

HEAVY METALS UPTAKE BY HYPERACCUMULATING FLORA IN SOME SERPENTINE SOILS OF KOSOVO

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ABSTRACT

Ultramafics represent magmatic or metamorphic rocks which are characterized by high concentrations of Mg, Fe, Ni, Cr and Co and low concentrations of Ca, and K. Serpentine soils are weathered products of a range of ultramafic rocks composed of ferromagnesian silicates. The aim of this study was to determine the content of heavy metals in some of serpentine soils of Kosovo and heavy metals uptake by entire associated flora. Furthermore, another objective of this study was finding out bioavailable Ca/Mg relationship, which is very important indicator for plants' development. The sampling was conducted in June 2014. A total of three serpentine areas have been surveyed and 7 soil samples have been taken in various depths of soil profiles. Those samples were analyzed for total Ca, Cd, Co, Cr, Cu, Mn, Ni, Pb, Fe and Zn. Results showed that each site exhibited a high concentration of at least one metal. The maximum concentrations of metals in soils Dry Matter (DM) were 108.9 mg kg⁻¹ Cd, 95.8 mg kg⁻¹ Co, 1206 mg kg⁻¹ Cr, 24 mg kg⁻¹ Cu, 2570 mg kg⁻¹ Ni, 21.7 mg kg⁻¹ Pb, 39 mg kg⁻¹ Zn, and 51563 mg kg⁻¹ Co, 1206 mg kg⁻¹ cr, 24 mg kg⁻¹ Cu, 2570 mg kg⁻¹ Ni, 21.7 mg kg⁻¹ Pb, 39 mg kg⁻¹ Zn, and 51563 mg kg⁻¹ cr, he serpentine soils at all sites were characterized by elevated levels of heavy metals, which showed typical properties of ultramafic environments. Nickel Total at studied areas varied between 1543 and 2570 mg kg⁻¹, while the highest Ni concentration was found in aerial part of Alyssum markgrafii (4038 mgkg⁻¹),

Based on our findings on the field we concluded that there is a close relationship between the quantity of Ni in soil and Ni uptake in plants.

Keywords: Serpentine soils, Heavy metals, Ultramafics, Metals bioavailabillity

1. Introduction

Ultramafics represent magmatic or metamorphic rocks which are characterized by high concentrations of Mg, Fe, Ni, Cr and Co and low concentrations of Ca and K containing less than 45% silica (SiO₂).

The term "serpentine" strictly speaking refers only to the serpentine group of minerals (including antigorite and chrysolite) with the general formula $Mg_3Si_2O_5(OH)_{4}$, 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 "serpentines". Serpentines are rocks that form as a result of metamorphism or metasomatism of primary magnesium–iron silicate minerals.

Throughout the territory of Kosovo there are many basic and ultra-basic rocks, commonly known as "serpentines". The aim of this study was to determine the content of heavy metals and other macro elements in some of serpentine soils, and as well as finding out bioavailable Ca/Mg relationship, which is very important indicator for normal plants development. The studied areas of serpentine soils include three separate zones with an area of 15.67 km² comprising thus 7.66 % out of total serpentine soils in Kosovo.

1.1. Chemical properties of serpentine soils - the serpentine factor

The serpentine factor may be defined as the causal factor (physical or chemical) related to the infertility of serpentine soils. The general infertility of serpentine soils may also be defined as the serpentine problem" (Brooks, 1987). Kazakou *et al.* in 2010 presented three of the most popular hypotheses in order to explain the edaphic factors which may control serpentine floras: 1) Low availability of calcium in relation to magnesium; 2) Deficiency of essential macronutrients; 3) High levels of phytotoxic heavy metals (Ni, Cr, Co, Mn).

