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Dynamics of soil chemistry in different serpentine habitats of Serbia

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Abstract: To enhance understanding of edaphic conditions in serpentine habitats, a thorough investigation of the chemical and mechanical properties of three soils from disjunct ultramafic outcrops in the central Balkans was undertaken. Soil from a nearby chemically contrasting limestone habitat was also analyzed. Three plant species differently associated with serpentine habitats (*Halacsya sendtneri*, *Cheilanthes marantae* and *Seseli rigidum*) were references for site and soil selection. Twenty elements were determined, and fourteen were measured in seven sequentially extracted soil fractions. The quantified soil properties included pH, levels of free $CaCO_3$, organic matter, P_2O_5, K_2O, N, C, S, cation exchange capacity, total organic carbon, field capacity and soil mechanical composition. The usual harsh components for plant growth in serpentine soil, such as elevated Mg:Ca ratios, high levels of Ni, Cr or Co, were significantly lower in the available fractions. There was a significant positive correlation of organic matter and field capacity, with most available Ca (70–80 %) found in the mobile, rather than the organically bound fraction.

Keywords: ultramafic; serpentine soil; sequential extraction; metal availability; Mg:Ca ratio.

INTRODUCTION

Serpentine soils form on hydrothermally altered ultramafic rocks which cover a little less than 1 % of the exposed surface of the Earth.[1] The elemental composition and physical properties of the parent rock make serpentine soils

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different from the majority of those formed over other bedrocks found on the continental crust.[2] Uncommon chemical/physical soil qualities generate a complex edaphic factor. The distinctive style of plant life on serpentine is most often easily noticeable by the barren landscape, different vegetation composition, and specific plant growth habits. Together with disjunct parent rock distribution, adverse edaphic conditions strongly force adaptation in plant populations.[3,4] The disadvantages of serpentine soils also become apparent in agriculture, where they are known to inhibit crop yields. The specific plant community affiliated to serpentine is defined as low-productive, and comprises a high number of endemics.[1,5,6]

Even though soils derived from ultramafic rocks are diverse in profile and composition, they generally display several similar features that define their hostility towards plant life.[7] Typically shallow and rocky, serpentine habitats are often extremely xeric, and prone to high temperatures and erosion.[8] The usual soil chemical restraints responsible for generating a "serpentine syndrome" response in plants are: unfavorable Mg:Ca quotient, toxic Mg levels, variously elevated contents of Ni, Cr, Co and other trace metals, and typically low levels of Ca, N, P and/or K.[6,9–11] Being physically present in the soil, does not necessarily make an element available for plant uptake, as interdependent soil qualities often impact their mobility in soil–plant systems.[12,13] Despite the potential redistribution of elements between the newly-emptied phases,[14] sequential extraction of soil metals still gives a detailed insight into elemental availability and can provide estimates of mobility, reactivity and toxicity.[13,15–17] Evaluating the strength of metal adsorption bonds is important in health hazard surveying and monitoring, and in understanding the ecology of serpentine outcrops.[18,19] Serpentine habitats are relatively abundant on the Balkan Peninsula and have been reviewed a number of times with focus on different components of plant life inhabiting them.[20–27] Furthermore, the Balkan's ultramafics are a spot of refugia and speciation, and host a significant number of endemic plant species.[22] The uniqueness of the biota in the serpentine emphasizes the need for its inclusion in the overall plans for biodiversity conservation.[26]

For elucidating the role of individual chemical components of soil in the evolution of a serpentine habitat, metal availability was investigated *via* a seven-stage sequential extraction and the soil was scanned for 20 elements within each fraction. The dynamic changes of these soil characteristics could play a significant role in shaping the edaphic conditions in serpentine habitats. The aim of the study was to compare these soil properties among three differently vegetated serpentine habitats in central/western Serbia (Brđani Gorge – grassland, Ravnik – conifer forest, and Đetinja River Gorge – sparsely vegetated talus). The plan was to identify components of the edaphic factor that are of greater importance for the development of serpentine habitats. The determined features were compared with a limestone-derived soil (Ovčar Banja), which was regarded as "contrasting" with respect to several factors that are important for plant physiology and adaptation. Some plant species find both these habitats suitable for growth. These species, namely *Halacsya sendtneri*, *Cheilanthes marantae* and *Seseli rigidum*, were selected as reference species for site and soil comparisons.

