Europe. *Thlaspi* was divided by Meyer (Feddes Repert. 84: 449-470. 1970) into 12 genera largely based on seed anatomy. Molecular data provide some support for the recognition of some of Meyer's segregates (e.g., *Microthlaspi* F. K. Meyer and *Noccaea* Moench), but such studies are not comprehensive and therefore the traditional broader concept of the genus is tentatively retained in this treatment (Shu, X. M. 2001).

Thlaspi kovatsii Heuffel, *Flora* (*Regensb.*) **36**: 624 (1853) (*T. avalanum* Pančić, *T. affine* Schott & Kotschy, *T. trojagense* Zapal.). Stock with stoloniferous branches; stems 8-25(-60) cm, erect. Rosette-leaves broadly elliptical to suborbicular, long-petiolate; cauline leaves ovate-oblong, obtuse, with obtuse auricles. Inflorescence much elongating in fruit. Sepals 2-2·5 mm; petals (3·5-)4-5 mm, white. Silicula 5-7 mm, narrowly obovate to triangular-obcordate, emarginate above; style 1-2 mm, exceeding the notch. Seeds 3-8 in each loculus. 2n = 14. *Carpathians; Balkan peninsula*. Al BH Bu ?Cg Gr Hu Mk Rm Sr Uk(U) [Cs] (Clapham and Akeroyd 1993) (Appendix 2, Figure D).

Thlaspi praecox Wulfen in Jacq., Collect. Bot. 2: 124 (1789) (*T. affine* sensu Boiss., non Schott & Kotschy). Stems (5-)10-20(-35) cm, erect, glabrous, more or less glaucous. Rosette-leaves oblong to broadly ovate, petiolate, often violet beneath; cauline leaves ovate-oblong, amplexicaul with obtuse auricles; all entire or sinuate-denticulate, coriaceous. Inflorescence much elongating in fruit. Sepals 2-3 mm, violet-tipped; petals 5-7 mm, narrow, white. Silicula usually 7-9 mm, narrowly obcordate; angle at base of notch not more than 90°; style (1-)2-3.5 mm, always exceeding the notch at least slightly. Seeds 2-4 in each loculus. Stony, shady slopes and dry grassland. S. Europe,from S. E. France to Krym. Al Au Bu Ga Gr It Ju Rs(K) Tu (Clapham and Akeroyd 1993).

Alyssum L., Sp. Pl. 2: 650. 1753.

Herbs annual, biennial, perennial, or rarely subshrubs. Trichomes stellate, stalked or sessile, with 2-6 minute basal branches from which originate up to 30, simple or branched rays, sometimes trichomes lepidote, rarely mixed with simple and forked. Stems erect or decumbent, simple or branched. Basal leaves petiolate or sessile, rosulate or not rosulate, simple, entire. Cauline leaves petiolate or sessile, cuneate or attenuate, not auriculate, entire. Racemes few to many flowered, dense or lax, ebracteate, corymbose or in panicles, elongated or not in fruit. Fruiting pedicels ascending, divaricate, or reflexed. Sepals ovate or oblong, base of lateral pair not saccate. Petals yellow, white, or rarely pink; blade suborbicular, obovate, or spatulate, apex obtuse or emarginate, glabrous or pubescent outside. Stamens 6, tetradynamous; filaments wingless or uni- or bilaterally winged, appendaged or not, toothed or toothless; anthers ovate or oblong, apiculate or not at apex. Nectar glands 4, lateral, 1 on each side of lateral stamen; median glands absent. Ovules 1 or 2(or 4-8) per ovary; placentation apical or parietal. Fruit dehiscent silicles, oblong, ovate, obovate, elliptic, obcordate, or rarely globose, strongly latiseptate or rarely inflated, sessile; valves veinless, pubescent or glabrous, smooth; replum rounded; septum complete, membranous, translucent, veinless; style distinct; stigma capitate, entire. Seeds biseriate, winged or wingless, orbicular or ovate, flattened; seed coat smooth or minutely reticulate, mucilaginous or not when wetted; cotyledons accumbent or incumbent. About 170 species: primarily in SW Asia and SE Europe (Shu, T. Q. 2001 2001).

Alyssum murale Waldst. & Kit., *Pl. Rar. Hung.* **1**: 5 (1799) (*A. argenteum* auct. balcan., *A. argenteum* sensu E. I. Nyárády, non All., *A. decipiens* E. I. Nyárády, *A. gracile* Form., *A. orphanides* Janka ex E. I. Nyárády, *A. punctatum* E. I. Nyárády). Caespitose perennial 25-70 cm, with long non-flowering stems or dense leaf-rosettes. Basal leaves obovate- or oblanceolate-spathulate; cauline leaves usually 10-20 × 3-6 mm, lanceolate or oblanceolate, grey-green above, white or grey beneath, larger then the basal leaves. Petals 2-3·5 mm, entire, rarely emarginate. Silicula 2·5-5 × 1·5-4 mm, obtuse to emarginate, pubescent, the hairs with 6-10(-13) rays; valves flat, but often undulate; style 0·5-2 mm. Seeds c. 3 mm; wing 0·2-0·8 mm wide. $2n = 30$. *S. E. Europe*. AE Al Ar BH Bu Cg -Cr Ct -Cy Gr Mk Mo Rm Sr Tu(A) ?Tu(E) Uk(K U) [Cs Ge] (Ball and Dudley 1993) (Appendix 2, Figure E).

Alyssum montanum L., *Sp. Pl.* 650 (1753) (incl. *A. thessalum* Halácsy, *A. vourinonense* T. R. Dudley & Rech. fil.). Procumbent to erect, green to almost white perennial 5-25 cm, with non-flowering rosettes or short stems. Basal leaves oblong or oblanceolate-spathulate, the upper linear or linear-spathulate; hairs 0·4-0·7 mm in diameter, 6- to 24-rayed. Raceme elongate in fruit; pedicels 4-6 mm, patent or erecto-patent. Sepals 2·5-3·5 mm; petals emarginate. Silicula 3-5·5 mm; valves inflated, with flattened margin. Seeds 1·5-2 mm; wing 0·2-0·4 mm wide. *Most of Europe except the north, much of the west and the islands*. Al Au(A) Be(B) BH Bu Cg Cs Ct Es Ga(F) Ge Gr He Hs(A S) Hu It La Lt Mk Mo Po Rf Rm Sk Sl Sr -Tu(A) Tu(E) Uk(K U) (Ball and Dudley 1993) (Appendix 2, Figure C).

Alyssum repens Baumg., Enum. Stirp. Transs. 2: 237 (1816) (*A. virescens* Halácsy). Diffuse or erect perennial up to 60 cm, the non-flowering stems terminated by rosettes; indumentum dimorphic, grey-green. Basal leaves obovate-spathulate; cauline leaves lanceolate or linear-lanceolate, acute. Raceme elongate in fruit; pedicels 4·5-10 mm, patent. Sepals 2·5-4 mm; petals 4·5-7 mm, emarginate, usually glabrous on the back. Silicula 3-6 mm, suborbicular to orbicular-obovate, pubescent, truncate or emarginate; valves inflated, with a narrow, flattened margin; style 1·5-3·5 mm. Seeds 1·5-2 mm; wing usually 0·1-0·2 mm wide. S. E. & E. C. Europe. Al Au Bu Gr Ju Rm Rs(K) Tu (Ball and Dudley 1993).

Cardamine L., Sp. Pl. 2: 654. 1753.

Herbs annual, biennial, or rhizomatous or tuberous perennial. Trichomes absent or simple. Stems erect or prostrate, leafy or rarely leafless and plant scapose. Basal leaves petiolate, rosulate or not, simple and entire, toothed, or 1-3-pinnatisect, or palmately lobed, sometimes trifoliolate, pinnately, palmately, or bipinnately compound. Cauline leaves alternate, (rarely opposite or whorled), simple or compound as basal leaves, petiolate or sessile and base cuneate, attenuate, auriculate, or sagittate, margin entire, dentate, or variously lobed. Racemes ebracteate or rarely bracteate throughout or only basally, corymbose or in panicles, elongated in fruit. Fruiting pedicels slender or thickened, erect, divaricate, or reflexed. Sepals ovate or oblong, base of lateral pair saccate or not, margin often membranous. Petals white, pink, purple, or violet, never yellow, rarely absent; blade obovate, spatulate, oblong, or oblanceolate, apex obtuse or emarginate; claw absent or strongly differentiated from blade, longer or shorter than sepals. Stamens 6 and tetradynamous, rarely 4 and equal in length; anthers ovate, oblong, or linear, obtuse at apex. Nectar glands confluent and subtending bases of all stamens; median glands 2 or rarely 4 or absent; lateral glands annular or semiannular. Ovules 4-50 per ovary. Fruit dehiscent siliques, linear or rarely narrowly oblong or narrowly lanceolate, latiseptate, sessile; valves papery, not veined, glabrous (or very rarely hairy), smooth or torulose, dehiscing elastically acropetally, spirally or circinately coiled; replum strongly flattened; septum complete, membranous, translucent; style distinct or rarely obsolete; stigma capitate, entire. Seeds uniseriate, wingless, rarely margined or winged, oblong or ovate, flattened; seed coat smooth, minutely reticulate, colliculate, or rugose; mucilaginous or not when wetted; cotyledons accumbent or very rarely incumbent. About 200 species: worldwide (Shu, S. M. Q. 2001).

Cardamine plumieri Vill., *Prosp. Pl. Dauph.* 38 (1779). Biennial or perennial 5-12(-20) cm; almost glabrous but not glaucous. Lowest leaves hederiform with 3-5 obtuse lobes; intermediate cauline leaves ternate with stalked, obovate lateral leaflets and a long terminal leaflet; upper cauline leaves pinnate with ternate, obtuse leaflets. Petals 6-8 mm, rounded or slightly emarginate at the apex, white. Siliqua 18-25 × (0·5-)1-1·5 mm, erect; style 1-2 mm. *Shady rocks and screes. S. Europe, from S. E. France to N. Greece.* Al BH Co Ga(F) Gr He It (Jones *et al.* 1993) (Appendix 2, Figure B).

Rorippa Scopoli, Fl. Carniol. 520. 1760.

Herbs annual, biennial, or perennial, usually of wet or aquatic habitats. Trichomes absent or simple. Stems erect or prostrate, simple or branched, leafy. Basal leaves petiolate, rosulate or not, simple, entire, dentate, sinuate, lyrate, pectinate, or 1-3-pinnatisect. Cauline leaves petiolate or sessile, cuneate, attenuate, auriculate, or sagittate at base, entire, dentate, pinnatifid, or pinnatisect. Racemes ebracteate or rarely bracteate throughout, elongated in fruit. Sepals ovate or oblong, erect or spreading, base of lateral pair not saccate or rarely saccate, margin often membranous. Petals yellow, sometimes white or pink, rarely vestigial or absent; blade obovate, spatulate, oblong, or oblanceolate, apex obtuse or emarginate; claw sometimes distinct, often shorter than sepals. Stamens 6 and tetradynamous, rarely 4 and equal in length; anthers ovate or oblong, obtuse or rarely apiculate at apex. Nectar glands confluent, often subtending bases of all stamens; median glands narrow; lateral glands semiannular and intrastaminal, or annular. Ovules 10-300 per ovary. Fruit dehiscent siliques or silicles, linear, oblong, ovoid, ellipsoid, or globose, terete or slightly latiseptate, sessile or rarely shortly stipitate; valves 2(or 3-6), papery or leathery, veinless or obscurely veined, smooth or torulose; replum rounded; septum complete or rarely perforated, membranous, translucent, veinless; style obsolete or distinct; stigma capitate, entire or slightly 2-lobed. Seeds biseriate or rarely uniseriate, wingless or rarely winged, oblong, ovoid, or ellipsoid, plump; seed coat reticulate, colliculate, rugose, tuberculate, or foveolate, mucilaginous or not when wetted; cotyledons accumbent. About 75 species: worldwide (Shu, H. C. 2001).

Rorippa lippizensis (Wulfen) Reichenb., *Icon. Fl. Germ.* **2**: 15 (1837) (*Nasturtium lippizense* (Wulfen) DC.). Perennial 10-20 cm, shortly pubescent at the base. Lower leaves in a rosette, with long petioles, orbicular-ovate, entire, or pinnatifid with 1-4 pairs of narrow lateral lobes and a large terminal lobe; cauline leaves sessile, with narrow, amplexicaul auricles, pinnatifid with 1-4 pairs of linear to lanceolate lobes. Petals (3-)4-5 mm. Fruit 10-20 × c. 1 mm, longer than the erect or ascending pedicels; style 0·5-1·5 mm. 2n = 32. *Balkan peninsula and N. W. Jugoslavia*. Al BH Bu Cg Ct Gr It Mk Rm Sl Sr Tu(E) (Valentine and Jonsell 1993).

Aethionema R.Br. in Aiton, Hort. Kew. ed. 2, 4: 80. 1812. Benth. & Hook. f., l.c. 88; Schulz in Engl. & Prantl, l.c. 440; Hedge in Davis, l.c. 314; in Rech. f., l.c. 102.

Perennial or annual herbs, often woody below, branched, erect or suberect, leafy, glabrous or rarely papillose. Leaves simple, usually sessile or subsessile, oblong or linear, glaucous. Racemes corymbose, usually many flowered, ebracteate. Flowers mediocre, rose, lilac or white, rarely yellowish; pedicls filiform, usually spreading in fruit. Sepals oblong, obtuse, rounded at apex; inner ±saccate at base; outer often somewhat hooded at apex. Petals obovate, cuneate or clawed, rarely oblong; claw 1-3-nerved. Stamens 6; filaments of longer stamens append-aged, dilated or linear; anthers often apiculate, ovate-orbicular. Lateral nectar glands in pairs, minute, semiglobose; middle usually absent. Ovary ± ellipsoid with narrowly flattened margin, 1-2-locular with 1-2 (rarely 3-4) ovules in each locule; stigma capitate, sub-sessile or on distinct short style. Siliculae ovate, elliptic or suborbicular, laterally flattened, usually winged, dehiscent, (rarely heterocarpic with dehiscent and indehiscent fruits), 1-4-seeded; apex generally deeply notched or emarginate; wing entire or variously dentate; seed ovate, brown, often minutely papillose; radicle incumbent, oblique or accumbent. About 50 species, chiefly in the Mediterranean region (Jafri 1973).

Aethionema saxatile (L.) R. Br. in Aiton, *Hort. Kew.* ed. 2, **4**: 80 (1812) (*A. creticum* Boiss. & Heldr., *A. subcapitatum* Bornm.; incl. *A. gracile* DC., *A. pyrenaicum* Bout.). Perennial up to 35 cm, ascending or erect, simple or branched. Lower leaves more than 5 mm, ovate to suborbicular or oblong and obtuse, the upper narrower, often acute. Sepals 1-3(-3·8) mm; petals 2-6·5(-8·5) mm, white, purplish or lilac. Silicula 5-10 mm, obovate to suborbicular, emarginate, sometimes a little broader than long, up to 8-seeded, in lax or rather dense racemes; unilocular siliculae 3-6·5 mm, the wing 1-3 mm wide at the apex; bilocular fruits 4·5-10 mm, the wing 1·5-4 mm wide at the apex; style 0·2-2 mm, usually shorter than but sometimes equalling or exceeding notch. *S. & S. C. Europe, mainly in the mountains*. AE(G) Ag Al Au(A) BH Bu Cr Ct Ga(F) Ge Gr He Hs(S) Hu It Ma Mk Rm Sa Si Sk Sr Tu(A) ?Tu(E) (Chater and Akeroyd 1993).

Isatis L., Sp. Pl. 2: 670. 1753.

Herbs annual, biennial, or perennial, often glaucous. Trichomes absent or simple. Stems erect, simple at base, paniculately branched above. Basal leaves petiolate or rarely sessile, rosulate or not, simple, entire, dentate, or pinnately lobed. Cauline leaves sessile and auriculate, sagittate, or amplexicaul at base, rarely petiolate and attenuate, entire or dentate. Racemes many flowered, ebracteate, forming panicles, elongated considerably in fruit. Fruiting pedicels filiform, often thickened and clavate at apex, reflexed. Sepals ovate or oblong, erect or ascending, base of lateral pair not saccate. Petals yellow, as long as or longer than sepals; blade obovate, spatulate, oblong, or oblanceolate, apex obtuse or subemarginate; claw absent. Stamens 6, slightly tetradynamous; anthers ovate or oblong, apiculate or obtuse at apex. Nectar glands confluent, or 4 and median and lateral pairs distinct. Ovules 1(or 2) per ovary, subapical. Fruit indehiscent, samaroid siliques or silicles, oblong, ovate, obovate, cordate, elliptic, oblanceolate, spatulate, or orbicular, strongly angustiseptate, sessile, prominently winged all around or distally, 1(or 2)-seeded, glabrous or hairy, smooth; seed-bearing locule papery or corky, prominently or obscurely 1- or 3-veined, sometimes keeled or shortly winged; valves and replum united; gynophore, style, and septum absent; stigma capitate, entire. Seeds wingless, narrowly oblong, plump; seed coat smooth, not mucilaginous when wetted; cotyledons incumbent or accumbent. About 50 species: primarily in C and SW Asia (Shu, S. L. 2001).

Isatis tinctoria L., Sp. Pl. 2: 670. 1753. *Isatis indigotica* Fortune; *I. oblongata* de Candolle var. *yezoensis* (Ohwi) Y. L. Chang; *I. tinctoria* var. *indigotica* (Fortune) T. Y. Cheo & K. C. Kuan; *I. tinctoria* var. *yezoensis* (Ohwi) Ohwi; *I. yezoensis* Ohwi. Herbs biennial, (30-)40-100(-150) cm tall. Stems branched above, glabrous and somewhat glaucous, or hirsute, often paniculately branched. Basal leaves rosulate; petiole 0.5-5.5 cm; leaf blade oblong or oblanceolate, (2.5-)5-15(-20) × (0.5-)1.5-3.5(-5) cm, base attenuate, margin entire, repand, or dentate, apex obtuse. Middle cauline leaves sessile; leaf blade oblong or lanceolate, rarely linear-oblong, (1.5-)3-7(-12) × (0.2-)0.8-2.5(-3.5) cm, base sagittate or auriculate and with acute or obtuse auricles, margin entire, apex acute. Fruiting pedicels slender, considerably thickened and subclavate at apex, 5-10 mm. Sepals oblong, 1.5-2.8 × 1-1.5 mm, glabrous. Petals yellow, oblanceolate, 2.5-4 × 0.9-1.5 mm, base attenuate, apex obtuse. Filaments 1-2.5 mm; anthers oblong, 0.5-0.7 mm. Fruit black or dark brown when mature, oblong-oblanceolate, elliptic-obovate, or rarely oblong, (0.9-)1.1-2(-2.7) cm × 3-6(-10) mm, often broader above middle, glabrous or pubescent, winged all around, base cuneate, margin sometimes slightly constricted, apex subacute, rounded, or rarely subemarginate; locule 3-6(-10) mm, with a distinct midvein and inconspicuous lateral veins; apical wing 3.5-5(-7) mm wide. Seeds light brown, narrowly oblong, 2.3-3.5(-4.5) × 0.8-1 mm. Fl. Apr-Jun, fr. May-Jul. 2n = 14, 28*. Ag Al Ar Au Be dBe(L) BH Br Bu Co Ct Da Es Fe Ga(F) Ge Gr He Ho Hs(S) Hu It La LS Lt Ma Mk Mo No Po Rf Rm Sa Si(S) Sk Sl Sr Su Tu(A E) Uk(K U) [Au(A) By nCa(H) Co Cs Fe Ge He Hs(S) Md(M) No] (Shu, S. L. 2001).

2. AIMS

- Determine the basic habitat characteristics of the selected taxa from the Brassicaceae family, including information on the type of vegetation and/or community, altitude, slope, geological and pedological substrate and climate.

- Identify the basic physical (texture) and chemical (pH in H_2O, pH in 1N KCl, percent of organic matter, contents of P_2O_5 and K_2O) features of serpentine soil of the study sites

- Determine the concentrations of the macro (Ca, Mg, Fe) as well as trace elements (Ni, Mn, Zn, Cu, Cr, Pb, Co, Cd) in soils that are developed over serpentinite substrates at several locations in the northwestern, western, southwestern and central Serbia

- Examine the concentrations of the macro and trace elements in underground and aboveground parts (stems and/or leaves) of plants of the family Brassicaceae that inhabit serpentinite habitats in Serbia

- Based on the obtained results, the assessment of the capacity of some plant species of the family Brassicaceae for tolerance and/or bioaccumulation of certain trace elements (with special attention to nickel and zinc)

- Identificationn and discovery of new potential hyperaccumulator species in the family Brassicaceae from Serbia

3. MATERIAL AND METHODS

3. 1. Material

The distribution of ultramafic bedrocks occurrence, locations (latitude and longitude) and habitat characteristics (altitude, vegetation alliances, type of ultramafic rocks) of the studied species populations are shown in Figure 3 and Table 2.

The sample selection was based on the presence at each site of large populations of the plants. Plant and soil samples were collected in May of 2010 and May of 2011 from 30 sampling points, SP1–SP30 (Table 2).

Soil samples were chosen by eye based on the presence of the 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 after that dried at 40^{o}C for 3 days.

Plant species were sampled according to their abundance and biomass, 5–10 replicate of each investigated population. All the plant samples were collected, separated into roots, shoots or leaves, and washed carefully with distilled water to remove soil particles. Dry weights were obtained after drying at 40^{o}C for 3 days. Plants have been identified with the help of local flora (Diklić 1973).

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).