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 topic was elaborated in detail by Krause, (1958) and has been dealt with over the past 150 years. Part of this toxicity comes from the very high magnesium concentration in some soils, containing up to 36% of MgO. As per my results obtained at examined areas, the total Mg content ranges from 16 to 19.4%.

b) Deficiency of Calcium

The concentration of calcium in serpentine soils is extremely low and in some cases is <100 mg kg⁻¹. However, low calcium levels are not the primary cause of serpentine infertility. It is rather the low Ca/Mg quotients that primarily cause this serpentine syndrome. Experiments conducted by Proctor, (1971) have proved that addition of calcium could largely reduce the incidence of nickel toxicity symptoms in vegetation. The Ca content in serpentine soils of Kosovo varies from 628.83 to 5422.27 mgkg⁻¹.

c) Unfavorable Ca/Mg Quotient.

Loew & May (1901) first reported that poor productivity of serpentine soils is due to the low Ca:Mg ratio present in them. From their experiments, they concluded that the Ca:Mg ratio must be at least in unity for optimal growth of plants.(The bioavailable Ca/Mg ratio in studied serpentine soils of Kosovo is in range of 0.63-3.17)

2. Materials and methods

The sampling was conducted in June 2014. A total of three serpentine areas have been surveyed and 7 soil samples taken from various depths of soil profiles. In addition to this, the entire flora present at studied locations was collected, identified, air-dried, sieved to less than 2 mm and analyzed for heavy metals concentrations as well as for the content of Calcium and Magnesium. Soil samples were picked upon observing the presence of the plants. All soil samples were air-dried, ground and sieved to 2 mm, then transported in polyethylene bags to the laboratory for further analysis.

Those samples were analyzed for total Ca, Cd, Co, Cr, Cu, Mn, Ni, Pb, Fe and Zn. Results showed that each site exhibited a high concentration of one or more heavy metals.

The first site investigated was Vejshtine, situated in the south-west Kosovo, lyingat an altitude of 632 m above the sea level and belonging to the continental climate with an average annual precipitation of 604 mm. The average temperature is 10 °C with its lowest -1.3 °C in January and its highest 20.4 °C in July. The area is characterized by poor vegetation. In this area one soil profile was dug and two soil samples were taken, one for each horizon. The depth of profile was 0.6 m with bedrock lying underneath. The second

investigated site was Çabër, situated in the southern part of Kosovo at an elevation of 587 m, where one soil profile was dug and three samples of soil were taken. The third investigated site was Radoniq, a location situated in the western part of Kosovo, at an elevation of 455 m. In this sampling site one soil profile was dug and 2 soil samples were taken at two different horizons. All soil and plants' samples were air-dried, then ground and sieved to 2 mm or less. Subsequently, they were mineralized with a microwave digester. Conditions for mineralization were 6 ml HCl, 2 ml HNO₃, and 3 ml H₂O₂, per 0.5-g soil. Soils were air-dried and sieved to 2 mm. Total major (Ca, Mg) and trace elements (Ni, Cr, Cu, Zn, Co, Pb, Cd, Fe and Mn) were determined in mineralization solution by applying atomic absorption spectrophotometry. Ni, Ca and Mg bioavailability in different soil samples was extracted by Mehlich 3 method (Schroder *et al.*, 2010)

3. RESULTS AND DISCUSSIONS

a) Trace elements

The serpentine soils at all sites were characterized by elevated levels of heavy metals that show typical properties of ultramafic environments.

Results showed that each site exhibited a high concentration of at least one heavy metal. The maximum concentrations of Cd in soils dry matter (DM) were 108.9 mg kg⁻¹ varying between 8.9 and 108.9 mg kg⁻¹ The DM value was relatively high compared to the values generally observed in non-serpentine soils and considered toxic according to Kabata-Pendias and Pendias (1992). Its highest value was observed at Çabër location.