EXPERIMENTAL

Sampling locations

All four sampling habitats were located in central/western Serbia – Fig. 1 and Table I. The reference plant species, upon the presence of which the sites were selected, were *Halacsya sendtneri* (Boiss.) Dörfl. (Boraginaceae) – a strict serpentine endemic, *Cheilanthes marantae* (L.) Domin. (Pteridiaceae) – a preferential serpentinophyte/strong serpentine indicator, and *Seseli rigidum* Waldst. et Kit. (Apiaceae) – bodenvag, found on and off serpentine. Brđani Gorge hosted all three species, while another population of *H. sendtneri* was found in Ravnik, of *C. marantae* in Đetinja River Gorge, and of *S. rigidum* in Ovčar Banja limestone habitat. In each locality, three soil samples were taken from near the roots of the reference species, from a depth of 0 to 10 cm.

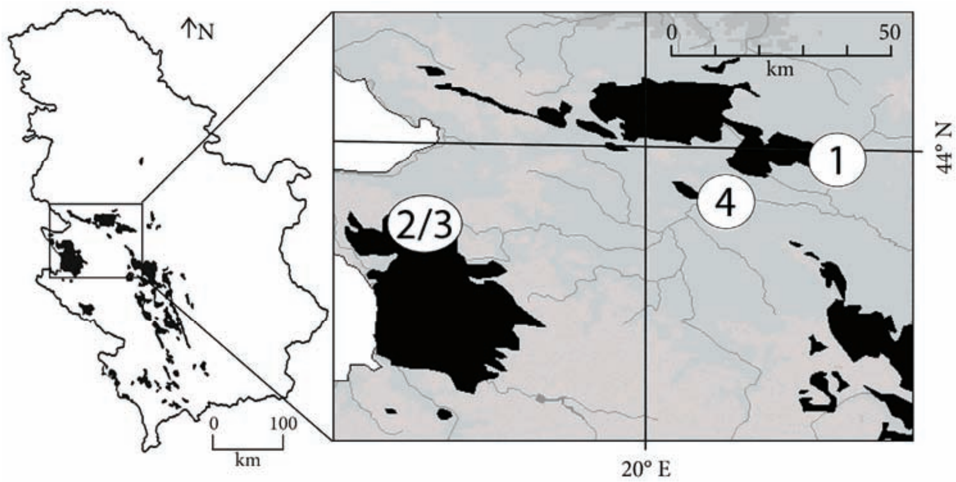

Fig. 1. Left: distribution of serpentine areas in Serbia (black). Right: close-up and position of researched habitats – see Table I for details.

Table I. Collection location substrate, location coordinates, altitude, aspect and slope

No.	Collection location	Substrate	Location coordinate		Altitude m	Aspect	
			Latitude	Longitude		Direction	Slope, °
1	Brđani Gorge (BR)	Serpentine	43°59′22.1″	20°25′34.2″	383	SW	45
2	Ravnik (RA)	Serpentine	43°51’39.5″	19°35′4.7″	616	S–SW	30
3	Đetinja River Gorge (DJ)	Serpentine	43°51′22.9″	19°35′40.1″	600	E–SE	65
4	Ovčar Banja (OB)	Limestone	43°54′0.8″	20°11’47.6″	294	NW	70

Physical and chemical analysis of soils

To survey soil morphological characteristics, soil samples were air-dried and milled to <2 mm, in accordance with ISO 11464:2006.[28] The particle size distribution was then determined by the pipette method. The size fractions were defined as coarse sand (200–2000 µm), fine sand (20–200 µm), silt (<20 µm) and clay (<2 µm). The soil form was determined according to the ISSS (International Society of Soil Science) soil texture classification.[29] The field capacity was calculated as the mass percent water retention of dry soil. The pH value was determined in a 1:5 (*V*/*V*) suspension of soil in 1 M KCl using a glass electrode by the ISO 10390:1994 method.[30] The organic matter content was measured by the ISO 14235:1998 sulfochromic oxidation method.[31] The free $CaCO_3$ content was determined by the ISO 10693:1995 volumetric method.[32] The available phosphorus (P_2O_5) and available potassium (K_2O) were determined by ammonium lactate extraction,[33] followed by spectrophotometry and flame photometry detection, respectively. The total N, S, and C were determined according to the AOAC 972.43:2000 method,[34] and the total organic carbon (*TOC*) according to ISO 10694:1995,[35] all by elemental analysis on a CHNS analyzer Vario EL III (Elementar, Germany). The cation exchange capacity (*CEC*) was measured using the ISO 11260:1994 method.[36]