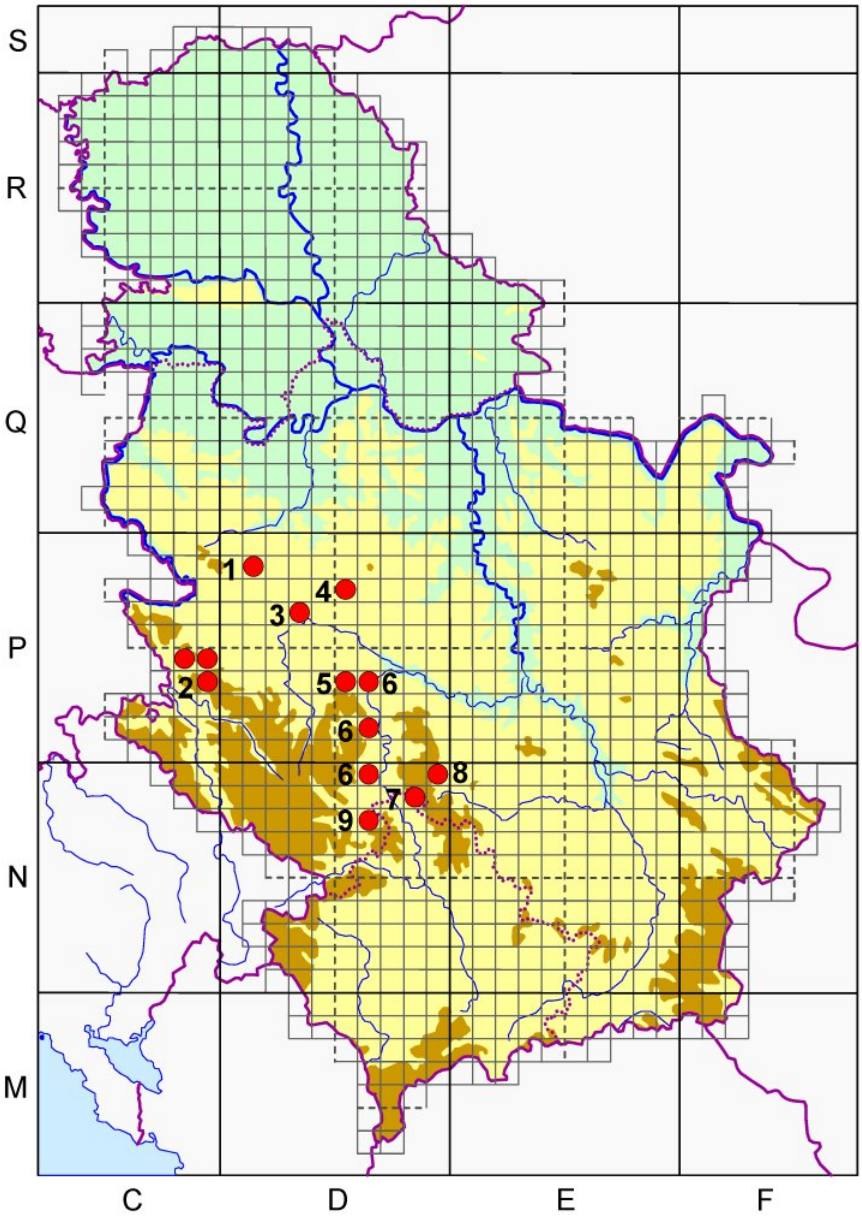

Figure 3. Map of Serbia showing locations (**1–9**) of the studied Brassicaceae plant populations (red circles) in Serbia – **1.** Mt Maljen, Divčibare (SP9, SP29, SP30); **2.** Mt Zlatibor (SP1, SP10, SP17, SP20, SP22, SP25, SP28); **3.** Požega, Tučkovo, Vrnčanska River Gorge (SP2, SP18, SP23); **4.** Gornji Milanovac, Brđani River Gorge (SP3, SP12, SP21); **5.** Kraljevo, Bogutovac, Gornja Lopatnica River Gorge (SP4, SP11, SP16, SP19); **6.** Ibar River Valley (Maglič ruins SP13, SP24), Ušće, Studenica river gorge (SP8), Raška, Trnavska river gorge (SP5, SP15); **7.** Mt Kopaonik, Šiljak (SP26); **8.** Mt Kopaonik, Paljevštica (Brzećka River Gorge) (SP6); **9.** Mt Rogozna (SP7, SP14, SP27)

Table 2. Latitude, longitude and altitude of the sampling points of different Brassicaceae species from the ultramafic areas of Serbia

Species	**Locality**	**Sample point**	**Latitude (N)**	**Longitude (E)**	**Altitude (m. a. s. l.)**	**Vegetation alliance of the studied location**	**Type of rocks**
Alyssum murale	Mt Zlatibor, Čelićko hill – Okolište	SP1	43.63250	19.73722	1090	*Quercion frainetto*	metamorphic (serpentinite)
Alyssum murale	Požega, Tučkovo village (Vrnčanska gorge)	SP2	43.91806	20.13639	410	*Quercion frainetto* *Orno-Ostryon*	metamorphic (serpentinite)
Alyssum murale	Gornji Milanovac, Brđani gorge	SP3	43.99083	20.42083	330	*Centaureo-Bromion fibrosi*	metamorphic (serpentinite)
Alyssum murale	Kraljevo, Bogutovac (Gornja Lopatnica gorge)	SP4	43.68028	20.46278	430	*Quercion frainetto* *Orno-Ostryon*	intrusive (harzburgite)
Alyssum murale	Raška (Trnavska river gorge)	SP5	43.28722	20.60194	420	*Quercion frainetto*	intrusive (harzburgite)
Alyssum murale	Mt Kopaonik, Paljevštica (Brzećka gorge)	SP6	43.33889	20.94222	680	*Orno-Ostryon*	intrusive (harzburgite)
Alyssum murale	Mt Rogozna (Izbice – Negotinac)	SP7	43.11278	20.60611	730	*Centaureo-Bromion fibrosi*	intrusive (harzburgite)
Alyssum murale	Ušće (Studenica river gorge)	SP8	43.46111	20.59806	400	*Centaureo-Bromion fibrosi*	intrusive (harzburgite)
Alyssum murale	Mt Maljen, Divčibare (Golubac)	SP9	44.11083	19.97500	950	*Fraxino orni-Ericion*	metamorphic (serpentinite)
Aethionema saxatile	Mt Zlatibor (Skakavac river)	SP10	43.74278	19.61167	840	*Centaureo-Bromion fibrosi*	metamorphic (serpentinite)

Species	Locality	Sample point	Latitude (N)	Longitude (E)	Altitude (m. a. s. l.)	Vegetation alliance of the studied location	Type of rocks
Aethionema saxatile	Kraljevo, Bogutovac (Gornja Lopatnica gorge)	SP11	43.66917	20.44167	440	*Quercion frainetto* *Orno-Ostryon*	intrusive (harzburgite)
Aethionema saxatile	Gornji Milanovac, Brđani gorge	SP12	43.99083	20.42083	330	*Centaureo-Bromion fibrosi*	metamorphic (serpentinite)
Alyssum montanum	Ibar river valley (Maglič ruins)	SP13	43.62583	20.55111	300	*Centaureo-Bromion fibrosi*	intrusive (harzburgite)
Alyssum montanum	Mt Rogozna (Pasji stream – Vinogor)	SP14	43.12361	20.63000	1120	*Centaureo-Bromion fibrosi*	intrusive (harzburgite)
Alyssum montanum	Raška (Trnavska river gorge)	SP15	43.28722	20.60194	420	*Quercion frainetto*	intrusive (harzburgite)
Alyssum repens	Kraljevo, Bogutovac (Gornja Lopatnica gorge)	SP16	43.68000	20.46806	450	*Quercion frainetto* *Orno-Ostryon*	intrusive (harzburgite)
Cardamine plumieri	Mt Zlatibor (Trčinoga – Ravna Kosa)	SP17	43.73139	19.63667	850	*Achnatherion calamagrostis*	metamorphic (serpentinite)
Cardamine plumieri	Požega, Tučkovo village (Vrnčanska gorge)	SP18	43.91806	20.13639	410	*Quercion frainetto* *Orno-Ostryon*	metamorphic (serpentinite)
Cardamine plumieri	Kraljevo, Bogutovac (Gornja Lopatnica gorge)	SP19	43.68000	20.46806	450	*Quercion frainetto* *Orno-Ostryon*	intrusive (harzburgite)
Erysimum linariifolium	Mt Zlatibor (Skakavac river)	SP20	43.74278	19.61167	840	*Centaureo-Bromion fibrosi*	metamorphic (serpentinite)
Erysimum linariifolium	Gornji Milanovac, Brđani gorge	SP21	43.99083	20.42083	330	*Centaureo-Bromion fibrosi*	metamorphic (serpentinite)

Species	**Locality**	**Sample point**	**Latitude (N)**	**Longitude (E)**	**Altitude (m. a. s. l.)**	**Vegetation alliance of the studied location**	**Type of rocks**
Erysimum carniolicum	Mt Zlatibor (Krivi Potok stream)	SP22	43.68639	19.70083	1020	*Achnatherion calamagrostis*	metamorphic (serpentinite)
Isatis tinctoria	Požega, Tučkovo village (Vrnčanska gorge)	SP23	43.91806	20.13639	410	*Quercion frainetto* *Orno-Ostryon*	metamorphic (serpentinite)
Isatis tinctoria	Ibar river valley (Maglič ruins)	SP24	43.62583	20.55111	300	*Centaureo-Bromion fibrosi*	intrusive (harzburgite)
Rorippa lippizensis	Mt Zlatibor (Prdavac stream)	SP25	43.73611	19.65806	900	*Achnatherion calamagrostis*	metamorphic (serpentinite)
Thlaspi kovatsii	Mt Kopaonik (Šiljak)	SP26	43.26222	20.78583	1550	*Chrysopogoni-Danthonion alpinae*	intrusive (harzburgite)
Thlaspi kovatsii	Mt Rogozna (Izbice – Negotinac)	SP27	43.11278	20.60611	730	*Centaureo-Bromion fibrosi*	intrusive (harzburgite)
Thlaspi praecox	Mt Zlatibor, Čelićko hill – Okolište	SP28	43.63250	19.73194	1090	*Quercion frainetto*	metamorphic (serpentinite)
Thlaspi praecox	Mt Maljen, Divčibare (Crni vrh)	SP29	44.09611	19.99639	850	*Fraxino orni-Ericion*	metamorphic (serpentinite)
Thlaspi praecox	Mt Maljen, Divčibare (Golubac)	SP30	44.10333	19.98667	950	*Fraxino orni-Ericion*	metamorphic (serpentinite)

3. 2. Reagents

1) Deionized water
2) Potassium chloride (KCl), Zorka Pharma, Serbia
3) Sodium hexamethaphosphate $(NaPO_3)_6$, Merck Millipore, India
4) Nitric acid (HNO_3) 68%, Zorka Pharma, Serbia
5) Lactic acid ($C_3H_6O_3$), Kemika, Croatia
6) Acetic acid (CH_3COOH), Zorka Pharma, Serbia
7) Ammonium acetate (CH_3COONH_4), Zorka Pharma, Serbia
8) Potassium di-hydrogen phosphate (KH_2PO_4), Carlo Erba, Italy
9) Ammonium molibdate tetra-hydrate $(NH_4)_6Mo_7O_{24}$ x $4H_2O$, Merck, Germany
10) Sulphuric acid (H_2SO_4) 96%, Fisher Chemicals, UK
11) Ascorbic acid ($C_6H_8O_6$), Sinex, Serbia
12) Tin chloride di-hydrate ($SnCl_2$ x $2H_2O$), Merck, Germany
13) Hydrochloric acid (HCl) 37%, Zorka Pharma, Serbia
14) Strontium chloride hexa-hydrate ($SrCl_2$ x $6H_2O$), Merck, Germany
15) Lithium chloride ($LiCl_2$), Sigma Chemicals, USA
16) Potassium dichromate ($K_2Cr_2O_7$), Merck, Germany
17) Silver sulphate (Ag_2SO_4), Merck, Germany
18) Phenyl anthranilic acid ($C_{13}H_{11}NO_2$), Merck, Germany
19) Ammonium iron (II) sulphate ($(NH_4)_2Fe(SO_4)_2$ x $6H_2O$), Zorka Pharma, Serbia
20) Standard solutions for atomic absorption spectrophotometry, Carlo Erba, Italy
21) Standard buffer solution pH 4, Carlo Erba, Italy
22) Standard buffer solution pH 7, Carlo Erba, Italy

3. 3. Equipment

1) Rotating shaker
2) pH meter Iskra MA 5730, Slovenia
3) Spectrophotometer Ultrospec 2000 UV/Vis, UK
4) Atomic absorption spectrophotometer Pye Unicam SP 192, UK
5) Analytical balance Adventurer Pro 260/0.0001, Ohaus, Germany

6) Quantitative filter paper Sartorius Stedim, Germany
7) Laboratory glassware

3. 4. Procedures

3.4.1. Soil analysis

1. Determination of particle size distribution

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) is fractionated by dry sieving. The pipette method was used for the determination of clay (< 2 µm) and silt (< 50 µm) fractions (Van Reeuwijk 1995). The USDA classification was applied to determine soil textural classes (Rowell 1997).

2. Determination of soil actual and exchangeable pH values

Soil actual and exchangeable pH was determined by the method proposed by McKeague (1978). For pH determination, 10 g of air-dried, ground and sieved (< 2 mm) soil samples was weighed and transferred to 50 ml glass beakers. After that, 25 ml of deionized water and 25 ml of 1 N KCl solution were added, for determination of actual and exchangeable pH, respectively. The samples were stirred with glass rods. After 60 min, pH was determined by putting pH-meter electrode into the suspension and reading pH value after 30 sec.

3. Determination of available P_2O_5 and K_2O in soil

Available P_2O_5 and K_2O were measured by following the method of Egner *et al.* (1960). Weighed 5 g of air-dried, ground and sieved (< 2 mm) soil were transferred to 200 ml plastic bottles. The extraction was performed in 100 ml of 10 times diluted AL solution by 2 h rotation. The original AL solution (pH 3.2) contained 12 M lactic acid, 28.5 M acetic acid and 10 M NH_4-acetate. The extracts were filtered through slow filtration quantitative paper.

The series of standard solutions was made using ground standard solution containing 1 g l^{-1} P_2O_5 and 1 g l^{-1} K_2O. Ground standard is made by dissolving 1.917 g of KH_2PO_4 and 0.534 g of KCl which were previously dried for 2 h at 105ºC in a normal vessel of 1000 ml with deionized water. The series included standards corresponding to 0 to 40 mg of P_2O_5 and K_2O per 100 g soil.

For phosphorus determination, 5 ml from each sample extract and from each standard was transferred to glass tubes. To each tube 7.5 ml of molibdate reagent was added. The molibdate reagent contained 0.3 % ammonium-molibdate dissolved in 0.8 N H_2SO_4. At the end, 0.5 ml of freshly made 1 % ascorbic acid solution in 1 % $SnCl_2$ dissolved in 5 % HCl was added to each tube. Blue color was allowed to develop for 30 min. The absorbance was determined at 750 nm using Ultrospec 2000 UV/Vis spectrophotometer. Phosphorus concentrations in the samples were calculated using Origin 7.0 program.

Potassium concentration was determined in the same extract as phosphorus, using the same standard series. The method used was FES (flame emmision spectrophotometry) by Pye Unicam SP 192 atomic absorption spectrophotometer. Potassium concentrations in the samples were calculated using Origin 7.0 program.

4. Determination of exchangeable Ca and Mg in soil

Exchangeable Ca and Mg were determined according to Van Reeuwijk (2002). For the extraction, 2 g of air-dried, ground and sieved (< 1 mm) soil samples was transferred to 200 ml plastic bottles. The extraction was performed with 100 ml of 1 N ammonium acetate for 30 min on rotor shaker. The extracts were filtered through slow filtration quantitative paper. From each extract, 2 ml was transferred to 50 ml normal vessels. To each vessel 1 ml of Sr or La solution (50 mg Sr/La per 1 ml) was added and the volume was adjusted to the mark with deionized water.

The absorbance was determined by atomic absorption spectrophotometry (Pye Unicam SP 192). The concentration range of standard series for Ca was between 0 and 4 mg Ca l^{-1}, and for Mg between 0 and 1 mg Mg l^{-1}. The concentrations in the samples were calculated using Origin 7.0 program.

5. Determination of organic matter

Organic matter concentration was determined by dichromate digestion based on FAO (1974) procedure. Between 0.05 and 0.5 g of air dried, ground and sieved (< 0.2 mm) soil was weighed and transferred to 100 ml Erlenmeyer flask. To each flask, 10 ml of 0.4 N $K_2Cr_2O_7$ (potassium dichromate) solution and 0.1 g of $AgSO_4$ were added. The flask was coverred with a small funnel and warmed gradually to the boiling point. The boiling was allowed to last 5 min. After cooling, 30 ml dionized water was added and a few drops of the indicator phenylanthranilic acid. The content was titrated with 0.1 $(NH_4)_2Fe(SO_4)_2$ x 6 H_2O (ammonium ferrosulphate) until the purple colour turned into bright green.

Humus content was calculated according to the formula:

$[(a-b) \times F \times 0.0003 \times 100]/P \times 1.724 = \%$ humus

a - ml of 0.1 N ammonium ferrosulphate utilized for the titration of blank

b - ml of 0.1 N ammonium ferrosulphate utilized for the titration of sample extract

F - normality factor of ammonium ferrosulphate solution determined with standard potassium dichromate solution

P - soil weight (g)

6. Determination of available (potentially leachable) metals in soil

Extraction of available (potentially leachable) metals in soil was performed according to the procedure recommended by Garcia *et al.* (1979). Five grams of air-dried, ground and sieved (< 0.2 mm) soil was transferred to plastic bottles and extracted with 50 ml 0.1 N HCl overnight. The extracts were filtered through slow filtrating quantitative paper. The contents of Fe, Zn, Mn, Cu, Ni, Cr, Pb, Cd and Co were determined by measuring absorbances with Pye Unicam SP 192 atomic absorption spectrophotometer. The series of standard solutions for these metals were made from 1 g l^{-1} solutions purchased from Carlo Erba, Italy. The concentrations were calculated using Origin 7.0 program.

7. Determination of total metal concentrations in soil

The mineralization was performed by slightly modified wet procedure described by ISO 11466, 1995 and ISO 11047 1998. Approximately 3 g of air-dried, ground and sieved (< 0.2 mm) soil was weighed and transferred to reaction vessel. Each sample was moistened with 0.5 to 1.0 ml deionised water. Hydrochloric acid was added (21 ml) and then 7 ml of nitric acid, drop by drop, and the contents were left overnight for slow oxidation. Next day, the reaction mixture was heated until the boiling begun. The samples were boiled for 2 h, ensuring that the steams did not pass the 2/3 of the height of the reaction vessel. The mixture was allowed to cool. Additional 25 ml of nitric acid was added. The content was filtrated through filter paper into 100 ml normal vessel. The volume was adjusted to the mark.

Absorbances are determined by atomic absorption spectrophotometry (Pye Unicam SP 192). The series of standard solutions for these metals were made from 1 g l^{-1} solutions purchased from Carlo Erba, Italy. The concentrations were calculated using Origin 7.0 program.

3.4.2. Plant analysis

8. Determination of P_2O_5 content in plants

Dried and ground plant material was weighed (0.5 g) and transferred to reaction vessels for mineralization. The samples were mineralized by wet procedure, with boiling mixture of nitric and sulphuric acids.

Phosphorus concentration in the mineralized samples was determined by modified molybdenum blue method described by Chen *et al.* (1956).

9. Determination of K in plants

Dried and ground plant material was weighed (0.5 g) and transferred to reaction vessels for mineralization. The samples were mineralized by wet procedure, with boiling mixture of nitric and sulphuric acids.

Potassium concentration was determined by FES (flame emission spectrophotometry) by Pye Unicam SP 192 atomic absorption spectrophotometer. The concentration range of standard series for K was between 0 and 100 mg K $l^{-}1$. Potassium concentrations in the samples were calculated using Origin 7.0 program.

10. Determination of heavy metals and trace elements in plants

Dried and ground plant material was weighed (1 g) and transferred to reaction vessels for mineralization. The mineralization was performed by slightly modified wet procedure described by ISO standard 6636/2 1981.

Absorbances were determined in filtered samples adjusted to 50 ml volume, by AAS method, using the same standards as for the determination of the metals in soil samples. Concentrations were calculated using Origin 7.0 program.

3. 5. Data analysis

Each analysis of the soil and plant material was performed with three replicates. In addition, each of the chemical analyses was run with also 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, 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}. Data analysis was performed using STATISTICA for Windows 5.1. workpackage.

4. RESULTS

4. 1. Soil Characteristics

Physical characteristics of 30 ultramafic soil samples are given in Table 3. The textural classes of the studied samples range from silty loam and sandy loam to loam, but most of the samples fall into the sandy loam class. Concerning the particle size distribution, percentage of coarse sand (particle size 2.00–0.2 mm) varies in the range from 6.3% (SP29) to 66.7% (SP2, SP23), while percentage of fine sand (particle size 0.2–0.05 mm) was from 6.8% (SP3, SP12, SP21) to 26.7% (SP9, SP30). The smallest percent of the silt (particle size 0.05–0.002 mm) was 14.4% (SP5) and the largest one 68.1% (SP1). Percentage of clay (particle size < 0.002 mm) in soil samples was between 6.3% (SP17) and 18% (SP9, SP30).

Chemical soil properties (pH in H_2O, pH in 1 N KCl, percentage of organic matter, and concentrations of P_2O_5, K_2O) are shown in Table 4. The pH in H_2O of the soil samples varied from moderately acidic - 6.2 (SP1, SP28, SP30) to moderately alkaline - 7.8 (SP8), while pH in 1 N KCl the values were acidic - 5.1 (SP1, SP28) to neutral - 7.1 (SP7, SP8). Percentage of organic matter differed significantly among the sampling points and it was in the range from 0.7% (SP5, SP9) to 17.9% (SP1, SP28). The contents of P_2O_5 varied from <0.1 mg kg^{-1} (SP14) to 275 mg kg^{-1} (SP8) and K_2O from 14 mg kg^{-1} (SP9) to 268 mg kg^{-1} (SP26).

Concerning the concentrations of major elements (Fe, Ca, Mg) in soil samples (Table 5), the soils were more or less of typical ultramafic composition. The concentration of the available Fe varied from 110 mg kg^{-1} (SP6) to 950 mg kg^{-1} (SP5). Content of available Ca was from low - 310 mg kg^{-1} (SP2) to high - 5100 mg kg^{-1} (SP22), while available Mg content was from moderate - 500 mg kg^{-1} (SP6) to high - 5507 mg kg^{-1} (SP29).

The available Ca/Mg ratios in 23 soil samples are relatively low (0.20–0.97), while in six serpentine soil samples (SP7, SP8, SP10, SP20, SP22, SP26) higher values of bioavailable Ca compared to Mg (Ca/Mg ratios 1.01 and 2.01) would indicate lower Ca deficiency stress for the plants. Only in SP6, the Ca/Mg ratio was very high (6.22).