Site	mg kg ⁻¹ DM										% DM		
	Zn	Cd	Со	Cu	Ni	Pb	Mn	Cr	Fe	Са	Mg	рН	
Vejshtine HA	32.7	8.9	62.9	24.0	1543	21.7	1013	1150	4.87	0.37	17.90	8.58	
Vejshtine HB	26.6	84.3	71.5	21.4	2008	9.1	780	1206	5.06	0.54	18.70	8.64	
Çaber HA	39.0	103.1	82.8	14.4	2235	11.2	910	680	5.15	0.18	17.80	7.95	
Çaber HB	31.7	108.9	95.8	10.0	2570	9.2	898	218	4.50	0.07	19.20	7.88	
Çaber HC	25.7	94.8	54.2	5.4	1865	9.0	563	260	3.65	0.17	19.30	7.97	
Radoniq HA	31.7	67.2	78.8	9.2	2234	20.7	965	770	3.93	0.18	16.00	7.58	
Radoniq HB	20.4	66.8	49.5	8.3	1576	9.3	508	399	2.43	0.06	19.40	7.79	

Table 1. Heavy metals, Ca and Mg concentrations and pH in soils

Cr concentrations in soils were also elevated and varied from 218 to 1206 mg kg⁻¹ of DM.

Total Ni availability at studied areas varied between 1543 and 2570 mg kg⁻¹ respectively, whereas bioavailable Ni is at range of 37.71 mg kg⁻¹ and 101.04 mg kg⁻¹ respectively (Çabër).

The Mn content at all sites was rather homogenously distributed and varied between 508-1013 mg kg⁻¹

Co presence in soil varied from 49.5-95.8 mg kg⁻¹, being thus within the normal range for mafic rocks. Relatively easy interactions of Co with all metals that are associated geochemically or biochemically with Fe have a significant impact on its behavior in soils and its phytoavailability (Kabata-Pendias, 2011.

Iron concentration at all sampling sites showed typical ultramafic characteristics and varied from 2.43-5.15%, while most of the total Cu, Zn, and Pb concentrations in analyzed serpentine soil samples fall within the normal soils' range.

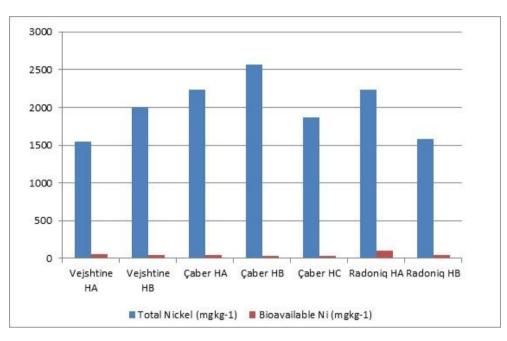
Soil Ca ranged from 0.06 - 0.54%, whilst total Mg concentrations were 16-19.4%. As we have anticipated, all serpentine soils were characterized by extremely low Ca/Mg quotients in the range of 0.003-0.02.

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.

There are several factors that contribute to phytotoxicity of heavy metals and their mobility through the soil horizons. One of the most important factors is the pH of the soil. In this study the pH of all soil samples

is predominantly basic and varies from 7.58 to 8.64, which shows that heavy metals mobility through the horizons of soil profile is very low when it comes to the pH readings.

If we refer to the papers published by (Bani *et al.*, 2010; Bani *et al.*, 2013) serpentine soils of Albania do not show any big difference in Ni content (1370-3240 mg kg⁻¹⁾ and 1658-3077) when compared to Kosovo's serpentine regions (1543-2570 mg kg⁻¹). Furthermore, if we compare the general presence of heavy metals in soils of Albania based on (Shallari *et al.*, 1998) paper, there is no big difference in their contents either.