Total and sequential extraction of metals

The soil pseudo-total (hereinafter: total) elemental content was gained by hotplate *aqua regia* digestion.[37] The samples were dried to constant mass at 105 °C, then well mixed and weighed to 0.5 g (±0.0001). The digestion was performed in 12 mL of *aqua regia* on a hotplate at 110 °C for 3 h. After evaporation to near dryness, the samples were diluted with 20 mL of 2 % HNO_3 (*p.a.*, Carlo Erba), then filtered and brought to 100 mL with double-distilled water.

Procedure for the sequential extraction is given in the Supplemetary material to this paper.

Data analysis

Linear correlations were determined using the two-tailed Pearson correlation, with the appropriate level of significance indicated in each case. All significance tests were performed by One-Way ANOVA, followed by the Post-hoc Tukey HSD test. Principal components analysis (PCA) was performed and the scores for different soil collection locations and loadings for each variable included in the calculation were plotted. The original variables found to be the most plant-relevant for the edaphic factor were included in the PCA. These were *TOC*, organic matter, *CEC*, S, K_2O, C:N, P_2O_5, field capacity, $CaCO_3$, pH-KCl, sums of available Ca, Cr, Cu, Mg, Ni and Zn. Eigenvalues were extracted from the correlation matrix. All statistical analyses were performed with IBM SPSS software.[41]

RESULTS AND DISCUSSION

Mg:Ca ratio

High Mg:Ca ratios (>> 1.0) are typical of serpentine soils, but are not usually encountered in non-serpentine habitats.[2] Being competitive for uptake into root,[42] the Ca and Mg contents and their balance are some of the most important factors behind the harshness of serpentine to plant life.[1] Both elements are macronutrients, but plants need higher amounts of Ca than of Mg for proper functioning.[42] Such a requirement is primarily due to the role of Ca in maintaining the structural integrity of the cell walls. This is especially true for dicotyledonous plants and their pectin-rich Type I cell wall that has a high Ca requirement. The Type II wall of commelinoid monocotyledonous, and Type III wall of Pteridophytes have somewhat different structures and, consequently, lower Ca requirements and higher tolerances towards extreme Mg:Ca ratios.[43,44] These pre-adaptations lead towards specific and low-productive vegetation patterns in serpentine.

In serpentine soils of sites RA and BR, the Mg:Ca ratio fell under 1 only slightly (0.9–0.7) in the final plant-available fraction (F4), while in DJ soil, 2.8 was the lowest detected ratio. Subsequent available fractions differed only moderately in their ratios in each serpentine soil. The difference was however very large when the available fractions were compared with the ratio of total contents, which ranged from 28 to 206 (Fig. 2). The available amounts of metals in soil were therefore more relevant, and considerably less harsh for plant growth than their total contents. This is an important consideration regarding reasons for low productivity on these soils.

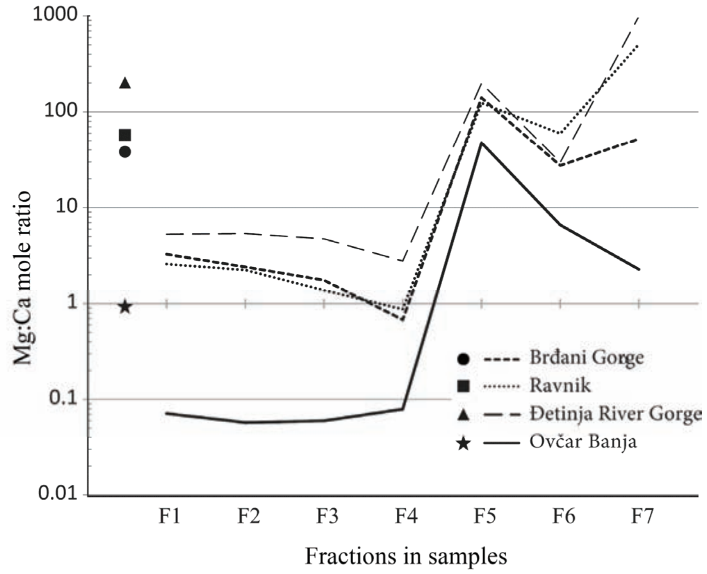

Fig. 2. Mean Mg:Ca mole ratio in *aqua regia* extract (filled shapes), and in each fraction (lines).