Table 3. Particle size distribution 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	7.2	12.9	68.1	11.8	silty loam
SP2	66.7	10.6	14.6	8.1	sandy loam
SP3	10.6	6.8	67.5	15.1	silty loam
SP4	46.7	14.2	30.7	8.4	sandy loam
SP5	52.1	19.5	14.4	14.0	sandy loam
SP6	44.0	18.8	22.0	15.2	sandy loam
SP7	46.8	15.5	24.3	13.4	sandy loam
SP8	29.9	17.7	40.2	12.2	loam
SP9	6.9	26.7	48.4	18.0	loam
SP10	45.7	17.9	24.9	11.5	sandy loam
SP11	46.7	14.2	30.7	8.4	sandy loam
SP12	10.6	6.8	67.5	15.1	silty loam
SP13	46.8	16.7	25.0	11.5	sandy loam
SP14	43.3	13.1	26.8	16.8	sandy loam
SP15	25.9	23.3	39.2	11.6	loam
SP16	7.9	14.1	65.5	12.5	silty loam
SP17	42.1	14.8	36.8	6.3	sandy loam
SP18	17.9	14.4	57.0	10.7	silty loam
SP19	7.9	14.1	65.5	12.5	silty loam
SP20	45.7	17.9	24.9	11.5	sandy loam
SP21	10.6	6.8	67.5	15.1	silty loam
SP22	22.2	11.3	57.1	9.4	silty loam
SP23	66.7	10.6	14.6	8.1	sandy loam
SP24	30.3	16.3	36.1	17.3	loam
SP25	23.0	12.6	56.1	8.3	silty loam
SP26	35.1	17.5	38.3	9.1	sandy loam
SP27	36.2	16.9	21.6	15.3	sandy loam
SP28	30.3	16.3	36.1	17.3	loam
SP29	6.3	23.7	42.4	10.0	loam
SP30	6.9	26.7	48.4	18.0	loam

Table 4. pH, organic matter P_2O_5 and K_2O concentrations (mg kg^{-1}) in soil samples. Concentrations are expressed as means ± standard deviations

Sample point	**pH soil in H_2O**	**pH soil in 1 N KCl**	**Organic matter %**	**P_2O_5 (available)**	**K_2O (available)**
SP1	6.2	5.1	17.9±0.7	6.8±0.0	155±1
SP2	7.0	5.8	10.1±0.4	5.3±0.6	25±1
SP3	6.5	5.6	8.9±0.6	8.1±0.6	219±1
SP4	7.3	6.3	0.9±0.4	18.6±0.6	85±4
SP5	7.6	7.0	0.7±0.1	24.6±4.2	64±4
SP6	7.6	7.0	3.3±0.4	17.6±2.7	59±1
SP7	7.6	7.1	2.4±0.1	15.1±0.7	135±4
SP8	7.8	7.1	5.1±0.7	275±22	242±6
SP9	7.6	7.0	0.7±0.1	1.7±0.3	14±1
SP10	7.1	6.2	1.8±0.2	4.9±0.0	25±1
SP11	7.3	6.3	0.9±0.4	18.6±0.6	85±4
SP12	6.5	5.6	8.9±0.6	8.1±0.6	219±1
SP13	7.4	6.4	3.7±0.3	6.8±1.3	59±1
SP14	7.7	6.9	12.1±1.5	<0.1	141±3
SP15	7.6	6.9	4.2±0.5	31.5±4.7	156±6
SP16	7.1	6.0	2.6±0.5	4.9±0.0	49±1
SP17	7.2	6.3	7.1±0.8	5.3±0.6	52±1
SP18	7.1	6.0	4.6±0.2	6.3±0.7	87±1
SP19	7.1	6.0	2.6±0.5	4.9±0.0	49±1
SP20	7.1	6.2	1.8±0.2	4.9±0.0	25±1
SP21	6.5	5.6	8.9±0.6	8.1±0.6	219±1
SP22	6.7	5.8	8.0±1.4	7.0±0.2	110±5
SP23	7.0	5.8	10.1±0.4	5.3±0.6	25±1
SP24	7.1	6.1	2.9±0.8	5.3±0.6	149±2
SP25	7.0	5.9	5.0±1.2	4.9±0.0	35±1
SP26	6.6	5.8	16.5±1.7	40.2±3.3	268±3
SP27	7.1	6.0	6.2±0.4	34.5±2.7	189±5
SP28	6.2	5.1	17.9±0.7	6.8±0.0	155±1
SP29	6.6	5.7	8.6±0.4	41.2±6.5	219±10
SP30	6.2	5.2	7.5±0.1	20.5±3.4	121±4

Table 5. Major elements (Fe, Ca, Mg) concentrations (mg kg^{-1}) in soil samples. Concentrations are expressed as means ± standard deviations

Sample point	Fe (available)	Ca (available)	Mg (available)	Ca/Mg
SP1	120±10	1360±170	4070±80	0.33
SP2	670±20	310±0	3800±120	0.08
SP3	150±10	2140±160	3870±90	0.55
SP4	270±10	900±50	4600±230	0.20
SP5	950±50	880±50	1240±40	0.71
SP6	110±20	3110±70	500±20	6.22
SP7	540±20	1080±40	810±10	1.33
SP8	120±10	1760±60	1750±40	1.01
SP9	610±10	1230±70	2320±60	0.53
SP10	580±10	2190±80	1480±80	1.48
SP11	270±10	900±50	4600±230	0.20
SP12	150±10	2140±160	3870±90	0.55
SP13	590±10	1140±50	2390±100	0.48
SP14	130±10	970±10	2490±60	0.39
SP15	490±10	750±30	1110±30	0.68
SP16	500±10	1300±40	2910±430	0.45
SP17	380±10	2010±50	3320±120	0.61
SP18	150±10	2350±0	3530±70	0.67
SP19	500±10	1300±40	2910±430	0.45
SP20	580±10	2190±80	1480±80	1.48
SP21	150±10	2140±160	3870±90	0.55
SP22	160±10	5100±280	2540±100	2.01
SP23	670±20	310±0	3800±120	0.08
SP24	170±10	2560±50	2650±140	0.97
SP25	330±10	1300±160	3510±30	0.37
SP26	160±10	1230±60	1160±30	1.06
SP27	400±10	840±60	970±30	0.87
SP28	120±10	1360±170	4070±80	0.33
SP29	209±14	1541±129	5507±90	0.28
SP30	806±7	1396±66	2867±12	0.49

Total and available concentrations of trace elements (Ni, Zn) in the soils are presented in Table 6. The total and available Ni concentrations varied from 1116 mg kg^{-1} (SP26) to 2272 mg kg^{-1} (SP15) and from 67 mg kg^{-1} (SP4) to 506 mg kg^{-1} (SP29) respectively. Total concentrations of Zn at all sites were above 30 mg kg^{-1}, lying in the range between 34 mg kg^{-1} (SP5) and 201 mg kg^{-1} (SP13), while content of available Zn was between 0.4 mg kg^{-1} (SP2) and 84.7 mg kg^{-1} (SP13).

Total and available trace elements (Mn, Cu) contents in the soils are presented in Table 7. Total and available Mn concentrations varied from 910 mg kg^{-1} (SP5) to 5650 mg kg^{-1} (SP17) and from 84 mg kg^{-1} (SP2) to 803 mg kg^{-1} (SP9) respectively. The total Cu concentration varied from 0.8 mg kg^{-1} (SP2) to 31.1 mg kg^{-1} (SP4), while available Cu contents were below <0.1 mg kg^{-1} in 20 soil samples, and only in sample point SP9 it was much higher (4.7 mg kg^{-1}).

Contents of the total and available trace elements (Cr, Co) in the soil samples are given in Table 8. The total Cr concentration varied from 174 mg kg^{-1} (SP2) to 1580 mg kg^{-1} (SP1, SP28), while available Cr reached 43.5 mg kg^{-1} (SP7). Content of total Co was between 81 mg kg^{-1} (SP8) and 241 mg kg^{-1} (SP1), except in the case of the SP9 where it was below 0.1 mg kg^{-1}. Available Co was below 0.1 mg kg^{-1} at 20 sample points and in the rest of the samples it varied from 36.1 mg kg^{-1} (SP5) to 82.0 mg kg^{-1} (SP7).

Contents of the total and available trace elements (Cd, Pb) in the soil samples are given in Table 9. Contents of total Cd were below <0.1 mg kg^{-1} in 14 soil samples, in the rest samples it varied from 2.3 mg kg^{-1} (SP2, SP3) to 4.5 mg kg^{-1} (SP30), while contents of available Cd were very low (below <0.1 mg kg^{-1}) at all but three samples points (SP9, SP16, SP29, SP30) (2.4, 1.3 mg kg^{-1} and 0.4 mg kg^{-1} respectively). Finally, the total and available Pb concentrations varied from 11.5 mg kg^{-1} (SP2) to 130 mg kg^{-1} (SP26) and from 0.5 mg kg^{-1} (SP6) to 38.4 mg kg^{-1} (SP15) respectively. At 19 sample points, available Pb concentration was below 0.1 mg kg^{-1}.

Table 6. Trace elements (Ni, Zn) concentrations (mg kg^{-1}) in soil samples. Concentrations are expressed as means ± standard deviations

Sample point	**Ni (total)**	**Ni (available)**	**Zn (total)**	**Zn (available)**
SP1	1699±276	181±12	124±6	11.2±0.4
SP2	1843±43	75±2	38±1	0.4±0.1
SP3	1544±64	171±6	126±4	12.2±0.6
SP4	1158±61	67±6	92±6	1.8±0.1
SP5	2269±38	155±1	34±1	9.2±0.2
SP6	2125±8	155±5	37±1	2.9±0.3
SP7	2091±38	181±2	35±1	6.4±0.5
SP8	1669±31	140±5	43±2	10.2±0.5
SP9	1489±41	443±80	119±12	26.5±6.6
SP10	1552±52	73±2	35±5	1.2±0.1
SP11	1158±61	67±6	93±6	1.8±0.1
SP12	1544±64	171±6	126±4	12.2±0.6
SP13	1463±44	122±7	201±9	84.7±3.9
SP14	1922±34	201±1	45±1	5.2±0.4
SP15	2272±75	218±1	46±2	12.7±0.1
SP16	1592±38	142±8	72±4	2.3±0.2
SP17	1697±70	127±4	56±5	2.9±0.1
SP18	1574±60	130±6	54±4	2.7±0.1
SP19	1592±38	142±8	72±4	2.3±0.2
SP20	1552±52	73±2	35±5	1.2±0.1
SP21	1544±64	171±6	126±4	12.2±0.6
SP22	1340±77	157±5	92±8	4.5±0.1
SP23	1843±43	75±2	39±1	1.4±0.1
SP24	1490±88	132±2	59±6	10.1±0.9
SP25	1548±58	130±6	70±2	2.1±0.1
SP26	1116±34	157±6	53±1	22.6±1.0
SP27	1970±15	152±4	41±1	7.5±0.3
SP28	1699±276	181±12	124.±7	11.2±0.4
SP29	1501±45	506±25	108±1	15.9±0.3
SP30	1237±8	340±16	78±1	10.3±0.3

Table 7. Trace elements (Mn, Cu) concentrations (mg kg^{-1}) in soil samples. Concentrations are expressed as means ± standard deviations

Sample point	Mn (total)	Mn (available)	Cu (total)	Cu (available)
SP1	4972±383	304±10	18.2±2.5	<0.1
SP2	1223±82	84±2	0.8±0.6	<0.1
SP3	2717±153	241±2	14.5±0.6	<0.1
SP4	1456±63	98±3	31.1±0.8	1.6±0.1
SP5	910±34	371±22	9.7±0.6	0.1±0.1
SP6	1186±14	460±15	8.9±0.2	<0.1
SP7	1160±29	538±7	12.3±0.3	0.1±0.1
SP8	1070±15	473±14	12.5±0.5	<0.1
SP9	2176±175	803±19	14.8±0.4	4.7±0.6
SP10	1716±84	131±3	4.0±0.3	<0.1
SP11	1456±63	98±3	31.1±0.8	1.6±0.1
SP12	2717±153	241±2	14.5±0.6	<0.1
SP13	1671±98	126±5	11.3±0.6	1.0±0.1
SP14	1674±27	588±21	18.1±0.4	<0.1
SP15	1262±30	534±12	10.4±0.2	<0.1
SP16	2857±78	192±7	18.9±0.8	1.7±0.1
SP17	5650±372	264±11	18.2±0.5	<0.1
SP18	2631±165	203±5	12.3±0.7	<0.1
SP19	2857±78	192±7	18.9±0.8	1.7±0.1
SP20	1716±84	131±3	4.0±0.3	<0.1
SP21	2717±153	241±2	14.5±0.6	<0.1
SP22	3199±114	220±6	20.4±0.2	<0.1
SP23	1223±82	84±2	1.0±0.6	<0.1
SP24	2236±112	211±2	12.6±0.5	0.9±0.1
SP25	2682±90	152±12	15.5±0.6	<0.1
SP26	1387±13	547±4	16.1±0.8	<0.1
SP27	1550±17	765±13	17.4±0.9	1.4±0.1
SP28	4972±383	304±10	18.2±2.5	<0.1
SP29	2037±81	630±8	11.8±0.2	<0.1
SP30	2265±102	721±26	14.6±0.1	<0.1

Table 8. Trace elements (Cr, Co) concentrations (mg kg^{-1}) in soil samples. Concentrations are expressed as means ± standard deviations

Sample Point	Cr (total)	Cr (available)	Co (total)	Co (available)
SP1	1580±73	<0.1	241±36	<0.1
SP2	174±3	<0.1	120±7	<0.1
SP3	919±28	<0.1	155±3	<0.1
SP4	270±12	<0.1	92±2	<0.1
SP5	341±19	20.5±0.1	90±9	36.1±1.2
SP6	608±16	19.6±0.7	108±2	52.0±1.3
SP7	789±31	43.5±0.1	102±2	82.0±2.7
SP8	317±8	13.6±0.1	81±6	41.1±0.7
SP9	366±12	14.4±0.9	<0.1	<0.1
SP10	244±6	<0.1	133±2	<0.1
SP11	270±12	<0.1	92±2	<0.1
SP12	919±28	<0.1	155±3	<0.1
SP13	868±26	<0.1	126±1	<0.1
SP14	610±26	16.8±0.8	115±7	55.4±0.7
SP15	349±11	19.6±0.8	104±11	58.5±1.1
SP16	357±12	<0.1	176±3	<0.1
SP17	359±10	<0.1	157±2	<0.1
SP18	310±5	<0.1	139±4	<0.1
SP19	357±12	<0.1	176±3	<0.1
SP20	244±6	<0.1	133±2	<0.1
SP21	919±28	<0.1	155±3	<0.1
SP22	1511±116	<0.1	177±3	<0.1
SP23	174±3	<0.1	120±7	<0.1
SP24	407±18	<0.1	143±6	<0.1
SP25	1042±80	<0.1	164±5	<0.1
SP26	625±26	16.8±0.8	88±1	53.6±0.8
SP27	731±9	27.6±2.0	105±3	56.6±0.8
SP28	1580±73	<0.1	241±36	<0.1
SP29	454±11	15.0±2.1	211±3	37.5±0.8
SP30	578±11	20.0±0.0	192±2	40.3±2.3

Table 9. Trace elements (Cd, Pb) concentrations (mg kg^{-1}) in soil samples. Concentrations are expressed as means ± standard deviations

Sample point	Cd (total)	Cd (available)	Pb (total)	Pb (available)
SP1	2.4±0.3	<0.1	75.2±6.4	<0.1
SP2	2.3±0.1	<0.1	11.5±1.3	5.3±0.6
SP3	2.3±0.1	<0.1	66.8±0.8	<0.1
SP4	2.6±0.1	<0.1	15.9±3.3	<0.1
SP5	4.2±0.3	<0.1	43.8±2.9	15.6±0.9
SP6	4.0±0.1	<0.1	26.6±1.6	0.5±0.1
SP7	3.4±0.1	<0.1	33.9±0.9	5.1±0.5
SP8	2.6±0.1	<0.1	28.1±1.7	2.5±0.5
SP9	4.1±0.1	1.3±0.1	95.4±8.1	17.6±0.9
SP10	<0.1	<0.1	22.0±1.6	<0.1
SP11	<0.1	<0.1	15.9±3.3	<0.1
SP12	<0.1	<0.1	66.8±0.8	<0.1
SP13	<0.1	<0.1	49.2±4.8	<0.1
SP14	3.2±0.2	<0.1	75.5±2.4	3.7±0.5
SP15	3.4±0.1	<0.1	120±5	38.4±1.0
SP16	<0.1	2.4±0.1	29.5±3.7	<0.1
SP17	<0.1	<0.1	23.5±5.5	<0.1
SP18	<0.1	<0.1	26.7±4.4	<0.1
SP19	<0.1	<0.1	29.5±3.7	<0.1
SP20	<0.1	<0.1	22.0±1.6	<0.1
SP21	<0.1	<0.1	66.8±0.8	<0.1
SP22	<0.1	<0.1	66.1±2.9	<0.1
SP23	<0.1	<0.1	11.5±1.3	5.3±0.6
SP24	<0.1	<0.1	54.1±2.1	<0.1
SP25	<0.1	<0.1	30.7±3.7	<0.1
SP26	2.5±0.1	<0.1	130±6	25.5±1.3
SP27	3.9±0.2	<0.1	50.9±0.6	7.8±0.4
SP28	<0.1	<0.1	75.2±6.4	<0.1
SP29	3.5 ±0.1	0.4±0.1	77.4±2.1	<0.1
SP30	4.5±0.2	0.4±0.2	95.2±2.7	<0.1

4. 2. Chemical composition of the plant material

Concentrations of P_2O_5, K_2O and major element (Fe) in the roots, shoots and leaves of *A. murale* populations are presented in Table 10. Concentrations of P_2O_5 varied from 500 mg kg^{-1} (SP3) to 2550 mg kg^{-1} (SP5) in the roots of *A. murale*, while in the shoots it was quite similar and within the range from 610 mg kg^{-1} (SP2) to 3240 mg kg^{-1} (SP9). K_2O contents in the roots varied from 1310 mg kg^{-1} (SP9) to 15950 mg kg^{-1} (SP8) and in the shoots from 1230 mg kg^{-1} (SP9) to 17440 mg kg^{-1} (SP8) Concentrations of Fe in all plant samples (except in the leaves of SP10) were generally below 1000 mg kg^{-1}; in the roots it was from 70 mg kg^{-1} (SP5) to 930 mg kg^{-1} (SP1), in the shoots it varied from 160 mg kg^{-1} (SP8) to 720 mg kg^{-1} (SP1) and in the leaves it was in the range of 200 mg kg^{-1} (SP5) to 1010 mg kg^{-1} (SP1).

Major elements (Ca, Mg) contents in the roots, shoots and leaves of *A. murale* populations are given in Table 11. 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 and varied between 3400 mg kg^{-1} (SP9) and 7920 mg kg^{-1} (Sp2) in the roots, from 8130 mg kg^{-1} (SP9) to 36920 mg kg^{-1} (SP5) in the shoots and from 17190 (SP3) mg kg^{-1} to 73250 mg kg^{-1} (SP6) in the leaves of these plants. On the contrary, concentration of available Mg in the soil samples was followed by only slightly higher Mg content in all plants samples. The concentration of this element varied from 2750 mg kg^{-1} (SP9) to 5420 mg kg^{-1} (SP1) in the roots, from 2220 mg kg^{-1} (SP9) to 9260 mg kg^{-1} (SP4) in the shoots and from 4260 mg kg^{-1} (SP5) to 11800 mg kg^{-1} (SP4) in the leaves.

In the roots, shoots and leaves of all *A. murale* samples Ca/Mg ratio >1 was noticed. In the roots this ratio was quite low (from 1.04 to 2.25), in the shoots it was much higher (from 1.53 to 9.25), but the highest value was noticed in *A. murale* leaves in SP6 (12.83) and SP5 (15.23). High Ca contents in the species plant tissues are the consequence of 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 a characteristic of serpentines, which would indicate lower Ca deficiency stress for the plants.

Table 10. P_2O_5 and K_2O and major element (Fe) concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations

Species	**Sample point**	**P_2O_5 root**	**P_2O_5 shoot**	**K_2O root**	**K_2O shoot**	**Fe root**	**Fe shoot**	**Fe leaves**
Alyssum murale	SP1	550±70	1190±30	8710±410	8890±190	930±140	720±150	1010±190
Alyssum murale	SP2	705±80	610±210	12990±630	12090±480	340±60	640±130	490±30
Alyssum murale	SP3	500±140	820±40	9730±1090	9550±380	500±120	250±20	270±20
Alyssum murale	SP4	960±70	1000±40	10390±170	13380±70	410±30	610±40	660±70
Alyssum murale	SP5	2550±50	2020±50	15220±540	14680±190	70±40	410±30	200±30
Alyssum murale	SP6	1090±140	1110±50	9660±130	7470±240	710±50	520±20	540±20
Alyssum murale	SP7	1910±110	1400±30	12110±220	15350±270	320±50	600±40	450±40
Alyssum murale	SP8	2340±190	1560±80	15950±470	17440±120	110±10	160±20	320±10
Alyssum murale	SP9	2080±20	3240±780	1310±90	1230±50	800±240	200±70	460±30

Table 11. Major elements (Ca, Mg) concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations

Species	Sample point	Ca root	Ca shoot	Ca leaves	Mg root	Mg shoot	Mg leaves	Ca/Mg root	Ca/Mg shoot	Ca/Mg leaves
Alyssum murale	SP1	5610±70	8140±250	19540±1900	5420±230	4370±80	6480±840	1.04	1.86	3.02
Alyssum murale	SP2	7920±480	18250±650	35350±2320	3940±120	8330±740	9340±510	2.01	2.19	3.78
Alyssum murale	SP3	6460±640	10650±930	17190±7880	3950±300	4650±360	5150±830	1.64	2.29	3.34
Alyssum murale	SP4	4810±270	14210±490	19360±880	3740±180	9260±130	11800±790	1.29	1.53	1.64
Alyssum murale	SP5	6180±250	36920±890	64900±5240	3050±160	3990±120	4260±1130	2.03	9.25	15.23
Alyssum murale	SP6	5980±50	34590±1550	73250±6110	5040±50	4970±60	5710±2050	1.19	6.96	12.83
Alyssum murale	SP7	7670±170	33990±500	61040±5400	3750±40	8110±20	9650±2490	2.05	4.19	6.33
Alyssum murale	SP8	6860±40	35700±1070	64900±2570	3050±160	6870±130	7750±390	2.25	5.20	8.37
Alyssum murale	SP9	3400±610	8130±1450	17280±7930	2750±1090	2220±880	6390±810	1.24	3.66	2.70

Concentrations of P_2O_5, K_2O and major element (Fe) in the roots and shoots of other investigated Brassicaceae plant species are presented in Table 12. Concentrations of P_2O_5 varied from 430 mg kg^{-1} (*A. saxatile* at SP12) to 3530 mg kg^{-1} (*T. praecox* at SP28) in the roots; in the shoots it was quite similar and in the range of 600 mg kg^{-1} (*A. saxatile* at SP12) and 3400 mg kg^{-1} (*E. linariifolium* at SP20). K_2O contents were much higher both in the roots and shoots of all populations; in the roots it varied from 6250 mg kg^{-1} (*T. praecox* at SP29) to 32460 mg kg^{-1} (*I. tinctoria* at SP24) and in the shoots from 10820 mg kg^{-1} (*A. repens* at SP16) to 41950 mg kg^{-1} (*I. tinctoria* at SP23). The lowest concentrations of Fe were in the roots of *A. saxatile* at SP12 (340 mg kg^{-1}) and in the shoots of *E. linariifolium* at SP21 (150 mg kg^{-1}), while the highest (both in the roots and in the shoots) were detected in *T. praecox* at SP30 (19390 mg kg^{-1} and 3880 mg kg^{-1} respectively).

Major elements (Ca, Mg) contents in the roots and shoots of other investigated Brassicaceae plant species are given in Table 13. Concentrations of Ca in the roots and shoots of all the investigated plant samples were several times higher than available Ca content (310–5100 mg kg^{-1}) in the soil samples and varied between 1600 mg kg^{-1} (*E. carniolicum* at SP22) and 8590 mg kg^{-1} (*A. repens* at SP16) in the roots, and from 2030 mg kg^{-1} (*C. plumieri* at SP17) to 43410 mg kg^{-1} (*A. montanum* at SP15) in the shoots. Mg content varied from 3370 mg kg^{-1} (*A. montanum* at SP13) to 20220 mg kg^{-1} (*T. praecox* at SP30) in the roots and from 4860 mg kg^{-1} (*E. carniolicum* at SP22) to 15070 mg kg^{-1} (*I. tinctoria* at SP23) in the shoots.

Generally, the concentrations of Ca were 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 the roots and shoots of some samples of *A. montanum*, *A. repens*, *E. linariifolium* as well as *R. lippizensis* and *T. praecox* (shoots only) the Ca/Mg ratio >1 (1.12–6.06) was noticed. Higher Ca contents in the roots and/or shoots of these five 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 Ca/Mg ratios that are a characteristic of serpentines.