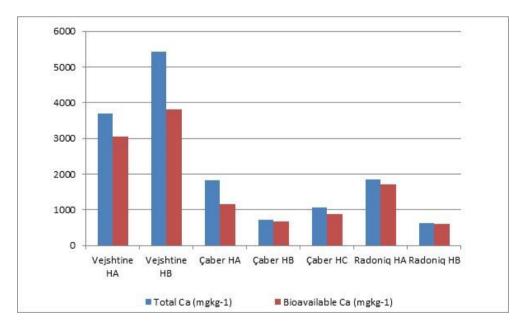


Figure 1. Total and Bioavailable Nickel proportion in serpentine soils

Figure 2. Total and Bioavailable Calcium proportion in serpentine soils

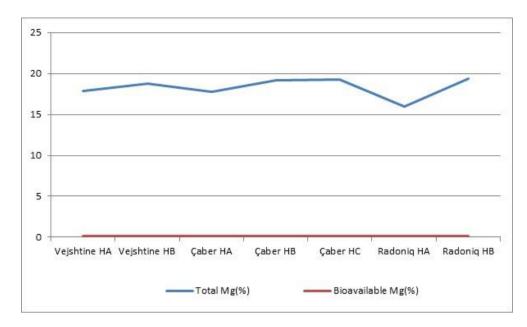


Figure 3. Total and Bioavailable Magnesium proportion in serpentine soils

Standard deviation of Nickel content in soil samples

Even though the serpentine soils are characterized by elevated levels of Nickel, Chromium and Cobalt, my attention will be focused on calculation of total Nickel content in soil and Ni uptake by hyperaccumulators plants

The formula for sample standard deviation

$$s = \sqrt{\frac{1}{N-1}} \sum_{i=1}^{N} (xi \cdot \overline{x})^2$$

The mean of Nickel content in soil is:

(1543+2008+2235+2570+1865+2234+1576)/7=2004 mg kg⁻¹

So, *x*=2004

Following subtraction and squaring the sample variance is: 140276

Whose square root is: s=374

Ni uptake by the hyperaccumulator plants species

Table 2 shows analysis performed on aerial part of plants. The highest Ni concentrations were recorded in *Alyssum markgrafii* which was the dominant species in all serpentine sites examined in this study. Ni uptake at *Alyssum markgrafii* varied from 1585.6-4038.5 mg kg⁻¹ which is lower than Ni concentration found in the same species of serpentines of Albania (from 5234 to 12691 mg kg⁻¹) (Bani *et al.*, 2013). Ni uptake at *Thlaspi sp*. is 798.7 mg kg⁻¹. If we compare this number with those from other serpentine regions of the Balkans we will see that Ni concentration of *Thlaspi sp*. found in Kosovo is lower than in Albania (1360 mg kg-1) or *Thlaspi species* found in Bulgaria (3400 mg kg⁻¹) (Bani *et al.*, 2010; Pavlova *et al.*, 2010).The reason why Ni uptake is so low at *Thlaspi sp*. is delay in sample collection, as the maximum content of Ni in hyperaccumulator plants is in blooming stage, which is in early May for this species, whereas the sample collection happened in mid-June.Calcium concentrations in aerial part of *Alyssum markgrafii* were very high despite low concentration of Ca in soil. In the present work we obtained readings as high as 2.03% for *A. markgrafii* and 1.48 % for *Thlaspi sp*.

The Mg concentration for the *Alyssum markgrafii* was in the range 0.23-0.62%, while the one for *Thlaspi* was lower (0.17%). This may be simply a reflection of the extraordinary uptake of Ca by both species.

Basically the Ca/Mg ratios to Nickel hyperaccumulator plants are greater than one (>1), which is in contrast with serpentine associated soils. This ratio was in the range 2.21-6.78 for *Alyssum markgrafii*, and 8.70 for *Thlaspi*. Positive Ca/Mg ratio has also been observed at Albanian *Alyssum markgraffi* (1.01) (Shallari *et al.*, 1998).

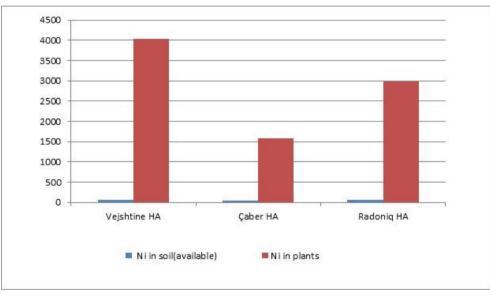
High Calcium contents in these hyperaccumulator plants are the consequence of the unusual ability of these plants to accumulate high Ca concentration in their tissues, even from the soils with low Ca/Mg ratio, which is a characteristic feature of serpentine soils. Thanks to this capability these plants are able to lower their Ca deficiency stress on their own.