In the serpentine soils studied herein, the Mg:Ca ratios showed similar trends of fractional distribution (Fig. 2). However, among habitats, the ratios were most noticeably different in the available fractions, as well as in the residual fraction. While the ratio in the residual fraction reflects the specific rock mineralogy, the difference is also governed by other factors in the available fractions. The amounts of available Ca could increase due to organic matter accumulation,[45] thus decreasing the available ratio with Mg. A decreasing trend was confirmed here for serpentine soils where the Mg:Ca ratio of available fractions was negatively correlated with level of organic matter (R = –0.832; p = 0.005). Soil from the RA site contained the most organic matter (8.98 %) and the lowest Mg:Ca ratio (2.4), site DJ had the least organic matter (3.71 %) and the highest ratio (5.2). Soil from the BR site had values between the other two serpentine soils. The Mg:Ca ratio in the available fractions was also negatively correlated with *TOC*, C, N and K_2O (R = –0.799, –0.803, –0.844 and –0.889, respectively, $p < 0.01$) and S and P_2O_5 (R = –0.765 and –0.717, respectively, $p < 0.05$). The bulk of available Ca that contributed to the trend in the ratio, however, did not originate from the organically bound fraction (F4) but from the water-soluble and exchangeable fraction (F1), containing 70 to 80 % of the bioavailable Ca. The significant positive correlation of organic matter with field capacity (R = 0.802, p = 0.09) showed that perhaps the field capacity played an important role, not only in drought relief, but also in enabling higher Ca availability and preventing its leaching. The results imply that a harsh Mg and Ca balance is strongly influenced and alleviated by the increased presence of organic debris originating from native vegetation. Although not necessarily bound in organic matter, Ca could be held in the water-soluble phase because of increasing the soil water holding capacity. The change towards a more favorable Mg:Ca regime, however, is not easily achieved in serpentine habitats as a low ratio is actually identified as one of the main reasons for low-productivity and scarce vegetation.[1]

Metal load and fertility

The elemental distribution in the available fractions (Fig. 3) was very similar among the serpentine soils, despite differences in soil development level, vegetation type/structure, and variation in some chemical properties (Tables II and III). Statistically significant differences were observed only in contents of Co in the F3 and F4 fractions, and of Ca in the F7 fractions (F = 6.881, 9.302 and 8.081, respectively, $p < 0.05$). Among the three serpentine habitats, the total Co, Fe, and Sc contents were significantly lower in soil from the RA site (F = 7.627, 8.521 and 7.620, respectively, $p < 0.05$). In the available pools (sum of F1 to F4), the soil from the RA site was significantly lower in Co (F = 11.325, $p < 0.05$), and higher in Mg and Zn (F = 5.850 and 5.457, respectively; $p < 0.05$) than the others. Other discrepancies included significantly lower available Al in the soil from the DJ than in the soil from the BR site (F = 16.959; $p < 0.05$), and the available Cr that was significantly lower in the soil from the DJ than in the soil from the RA site (F = 13.353; $p < 0.05$). No detectable amounts of Ag, B, Be, Cd, Mo or Pb were found in any of the examined soil samples (including the limestone soil). The total Ni content was negatively correlated with the organic matter, C, *CEC*, N, C, S, *TOC*, K_2O and P_2O_5 (R = –0.928, –0.956, –0.850, –0.940,