Table 12. P_2O_5, K_2O and major element (Fe) concentrations (mg kg^{-1}) in plant tissues of different Brassicaceae species. Concentrations are expressed as means ± standard deviations

Species	Sample point	P_2O_5 root	P_2O_5 shoot	K_2O root	K_2O shoot	Fe root	Fe shoot
Aethionema saxatile	SP10	2400±200	2830±150	11240±180	14110±60	870±10	300±10
Aethionema saxatile	SP11	1900±60	2100±200	17680±820	24970±200	530±30	260±20
Aethionema saxatile	SP12	430±60	600±0	11740±670	15410±130	340±20	670±140
Alyssum montanum	SP13	1200±300	740±30	10610±930	12240±390	700±70	1820±20
Alyssum montanum	SP14	1420±110	1370±70	13230±180	14510±160	1860±90	1370±30
Alyssum montanum	SP15	1520±50	1090±60	14670±240	13450±210	1310±50	450±20
Alyssum repens	SP16	660±100	810±60	10400±480	10820±250	470±40	570±30
Cardamine plumieri	SP17	2800±260	1900±170	16870±480	18910±200	1490±80	1000±100
Cardamine plumieri	SP18	1600±100	2000±170	28790±730	38720±860	1400±90	1330±30
Cardamine plumieri	SP19	1200±100	1200±300	19850±480	27460±880	1600±90	1320±40
Erysimum linariifolium	SP20	3430±210	3400±200	11720±140	13930±190	800±60	860±30
Erysimum linariifolium	SP21	1200±300	2000±200	12020±550	21950±1180	450±100	150±50
Erysimum carniolicum	SP22	1730±110	1990±230	16340±60	18430±610	960±50	1070±80
Isatis tinctoria	SP23	1130±100	2000±0	29950±1520	41950±620	740±80	760±30
Isatis tinctoria	SP24	1900±100	2100±200	32460±290	35790±3800	800±70	730±50
Rorippa lippizensis	SP25	1170±150	1770±230	14530±490	16820±460	1630±60	450±20
Thlaspi kovatsii	SP26	870±60	460±40	20940±280	27250±250	1020±120	890±30
Thlaspi kovatsii	SP27	490±20	300±30	11570±460	19490±850	1720±80	470±60
Thlaspi praecox	SP28	3530±250	1200±200	16160±340	21340±160	1370±70	690±20
Thlaspi praecox	SP29	24207±600	690±40	6250±420	41370±370	12710±650	1220±30
Thlaspi praecox	SP30	2560±90	1220±360	9030±420	37940±1309	19390±10	3880±420

Table 13. Major elements (Ca, Mg) concentrations (mg kg^{-1}) in plant tissues of different Brassicaceae species. Concentrations are expressed as means ± standard deviations

Species	Sample point	Ca root	Ca shoot	Mg root	Mg shoot	Ca/Mg root	Ca/Mg shoot
Aethionema saxatile	SP10	4450±280	9660±90	6840±120	10640±150	0.65	0.91
Aethionema saxatile	SP11	4010±320	8710±270	5430±170	12760±300	0.74	0.68
Aethionema saxatile	SP12	3490±280	4950±290	5820±270	9460±290	0.60	0.52
Alyssum montanum	SP13	7280±320	20710±480	3370±180	11280±490	2.16	1.84
Alyssum montanum	SP14	6580±110	34890±1480	9920±200	11150±190	0.66	3.13
Alyssum montanum	SP15	7900±140	43410±1290	10080±340	7160±30	0.78	6.06
Alyssum repens	SP16	8590±410	14310±390	5020±200	5990±50	1.71	2.39
Cardamine plumieri	SP17	6910±330	2030±160	10790±320	12170±320	0.64	0.17
Cardamine plumieri	SP18	6770±310	4440±180	10020±260	14080±90	0.68	0.32
Cardamine plumieri	SP19	4320±120	7720±310	10290±490	13290±200	0.42	0.58
Erysimum linariifolium	SP20	7590±330	3750±170	6330±130	7540±240	1.20	0.50
Erysimum linariifolium	SP21	7590±340	12160±360	4100±170	5010±370	1.85	2.43
Erysimum carniolicum	SP22	1600±210	3900±130	5230±540	4860±170	0.31	0.80
Isatis tinctoria	SP23	4240±310	8730±460	10670±120	15070±140	0.40	0.58
Isatis tinctoria	SP24	4190±150	4110±270	8070±80	8330±80	0.52	0.49
Rorippa lippizensis	SP25	6800±270	8650±270	9950±290	7710±290	0.68	1.12
Thlaspi kovatsii	SP26	3410±90	6020±40	6760±230	7160±30	0.50	0.84
Thlaspi kovatsii	SP27	4030±150	5390±80	10110±210	7310±250	0.39	0.74
Thlaspi praecox	SP28	3250±220	7690±110	6370±370	7040±430	0.51	1.09
Thlaspi praecox	SP29	5770±350	4400±120	17060±1110	7220±540	0.34	0.61
Thlaspi praecox	SP30	3580±560	6150±230	20220±210	10370±230	0.18	0.59

Trace elements (Ni, Zn) concentrations in *A. murale* plants are shown in Table 14. Contents of Ni were lying in the range 241 mg kg^{-1} (SP8) to 2926 mg kg^{-1} (SP9) in the roots, from 549 mg kg^{-1} (SP8) to 6793 mg kg^{-1} (SP9) in the shoots and from 733 mg kg^{-1} (SP8) to 13160 mg kg^{-1} (SP9) in the leaves. The concentration of Zn was the highest in the roots, from 31.7 mg kg^{-1} (SP7) to 564 mg kg^{-1} (SP2). Both in the shoots (from 22.6 mg kg^{-1} (SP7) to 287 mg kg^{-1} (SP2)) and in the leaves (from 11.8 mg kg^{-1} (SP5) to 115 mg kg^{-1} (SP1)) the concentrations were much lower.

Contents of trace elements (Mn, Cu) in *A. murale* plants are shown in Table 15. The lowest concentrations of Mn in *A. murale* samples were in the roots, from 4.4 mg kg^{-1} (SP4) to 29 mg kg^{-1} (SP1). In the shoots it varied from 5 mg kg^{-1} (SP9) to 107.2 mg kg^{-1} (SP8) and in the leaves it was in the range from 15.7 mg kg^{-1} (SP4) to 126.1 mg kg^{-1} (SP8). Contents of Cu were very variable; in the roots and shoots it varied from <0.1 mg kg^{-1} (SP5) to 1044 mg kg^{-1} (SP2) and from <0.1 mg kg^{-1} (SP5) to 1127 mg kg^{-1} (SP3) respectively, while in the leaves of four samples this element was found only in traces (up to 7.6 mg kg^{-1} at SP8).

Trace elements (Cr, Co) contents in *A. murale* roots, shoots and leaves are presented in Table 16. Contents of Cr and Co in all the investigated plants parts were very low. Only in some plant samples small amounts of Cr (up to 27.5 mg kg^{-1} in roots and shoots at SP7 and SP8) were found. It is evident that Co contents in all the plant samples were below 0.1 mg kg^{-1}.

Concentrations of trace elements (Cd, Pb) in *A. murale* roots, shoots and leaves are presented in the Table 17. Contents of Cd and Pb in all the investigated plants parts were very low. Only in some plant samples small amounts of Pb (up to 19.3 mg kg^{-1} in leaves at SP2) were found. Pb contents in all the plant samples were below 0.1 mg kg^{-1}.

Table 14. Trace elements (Ni, Zn) concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations

Species	Sample point	Ni root	Ni shoot	Ni leaves	Zn root	Zn shoot	Zn leaves
Alyssum murale	SP1	1712±107	2177±32	7664±713	466±50	223±16	115±18
Alyssum murale	SP2	1643±142	2198±85	5026±495	564±82	287±32	21.6±6.6
Alyssum murale	SP3	1813±248	2422±40	6478±1162	101±21	277±27	75.9±6.4
Alyssum murale	SP4	777±23	1680±38	2031±277	248±27	113±9	19.7±4.9
Alyssum murale	SP5	508±48	687±39	943±47	68.4±1.7	54.5±2.5	11.8±1.1
Alyssum murale	SP6	877±14	1820±14	4459±171	221±10	86.8±5.9	21.3±2.8
Alyssum murale	SP7	862±22	1184±51	1616±114	31.7±6.2	22.6±6.6	20.4±2.2
Alyssum murale	SP8	241±19	549±27	733±32	44.0±4.4	43.3±6.1	26.1±0.4
Alyssum murale	SP9	2926±472	6793±1201	13160±1652	50.9±6.2	46.8±4.3	33.3±7.6

Table 15. Trace elements (Mn, Cu) concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations

Species	Sample point	Mn root	Mn shoot	Mn leaves	Cu root	Cu shoot	Cu leaves
Alyssum murale	SP1	29.0±2.5	37.6±4.3	93.8±3.7	923±58	573±13	<0.1
Alyssum murale	SP2	14.6±2.6	50.6±3.3	30.0±3.9	1044±60	849±30	<0.1
Alyssum murale	SP3	7.9±2.6	15.8±1.3	19.7±8.1	159±19	1127±49	<0.1
Alyssum murale	SP4	4.4±1.1	46.8±1.3	15.7±7.3	236±18	440±31	<0.1
Alyssum murale	SP5	11.8±1.8	31.8±2.7	18.5±1.9	<0.1	<0.1	2.5±0.6
Alyssum murale	SP6	41.8±3.1	77.8±0.8	87.4±6.1	1023±60	354±7	7.3±0.6
Alyssum murale	SP7	22.0±1.8	69.1±3.7	68.2±4.2	84±7	78±2	5.0±1.0
Alyssum murale	SP8	22.7±1.8	107.2±5.9	126.1±2.9	89±7	18.3±1.7	7.6±1.0
Alyssum murale	SP9	11.8±9.5	5.0±1.7	39.2±4.9	3.6±0.6	3.9±0.6	3.2±0.7

Table 16. Trace elements (Cr, Co) concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations

Species	Sample point	Cr root	Cr shoot	Cr leaves	Co root	Co shoot	Co leaves
Alyssum murale	SP1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP5	13.4±0.2	27.2±0.4	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP6	13.7±0.2	27.5±0.4	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP7	27.5±0.2	27.2±0.4	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP8	27.5±0.4	27.5±0.4	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Table 17. Trace elements (Cd, Pb) concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues. Concentrations are expressed as means ± standard deviations

Species	Sample point	Cd root	Cd shoot	Cd leaves	Pb root	Pb shoot	Pb leaves
Alyssum murale	SP1	5.4±1.7	<0.1	16.5±1.3	<0.1	<0.1	<0.1
Alyssum murale	SP2	6.4±1.4	7.2±1.0	19.3±0.9	<0.1	<0.1	<0.1
Alyssum murale	SP3	3.5±0.1	<0.1	16.0±0.5	<0.1	<0.1	<0.1
Alyssum murale	SP4	<0.1	<0.1	16.0±0.1	<0.1	<0.1	<0.1
Alyssum murale	SP5	<0.1	6.9±1.8	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP6	4.5±0.7	<0.1	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP7	<0.1	4.0±0.6	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP8	12.2±0.2	4.8±1.2	<0.1	<0.1	<0.1	<0.1
Alyssum murale	SP9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Trace elements concentrations (Ni, Zn) in the roots and shoots of other investigated Brassicaceae plant species are given in Table 18. In plant samples concentrations of accumulated Ni was in the range < 0.1–1946 mg kg^{-1} in the roots and in the range < 0.1–11671 mg kg^{-1} in the shoots. In *A. saxatile* (SP10, SP12) and *R. lippizensis* (SP25) Ni was found only in traces, while in the roots (up to 657 mg kg^{-1}) and shoots (up to 676 mg kg^{-1}) of *A. repens* at SP16 Ni contents were high and very unusual for the species that belong to section *Alyssum*. Contrary, detected Ni content was several times higher both in the shoots and roots of *T. praecox* (SP29, SP30) and *T. kovatsii* (SP26, SP27), with values up to 1946 mg kg^{-1} in the roots and 11671 mg kg^{-1} in the shoots. Besides in *Thlaspi* species, contents of Zn were laying in the range 12.3–897 mg kg^{-1} in the roots and 7.3–87.2 mg kg^{-1} in the shoots of most of the analysed samples. Comparing to low Zn content in soil samples, in the roots and shoots of all plant samples Zn concentration was much higher. The highest concentrations of this trace element were detected in the roots and shoots of four samples of *Thlaspi* species with a range 467–897 mg kg^{-1} and 658–1086 mg kg^{-1} respectively.

Contents of trace elements (Mn, Cu) in the roots and shoots of other investigated Brassicaceae plant species are shown in Table 19. Although Mn content in soil samples was relatively high, its concentration in plant tissues was several times lower; in the roots Mn content was in the range from 7.6 mg kg^{-1} (*A. repens* at SP16) to 960 mg kg^{-1} (*T. praecox* at SP30) and in the shoots it varied from 13.2 mg kg^{-1} (*A. repens* at SP16) to 120 mg kg^{-1} (*C. plumieri* at SP17). In the roots of *T. praecox* (SP30) much higher concentration (460 mg kg^{-1}) was recorded, while in the shoots of the same species (SP29) this element was found only in traces. Contents of Cu were generally low; in the roots and shoots it were from <0.1 mg kg^{-1} to 78.6 mg kg^{-1} and from <0.1 mg kg^{-1} to 50.8 mg kg^{-1} respectively. Only in the roots and shoots of *A. repens* (SP16) much higher concentrations (178 mg kg^{-1} and 143 mg kg^{-1}) of this element were recorded.

Concentrations of four trace elements (Cr, Co, Cd, Pb) in the roots and shoots of other investigated Brassicaceae plant species are presented in Tables 20 and 21. Contents of Cr and Co in most of the investigated plants were below 0.1 mg kg^{-1}. In only 8 root and shoot samples higher Cr contents were found (up to 91.1 mg kg^{-1}), as well as in the case of *Thlaspi* samples where Co content in roots and shoots was up to 114 mg kg^{-1}. In 11 plant samples Cd was detected in higher concentrations (up to 53.3 mg kg^{-1}), while Pb content was higher in five plant samples (up to 67.4 mg kg^{-1}).

Table 18. Trace elements (Ni, Zn) concentrations (mg kg^{-1}) in plant tissues of different Brassicaceae species. Concentrations are expressed as means ± standard deviations

Species	**Sample point**	**Ni root**	**Ni shoot**	**Zn root**	**Zn shoot**
Aethionema saxatile	SP10	20.4±4.2	<0.1	44.8±3.9	52.9±2.5
Aethionema saxatile	SP11	84.3±4.6	41.8±13.6	12.7±1.5	11.2±2.7
Aethionema saxatile	SP12	<0.1	<0.1	12.3±1.8	7.3±0.8
Alyssum montanum	SP13	296±7	350±13	19.9±3.0	63.7±2.5
Alyssum montanum	SP14	31.6±17.9	11.6±4.1	22.8±1.8	32.9±3.0
Alyssum montanum	SP15	96.4±17.5	69.4±9.3	30.7±3.3	20.2±2.0
Alyssum repens	SP16	657±78	676±8	62.3±0.4	50.9±2.5
Cardamine plumieri	SP17	76.5±7.6	30.2±0.3	80.1±2.3	87.2±3.4
Cardamine plumieri	SP18	111±9	99.8±16.2	33.3±1.9	30.5±2.2
Cardamine plumieri	SP19	84.1±12.9	72.6±16.1	57.9±2.6	44.8±1.0
Erysimum linariifolium	SP20	22.7±7.1	42.6±4.3	47.9±7.1	111±20
Erysimum linariifolium	SP21	91.2±9.2	41.8±8.3	25.9±4.0	31.7±1.9
Erysimum carniolicum	SP22	10.5±8.4	12.9±4.1	47.0±6.2	67.2±4.3
Isatis tinctoria	SP23	81.6±7.2	89.6±31.0	29.5±7.7	18.8±3.7
Isatis tinctoria	SP24	113±7	152±1	62.0±4.8	22.6±2.4
Rorippa lippizensis	SP25	104±10	<0.1	107±9	77.6±7.5
Thlaspi kovatsii	SP26	1006±23	3379±92	502±3	1059±44
Thlaspi kovatsii	SP27	916±37	3082±80	75.5±5.8	87.1±4.3
Thlaspi praecox	SP28	141±7	756±6	467±40	917±90
Thlaspi praecox	SP29	1769±293	11671±301	897±3	1086±152
Thlaspi praecox	SP30	1946±93	9244±505	874±12	658±148

Table 19. Trace elements (Mn, Cu) concentrations (mg kg^{-1}) in plant tissues of different Brassicaceae species. Concentrations are expressed as means ± standard deviations

Species	**Sample point**	**Mn root**	**Mn shoot**	**Cu root**	**Cu shoot**
Aethionema saxatile	SP10	25.7±2.9	16.2±1.6	<0.1	3.7±0.4
Aethionema saxatile	SP11	25.4±0.8	35.2±0.1	2.1±0.1	3.2±0.01
Aethionema saxatile	SP12	16.8±3.1	20.0±3.9	5.7±0.3	4.6±0.1
Alyssum montanum	SP13	10.1±1.7	49.0±5.0	1.2±0.1	28.8±4.9
Alyssum montanum	SP14	83.0±6.6	72.6±5.7	3.8±0.6	6.5±1.1
Alyssum montanum	SP15	46.7±3.9	25.2±1.0	14.3±0.6	8.7±0.5
Alyssum repens	SP16	7.6±2.3	13.2±1.4	178±12	143±9
Cardamine plumieri	SP17	125±3	120±6	<0.1	<0.1
Cardamine plumieri	SP18	96.6±6.6	100±2	6.5±0.0	5.4±0.1
Cardamine plumieri	SP19	76.4±5.0	90.0±5.4	3.2±0.1	3.2±0.1
Erysimum linariifolium	SP20	15.2±3.4	33.9±2.8	<0.1	16.4±7.5
Erysimum linariifolium	SP21	34.4±2.6	26.4±1.5	3.2±0.1	3.2±0.1
Erysimum carniolicum	SP22	23.5±5.1	23.9±3.4	10.5±2.9	19.1±2.8
Isatis tinctoria	SP23	40.6±3.7	43.5±1.6	17.7±1.9	16.0±2.0
Isatis tinctoria	SP24	31.5±2.0	31.5±1.8	3.2±0.1	21.0±1.8
Rorippa lippizensis	SP25	63.5±9.8	17.4±0.8	78.6±7.1	19.8±2.2
Thlaspi kovatsii	SP26	75.0±11.7	60.9±4.5	10.8±0.7	6.6±0.1
Thlaspi kovatsii	SP27	87.0±1.6	22.2±5.4	1.4±0.6	9.6±1.2
Thlaspi praecox	SP28	62.7±4.6	49.2±1.4	<0.1	<0.1
Thlaspi praecox	SP29	158±12	<0.1	71.0±1.1	48.9±0.2
Thlaspi praecox	SP30	460±36	102±5	78.0±6.3	50.8±1.4

Table 20. Trace elements (Cr, Co) concentrations (mg kg^{-1}) in plant tissues of different Brassicaceae species. Concentrations are expressed as means ± standard deviations

Species	**Sample point**	**Cr root**	**Cr shoot**	**Co root**	**Co shoot**
Aethionema saxatile	SP10	<0.1	<0.1	<0.1	<0.1
Aethionema saxatile	SP11	<0.1	<0.1	<0.1	<0.1
Aethionema saxatile	SP12	<0.1	<0.1	<0.1	<0.1
Alyssum montanum	SP13	<0.1	<0.1	<0.1	<0.1
Alyssum montanum	SP14	72.0±7.8	83.3±0	<0.1	<0.1
Alyssum montanum	SP15	46.3±8.0	32.1±8.2	<0.1	<0.1
Alyssum repens	SP16	<0.1	<0.1	<0.1	<0.1
Cardamine plumieri	SP17	<0.1	<0.1	<0.1	<0.1
Cardamine plumieri	SP18	<0.1	<0.1	<0.1	<0.1
Cardamine plumieri	SP19	<0.1	<0.1	<0.1	<0.1
Erysimum linariifolium	SP20	<0.1	<0.1	<0.1	<0.1
Erysimum linariifolium	SP21	<0.1	<0.1	<0.1	<0.1
Erysimum carniolicum	SP22	<0.1	<0.1	<0.1	<0.1
Isatis tinctoria	SP23	<0.1	<0.1	<0.1	<0.1
Isatis tinctoria	SP24	<0.1	<0.1	<0.1	<0.1
Rorippa lippizensis	SP25	<0.1	<0.1	<0.1	<0.1
Thlaspi kovatsii	SP26	75.9±0.6	29.9±0.5	<0.1	10.3±2.4
Thlaspi kovatsii	SP27	91.1±0.9	30.2±0.4	<0.1	<0.1
Thlaspi praecox	SP28	<0.1	<0.1	<0.1	<0.1
Thlaspi praecox	SP29	<0.1	<0.1	86.9±2.8	<0.1
Thlaspi praecox	SP30	<0.1	<0.1	114±5	23.2±5.7

Table 21. Trace elements (Cd, Pb) concentrations (mg kg^{-1}) in plant tissues of different Brassicaceae species. Concentrations are expressed as means ± standard deviations

Species	**Sample point**	**Cd root**	**Cd shoot**	**Pb root**	**Pb shoot**
Aethionema saxatile	SP10	<0.1	<0.1	<0.1	<0.1
Aethionema saxatile	SP11	<0.1	<0.1	<0.1	<0.1
Aethionema saxatile	SP12	<0.1	<0.1	<0.1	<0.1
Alyssum montanum	SP13	<0.1	<0.1	<0.1	<0.1
Alyssum montanum	SP14	<0.1	<0.1	<0.1	<0.1
Alyssum montanum	SP15	7.0±0.7	0.8±0.1	<0.1	<0.1
Alyssum repens	SP16	2.8±0.7	0.2±0.1	<0.1	<0.1
Cardamine plumieri	SP17	<0.1	<0.1	<0.1	<0.1
Cardamine plumieri	SP18	<0.1	<0.1	<0.1	<0.1
Cardamine plumieri	SP19	<0.1	<0.1	<0.1	<0.1
Erysimum linariifolium	SP20	<0.1	<0.1	<0.1	<0.1
Erysimum linariifolium	SP21	<0.1	<0.1	<0.1	<0.1
Erysimum carniolicum	SP22	<0.1	<0.1	<0.1	<0.1
Isatis tinctoria	SP23	<0.1	<0.1	<0.1	<0.1
Isatis tinctoria	SP24	<0.1	<0.1	<0.1	<0.1
Rorippa lippizensis	SP25	<0.1	<0.1	<0.1	<0.1
Thlaspi kovatsii	SP26	8.4±0.1	6.7±0.7	67.4±5.3	<0.1
Thlaspi kovatsii	SP27	<0.1	9.9±0.6	41.0±4.8	40.9±5.4
Thlaspi praecox	SP28	<0.1	<0.1	<0.1	<0.1
Thlaspi praecox	SP29	34.0±7.8	9.3±0.9	13.3±0.5	<0.1
Thlaspi praecox	SP30	53.3±5.2	12.3±0.1	14.3±1.4	<0.1

4. 3. Comparison among metal concentrations in soils and plant tissues and assessment of the accumulation capacity of the Brassicaceae plant species

Comparison among available concentrations of Fe in soils and in *Alyssum murale* s.l. plant tissues (roots, shoots and leaves) and assessment of accumulation capacity for Fe are presented in Figure 4(a) and Table 22. Ratio between Fe concentrations in root and soil revealed great variation among analysed samples; the lowest ratio was 0.1 (SP5) and highest one 7.8 (SP1). Translocation factor was between 0.5 (SP3) and 2.9 (SP5, SP8), while Fe accumulation factor also showed great variation; the lowest one was 0.2 (SP5) and the highest was 8.4 (SP1). It is interesting to note that in the case of *A. murale* at SP5, root/soil ratio was the lowest, TF was the highest, and AF was the lowest. In the case of *A. murale* at SP1 the opposite trend was noticed; root/soil ratio was the highest, TF was the lowest, and AF was the highest of all the samples analysed.