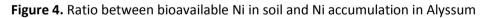
Comparison of available concentrations of Ni in soils and in Alyssum markgrafii plant tissues (aerial part) as well as assessment of accumulation capacity for Ni are presented in Figure 4. Ratio between Ni concentrations in aerial part of alyssum and its concentration in soil revealed variation among analyzed samples; the lowest ratio was 40.8 (Caber) and highest one 76.6 (Vejshtine). The accumulation factor (AF) is the ratio between trace elements concentration in plant tissues and their bioavailable concentration in the corresponding soil.

Accumulation factor is calculated with relation:

AF = Cp/Cs

where: Cp is the mean metal concentration in alyssum markgraffi sample (mg kg⁻¹) and Cs is the mean metal concentration available in soil sample (mg kg⁻¹).





Standard deviation of Nickel uptake by alyssum markgrafii

The mean of Nickel uptake by alyssum markgrafii is:

(4038.5+1585.6+2980.2)/3=2868.1 mg kg⁻¹

$$s=\sqrt{\frac{1}{N-1}}\sum_{i=1}^{N}(xi-\overline{x})2$$

Following subtraction and squaring the sample variance will be 1531604, whose square root is: s= 1230

Site	Species	mg kg ⁻¹									% DM		
		Zn	Cd	Mn	Ni	Cu	Pb	Мо	Cr	Со	Са	Mg	
	Alyssum alysoides	15.05	< 1	16.39	8.1	2.89	5.28	< 1	4.36	< 1	1.47	0.2	
	Alyssum markgrafii	143.29	< 1	38.2	4038.5	2.74	6.11	14.26	38.1	< 1	0.51	0.2	
	Asperula Arvensis	24.57	< 1	31.59	13.13	5.11	5.75	< 1	8.58	< 1	0.61	0.2	
	Bromus inermis Lays.	19.04	< 1	83.89	30.19	3.48	5.44	< 1	36.35	3.18	0.19	0.2	
	Carex Paniculata	16.86	< 1	68.11	75.72	4.15	7.63	< 1	72.39	4.4	0.19	0.6	
	Corydalis lutea	23.57	1.5	136.11	15.59	4.68	7.63	< 1	9.75	< 1	0.45	0.2	
	Crepis aurea	23.06	< 1	65.45	96.71	8.07	12.76	< 1	39.73	3.49	2.13	0.4	
	Dorycnium pentaphyllum	19.34	1.4	40.35	28.68	6.5	5.79	19.09	5.3	< 1	0.76	0.1	