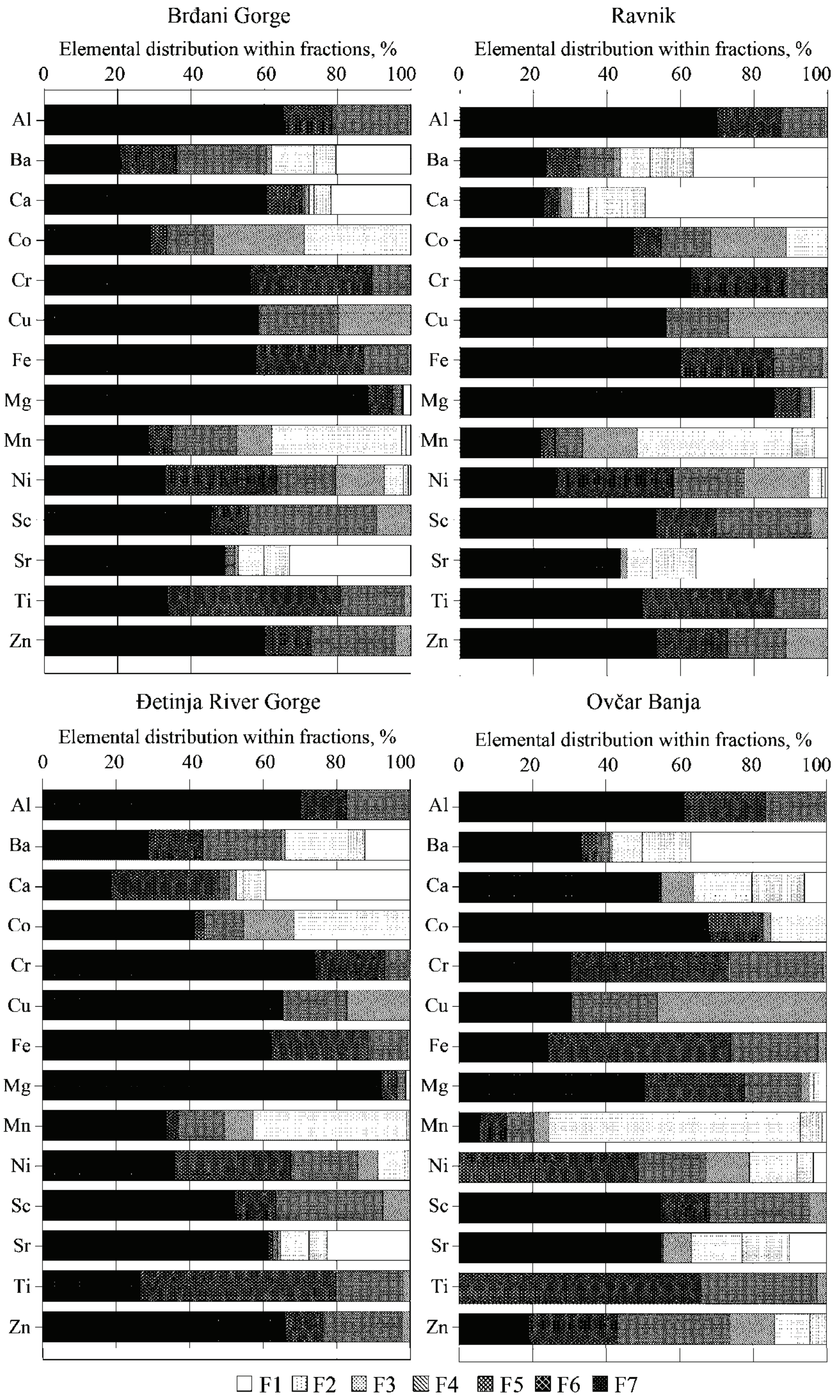

Fig. 3. Distribution of 14 elements in seven sequentially extracted soils fractions, %.

–0.956, –0.919, –0.955, –0.846 and –0.806, respectively, $p < 0.01$). Curiously, no relation of the sum of the available Ni was found with these chemical properties of soil, or with the total Ni content. However, there were positive correlations of the available Zn with organic matter, C, *TOC*, K_2O and P_2O_5 (R = 0.909, 0.963, 0.964, 0.800 and 0.863, respectively, $p < 0.01$). The positive correlation with the amount of organic matter is a reasonable finding since soil organic matter is expected to increase Zn accumulation in the surface horizons of most soils.[13]