Comparison among available contents of Ca in soils and in *Alyssum murale* s.l. roots, shoots and leaves, as well as assessment of accumulation capacity for Ca are given in Figure 4(b) and Table 22. Ratio between Ca concentrations in root and soil also showed great variation among analysed samples; the lowest ratio was 1.9 (SP6) and highest one 25.6 (SP2). Translocation factor was between 2.7 (SP3) and 12.2 (SP6), while Ca accumulation factor also showed great variation; the lowest one was 8.0 (SP3) and the highest was 114 (SP2). In the case of *A. murale* at SP2, root/soil ratio was the highest, TF was quite low, and AF was again the highest of all the samples analysed. In the case of *A. murale* at SP6 the opposite trend was noticed, since the root/soil ratio was the lowest and TF was the highest. *A. murale* at SP3 showed the lowest values both for TF and AF among all the samples.

Comparison among available concentrations of Mg in soils and in *Alyssum murale* s.l. plant tissues and assessment of accumulation capacity for Mg are shown in Figure 4(c) and Table 22. The lowest ratio between Mg concentrations in root and soil was 0.5 (SP7) and highest one 10.1 (SP6). Translocation factor was between 1.1 (SP6) and 3.2 (SP4), while Mg accumulation factor showed great variation; the lowest one was 1.2 (SP7) and the highest was 11.4 (SP6). In the case of *A. murale* at SP6, root/soil ratio was the highest, TF was the lowest, and AF was again the highest. The opposite trend was noticed in the case of *A. murale* at SP7, since the root/soil ratio was the lowest, TF was among the highest and AF was the lowest of all the samples analysed.

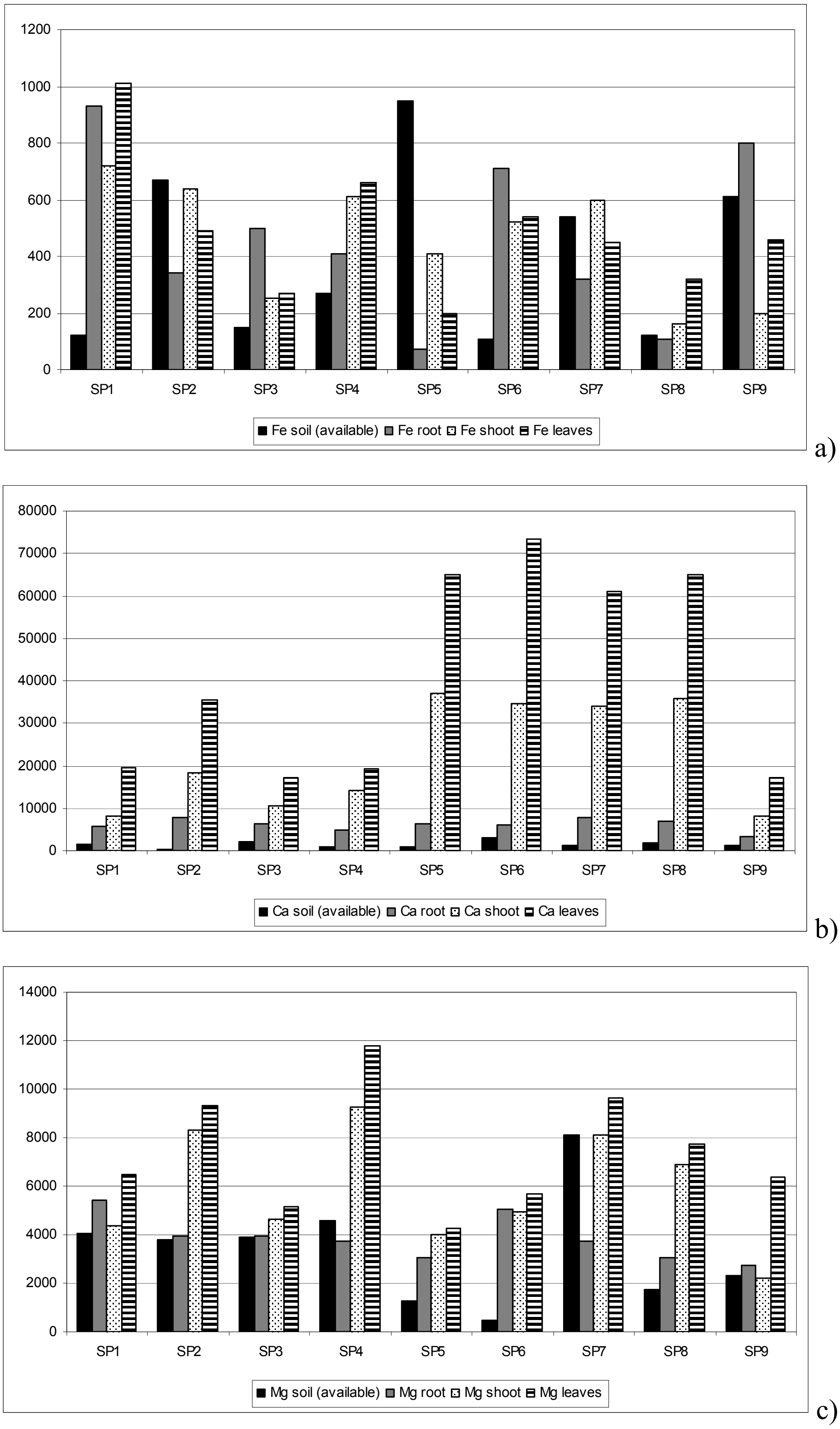

Figure 4. Comparison among available concentrations (mg kg^{-1}) of major elements - Fe **(a)**, Ca **(b)**, Mg **(c)** in soils and concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues (roots, shoots and leaves).

Table 23. Accumulation potential for Fe, Ca and Mg of *Alyssum murale* s.l. samples. The translocation factor (TF) is the leaves-to-root ratio of trace elements concentration. The accumulation factor (AF) is the ratio between trace elements concentration in leaves and their bioavailable concentration in the corresponding soil

Species	**Sample point**	**Fe root/soil**	**Fe TF**	**Fe AF**	**Ca root/soil**	**Ca TF**	**Ca AF**	**Mg root/soil**	**Mg TF**	**Mg AF**
Alyssum murale	SP1	7.8	1.1	8.4	4.1	3.5	14.4	1.3	1.2	1.6
Alyssum murale	SP2	0.5	1.4	0.7	25.6	4.5	114	1.0	2.4	2.5
Alyssum murale	SP3	3.3	0.5	1.8	3.0	2.7	8.0	1.0	1.3	1.3
Alyssum murale	SP4	1.5	1.6	2.4	5.3	4.0	21.5	0.8	3.2	2.6
Alyssum murale	SP5	0.1	2.9	0.2	7.0	10.5	73.8	2.5	1.4	3.4
Alyssum murale	SP6	6.5	0.8	4.9	1.9	12.2	23.6	10.1	1.1	11.4
Alyssum murale	SP7	0.6	1.4	0.8	7.1	8.0	56.5	0.5	2.6	1.2
Alyssum murale	SP8	0.9	2.9	2.7	3.9	9.5	36.9	1.7	2.5	4.4
Alyssum murale	SP9	1.3	0.6	0.8	2.8	5.1	14.0	1.2	2.3	2.8

Table 23. Accumulation potential for Ni, Zn and Mn of *Alyssum murale* s.l. samples. The translocation factor (TF) is the leaves-to-root ratio of trace elements concentration. The accumulation factor (AF) is the ratio between trace elements concentration in leaves and their bioavailable concentration in the corresponding soil

Species	**Sample point**	**Ni root/soil**	**Ni TF**	**Ni AF**	**Zn root/soil**	**Zn TF**	**Zn AF**	**Mn root/soil**	**Mn TF**	**Mn AF**
Alyssum murale	SP1	9.5	4.5	42.3	41.6	0.25	10.3	0.10	3.2	0.31
Alyssum murale	SP2	21.9	3.1	67.0	1411	0.04	54.0	0.17	2.1	0.36
Alyssum murale	SP3	10.6	3.6	37.9	8.3	0.75	6.2	0.03	2.5	0.08
Alyssum murale	SP4	11.6	2.6	30.3	138	0.08	10.9	0.04	3.6	0.16
Alyssum murale	SP5	3.3	1.9	6.1	7.4	0.17	1.3	0.03	1.6	0.05
Alyssum murale	SP6	5.7	5.1	28.7	76.5	0.10	7.3	0.09	2.1	0.19
Alyssum murale	SP7	4.8	1.9	8.9	5.0	0.64	3.2	0.04	3.1	0.13
Alyssum murale	SP8	1.7	3.0	5.2	4.3	0.59	2.6	0.05	5.6	0.27
Alyssum murale	SP9	6.6	4.5	29.7	1.9	0.65	1.3	0.01	3.3	0.05

Comparison among available concentrations of Ni in soils and in *Alyssum murale* s.l. plant tissues (roots, shoots and leaves) and assessment of accumulation capacity for Ni are presented in Figure 5(a) and Table 23. Ratio between Ni concentrations in root and soil revealed great variation among analysed samples; the lowest ratio was 1.7 (SP8) and highest one 21.9 (SP2). Translocation factor was between 1.9 (SP5, SP7) and 5.1 (SP6), while Ni accumulation factor also showed great variation; the lowest one was 5.2 (SP8) and the highest was 67.0 (SP2). In the case of *A. murale* at SP2 both root/soil ratio and AF was the highest, while TF was of medium value, while at SP8 both root/soil ratio and AF was the lowest of all the samples analysed.

Comparison among available contents of Zn in soils and in *Alyssum murale* s.l. roots, shoots and leaves, as well as assessment of accumulation capacity for Zn are given in Figure 5(b) and Table 23. Ratio between Zn concentrations in root and soil showed extremely great variation among analysed samples; the lowest ratio was 1.9 (SP9) and extremely high value was 1411 (SP2). Translocation factor was between 0.04 (SP2) and 0.75 (SP3), while Zn accumulation factor also showed great variation; the lowest one was 1.3 (SP5, SP9) and the highest was 54 (SP2). In the case of *A. murale* at SP2, root/soil ratio was the highest, TF was the lowest, and AF was the highest of all the samples analysed. In the case of *A. murale* at SP9 the opposite trend was noticed, since the root/soil ratio was the lowest, TF was among the highest and AF was again the lowest. Having in mind that very low values of TF in all *A. murale* samples were noticed, low concentrations of Zn in leaves and low AF values of these samples are to be expected.

Comparison among available concentrations of Mn in soils and in *Alyssum murale* s.l. plant tissues and assessment of accumulation capacity for Mn are shown in Figure 5(c) and Table 23. The lowest ratio between Mn concentrations in root and soil was 0.01 (SP9) and highest one 0.17 (SP2). Translocation factor was between 1.6 (SP5) and 5.6 (SP8), while Mn accumulation factor showed very low values in the entire sample; the lowest one was 0.08 (SP3) and the highest was 0.36 (SP2). Generally speaking, both root/soil ratios and values of AF for Mn in all *A. murale* samples were very low, and only TF factors showed much higher values.

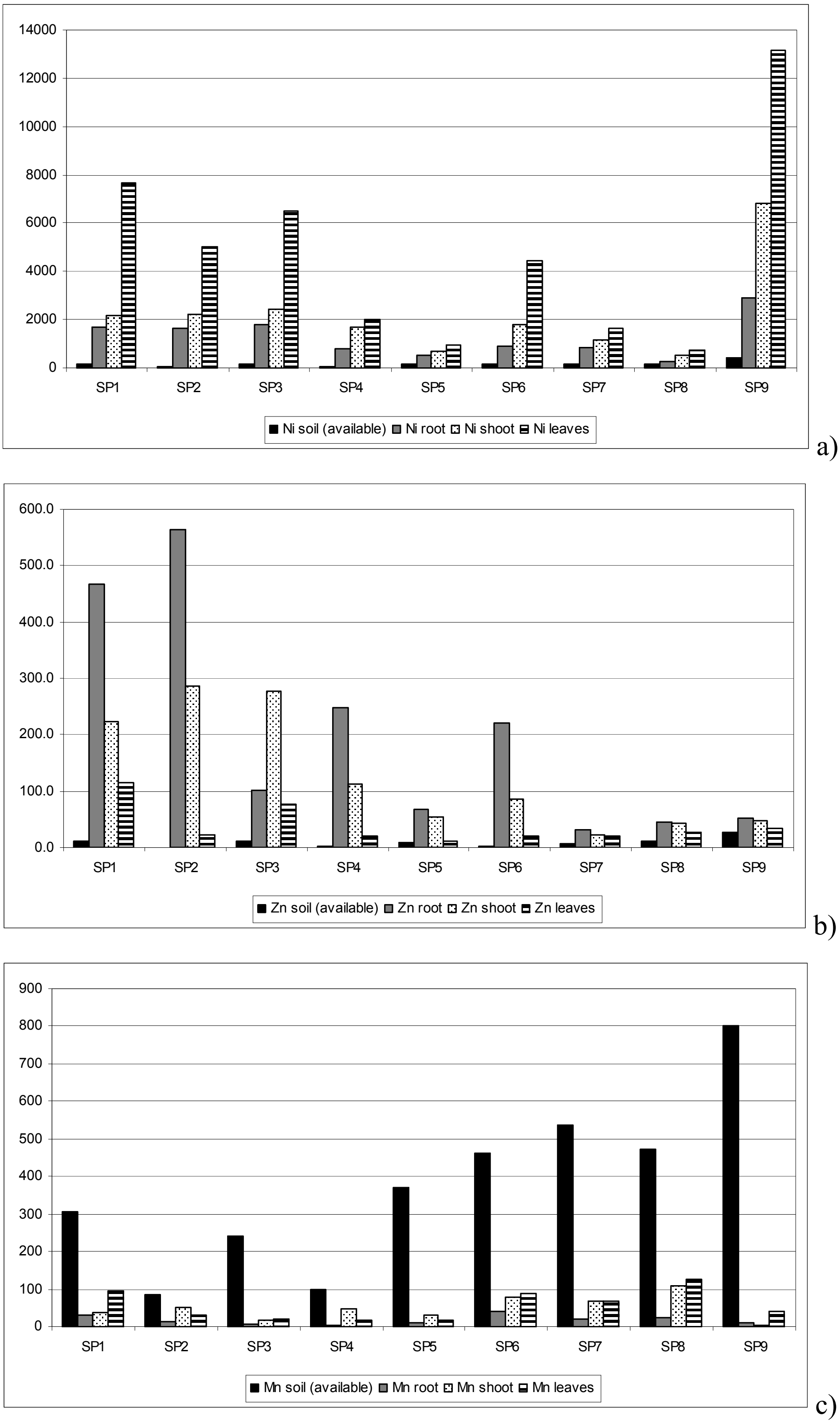

Figure 5. Comparison among available concentrations (mg kg^{-1}) of trace elements - Ni **(a)**, Zn **(b)**, Mn **(c)** in soils and concentrations (mg kg^{-1}) in *Alyssum murale* s.l. plant tissues (roots, shoots and leaves).

Comparison among available contents of Fe in soils and in other Brassicaceae plant tissues (roots, shoots and leaves) and assessment of accumulation capacity for Fe are presented in Figure 6(a) and Table 24. Ratio between Fe concentrations in root and soil revealed extremely great variation among analysed samples; the lowest ratio was 0.9 in *A. repens* (SP16) and highest were in *T. praecox* 60.8 and 24.1 (SP29 and SP30 respectively). Translocation factor was between 0.1 in *T. praecox* (SP29) and 2.6 in *A. montanum* (SP13), while Fe accumulation factor also showed great variation; the lowest one was 0.5 *A. saxatile* (SP1) and the highest was in *A. montanum* 10.5 (SP14). It is interesting to note that in the sample of *T. praecox* at SP29, root/soil ratio was the highest, TF was the lowest, and AF was of medium value.

Comparison among available concentrations of Ca in soils and in other Brassicaceae plant roots, shoots and leaves, as well as assessment of accumulation capacity for Ca are given in Figure 6(b) and Table 24. Ratio between Ca concentrations in root and soil also showed great variation among analysed samples; the lowest ratio was 0.3 in *E. carniolicum* (SP22) and highest one 13.7 in *I. tinctoria* (SP23). Translocation factor was between 0.3 in *C. plumieri* (SP17) and 5.5 in *A. murale* (SP15), while Ca accumulation factor also showed great variation; the lowest one was 0.8 in *E. carniolicum* (SP22) and the highest was 57.9 in *A. murale* (SP15). In the samples of *A. montanum* at SP14 and SP15, both TF and AF values were the highest of all the samples analysed. Also in *I. tinctoria* root/soil ratio and AF factor were also very high. Although TF was of medium value in *E. carniolicum* (SP22), both root/soil ratio and AF factor were extremely low.

Comparison among available contents of Mg in soils and in other Brassicaceae plant tissues and assessment of accumulation capacity for Mg are shown in Figure 6(c) and Table 24. The lowest ratio between Mg concentrations in root and soil was 1.1 in *E. linariifolium* (SP21) and highest one 10.4 in *T. kovatsii* (SP27). Translocation factor was between 0.4 in *T. praecox* (SP29) and 3.4 in *A. montanum* (SP13), while Mg accumulation factor showed great variation; the lowest were 1.3 in *E. linariifolium* and *T. praecox* (SP21 and SP29 respectively) and the highest was 7.5 in *T. kovatsii* (SP27). In the case of *T. kovatsii* (SP27), root/soil ratio was the highest, TF was the lowest, and AF was again the highest, while in the sample of *T. praecox* (SP29) both TF and AF values were the lowest of all the samples analysed.

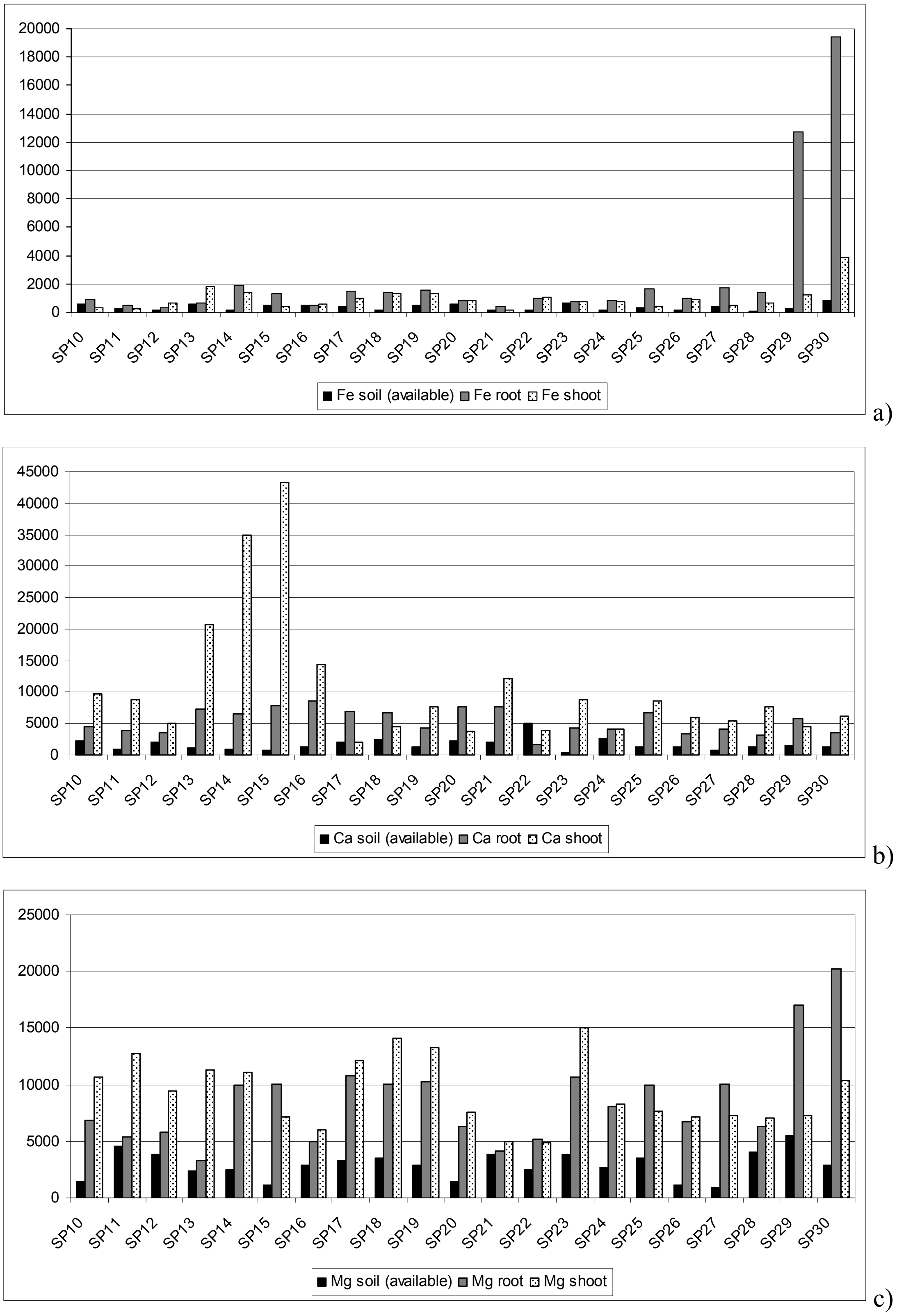

Figure 6. Comparison among available concentrations (mg kg^{-1}) of major elements - Fe **(a)**, Ca **(b)**, Mg **(c)** in soils and concentrations (mg kg^{-1}) in other Brassicaceae plant tissues (roots and shoots).