	Echium rubrum	31	< 1	98.55	69.61	8.33	23.84	< 1	87.72	4.31	1.97	0.4	
	Euphorbia helioscopia	28.06	< 1	60.89	50.15	6.62	7.3	< 1	13.27	3.03	1.21	0.3	
	Fumana procumbens	21.75	< 1	60.92	66.96	6.58	9.26	< 1	46.16	6.14	0.83	0.5	
Vejshtine	Galium cruciata	22.38	< 1	33.92	18.12	9.28	6.72	< 1	16.58	< 1	0.63	0.2	
	Helianthemum Numularium	20.53	< 1	48.49	21.42	8.86	7.32	< 1	25	< 1	0.83	0.2	
	Illecebrum verticillatum	27.02	1.8	82.78	11.63	3.37	8.39	< 1	20.19	< 1	0.49	0.2	
	Potentilla arenaria	34.73	< 1	129.41	102.03	7.49	14.86	< 1	68.37	4.85	0.91	0.6	
	Rumex acetosella	20.9	< 1	39.82	23.88	3.49	6.64	< 1	18.55	< 1	0.42	0.2	
	Sanguisorba minor scop.	17.6	< 1	37.55	13.53	5.09	7.25	< 1	12.22	< 1	0.87	0.4	
	Scorzonera humilis	36.77	< 1	59.64	25.77	7.89	9.36	< 1	27.8	< 1	1.17	0.2	
	Sedum album	9.47	< 1	21.32	22.42	3.97	5.74	< 1	11.97	< 1	1.17	0.2	
	Sedum atratum	20.83	< 1	51.71	54.35	6.81	17.68	< 1	79.7	3.74	3.35	0.6	
	Stipa pennata	16.71	< 1	31.9	8.23	5.21	6.48	< 1	92.72	< 1	0.22	0.0	
	Thalictrum minus	23.52	< 1	25.77	8.63	9.23	5.25	< 1	6.72	< 1	0.49	0.1	
	Thymus pulegioides	34.19	< 1	47.12	24.46	6.83	11.3	1.81	16.12	< 1	0.83	0.2	
	Alyssum markgrafii	30.84	< 1	70.24	1585.6	1.24	< 1	2.03	< 1	38.07	2.03	0.6	
	Astragalus onobrychis	13.63	< 1	82.77	64.96	4.47	2.14	7.41	< 1	24.51	0.38	0.7	
	Carex paniculata	17.95	< 1	49.1	48.22	3.48	< 1	8.49	< 1	28.22	0.15	0.3	
Çabër	Crepis aurea	20.37	< 1	35.88	21.98	6.57	< 1	7.18	< 1	16.87	1.24	0.5	
	Dorycnium pentaphyllum	30.52	< 1	79.56	40.3	7.98	< 1	7.01	< 1	14.47	0.72	0.3	
	Echium rubrum	14.52	< 1	56.22	63.1	5.51	< 1	6.34	< 1	49.2	1.66	0.4	
	Euphorbia amygdaloides	17.36	< 1	36.78	44.31	3.73	3.1	9.36	< 1	23.3	0.93	0.4	
	Illecebrum verticillatum	15.74	< 1	106.45	32.15	3.33	< 1	9.17	< 1	29.98	0.36	0.3	