TABLE II. Chemical and morphological properties of soil; mean ± *SE*

Property/locality	Brđani Gorge	Ravnik	Đetinja River Gorge	Ovčar Banja
pH-KCl	6.2±0.2	6.2±0.3	6.5±0.1	7.2±0.2
$CaCO_3$ / %	0.57	0.48	0.51	14.66
Organic matter, %	7.38	8.98	3.71	5.00
P_2O_5 / mg 100g^{-1}	2.5±0.4	4.2±1.5	2.0±0.3	3.2±0.3
K_2O / mg 100g^{-1}	17.1±2.7	17.4±2.8	6.8±0.4	13.2±2.1
CEC / cmol+ kg^{-1}	6.1±1.6	8.2±1.7	4.0±0.6	4.4±0.7
N / %	0.53	0.54	0.22	0.34
C / %	5.31	12.63	1.92	6.00
TOC / %	5.16	12.55	1.87	3.00
S / %	0.09	0.15	0.07	0.09
C:N	9.70	20.85	9.03	15.58
Clay, %	7.8	9.9	5.3	7.1
Silt, %	21.8	10.9	17.8	26.6
Fine sand, %	33.1	21.9	29.7	41.5
Coarse sand, %	37.4	57.4	47.2	24.8
Field capacity, %	93	108	70	71
Total mass Mg:Ca	16.8	32.8	125.1	0.6
Total molar Mg:Ca	27.7	54.1	206.3	0.9

The K_2O levels of soil from the DJ site were found to be significantly lower than those in the other two serpentine sites ($F = 7.127$, $p < 0.05$), which places the DJ samples in the poor class of soil, while in other habitats, the soils were classified as optimal with regards to their K_2O levels. According to the classification of agricultural soils, all four soils were in the class of very poor soils in terms of the available P_2O_5 (< 5 mg 100 g^{-1}).[46] Contrary to this, all the examined soils were considered to be well supplied with total N (> 0.2 %).[47] In addition, a significantly lower field capacity was found for the soil from DJ site ($F = 11.734$, $p < 0.01$). Significantly elevated C:N ratios in soils occur through a low rate of organic matter decomposition and/or low N level.[48] In the soil from the RA habitat especially, it was clearly the high level of organic matter that caused the high C:N ratio ($F = 7.651$, $p < 0.05$). In the soils from the DJ and BR sites, the C:N ratios were comparable although their organic matter was considerably different. This confirms the hypothesis that serpentine soils can exhibit both higher and lower C:N ratios compared with similar non-serpentine soils,[2,49] even if the organic matter levels are dissimilar.

TABLE III. Eigenvalues, percentage and cumulative percentage of variance explained by the first two components, and factor loadings of the variables

Parameter	PC 1	PC 2
Eigenvalue	7.381	5.243
Variance, %	46.1	32.8
Cumulative var., %	46.1	78.9
TOC	0.951	–0.370
organic matter	0.933	0.026
CEC	0.924	–0.121
S	0.914	0.085
K_2O	0.870	0.154
C:N	0.851	0.505
P_2O_5	0.799	0.202
available Mg	0.751	–0.517
available Cr	0.620	–0.250
Field capacity	0.401	–0.168
available Zn	0.165	0.986
available Ca	0.144	0.969
available Cu	0.035	0.965
$CaCO_3$	0.064	0.963
available Ni	0.244	–0.744
pH-KCl	–0.486	0.735

Consistently and significantly lower amounts of soil organic matter, K_2O, P_2O_5, field capacity, and high Mg:Ca ratio promoted the infertility of the DJ soil. Similar causes of infertility were also identified in attempts at re-vegetating the low water-holding capacity and low nutrient roadcuts on serpentine.[50] However, the availability of potentially toxic metals typical of serpentine was also found to be lower in the DJ soil as the pH remained high, and *CEC* remained low compared with the other investigated sites. This implies that vegetation scarcity in this serpentine site is primarily due to low soil fertility and unfavorable Mg and Ca contents, rather than toxic contents of micronutrients (Ni and Zn) or other non-essential metals (Al, Co and Cr). The availability of elements (Al, Ba, Ca, Cr, Cu, Mg, Ni and Zn) was shown to be increased in the more developed grassland (BR) and forest (RA), as were the factors of fertility, including improvement in the Mg:Ca ratio.

Limestone vs. *serpentine contrast*

As expected, the limestone soil from Ovčar Banja differed from the three serpentine soils in many characteristics. A slightly alkaline pH was found in limestone (7.2), and mildly acidic pH in serpentine (6.2–6.5). Free $CaCO_3$ reached far higher concentrations in limestone, with significantly more fine sand and less coarse sand (Table II). This classifies limestone soil as loam, in contrast to the sandy loams in serpentine. The only two elements without significant differences in total extracts from the contrasting soils were Ti and Sc, both physiologically rather irrelevant. The available Mg:Ca mole ratio in limestone averaged in the Ovčar Banja soil at 0.06, and around 1.0 in the total extraction. The available pools of Co, Mg, and Ni were significantly lower (F = 11.057, 11.064 and 15.441, respectively, $p < 0.01$), and Ba, Ca, Cu, Sr, Ti and Zn significantly higher (F = 39.215, 10.120, 70.117, 11.632, 25.755 and 16.726; respectively, $p < 0.01$) in the limestone soil compared with the serpentine soils. The results suggest that the core differences between the limestone and serpentine soils surveyed in this study were in the expected disproportional properties (pH, Mg:Ca and metal load), but did not necessarily include the fertility factors (N, P_2O_5, K_2O).