Table 24. Accumulation potential for Fe, Ca and Mg of different Brassicaceae species. The translocation factor (TF) is the shoot-to-root ratio of trace elements concentration. The accumulation factor (AF) is the ratio between trace elements concentration in shoots and their bioavailable concentration in the corresponding soil

Species	Sample point	Fe root/soil	Fe TF	Fe AF	Ca root/soil	Ca TF	Ca AF	Mg root/soil	Mg TF	Mg AF
Aethionema saxatile	SP10	1.5	0.3	0.5	2.0	2.2	4.4	4.6	1.6	7.2
Aethionema saxatile	SP11	2.0	0.5	1.0	4.5	2.2	9.7	1.2	2.4	2.8
Aethionema saxatile	SP12	2.3	2.0	4.5	1.6	1.4	2.3	1.5	1.6	2.4
Alyssum montanum	SP13	1.2	2.6	3.1	6.4	2.8	18.2	1.4	3.4	4.7
Alyssum montanum	SP14	14.3	0.7	10.5	6.8	5.3	36.0	4.0	1.1	4.5
Alyssum montanum	SP15	2.7	0.3	0.9	10.5	5.5	57.9	9.1	0.7	6.5
Alyssum repens	SP16	0.9	1.2	1.1	6.6	1.7	11.0	1.7	1.2	2.1
Cardamine plumieri	SP17	3.9	0.7	2.6	3.4	0.3	1.0	3.3	1.1	3.7
Cardamine plumieri	SP18	9.3	1.0	8.9	2.9	0.7	1.9	2.8	1.4	4.0
Cardamine plumieri	SP19	3.2	0.8	2.6	3.3	1.8	5.9	3.5	1.3	4.6
Erysimum linariifolium	SP20	1.4	1.1	1.5	3.5	0.5	1.7	4.3	1.2	5.1
Erysimum linariifolium	SP21	3.0	0.3	1.0	3.6	1.6	5.7	1.1	1.2	1.3
Erysimum carniolicum	SP22	6.0	1.1	6.7	0.3	2.4	0.8	2.1	0.9	1.9
Isatis tinctoria	SP23	1.1	1.0	1.1	13.7	2.1	28.2	2.8	1.4	4.0
Isatis tinctoria	SP24	4.7	0.9	4.3	1.6	1.0	1.6	3.1	1.0	3.1
Rorippa lippizensis	SP25	4.9	0.3	1.4	5.2	1.3	6.7	2.8	0.8	2.2
Thlaspi kovatsii	SP26	6.4	0.9	5.6	2.8	1.8	4.9	5.8	1.1	6.2
Thlaspi kovatsii	SP27	4.3	0.3	1.2	4.8	1.3	6.4	10.4	0.7	7.5
Thlaspi praecox	SP28	11.4	0.5	5.8	2.4	2.4	5.7	1.6	1.1	1.7
Thlaspi praecox	SP29	60.8	0.1	5.8	3.7	0.8	2.9	3.1	0.4	1.3
Thlaspi praecox	SP30	24.1	0.2	4.8	2.6	1.7	4.4	7.1	0.5	3.6

Comparison among available contents of Ni in soils and in other Brassicaceae plant tissues (roots, shoots and leaves) and assessment of accumulation capacity for Ni are presented in Figure 7(a) and Table 25. The lowest ratio between Ni concentrations in root and soil was 0.1 in *E. carniolicum* (SP22), while highest were 6.4 and 6.0 in *T. kovatsii* (in SP26 and SP27 respectively). In *A. saxatile* at SP12, this ration could not be calculated, since the concentration of Ni in the roots was below 0.1 mg kg^{-1}. Translocation factor was between 0.4 in *A. montanum* (SP14) and *C. plumeri* (SP17) and 6.6 in *T. praecox* (SP29). Ni accumulation factor showed extremely great variation; the lowest one was 0.1 in *A. montanum* (SP14) and in *E. carniolicum* (SP22), while the highest was 27.2 in *T. praecox* (SP30). It was also not possible to calculate both TF and AF for Ni, since its content in the roots of *A. saxatile* (SP12) was below 0.1 mg kg^{-1}. In almost all *Thlaspi* samples the highest values of roots/soil ratios, TF and AF were calculated. Although TF was of medium value in *E. carniolicum* (SP22), both root/soil ratio and AF factor were extremely low, while in *A. montanum* (SP14) all three values were the lowest of all the analysed samples.

Comparison among available concentrations of Zn in soils and in other Brassicaceae plant roots, shoots and leaves, as well as assessment of accumulation capacity for Zn are given in Figure 7(b) and Table 25. Ratio between Zn concentrations in root and soil showed extremely great variation among analysed samples; the lowest ratio was 0.2 in *A. montanum* (SP13) and very high value was 87.4 in *T. praecox* (SP30). Translocation factor was between 0.4 in *I. tinctoria* (SP24) and 3.2 in *A. motanum* (SP13), while Zn accumulation factor also showed great variation; the lowest one was 0.6 in *A. saxatile* (SP12) and the highest one was 92.5 in *E. linariifolium* (SP20). In all *T. praecox* samples almost the highest values of roots/soil ratios and AF were calculated, while the lowest roots/soil and AF values and highest TF value were noticed in *A. montanum* (SP13).

Comparison among available contents of Mn in soils and in other Brassicaceae plant tissues and assessment of accumulation capacity for Mn are shown in Figure 7(c) and Table 25. The ratio between Mn concentrations in root and soil was up to 0.6 in all the analysed samples. Translocation factor was between 0.2 in *T. praecox* (SP30) and 4.9 in *A. montanum* (SP13), while Mn accumulation factor also showed very low values in all the sample (up to 0.5). It was also not possible to calculate both TF and AF for Mn, since its content in the shoots of *T. praecox* (SP29) was below 0.1 mg kg^{-1}. Generally speaking, both root/soil ratios and values of AF for Mn in all the Brassicaceae samples were very low, and only TF factors showed slightly higher values.

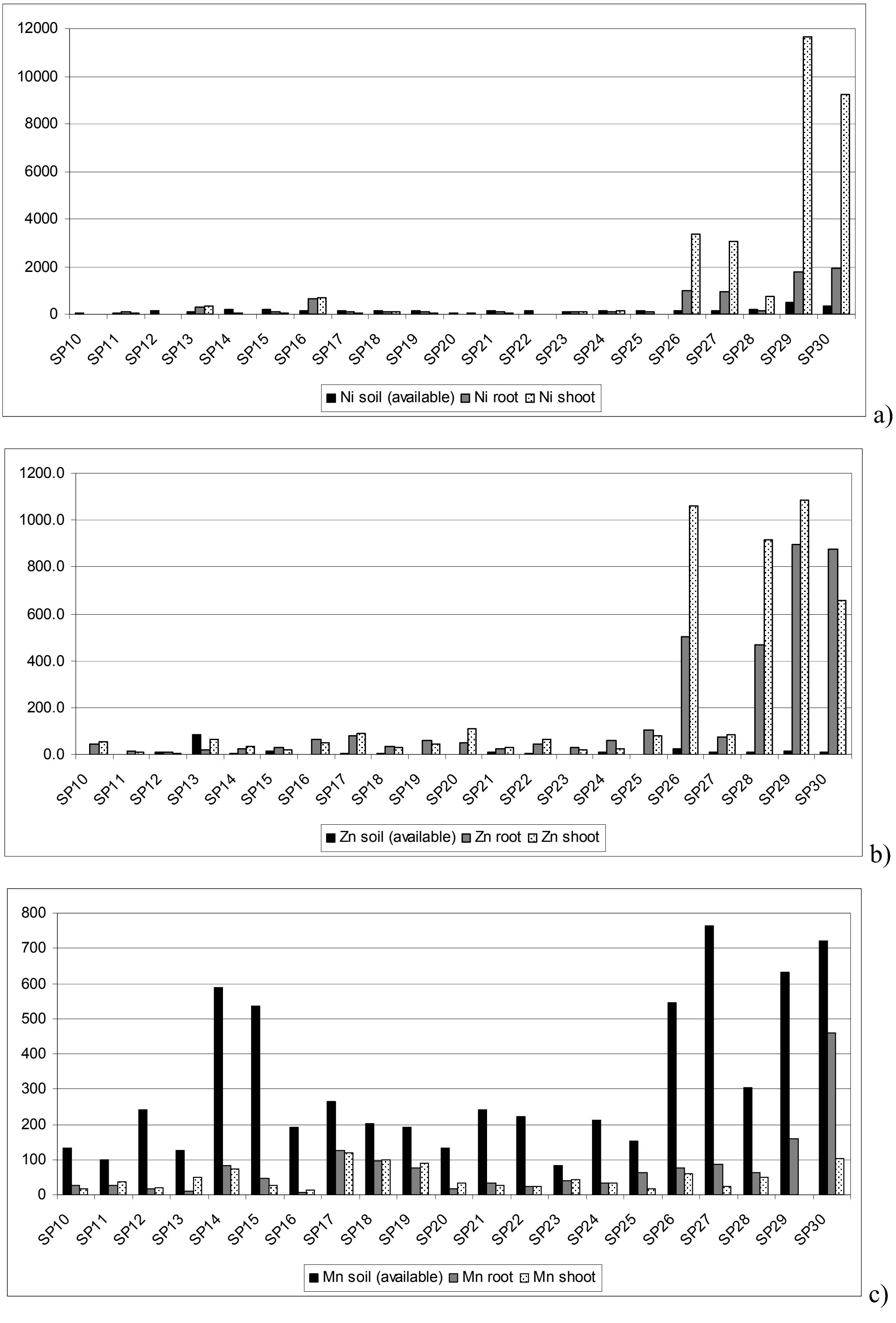

Figure 7. Comparison among available concentrations (mg kg^{-1}) of trace elements - Ni **(a)**, Zn **(b)**, Mn **(c)** in soils and concentrations (mg kg^{-1}) in other Brassicaceae plant tissues (roots and shoots).

Table 25. Accumulation potential for Ni, Zn and Mn of different Brassicaceae species. The translocation factor (TF) is the shoot-to-root ratio of trace elements concentration. The accumulation factor (AF) is the ratio between trace elements concentration in shoots and their bioavailable concentration in the corresponding soil

Species	Sample point	Ni root/soil	Ni TF	Ni AF	Zn root/soil	Zn TF	Zn AF	Mn root/soil	Mn TF	Mn AF
Aethionema saxatile	SP10	0.3	–	–	37.3	1.2	44.1	0.2	0.6	0.1
Aethionema saxatile	SP11	1.3	0.5	0.6	7.1	0.9	6.2	0.3	1.4	0.4
Aethionema saxatile	SP12	–	–	–	1.0	0.6	0.6	0.1	1.2	0.1
Alyssum montanum	SP13	2.4	1.2	2.9	0.2	3.2	0.8	0.1	4.9	0.4
Alyssum montanum	SP14	0.2	0.4	0.1	4.4	1.4	6.3	0.1	0.9	0.1
Alyssum montanum	SP15	0.4	0.7	0.3	2.4	0.7	1.6	0.1	0.5	0.1
Alyssum repens	SP16	4.6	1.0	4.8	27.1	0.8	22.1	0.1	1.7	0.1
Cardamine plumieri	SP17	0.6	0.4	0.2	27.6	1.1	30.1	0.5	1.0	0.5
Cardamine plumieri	SP18	0.9	0.9	0.8	12.3	0.9	11.3	0.5	1.0	0.5
Cardamine plumieri	SP19	0.6	0.9	0.5	25.2	0.8	19.5	0.4	1.2	0.5
Erysimum linariifolium	SP20	0.3	1.9	0.6	39.9	2.3	92.5	0.1	2.2	0.3
Erysimum linariifolium	SP21	0.5	0.5	0.2	2.1	1.2	2.6	0.1	0.8	0.1
Erysimum carniolicum	SP22	0.1	1.2	0.1	10.4	1.4	14.9	0.1	1.0	0.1
Isatis tinctoria	SP23	1.1	1.1	1.2	21.1	0.6	13.4	0.5	1.1	0.5
Isatis tinctoria	SP24	0.9	1.4	1.2	6.1	0.4	2.2	0.2	1.0	0.2
Rorippa lippizensis	SP25	0.8	–	–	51.0	0.7	37.0	0.4	0.3	0.1
Thlaspi kovatsii	SP26	6.4	3.4	21.5	22.8	2.1	48.1	0.1	0.8	0.1
Thlaspi kovatsii	SP27	6.0	3.4	20.3	10.7	1.2	12.4	0.1	0.3	0.1
Thlaspi praecox	SP28	0.8	5.4	4.2	42.5	2.0	83.4	0.2	0.8	0.2
Thlaspi praecox	SP29	3.5	6.6	23.1	59.8	1.2	72.4	0.3	–	–
Thlaspi praecox	SP30	5.7	4.8	27.2	87.4	0.8	65.8	0.6	0.2	0.1

4. 4. Relationships between metal concentrations

Nonparametric Spearman correlations and linear regression correlation test were performed to investigate correlations between metal concentrations. Correlations were evaluated using the bi-variation method, with two-tailed significance and Spearman R correlation coefficients.

In the soils of *Alyssum murale*, the correlation analysis found most significant positive mutual relationships in Ni-Zn and Ni-Mn, moderate positive correlation was in Zn-Mn, while low positive relationship was in Ca-Zn. The most significant negative correlations were in Mg-Mn and in Fe-Ca. In the roots of *Alyssum murale* the most significant positive correlations were in Fe-Ni, Fe-Mg and in Mg-Zn, while low positive relationship was in Fe-Zn, Mg-Ni and Mg-Mn. Moderate negative correlation was noticed in Fe-Ca (Table 26).

Table 26. Correlation coefficients (r) for soil and *Alyssum murale* s.l. The upper right part for root, the lower left part for soil; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

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

In the shoots of *Alyssum murale*, most significant positive correlations were in Ca-Mn and Mg-Mn, while moderate correlations were in Fe-Mg and Ni-Zn. The correlation analysis found most significant negative relationships between Ca-Ni and Ni-Mn and low negative relationship in Ca-Zn. In the leaves of *Alyssum murale*, most significant positive correlations were in Ni-Zn and low correlations in Fe-Ni and Fe-Mg. The most significant negative correlations were in Ca-Ni and Ca-Zn (Table 27).

Table 27. Correlation coefficients (r) for *Alyssum murale* s.l. The upper right part for leaves, the lower left part for shoot; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

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

Comparison of Ni-accumulating capacity of *Alyssum murale* leaves (Ni-concentration in leaves vs. available Ni concentration in soil) in analysed populations' revealed great variations. 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 28).

Table 28. 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≤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				

In the soils of *Alyssum montanum*, the correlation analysis found most significant positive mutual relationships in Fe-Zn, while most significant negative correlations were in Fe-Mn, Zn-Mn and Ca-Ni. In the soils of *Alyssum repens*, low positive correlation was only in Ni-Mn (Table 29).

Table 29. Correlation coefficients (r) for soil. The upper right part for *Alyssum montanum*, the lower left part for *Alyssum repens*; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	Fe	Ca	Mg	Ni	Zn	Mn
Fe	–	0.41	-0.26	-0.46	**0.90*****	**-0.92*****
Ca	-0.50	–	0.53	**-0.91*****	0.46	-0.52
Mg	-0.39	0.99	–	-0.50	-0.36	0.33
Ni	0.00	0.87	0.92	–	-0.47	0.45
Zn	0.11	-0.92	-0.96	-0.99	–	**-0.93*****
Mn	0.08	0.82	0.89	**1.00***	-0.98	–

In the soils of *Aethionema saxatile*, the correlation analysis found moderate negative mutual relationships in Mg-Ca and Fe-Zn. In the soils of *Cardamine plumieri*, the most significant positive relationship was in Mg-Ca and moderate one was in Ni-Mn. The most significant negative correlations were in Fe-Ca and Fe-Mg and only low negative correlation was in Ni-Zn (Table 30).

Table 30. Correlation coefficients (r) for soil. The upper right part for *Aethionema saxatile*, the lower left part *Cardamine plumieri*; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	Fe	Ca	Mg	Ni	Zn	Mn
Fe	–	0.03	-0.38	-0.53	**-0.88****	-0.08
Ca	**-0.97*****	–	**-0.66***	0.50	0.08	0.57
Mg	**-0.90*****	**0.95*****	–	-0.27	0.42	-0.53
Ni	0.45	-0.47	-0.43	–	0.62	0.58
Zn	-0.46	0.38	0.36	**-0.78***	–	0.07
Mn	-0.41	0.42	0.43	-0.50	**0.81****	–

In the soils of *Erysimum carniolicum*, the correlation analysis found low negative mutual relationships only in Mg-Ni, while in the soils of *Erysimum linariifolium*, low negative correlations were in Fe-Ni and Fe-Mn (Table 31).

Table 31. Correlation coefficients (r) for soil. The upper right part for *Erysimum carniolicum*, the lower left part for *Erysimum linariifolium*; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	Fe	Ca	Mg	Ni	Zn	Mn
Fe	–	0.96	0.74	-0.70	-0.98	0.92
Ca	0.20	–	0.90	-0.88	-0.99	0.99
Mg	-0.60	0.06	–	**-1.00***	-0.85	0.95
Ni	**-0.83***	0.14	0.77	–	0.82	-0.93
Zn	-0.77	0.38	0.71	0.77	–	-0.97
Mn	**-0.83***	0.14	0.77	1.00	0.77	–

In the soils of *Isatis tinctoria*, the correlation analysis found moderate positive mutual relationships only in Ca-Ni and low positive correlations in Ca-Mn and Ni-Mn. The most significant negative correlation was noticed in Zn-Mn and moderate negative correlations in Fe-Ca and Fe-Ni. In the soils of *Rorippa lippizensis*, there were no significant (positive or negative) correlations (Table 32).

Table 32. Correlation coefficients (r) for soil. The upper right part for *Isatis tinctoria*, the lower left part for *Rorippa lippizensis*; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	Fe	Ca	Mg	Ni	Zn	Mn
Fe	–	**-0.93****	0.64	**-0.94****	-0.58	-0.79
Ca	-0.03	–	-0.75	**0.93****	0.75	**0.81***
Mg	0.97	-0.27	–	-0.64	**-0.97*****	-0.76
Ni	-0.42	-0.89	-0.19	–	0.64	**0.88***
Zn	0.72	-0.71	0.87	0.33	–	0.81
Mn	-0.95	-0.29	-0.84	0.69	-0.46	–

In the soils of *Thlaspi kovatsii*, the low positive correlations were in Mg-Ca and Mg-Zn, while low negative correlation was in Fe-Zn. In the soils of *Thlaspi praecox*, the most significant positive correlation was noticed in Fe-Mn and moderate positive correlation in Mg-Zn (Table 33).

Table 33. Correlation coefficients (r) for soil. The upper right part for *Thlaspi kovatsii*, the lower left part for *Thlaspi praecox*; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	-0.54	-0.66	-0.66	**-0.83***	0.77
Ca	-0.12	–	**0.89***	0.54	0.71	-0.77
Mg	-0.50	0.47	–	0.31	**0.83***	-0.77
Ni	0.45	0.20	0.46	–	0.37	-0.77
Zn	-0.42	0.15	**0.88****	0.53	–	-0.60
Mn	**0.92*****	-0.32	-0.46	0.51	-0.37	–

In the roots of *Alyssum montanum* the most significant positive correlation was in Fe-Mn, while moderate one was in Mg-Zn. Highest negative correlation was noticed in Ni-Mn and moderate one was in Fe-Ni. In the shoots of *Alyssum montanum*, most significant positive correlation was in Fe-Zn, while moderate correlation was in Fe-Mg and low was in Mg-Zn and Mg-Mn. The correlation analysis found most significant negative relationship in Ca-Zn, moderate in Fe-Ca and low correlation in Mg-Ca (Table 34).

Table 34. Correlation coefficients (r) for *Alyssum montanum*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	**-0.87****	**0.83****	0.50	**0.92*****	0.53
Ca	-0.48	–	**-0.68***	-0.38	**-0.90*****	-0.43
Mg	0.63	0.25	–	0.18	**0.68***	**0.72***
Ni	**-0.88****	0.42	-0.62	–	0.48	-0.44
Zn	0.37	0.47	**0.87****	-0.22	–	0.43
Mn	**1.00*****	-0.48	0.64	**-0.90*****	0.36	–

In the roots of *Alyssum repens* moderate positive correlation was only in Fe-Mg, while in the shoots of *Alyssum repens* moderate positive correlations was only in Mn-Zn (Table 35).

Table 35. Correlation coefficients (r) for *Alyssum repens*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	0.34	-0.73	-0.80	0.54	0.55
Ca	0.42	–	0.40	-0.84	-0.61	-0.60
Mg	**1.00****	0.43	–	0.16	-0.97	-0.97
Ni	0.93	0.04	0.92	–	0.08	0.07
Zn	0.05	0.93	0.07	-0.33	–	**1.00****
Mn	0.96	0.65	0.96	0.79	0.33	–

In the roots of *Aethionema saxatile* the most significant positive correlation was in Fe-Ca, while low were in Mg-Zn and Fe-Mn. Low negative correlation was noticed in Fe-Ni. In the shoots of *Aethionema saxatile*, moderate positive correlation was in Ca-Zn, while moderate negative correlation was in Fe-Mg (Table 36).

Table 36. Correlation coefficients (r) for *Aethionema saxatile*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	-0.45	**-0.85****	0.00	-0.45	-0.63
Ca	**0.90*****	–	0.52	-0.87	**0.81****	-0.16
Mg	0.50	0.50	–	-0.87	0.40	0.58
Ni	**-0.81***	-0.66	-0.77	–	0.87	-0.50
Zn	0.54	0.62	**0.70***	-0.60	–	-0.35
Mn	**0.78***	0.63	0.32	-0.60	0.35	–

In the roots of *Cardamine plumieri* low positive correlations were in Mg-Zn and Ca-Mn, while low negative correlation was noticed in Ni-Zn. In the shoots of *Cardamine plumieri*, moderate positive correlations were in Mg-Ni and low were in Fe-Mg and Fe-Ni. The most significant negative correlations were in Mg-Zn and Ca-Mn, while moderate negative correlation was in Ni-Zn (Table 37).

Table 37. Correlation coefficients (r) for *Cardamine plumieri*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	Fe	Ca	Mg	Ni	Zn	Mn
Fe	–	0.65	**0.70***	**0.77***	-0.65	-0.48
Ca	-0.48	–	0.50	0.52	-0.47	**-0.90*****
Mg	0.53	0.28	–	**0.88****	**-0.98*****	-0.45
Ni	-0.36	0.03	-0.37	–	**-0.85****	-0.45
Zn	0.32	0.13	**0.73***	**-0.67***	–	0.47
Mn	-0.28	**0.77***	0.60	-0.10	0.50	–

In the roots of *Erysimum carniolicume* there were no significant (positive or negative) correlations, while in the shoots of *Erysimum carniolicum*, only low negative correlation was in Fe-Zn (Table 38).

Table 38. Correlation coefficients (r) for *Erysimum carniolicum*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	Fe	Ca	Mg	Ni	Zn	Mn
Fe	–	0.22	0.77	-0.11	**-1.00***	0.99
Ca	0.71	–	0.79	-0.99	-0.20	0.35
Mg	0.58	0.98	–	-0.71	-0.76	0.85
Ni	0.75	0.06	-0.11	–	0.09	-0.24
Zn	0.92	0.38	0.22	0.95	–	-0.99
Mn	0.99	0.80	0.69	0.65	0.86	–

In the roots of *Erysimum linariifolium* moderate positive correlations were in Fe-Zn and Mg-Zn. Moderate negative correlation was noticed in Ni-Zn and low was in Fe-Ni and Mg-Ni. In the shoots of *Erysimum linariifolium*, moderate positive correlations were in Fe-Zn and Fe-Mn and low in Fe-Mg, while low negative correlations were in Fe-Ca, Ca-Zn and Ca-Mn (Table 39).

Table 39. Correlation coefficients (r) for *Erysimum linariifolium*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	**-0.90***	**0.90***	0.00	**0.93****	**0.93****
Ca	0.37	–	-0.77	-0.03	**-0.89***	**-0.89***
Mg	1.00	0.37	–	0.37	0.77	0.77
Ni	**-0.83***	-0.49	**-0.83***	–	-0.26	-0.26
Zn	**0.94****	0.54	**0.94****	**-0.94****	–	1.00
Mn	-0.54	0.20	-0.54	0.71	-0.60	–

In the roots of *Isatis tinctoria* moderate positive correlation was noticed in Ni-Zn and low correlation in Mg-Mn, while low negative relationship was in Mg-Ni. In the shoots of *Isatis tinctoria*, there were only positive correlations - moderate in Mg-Mn and low in Ca-Mn (Table 40).