Table 2. Heavy metals, Ca, Mg concentrations in plants collected at serpentine sites

Juniperus communis	7.91	< 1	45.37	19.76	3.49	< 1	6.06	< 1	17.99	0.72	0.16
Linum tauricum	21.22	< 1	40.45	44.29	6.04	< 1	6.82	< 1	13.27	0.25	0.50
Potentilla argentea	20	< 1	48.12	27.6	5.21	< 1	7.22	< 1	8.74	0.69	0.39
Potentilla visianii	21.78	< 1	24.54	18.4	7.19	< 1	6.85	< 1	3.32	0.31	0.19
Sedum atratum	13.97	< 1	26.23	22.42	4.22	< 1	8.78	< 1	8.79	1.79	0.46
Silene pusilla W.et K.	9.1	< 1	73.16	28.28	3.59	< 1	9.06	< 1	14.12	0.94	0.43
Stachys officinalis	15.63	< 1	33.83	35.06	8.49	< 1	8.12	< 1	10.43	0.39	0.31
Thymus pulegioides	24.98	< 1	88.35	61.52	6.36	< 1	8.48	< 1	25.98	0.57	0.42
Trifolium dubium sibth.	7.56	< 1	33.93	34.12	4.6	< 1	7.23	< 1	12.99	0.61	0.48
Alyssum markgrafii	70.2	< 1	23.99	2980.2	2.73	< 1	5.17	8.69	11.28	1.56	0.23
Astragalus onobrychis	10.69	< 1	47.89	49.14	5.07	< 1	5.18	5.42	27.24	0.50	0.22
Carex paniculata	24.29	< 1	34.93	31.61	5.32	1.5	2.81	14.32	41.14	0.16	0.21
Chamomilla recutita	35.26	< 1	27.96	21.97	9.54	< 1	3.33	10.67	13.68	1.23	0.22
Cirsium canum	51.78	< 1	52.66	39.16	9.06	1.3	6.34	< 1	45.3	1.26	0.60
Crepis aurea	20.86	< 1	27.73	21.3	7.86	< 1	4.09	< 1	22.17	0.99	0.27
Dorycnium pentaphyllum	13.31	< 1	51.06	22.88	5.27	1.2	4.34	2.71	10.24	0.63	0.21
Galium verum	17.79	< 1	23.08	8.28	4.55	< 1	2.95	< 1	33.47	0.72	0.09
Gentista tinctoria	14.03	< 1	43.89	18.13	4.51	< 1	3.9	5.99	18.01	1.02	0.30
Hypericum perforatum	30.54	1.3	35.09	51.06	9.8	< 1	6.27	7.82	17.23	0.39	0.16
Melisa ciliate	19.62	< 1	52.96	10.2	3.05	< 1	5.08	< 1	9.6	0.10	0.06
Phleum Phleoides	18.83	< 1	16.5	7.84	3.83	< 1	3.7	< 1	7.05	0.09	0.03
Plantago lanceolata	27.68	< 1	30.81	21.92	5.65	< 1	4.62	4.62	29.45	0.54	0.33
Potentilla recta	20.19	2.4	31.69	10.2	5.67	< 1	6.37	< 1	7.45	1.60	0.19
Rumex acetosella	15.62	< 1	42.81	31.43	3.82	< 1	6.72	5.09	23.26	0.20	0.34
Sanguisorba minor scop.	15.22	< 1	25.69	11.47	4.71	< 1	6.29	11.8	6.47	1.30	0.22
Sedum ochroleucum	12.45	< 1	24.26	32.01	3.29	< 1	7.2	< 1	26.6	1.49	0.42
Solidago virgaurea	13.03	3.6	28.2	5.8	4.18	< 1	6.44	< 1	3.59	0.45	0.16
Stachys scardica	71.23	4.1	68.46	48.6	11.87	< 1	7.46	5.95	15.47	0.46	0.32
Thlaspi perfoliatum	593.24	< 1	38.2	798.7	2.79	< 1	3.54	14.3	38.1	1.48	0.17
Thymus pulegioides	31.42	3.1	60.24	178.21	5.87	< 1	5.63	< 1	21.97	0.52	0.41
Trifolium alpestre	13.99	< 1	85.61	16.23	4.74	< 1	6.85	< 1	4.64	0.96	0.26

4. Conclusions

Based on site surveys Kosovo serpentine flora is more peculiar when compared to adjacent nonserpentine areas. Presence of hyperaccumulator plants is not very diverse. *Alyssym markgrafii* and *Thlaspi sp* are the dominant species, with the latter one not being present in all serpentine areas, but rather found here and there. Based on our findings from the field we concluded that there is a close relationship between the quantity of Ni in soil and Ni uptake in plants, but not as close as it is in the serpentines of Albania. The Ni amount in plants is twice as much as in associated soil. These plants species can be used for phytostabilization and phytoremediation of contaminated areas by anthropogenic factor. Ultramafic areas in Kosovo, such as those examined for the present study, have been studied in less detail than many others in terms of phytoextraction, at least until recently; hence we have to intensify our research in this field in future.

Results from the field have proved that the following two out of three most popular hypotheses presented by Kazakou *et al.* 2010 have been met: Low availability of calcium in relation to magnesium; Deficiency of essential macronutrients and High levels of phytotoxic heavy metals (Ni, Cr, Co, Mn, Co).

Serpentine soils have properties that are highly unfavorable for most plants, amongst which, the heavy metal tolerance, especially to Ni, is considered to be the crucial factor for plant survival in certain serpentine soils (Kruckeberg 1954; Proctor and Woodell 1975).

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