Nickel and titanium in the Ovčar Banja limestone samples, as well as Mn in one sample from the locality, were higher in the summed six sequential fractions than in the *aqua regia* extract. Therefore, their content in the seventh (residual) fraction could be considered zero, leading to the conclusion that the actual total contents in Ovčar Banja soil were at least 25 % higher for Ti, and about 30 % higher for Ni.

Principal components analysis

Percentage eigenvalues, scores plot and factor loadings on the axes are given in Table III and shown in Figs. 4 and 5. The first two principal components accounted for 78.9 % of the variance in the 16 original variables. The first axis

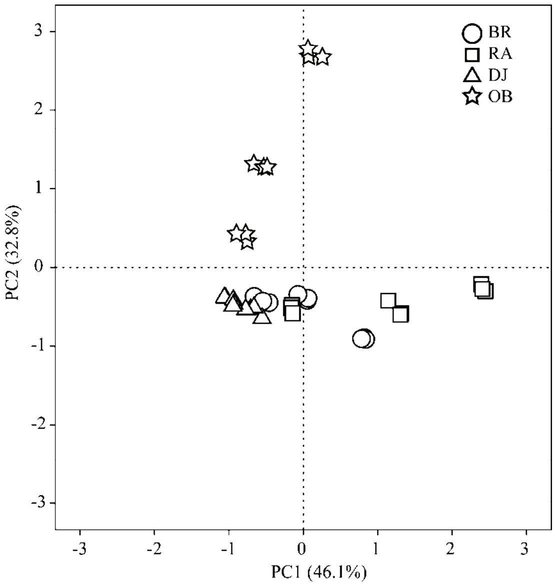

Fig. 4. Principal components analysis – scores plot of soils from the four sampled locations. The first two axes accounted for 78.9 % of the total variance.

explained 46.1 % of the variance and described the sites characterized by soil fertility markers (the fertility axis: P_2O_5, K_2O, S, N, organic matter, *TOC* and *CEC*). The second axis explained 32.8 % of the variance and was determined by other micro- and macronutrients that were elevated in limestone (Ca, Cu and Zn).

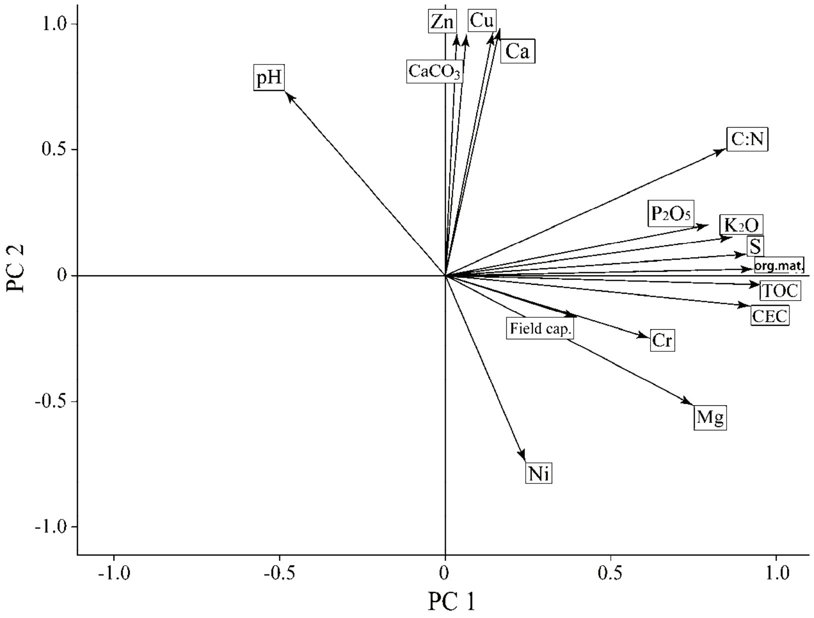

Fig. 5. Principal components analysis – loadings of the original variables on the axes.