Table 40. Correlation coefficients (r) for *Isatis tinctoria*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	0.14	0.60	0.09	-0.60	0.37
Ca	0.43	–	0.77	-0.77	-0.43	**0.89***
Mg	0.09	0.37	–	-0.71	-0.71	**0.94****
Ni	0.26	0.03	**-0.83***	–	0.43	-0.77
Zn	0.49	0.14	-0.77	**0.94****	–	-0.54
Mn	0.09	0.49	**0.89***	-0.71	-0.60	–

In the roots of *Rorippa lippizensis* low positive correlation was in Ni-Zn and moderate to low negative correlations in Fe-Ni and in Fe-Zn. In the shoots of *Rorippa lippizensis* only low positive correlation was in Ca-Mn (Table 41).

Table 41. Correlation coefficients (r) for *Rorippa lippizensis*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	0.13	0.94	–	0.48	0.22
Ca	-0.99	–	0.46	–	-0.81	**1.00***
Mg	-0.48	0.38	–	–	0.14	0.54
Ni	**-1.00****	0.99	0.49	–	–	–
Zn	**-1.00***	1.00	0.46	**1.00***	–	-0.76
Mn	-0.92	0.86	0.79	0.92	0.91	–

In the roots of *Thlaspi kovatsii* moderate positive correlation was noticed in Fe-Mg, while low negative relationship was in Ca-Ni. In the shoots of *Thlaspi kovatsii*, there were only positive correlations - moderate in Fe-Mn and Ni-Mn, while low were in Fe-Ca, Fe-Ni and Ca-Zn (Table 42).

Table 42. Correlation coefficients (r) for *Thlaspi kovatsii*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	**Fe**	**Ca**	**Mg**	**Ni**	**Zn**	**Mn**
Fe	–	**0.83***	-0.52	**0.89***	0.66	**0.94****
Ca	0.60	–	-0.41	0.71	**0.83***	0.77
Mg	**0.94****	0.54	–	-0.17	-0.20	-0.46
Ni	-0.77	**-0.83***	-0.71	–	0.71	**0.94****
Zn	-0.66	-0.77	-0.77	0.60	–	0.77
Mn	0.66	0.14	0.71	-0.37	-0.31	–

In the roots of *Thlaspi praecox* the most significant positive correlations were noticed in Fe-Mg, Fe-Ni, Fe-Mn, Mg-Ni, Mg-Mn and Ni-Mn, while low correlations were in Ca-Zn and in Zn-Mn. In the shoots of *T. praecox* the most significant positive correlation was in Fe-Mg, moderate was noticed in Fe-Mn and low correlation in Mg-Mn. The most significant negative correlation was in Ca-Ni, while low correlation was in Ca-Mn. (Table 43).

Table 43. Correlation coefficients (r) for *Thlaspi praecox*. The upper right part for shoots, the lower left part for roots; significant correlation coefficients are bolded (*p<0.05, **p<0.01, ***p<0.001)

Metal	Fe	Ca	Mg	Ni	Zn	Mn
Fe	–	-0.48	**0.92*****	0.43	-0.27	**0.94****
Ca	0.10	–	-0.30	**-0.95*****	-0.43	**-0.89***
Mg	**0.97*****	0.13	–	0.23	-0.23	**0.83***
Ni	**0.95*****	0.20	**0.97*****	–	0.35	0.71
Zn	0.53	**0.75***	0.47	0.50	–	-0.26
Mn	**0.93*****	0.27	**0.90*****	**0.93*****	**0.67***	–

5. DISCUSSION

5. 1. Soil Characteristics

On the basis of the analyses of physical soil characteristics that were done on 30 different ultramafic sites in northwestern, western, southwestern and central Serbia it can be concluded that textural classes of the studied samples range from silty loam to sandy loam. Generally speaking, texture of serpentine soils varies from heavy clays to light loams (Walker 1954). The physical conditions of serpentine soils prove inhospitable for many plants and serpentine outcrops are often steep and comparatively rocky, making them particularly vulnerable to erosion, which results in shallow soils. Silt and clay contents in serpentine soils are generally minimal (Brady *et al.* 2005), but in our study almost one third of the soil samples were of silty loam type.

The pH in H_2O of the serpentine soil samples varied from moderately acidic (6.2) to moderately alkaline (7.8) which is in agreement with the mean pH (6.8) of serpentine soils given by Brooks (1987) and the results from Albanian soils presented by Bani *et al.* (2010). Percent of organic matter in soils is low to moderate (up to 17.9%), which indicate to relatively good humidization. Concerning the content of P_2O_5 and K_2O, most of the soil samples are characterised by low level of these nutrients, which is a general characteristics of ultramafic soils (Whittaker 1954; Brooks 1987), but some of the samples had up to 275 mg kg^{-1} of P_2O_5 and up to 268 mg kg^{-1} K_2O which is in agreement with the results of the analysis of some other serpentine soils from Serbia (Licina *et al.* 2010).

The serpentine soils of 30 Serbian sites were characterized by generally elevated total concentrations of trace elements such as Ni, Mn, Co and Cr that are typical of such sites (Whittaker 1954; Brooks 1987). Total Ni content of the world serpentine soils are generally in range 500–8000 mg kg^{-1} (Ghaderian *et al.* 2007), 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 (170–3400 mg kg^{-1}, sometimes to above 100000 mg kg^{-1}) (Kabata Pendias 2011). 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, other Balkan Peninsula, Turkish and Iranian serpentines (Kostić *et al.* 1998; Licina *et al.* 2010; Obratov *et al.* 2008; Kazakou *et al.* 2010; Bani *et al.* 2010; Reeves *et al.* 2009; Shallari *et al.* 1998; Ghaderian *et al.* 2007; Ghaderian *et al.* 2007a). 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 (Bani *et al.* 2010; Reeves *et al.* 2009; Obratov *et al.* 2008), than in soil samples presented here.

Most of the total Cu, Zn, Cd and Pb concentrations in analyzed serpentine 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 is lying in the range from 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 as 27 mg kg^{-1} (Kabata-Pendias 2011). However, it had been reported that only about 0.005–0.13% of Pb in soil solution is available to plants (Davies 1995). Our results correspond to three other serpentine soils from the western (Mt Zlatibor), northwestern (Mt Divčibare) and central Serbia (Goč mountain) (Obratov *et al.* 2008), but total contents of Zn and Cu were much lower in our analyses then those presented for some other Balkan serpentines (Bani *et al.* 2010).

It is generally accepted that total soil metal concentration does not determine plant tissue concentrations, and that “plant available” concentration (extractable fraction) generally correlates closely with plant uptake (Adriano 2001). 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 (500–5507 mg kg^{-1}) and low to high available Ca content (310–5100 mg kg^{-1}). The Ca/Mg quotients for the available fraction in 23 samples are relatively low (0.20–0.97); this is in accordance with some other serpentine soils of the Apennine, where the concentrations of available Ca can be much lower than those of Mg (Lombini *et al.* 1998). In two serpentine soil samples (SP6, SP22) 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.* (2007) for the ultramafic soils in Iran. The higher Ca content compared to Mg in these two soil samples is probably attributable to weathering and leaching processes combined with biological activity at these locations (Alexander *et al.* 2007).

The content of the available Fe was high for the loamy and alkaline soils (Kabata-Pendias 2011), while available concentrations of Ni and Mn were high in soil samples and much higher than those presented by Ghaderian *et al.* (2007; 2007a) for ultramafic soils from Iran. Available Zn concentrations in analyzed soils were much higher than those presented for serpentine soils from Italy (Lombini *et al.* 1998). Available Cr was below 0.1 mg kg^{-1} in 18 sample points and in the rest of the samples it reached up to 43.5 mg kg^{-1}. These low values confirm low mobility of Cr in surface soil layer and its limited availability to plants (Lombini *et al.* 1998; Słowik *et al.* 2011).

5. 2. Chemical composition of the plant material

Calcium (Ca) and Magnesium (Mg) concentrations

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, concentration of available Mg in the soil samples was 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 in leaves. This is because Ca content in *A. murale* leaves is remarkably high, which is the consequence of the unusual ability of this plant to accumulate high Ca concentrations, even from the soils with high Mg/Ca quotients that are characteristic of serpentines. The similar results were presented for different plants (including *Alyssum* species) from Italy, Albania and Greece (Lesbos Island) respectively (Kazakou *et al.* 2010; Shallari *et al.* 1998; Lombini *et al.* 1998). According to Pandolfini and Pancaro (1992) serpentine plants must posses a mechanism to limit uptake of Mg and/or a high absorption capacity for selective Ca uptake.

In the roots and shoots of *A. montanum*, *A. repens*, *E. linariifolium* and only in shoots of *R. lippizensis* and *T. praecox* the Ca/Mg ratios > 1 (1.09–6.06) were noticed. Higher Ca contents in roots and shoots of these five species are the consequence of unusual ability of these plants to accumulate high Ca concentrations in their tissues, even from the soils with low Ca/Mg quotients that are characteristic of serpentines. *A.*

montanum, *A. repens*, *E. linariifolium*, *R. lippizensis* and *T. praecox* occur both on and off serpentine soils (Jovanović-Dunjić *et al.* 1973). According to Kruckenberg (1954) a major criterion for serpentine tolerance must be the capacity of serpentine plants to grow on soils of low calcium levels. Some native species occur both on and off serpentine, and it might be expected to find, within species of this latter type, some populations adapted to serpentine and others not so adapted. The demonstration of such serpentine and nonserpentine races, if they do exist, would provide some basis for a genetic interpretation of serpentine endemism.

In the plant tissues of the rest of investigated Brassicaceae species, the contents of Ca were lower than Mg (Ca/Mg ratio < 1) and it seems that all these plants might be tolerant to low Ca/Mg level in the soils, as well as in their roots and shoots. Walker *et al.* (1955) surmised that serpentine-tolerant species survive on soils with depleted levels of Ca because they are still able to absorb sufficient quantities of Ca without taking up excessive quantities of Mg.

Iron (Fe) concentrations

Concentrations of Fe in all *A. murale* plant samples were generally below 1000 mg kg^{-1}, and only in *A. murale* leaves at SP1 it was slightly above this value. According to Reeves *et al.* (1999) Fe content higher than 1000 mg kg^{-1} 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 *A. murale* plant tissues was significantly elevated in relation to normal values for plants – approximately 100, maximum of about 700 mg kg^{-1} (Larcher 1995). 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 (Narwal *et al.* 1991; Parida *et al.* 2003), 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 *Alyssum lesbiacum* at high Ni concentrations (Ingle *et al.* 2005).

In the roots and shoots of two *T. praecox* samples extremely high Fe values were found and this can not be only the matter of mistake or coincidence since all the plant samples were washed carefully with bidistilled water to remove soil particles. Bani *et al.* (2010) also recorded higher Fe concentrations in the leaves of several *Thlaspi* species from the Albanian, Greek and Bulgarian serpentine sites. Johnston and Proctor (1977) concluded that, where Fe is easily soluble, plants may take up a very large amount of Fe. This is clearly shown by vegetation grown in soils derived from serpentine, where grass contained Fe within the range of 2127–3580 mg kg^{-1}. Moreover, Babalonas *et al.* (1984) detected Fe concentration up to 9070 mg kg^{-1} in the leaves of serpentine plants from Greece and concluded that high Fe content of plants is not a unique characteristic of the serpentine flora in Bosnia or Greece and it may probably be applicable to all the serpentine flora of the Balkans.

Concerning the rest of investigated plants, in six root samples as well as in six shoot samples, Fe contents above 1000 mg kg^{-1} were recorded. Generally speaking Fe contents in the rest of Brassicaceae plant tissues were similar to those observed by Lombini *et al.* (1998) in the North Apennines serpentine plants and elevated in relation to the natural Fe content of fodder plants which ranges 18–1000 mg kg^{-1} (Kabata-Pendias 2011).

Nickel (Ni) concentrations

The total quantity of Ni in tissues of all nine *A. murale* samples studied was high to extremely high (up to 13160 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 67:1. 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 (Dudić *et al.* 2007). This is completely in accordance with the finding of McNear *et al.* (2010) 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.

On the ultramafic slopes of Goč mountain (central Serbia), plants of *A. markgrafii* contained from 5125 mg·kg^{-1} to 6250 mg·kg^{-1} Ni (Obratov *et al.* 1997). Vinterhalter and Vinterhalter (2005) also proved that that under conditions of *in vitro* culture (seed collected from the same locality in Goč Mountain), *A. markgrafii* 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. Still, generally speaking, Ni contents in Serbian *A. murale* plant samples are lower then those recorded by Bani *et al.* (2010), since the concentrations of Ni in *A. murale* plants from several localities in the Balkans ranged from 4730 mg kg^{-1} up to 20100 mg kg^{-1}.

In *A. montanum* at SP13 and *A. repens* at SP16 Ni contents were high and very unusual for the species that belong to section *Alyssum*. Actually, this is the first time that such high Ni content was measured within any species that belongs to this section, since Reeves *et al.* (1983) measured only 22 mg kg^{-1} Ni in the leaves of *A. montanum* from herbarium material and concluded that *Alyssum* species from sect. *Alyssum*s consistently show unremarkable Ni levels even of serpentine endemics in this section. The higher values of Ni for some *Alyssum* and *Aethionema* species were presented by Reeves *et al.* (2001) from Turkey. Brooks *et al.* (1979) found that Ni contents in *A. montanum* and *A. repens* leaves were up to 5 mg kg^{-1} and 6 mg kg^{-1} respectively and 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. Based on their accumulation properties some populations of *A. montanum* and *A. repens* can be classified as Ni strong accumulators, since such plants can accumulate 100–999 mg kg^{-1} Ni in leaves (Brooks *et al.* 1979).

The Ni concentrations in the shoots of *Thlaspi* species were between 756 and 11671 mg kg^{-1}, which is in line with the previously reported status of some *Thlaspi* species from Turkey (e.g. *T. cyprium* Bornm, *T. elegans* Boiss., *T. jaubertii* Hedge, *T.*

ochroleucum Boiss. & Heldr., *T. oxyceras* (Boiss.) Hedge, *T. rosulare* Boiss. & Bal. etc.) as a strong Ni hyperaccumulators (Reeves 1988; Reeves and Adigüzel 2008). Additionaly, our results are more or less similar to the results for several *Thlaspi* species from Albania, Greece and Bulgaria presented by Bani *et al.* (2010), Kazakou *et al.* (2010).

All the rest of Brassicaceae species studied here had Ni concentrations in their roots and shoots lower than 100 mg kg^{-1}, considered to be the threshold of physiological evidence of toxicity in plants of serpentine habitats (Kazakou *et al.* 2008), confirming that no nickel hyperaccumulating but tolerant species were recorded among these plants. On ultramafic soils containing more than 1000 mg kg^{-1} Ni most of the plants that survive on these soils show Ni concentrations about 10 times higher than elsewhere, i.e. from about 2 to 80 mg kg^{-1} (Reeves and Adigüzel 2008) while the uptake of this element by Brassicaceae species generally falls in the range 20-150 mg kg^{-1} Reeves (1988). So the hypothesis suggested by Brooks (1987) that serpentine tolerant species are adapted to high heavy metal soil concentrations but restrict heavy metal accumulation in their leaves was confirmed excepted for *A. murale*, *A. montanum*, *A. repens*, *T. kovatsii* and *T. praecox*.

Zinc (Zn) concentrations

Comparing to low Zn content in soil samples, in the roots of all *A. murale* samples Zn concentration was the highest. Concentrations of Zn found in this study are in agreement with values reported for other Balkan populations of *A. murale* (Bani *et al.* 2010). It is assumed that species growing in serpentine soils generally act as excluders of Zn and those they can restrict transport of such metal to the shoots and maintain relatively low concentration of metal in leaves even at high concentrations in soils (Bani *et al.* 2010a).

Zinc uptake by other Brassicaceae species investigated was similar to that observed in normal plants on normal soils. This is not surprising, since the Zn concentrations in serpentine soils are not unusual. We note, however, that certain *Thlaspi* species have an extraordinary ability to accumulate Zn, to concentrations often exceeding 10,000 mg kg^{-1} (Reeves and Brooks 1983). In some species such as *T.*

caerulescens this can occur not only from Zn-rich soils but even from a variety of types of soil with normal Zn concentrations (Reeves *et al.* 2001). Among the species studied here *Thlaspi* was noteworthy for two of its shoot samples containing Zn at concentrations 1059 and 1086 mg kg^{-1} respectively.

Reeves and Brooks (1983) proved that many other European *Thlaspi* species are capable to accumulate nickel or zinc, or both, depending on the nature of the soils on which they are growing. Not only are the zinc levels very high (> 5000 mg kg^{-1}) for populations of *T. caerulescens* growing on zinc-rich soils, but levels above 1000 mg kg^{-1} are commonly found in specimens of this species taken from a wide variety of other substrates, including gneissic, argillaceous granite and calcareous soils. This accumulation of zinc to concentrations above 1000 mg kg^{-1} is not restricted only to *T. caerulescens*; at least 30 European species of *Thlaspi* seems to be capable of acting in this way. In addition, at least 12 European species occur on serpentine soils, and in these cases, hyperaccumulation of nickel/zinc is observed. The ability of the species *T. goesingense* Halacsy from Austria to accumulate nickel and cobalt from serpentines, and zinc from zinc-amended soils, was studied by Reeves and Baker (1984)

Manganese (Mn) and Copper (Cu) concentrations

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 of *A. murale* it was up to 126.1 mg kg^{-1} and in the shoots of *C. plumieri* up to 120 mg kg^{-1}. Broadhurst *et al.* (2004) presented much higher concentration of Mn in leaves of several ecotypes of *A. murale* species. Concentrations of Mn found in this study are in agreement with values reported for other species of the genera *Alyssum* and *Thlaspi* from the Balkan countries (Bani *et al.* 2010). It is assumed that species growing in serpentine soils generally act as excluders of Mn and can restrict transport of this element to the shoots and maintain relatively low concentration of manganese in aboveground parts even at high concentrations in soils (Bani *et al.* 2010a; Kazakou *et al.* 2010). Babalonas *et al.* (1984) found that lower Mn content in plants 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 reduces availability.

Despite the fact that available Cu content in all soil samples was very low, in *A. repens* and in the two *T. praecox* plant samples high concentrations of this element were found both in the roots and in the shoots. The accumulation of Cu by higher plant is not so common feature, and hyperaccumulation of copper is noticed in some terrestrial plants such as *Mimulus guttatus* DC. (Allen and Sheppard 1971) or *Helichrysum candolleanum* as both Ni and Cu hyperaccumulator (Nkoane *et al.* 2005), as well as in aquatic macrophytes (Welsh and Denny 1980). In plant tissues of almost all the rest Brassicaceae plant species this element was found only in traces which correspond to the results given by Bani *et al.* (2010). Cu has low mobility relative to other elements in plants and most of this metal appears to remain in root (Kabata-Pendias 2011).

Chrome (Cr) and Cobalt (Co) concentrations

Contents of Cr in all the investigated plant parts were very low, and only in some plant samples small amounts of Cr were found in the roots and shoots (up to 91.1 mg kg^{-1}). Chrome concentrations in some other Balkan *Thlaspi* and *Alyssum* plants were mainly below 10 mg kg^{-1} as expected for uncontaminated samples (Bani *et al.* 2010). Reeves *et al.* (1999) reported that concentrations of Cr in leaves are generally below 20 mg kg^{-1} and chromium uptake by plants is usually very slight (Brooks and Radford 1978). This can be explained by its by the fact that much of the Cr in ultramafic rocks and their derived soils is likely to be in the form of relatively insoluble minerals such as chromite and low solubility in the serpentine soil solution is the consequence of the relatively high pH values (Freitas *et al.* 2004; Kazakou *et al.* 2010). 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 (Antosiewicz 2004).

In the case of Co, only in the roots and shoots of three plant samples of the genus *Thlaspi* small concentrations of this element were recorded (up to 114 mg kg^{-1}). In the samples of *Alyssum* species cobalt was found only in traces, which is in contrast to the results of investigations done by Brooks and Radford (1978) on the leaves from herbarium materials of *A. murale* populations from Europe, where content of this element was between 4 and 34 mg kg^{-1} of dry leaf mass. These authors claimed that the normal Ni/Co ratio in ultramafic 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 and Co in plant tissues of two *Alyssum* species from ultramafic soils of Iran were generally low (Ghaderian *et al.* 2007). Cobalt concentrations seldom reach 10 mg kg^{-1} in plants on normal soils, and even on serpentine this level is not often exceeded. However, in many of the Ni hyperaccumulator populations studied from the Albania, Greece and Bulgaria content of Co was in the range 15–100 mg kg^{-1} and in the case of *T. kovatsii* Co attained 173 mg kg^{-1} (Bani *et al.* 2010).

5. 3. Assessment of the accumulation capacity of the Brassicaceae plant species

As previously discussed, serpentine-tolerant plants must undergo a variety of unfavorable chemical conditions. The adaptive mechanism(s) that confer to plants tolerance to soils with reduced quantities of Ca and high concentrations of Mg and heavy metals is still not well understood (Brady *et al.* 2005).

Since the major criterion for serpentine tolerance must be the capacity of serpentine plants to grow on soils of low calcium and high magnesium levels (Kruckenberg 1954), it might be appropriate to evaluate which of Brassicaceae plant species investigated are well adapted to serpentine and which are not so adapted. The demonstration of such serpentine and nonserpentine adaptive types, if they do exist, would provide some basis for a genetic interpretation of serpentine endemism.

Concerning the accumulation capacity for Ca, all Brassicaceae plants accumulated Ca from the corresponding soil by a factor > 1 (up to 25.6 in *A. murale* at SP2) and only in the case of *E. carniolicum* this factor was 0.3. TF was high (> 1) in most of Brassicaceae plant samples with the highest values recorded in *A. murale* and *A. montanum* plant samples (up to 12.2 in SP6), while only in the case of *C. plumieri* (SP17 and SP19) and *E. linariifolium* (SP20) TF values were < 1 (0.3, 0.7 and 0.5 respectively). AF values > 1 was recorded in most of the Brassicaceae plant samples, also with the highest values recorded in *A. murale* and *A. montanum* plant samples (AF value was extremely high in *A. murale* at SP2 - 114). Only in the case of *E. carniolicum* (AF values were < 1. On the basis of these calculations, it can be concluded that almost all of the investigated Brassicaceae plant species are capable to absorb, translocate and/or accumulate sufficient quantities of Ca from the serpentines, even if the available content of this essential element in corresponding soil is low.

In the case of Mg, all Brassicaceae plants also accumulated Mg from the corresponding soil by a factor > 1 (up to 10.1 in *A. murale* at SP6 and 10.4 in *T. kovatsii* at SP27) and only in *A. murale* at SP7 and SP4 this factor was < 1 (0.5 and 0.8 respectively). TF was high (> 1) in most of Brassicaceae plant samples with the highest values recorded in *A. murale* and *A. montanum* plants (up to 3.2 in SP4 and 3.4 in SP13), while in several plant samples TF values were < 1. AF values > 1 was recorded in all of the Brassicaceae plant samples, also with the highest value recorded in *A. murale* (AF value was 11.4 in SP6). It is evident that almost all of the investigated Brassicaceae plant species are more or less induced to absorb, translocate and/or accumulate Mg from the serpentines, probably as a direct consequence and adaptive trait to the high content of this element in corresponding serpentine soils.