The PCA confirmed that the prime differences among the serpentine habitats lay mostly in the fertility factors, which were explained by the fertility axis. These factors are dependent upon the richness and productivity of the vegetation, which gives rise to soil organic matter, and consequently to increased N, P and K levels. The soil from the BR habitat (Fig. 4; circles) was placed between the other two serpentine sites (DJ and RA), which may contribute to explaining the reason this habitat was able to host all three reference plant species. The Brđani Gorge habitat may therefore represent a midway condition in a plant habitat selection gradient.

CONCLUSIONS

The complexity of the edaphic factor in these surveyed serpentine ecosystems was the result of a fine balance of causally linked properties. The chemical composition of serpentine bedrock and soil, combined with different environmental factors, results in a variable vegetation composition, even in habitats physically similar to one another. A combination of these soil components continues to be an unconquerable obstacle for the majority of plants, which enables specific serpentine communities to sustain and develop.

It is clear from the present findings that some of the usual reasons for the harshness of serpentine environments, such as high levels of Ni, Cr or Co were not so intense in the bioavailable fractions of these metals. The task of identifying components with more importance becomes complicated when low-fertility components (N, P and K) and physical harshness (low field capacity, erosion and high temperatures) are included and combined with metal load. The low-fertility soil of Đetinja River Gorge maintained a low metal load through a lower *CEC* and higher pH. Simultaneously, its low productivity gave less organic matter, consequently leading to lower field capacity. This indicates a mechanism that prevents the Mg:Ca ratio from becoming more favorable. Conversely, the higher amount of organic matter in Brđani Gorge and Ravnik lowered Mg:Ca ratios and raised field capacity, while increasing the availability of certain metals (Al, Ba, Ca, Cr, Cu, Mg, Ni and Zn), thus increasing the metal load. Despite the more xeric conditions and higher metal availabilities, Brđani Gorge and Ravnik soils were actually less hostile with regard to the soil Mg and Ca availabilities, and consequently they hosted richer vegetation types. When comparing the soil from serpentine with the limestone soil, the differences in metal content and distribution of availabilities were large, but much smaller for other chemical and physical properties related to fertility.

The evolution of edaphic conditions in serpentine habitats is a slow and complex process. Once disturbed, they are not easily restored. The proportion of endemics determined in the serpentine flora of the Balkans is very high, and the area underlain by ultramafics is significant. In view of the fragility and value of serpentine habitats, we call for intensified research of the biota in this natural resource and protection of serpentine habitats as hotspots of diversity and endemism on the Balkan Peninsula.

ИЗВОД

ДИНАМИКА ХЕМИЈСКИХ ОДЛИКА ЗЕМЉИШТА РАЗЛИЧИТИХ СЕРПЕНТИНИТСКИХ СТАНИШТА У СРБИЈИ

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Ова студија бавила се стањем и развојем едафских услова у серпентинитским стаништима. Детаљно су проучене хемијске и механичке одлике три земљишта са дисјунктно дистрибуираних, али честих серпентинитских станишта централног Балкана. Компаративном анализом обухваћено је и једно просторно блиско, али хемијски и биолошки контрастно кречњачко земљиште. Узорковање је обављено на локалитетима одабраним према присутности референтних серпентинофилних биљака – *Halacsya sendtneri*, *Cheilanthes marantae* и *Seseli rigidum.* Утврђивано је двадесет, а квантификовано четрнаест метала у свакој од седам секвенцијално екстрахованих фракција земљишта. Поред тога, одређени су и: pH земљишта, нивои слободног $CaCO_3$, количине органске материје, P_2O_5, K_2O, N, C, S, капацитет размене катјона, укупни органски угљеник, капацитет задржавања воде, као и механички састав земљишта. Утврђено је да су типичне стресне карактеристике земљишта попут високог односа Mg:Ca, високих нивоа никла, хрома или кобалта, у значајној мери убажене у биодоступним фракцијама. Утврђена је висока корелација између нивоа органске материје и капацитета задржавања воде, као и то да се највећи део (70–80 %) доступног Ca налази у мобилној, а не органски-везаној фракцији земљишта.

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