In order to expose major challenges to plants growing on serpentine soils, Kazakou *et al.* (2008) summarized the hypotheses of mechanisms allowing serpentine species to tolerate low soil Ca/Mg quotients as:

Hypothesis a: serpentine species have greater absorption of Ca and lower absorption of Mg than non-serpentine species. This mechanism was supported by Walker *et al.* (1955), who suggested that serpentine plants developed a greater absorption efficiency for Ca relative to that of Mg than is normal in non-serpentine plants, i.e. the lower available Ca is better utilized. It has been proposed that selective Ca translocation to leaves and inhibited Mg translocation and sequestration by roots are important evolutionary adaptations in serpentine species (O'Dell *et al.* 2006).

Hypothesis b: serpentine species are more tolerant to high soil Mg concentrations. This mechanism was presented by Kruckeberg (1954), who suggested that some plants tolerate high Mg concentrations by restricting uptake; they require high soil concentrations before they begin to absorb Mg. Marrs and Proctor (1976) compared the tolerance of three races of *Agrostis stolonifer*a and found that the serpentine races had a higher Mg requirement and a higher tolerance of excess Mg than the non-serpentine races.

Hypothesis c: luxury consumption of Mg. The mechanism of luxury consumption of Mg (i.e. Mg uptake far in excess of that needed to promote immediate growth due to high cation availability in soil) was proposed by Madhok and Walker (1969) for the serpentine endemic *Helianthus bolanderi* ssp. *exilis*. These authors suggested that tolerance of the high Mg habitat involves not just the ability to withstand very high Mg levels but also a higher requirement for magnesium.

However, according to Kazakou *et al.* (2008) hypotheses a and b are not necessarily independent: a greater absorption efficiency of Ca relative to that of Mg could be brought about solely by plants having a lower absorption efficiency and greater tolerance of Mg. In Hypothesis c, the term 'luxury consumption' seems to be unsuitable, since the definition of luxury consumption almost seems corresponding to hypothesis b, i.e. the absorption efficiency of serpentine plants for Mg is lower, allowing more tolerance of high external levels. Still, Lombini *et al.* (2003) concluded that the mechanisms of tolerance to unfavourable Ca/Mg concentrations and their relationship to the resistance of serpentinophytes to the excess of metal ions that prevail in serpentine soils are still not clearly understood.

Concerning the accumulation capacity for Fe, all *A. murale* plants accumulated Fe from the corresponding soil by a factor < 1 and only in the case of SP6 and SP1 the values were up to 6.5 and 7.8 respectively; also both of *Thlaspi* species accumulated Fe from the corresponding soil by a factor distinctly > 1 (up to 60.8 in *T. kovatsii* at SP29). Only the roots of *A. montanum* (SP14), *C. plumieri* (SP18) and *E. carniolicum* (SP22) accumulated Fe from the corresponding soil by a factor distinctly > 1 (14.3, 9.3 and 6.0 respectively), while in the roots of all the rest plant samples accumulation of Fe was not found (Fe concentrations in soil and roots were almost equal or slightly > 1). TF was low (< 1) for the most of Brassicaceae plant samples (from 0.1 to 1.6), while only in the case of *A. saxatile* (SP12), *A. montanum* (SP13) and *A. murale* (SP5, SP8) and *E. carniolicum* TF values were much > 1 (2.0, 2.6 and 2.9 respectively). AF values > 1 was recorded in most of the Brassicaceae plant samples (up to 10.5 in *A. murale* at SP14).

Baker (1981) suggested four categories of plant-heavy metal relationships: excluders, indicators, accumulators and hyperaccumulators. In order to obtain in which of four above presented plant-heavy metal relationships investigated Brassicaceae plants can be classified, we calculated and presented their accumulation potential for Ni, Zn and Mn. Accumulation and transport characteristics for these trace elements of Brassicaceae species were estimated from the ratio between element concentrations in soil, roots, and shoots/leaves. The transport factor (TF) was calculated as the shoot-to-root ratio of heavy metal concentrations. The accumulation factor (AF) was calculated as the ratio between metal concentration in the shoot/leaves and the bioavailable concentration in the corresponding soil.

Concerning the accumulation capacity for Ni, all *A. murale* plants accumulated Ni from the corresponding soil by a factor > 1 and in the case of SP2 the value was up to 21.9; also both of *Thlaspi* species accumulated Ni from the corresponding soil by a factor distinctly > 1 (up to 6.4 in *T. kovatsii* at SP26). Only the roots of *A. montanum* (SP13) and *A. repens* accumulated Ni from the corresponding soil by a factor distinctly > 1 (2.4 and 4.6 respectively), while in the roots of all the rest plant samples accumulation of Ni was not found (Ni concentrations in soil and roots were almost equal or distinctly < 1). Both TF and AF values > 1 were recorded in all *A. murale* and both of *Thlaspi* species samples and the AF values were extremely high (up to 67 in *A. murale* at SP2 and up to 6.6 in *T. praecox* in SP29). TF and AF were low (< 1) for the most of Brassicaceae plant samples (from 0.1 to 0.9), while only in the case of *E. linariifolium* (SP20) and *E. carniolicum* TF values were > 1 (1.9 and 1.2 respectively). Both TF and AF values > 1 were recorded in *A. montanum* (SP4), *A. repens* and *I. tinctoria*.

Concerning the accumulation capacity for Zn roots of *A. murale* samples accumulated Zn from the corresponding soil by a factor distinctly > 1 (extremely high value of root/soil ratio was 1411 in SP2). In all *A. murale* samples TF < 1 were reported, while in most cases AF values distinctly > 1 were noticed (up to 54 at SP2). Roots of almost all the rest of Brassicaceae species accumulated Zn from the corresponding soil by a factor distinctly > 1 (up to 87.4 in *T. praecox* at SP30). TF was low (< 1 or slightly above this value) for the most of plant samples (from 0.4 to 1.4), and only in the case of *A. montanum* (SP13; 3.2), *E. linariifolium* (SP20; 2.3), *T. kovatsii* (SP26; 2.1) and *T. praecox* (SP28; 2.0) TF values were distinctly > 1. AF values > 1 were recorded in almost all the Brassicaceae plant samples (up to 92.5 in *E. linariifolium* at SP20) and only in the case of *A. saxatile* (SP11) and *A. montanum* (SP12) AF values were < 1 (0.6 and 0.8 respectively).

In the case of Mn roots of all Brassicaceae samples accumulated Zn from the corresponding soil by a factor distinctly < 1. In all *A. murale* samples TF > 1 were reported (up to 5.6 at SP8), while AF values distinctly < 1 were noticed in all Brassicaceae plant samples.

If hyperaccumulators are defined as those plants which contain in their tissue more than 1000 mg kg^{-1} dry weight of Ni or more than 10000 mg kg^{-1} dry weight of Zn or Mn (Baker and Brooks 1989), only *A. murale*, *T. kovatsii* and *T. praecox* belong to the group of Ni hyperaccumulators. Strong accumulators can accumulate 100–999 mg kg^{-1} Ni in leaves (Brooks *et al.* 1979) and based on their accumulation properties, some populations of *A. montanum*, *A. repens* and *I. tinctoria* can be classified as Ni strong accumulators.

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. (Brooks *et al.* 1979). If we apply this criterium for the selection of accumulator Brassicaceae species from Serbian serpentine sites, some populations of *A. montanum*, *E. linariifolium*, *T. kovatsii* and *T. praecox* can be identified as Zn accumulators.

Therefore, most Brassicaceae species studied here belong to the group of species tolerant to serpentine soils, and can be considered either as excluders (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 shoot/root metal concentration quotient is < 1.0) or as indicators (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) (Baker 1981; Baker and Brooks 1989).

5. 4. Analyses of correlations

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 (Robinson *et al.* 1996). 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* (Kaur *et al.* 2010).

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 (Lopez-Bucio *et al.* 2000). If constitutionally high organic acid synthesis in *A. murale* (McNear *et al.* 2010) would be increased by P-starvation that would lead to additionally increased transport within the plant.

In all plant parts (roots, 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) (Kazakou *et al.* 2010). However Bani *et al.* (2010) 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 shoot 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 (Robinson *et al.* 1999). The trait of *A. murale* to concentrate 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 towards 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.* (2001) found the same compartmentation 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 to prevent their incorporation in chlorophyll.

Contrary to Kaur *et al.* (2010) who found antagonism of Ni with Cu and Zn in *B. juncea*, in *A. murale* plants analysed 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 (Mihailovic and Drazic 2011) or *Trigonella corniculata* (Parida *et al.* 2003) 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 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 that 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 remain to be investigated in the analysed populations.

6. CONCLUSIONS

On the basis of the analyses of physical soil characteristics that were done on 30 different ultramafic sites (within nine locations) in northwestern, western, southwestern and central Serbia it can be concluded that textural classes of the studied samples range from silty loam to sandy loam. Silt and clay contents in serpentine soils are generally minimal but in our study almost one third of the soil samples were of silty loam classes.

The pH in H_2O of the serpentine soil samples varied from moderately acidic to moderately alkaline, while percent of organic matter in soils was low to moderate which indicate a relatively good humidization. Soils collected at Serbian sampling sites contained low level of P_2O_5, K_2O and Ca and high Fe and Mg contents which is also general characteristics of ultramafic soils.

The serpentine soils of 30 Serbian sites were characterized by generally elevated total concentrations of trace elements such as Ni, Mn, Co and Cr, while most of the total Cu, Zn, Cd and Pb concentrations in analyzed serpentine soil samples fall within the ranges for normal soils

Concerning the available concentration of major elements, the soils were more or less of typical ultramafic composition, with the moderate to high concentration of available Mg and low to high available Ca content. The Ca/Mg quotients for the available fraction in 23 samples were relatively low; the content of the available Fe was high for the loamy and alkaline soils.

Available concentrations of Ni, Zn and Mn were high in soil samples and much higher than those presented by some previous records from the Balkans and the Apennines. Available Cr contents were low which confirms low mobility of Cr in surface soil layer and its limited availability to plants.

Despite relatively low available Ca content in the soil samples, the concentrations of Ca in the tissues of *A. murale* populations were several times higher. Concentration of available Mg in the soil samples was 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 was noticed in leaves. In the roots and shoots of *A. montanum*, *A. repens*, *E. linariifolium* and only in shoots of *R. lippizensis* and *T. praecox* the Ca/Mg ratios > 1 were noticed. It was concluded that the higher Ca contents in roots and shoots of these five species are the consequence of unusual ability of these plants to accumulate high Ca concentrations in their tissues, even from the soils with low Ca/Mg quotients that are characteristic of serpentines. In the plant tissues of the rest of investigated Brassicaceae species, the contents of Ca were lower than Mg (Ca/Mg ratio < 1) and it seems that all these plants might be tolerant to low Ca/Mg level in the soils, as well as in their roots and shoots.

Generally speaking Fe concentrations in the most of Brassicaceae plant tissues were elevated in relation to the natural Fe content of fodder plants, which is in accordance to the results for some North Apennine serpentine plants. In the roots and shoots of two *T. praecox* samples extremely high Fe values were found and this can not be only the matter of mistake or coincidence. It has been concluded that high Fe content of plants is may probably be applicable to all the serpentine flora of the Balkans.

The total quantity of Ni in tissues of all nine *A. murale* samples studied was high to extremely high and in some cases, the ratio between concentration of Ni in leaves and in the corresponding soil was 67:1. Actually in all *A. murale* samples studied the concentration of Ni was higher in the above-ground parts compared to the root. The Ni concentrations in the shoots of *Thlaspi* species were high to extremely high, which is in line with the previously reported status of some *Thlaspi* species from Turkey. In *A. montanum* and *A. repens* Ni contents were high and very unusual for the species that belong to section *Alyssum*. Actually, this is the first time that such high Ni content was measured within any species that belongs to this section. All the rest of Brassicaceae species studied here had Ni concentrations in their roots and shoots lower than 100 mg kg^{-1}, confirming that no nickel hyperaccumulating but tolerant species were recorded among these plants. So the hypothesis that serpentine tolerant species are adapted to high heavy metal soil concentrations but restrict heavy metal accumulation in their leaves was confirmed except for *A. murale*, *A. montanum*, *A. repens*, *T. kovatsii* and *T. praecox*.

Correlation of Ni accumulating capacity with soil properties lead to a 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. It is possible that the increase of this capacity was necessitated by the absence of other vegetation at the infertile sites with toxic concentrations of Mg in order to fight away potential herbivores and pests.

Comparing to low Zn content in soil samples, in the roots of all *A. murale* samples Zn concentrations were the highest. Concentrations of Zn found in this study are in agreement with values reported for other Balkan populations of *A. murale*. Zinc uptake by other Brassicaceae species investigated was similar to that observed in normal plants on normal soils. We note, however, that certain *Thlaspi* species studied here was noteworthy for containing Zn at concentrations 1000 mg kg^{-1}.

Although Mn content in soil samples was relatively high, its concentration in plant tissues was noticeably lower; it is assumed that species growing in serpentine soils generally act as excluders of Mn and can restrict transport of this element to the shoots and maintain relatively low concentration of manganese in aboveground parts even at high concentrations in soils.

Despite the fact that available Cu content in all soil samples was very low, in *A. repens* and in the two *T. praecox* plant samples high concentrations of this element were found both in the roots and in the shoots; the accumulation of Cu by higher plant is not so common feature. In plant tissues of almost all the rest Brassicaceae plant species this element was found only in traces, since Cu has low mobility relative to other elements in plants and most of this metal appears to remain in root.

Contents of Cr in all the investigated plant parts were very low, and only in some plant samples small amounts of Cr were found in the roots and shoots. Much of the Cr in ultramafic rocks and their derived soils is likely to be in the form of relatively insoluble minerals such as chromite and low mobility of this metal in the serpentine soil solution is the consequence of the relatively high pH values.

In the case of Co, only in the roots and shoots of three plant samples of the genus *Thlaspi* small concentrations of this element were recorded. Cobalt concentrations seldom reach 10 mg kg^{-1} in plants on normal soils, and even on serpentine this level is not often exceeded.

If hyperaccumulators are defined as those plants which contain in their tissue more than 1000 mg kg^{-1} dry weight of Ni or more than 10000 mg kg^{-1} dry weight of Zn or Mn, only *A. murale*, *T. kovatsii* and *T. praecox* belong to the group of Ni hyperaccumulators. Strong accumulators can accumulate 100–999 mg kg^{-1} Ni in leaves and based on their accumulation properties, some populations of *A. montanum*, *A. repens* and *I. tinctoria* can be classified as Ni strong accumulators.

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. If we apply this criterion for the selection of accumulator Brassicaceae species from Serbian serpentine sites, some populations of *A. montanum*, *E. linariifolium*, *T. kovatsii* and *T. praecox* can be identified as Zn accumulators.

Therefore, most Brassicaceae species studied here belong to the group of species tolerant to serpentine soils, and can be considered either as excluders (plants that restrict transport of metals to the shoot, and maintain relatively low metal concentrations in the shoot; their shoot/root metal concentration quotient is < 1.0) or as indicators (plants that show an intermediate response to high soil metal concentrations; the plant/soil metal concentration quotient in indicators is approximately 1.0).

The serpentine tolerant *Alyssum* and *Thlaspi* species should be useful for soil stabilization on ultramafic substrate, and can be used to establish a vegetative cover at serpentine sites or where vegetation is scarce due to high levels of metal contamination. This applies particularly to sites of former mining activity. Some populations of *A. murale*, *T. praecox* and *T. kovatsii* display the best efficiency of Ni uptake, in terms of both the Ni concentrations attained and the biomass produced. Together with *Leptoplax emarginata* and *Bornmuellera tymphaea* studied from Greece, they are of potential utility in any attempt to use hyperaccumulation for extracting Ni from soil, whether for remediation of contaminated soil or for phytomining from natural serpentine soils of temperate regions.

Despite the fact that populations of *I. tinctoria* showed only low to moderate capacity to uptake, translocate or accumulate Ni and Zn in its roots and shoots, this biennial plant could be very good candidate for phytoextraction practice due to its well developed and deep roots, fast growth rate and high biomass producing. For the commercial application of phytoremediation of soil heavy metal, fast growing plants with high biomass and good metal uptake ability are needed. 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 heavy metals) could be a very convenient approach to phytoremediation and phytoextraction practices.

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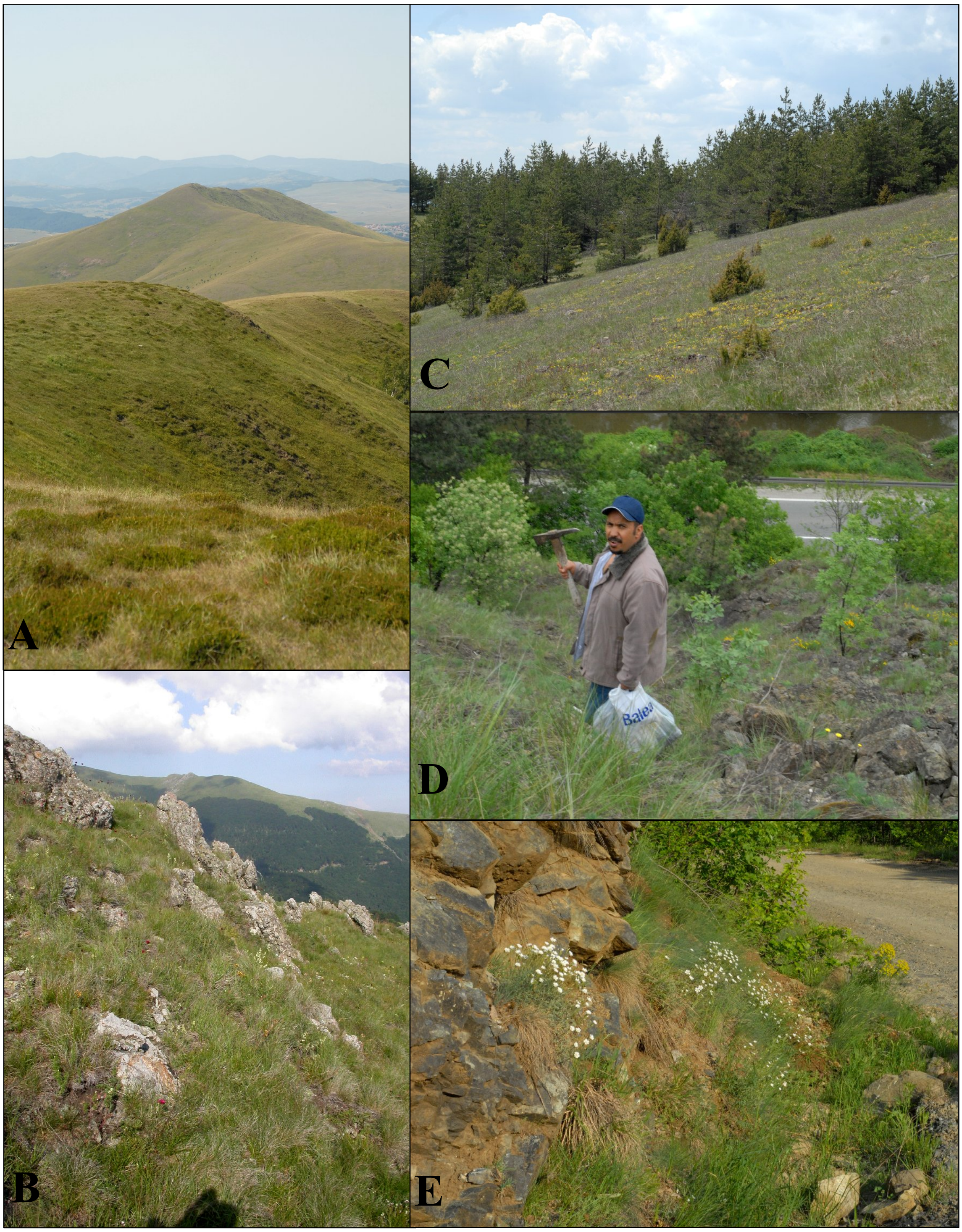
Appendix 1 – Investigated localities: **A.** Mt Zlatibor (Čelićko hill – Okolište); **B.** Mt Kopaonik (Šiljak); **C.** Mt Rogozna (Pasji stream – Vinogor); **D.** Gornji Milanovac (Brđani gorge); **E.** Kraljevo, Bogutovac (Gornja Lopatnica river gorge) (photos: M. Niketić).



Appendix 2 – Investigated plant species: **A.** *Erysimum linariifolium* (Brđani gorge); **B.** *Cardamine plumieri* (Mt Zlatibor); **C.** *Alyssum montanum* (Mt Rogozna); **D.** *Thlaspi kovatsii* (Mt Kopaonik); **E.** *Alyssum murale* (Mt Rogozna) (photos: M. Niketić).

Биографија аутора

Мастер биологије Ахмед Фараг Туми

Ахмед Фараг Туми рођен је 15. јуна 1965. године у Ласаби у Либији, где је завршио основну школу и гимназију.

Школске 1986/1987 године уписао је Биолошки факултет на Универзитету „7. октобар“ у Мисурати, где је дипломиро 1990. године.

Мастер студије завршио је 2006. године на Факултету Природних наука, Универзитет Јуба у Судану.

У периоду од 1991. до 1993. године био је запослен као асистент на Биолошком институту у Триполију у Либији.

Од 1994. до 2000. године радио је као шеф одељења за заштиту животне средине у Триполију.

У периоду од 2000. до 2007. године био је шеф Биолошке лабораторије у Триполију.

2009. добио је стипендију Либијске владе за израду докторске дисертације у Србији, када је и уписан на Биолошки факултет Универзитета у Београду.

Рад на докторској дисертацији започео је исте године, а део резултата свог истраживачког и научног рада објавио је у једном часопису од међународног значаја, док је други рад у часопису од међународног значаја прихваћен за штампу. Имао је и два саопштења на скуповима од међународног значаја.

Прилог 1.

Изјава о ауторству

Потписани-а ____________________Ахмед Фараг Туми______________________

број уписа ____

Изјављујем

да је докторска дисертација под насловом

Bioaccumulation potential of selected plant species of the family Brassicaceae

from serpentine habitats in Serbia

- резултат сопственог истраживачког рада,
- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

Потпис докторанда

У Београду, ____23.05.2013.___________

Ahmed Tumi

Прилог 2.

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора _________Ахмед Фараг Туми_________________________

Број уписа ____

Студијски програм ____Екологија, биогеографија и заштита биодиверзитета____

Наслов рада ____ Bioaccumulation potential of selected plant species of the family __________________Brassicaceae from serpentine habitats in Serbia ____________

Ментор ___др Гордана Томовић, доцент; др Марјан Никетић, виши научни сарадник___

Потписани __________________ Ахмед Фараг Туми _________________________

изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис докторанда

У Београду, ____23.05.2013.___________

Ahmed Tumi

____

Прилог 3.

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Bioaccumulation potential of selected plant species of the family Brassicaceae
from serpentine habitats in Serbia

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство

2. Ауторство - некомерцијално

3. Ауторство – некомерцијално – без прераде

4. Ауторство – некомерцијално – делити под истим условима

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(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

Потпис докторанда

У Београду, 23.05.2013.

Ahmed Tumi