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PHYTOACCUMULATION IN PLANTS OF MOUNTAIN GOC IN SERBIA

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ABSTRACT: The aim of this study was to determine possible phytoaccumulation of metals (Ca, Mg, Fe, Mn, Cu, Zn, Ni, Pb, Cd, Co, Cr) in the following plants growing on one specific serpentine site (mountain Goc, village Kamenica, central Serbia): *Chrysopogon gryllus* (L.) Trin.; *Cotinus coggygria* Scop.; *Dorycnium pentaphyllum* Scop. subsp. *herbaceum* (Vill.) Rouy; *Melica ciliata* L.; *Potentilla argentea* L.; *Sanguisorba minor* Scop.; *Teucrium chamaedrys* L. and *Teucrium montanum* L. The found metal concentration in the soil was in the descending order: Mg>Fe>Ca>Ni>Cr>Mn>Co>Zn>Pb>Cu>Cd. Concentrations of Ni and Cr in the investigated soil were higher then both. Also, according to the official regulation of Republic of Serbia, the concentration of Cd was above limit values. The highest concentrations of Mg, Mn, Pb, Co, and Cr were found in species *T. chamaedrys.* Zn, Ni and Cd domninated *P. argentea,* Ca in *S. minor*, Cu in *C. coggygria*, and Fe in species *T. montanum*. On the basis of these results the value for biological absorption coefficient for Ca was higher than one for all researched plants. The species *Ch. gryllus* and *P. argentea* showed the bioaccumulation potential of Zn. Our results indicate possible application of the studied plants, growing on soil formed on specific serpentine geological background, in the process of phytoaccumulation of metals.

Key words: plants, metals, phytoaccumulation.

INTRODUCTION:

Ultramafic (serpentine) type of soil, placed on ultramafic rocks, has been widely distributed in different parts of the world. Serpentine substrate surfaces cover quite large areas in the Balkans, more than in other parts of Europe (Brooks, 1987; Tatić et al., 1992). They may exist in the form of large blocks or small outcrops separated from other geological formations. They are spread from Central Bosnia, towards Western and Central Serbia, extending to North, Central and South-Eastern parts of Albania, reaching Epirus and Thessaly in Greece (Bani et al., 2010). There are some serpentine "islands" that can be considered fairly isolated, located in the North of Macedonia and North-Eastern Serbia and Greece. A small area of serpentine bedrock has been distributed in South-Western, South and Central parts of Bulgaria, mainly on Rhodopean mountains (Eastern and Central part) (Pavlova et al., 1998). Serpentine type of the soil located in Serbia occurs both on hills and mountains, covering a large area (~ 250 000 ha). (Stevanović et al., 2003).

Since the serpentine-type soil posesses properties disadvantageous for most of the plants, each serpentine soil has developed the distinctive vegetation communities. The unique features of serpentine soil led to the evolution of a characteristic flora, consisting of many endemics (Brooks, 1987). Therefore, they are specific species that are connected to the serpentine ground (serpentinophytes). Vegetation existing on serpentine soil is often sparse and patchy, presenting small number of species and individuals. Plant species found on serpentine soil have been generally divided into serpentine-tolerant and serpentine-endemic species. The first group known also as serpentine-facultative plants, survive on serpentine, but its growth is much better on other substrate, while the other group, known also as serpentinicolous or serpentine-obligate plants, grow exclusively on serpentine soil and have not been found on other substrates (Freitas et al., 2004).

General environment of serpentine-type soil is not amicable for many plants due to soil chemistry and other factors, named the "serpentine syndrome" (Kazakou et al., 2008; Kazakou et al., 2010). It comprehends: low availability of calcium (relative to magnesium), lack of essential macronutrients (P, N, K) and high levels of potentially phytotoxic elements (Ni, Cr, Co, sometimes Mn and/or Cu). The serpentine-tolerant plants ought to stand a variety of adverse physical and chemical conditions, and among others specially high concentration of some heavy metals. The extent of metal adsorption and its distribution in the plant, seems to be related to the capacity and rate of metal removal, residence time and release to the environment. Plant species posses capacity for removal and accumulation of metals. Evolution of metal tolerance of a plant, depends on the exposure of plant species to level of metals in the soil (Ernst, 2006).

Since ultramafic soil is located in many parts of Serbia covering substantial area, but there is a lack of information on its flora and biogeochemistry, particularly of small outcrops on serpentine localities. The aim of this study was to determine possible phytoaccumulation of metals (Ca, Mg, Fe, Mn, Cu, Zn, Ni, Pb, Cd, Co, Cr) in plants growing on a specific serpentine site on mountain Goc, village Kamenica in Central Serbia. The following plants were studied: *Chrysopogon gryllus* (L.) Trin.; *Cotinus coggygria* Scop.; *Dorycnium pentaphyllum* Scop. subsp.

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herbaceum (Vill.) Rouy; *Melica ciliata* L.; *Potentilla argentea* L.; *Sanguisorba minor* Scop.; *Teucrium chamaedrys* L. and *Teucrium montanum* L.

MATERIAL AND METHODS:

Study site

This researched serpentine soil was located near the village of Kamenica, in Central Serbia (Figure 1). This site belongs to the larger part of serpentine substrate, located in Western and Central Serbia, extending to North, Central and South-Eastern parts of Albania. There was a thin level of serpentine soil in Village Kamenica, with no clearly formed soil profile, containg fractions of sand and mixed stones and belongs to the deeper rendzina (leptosols).

The alltitude of investigated site was 352-410 m and is centered on 43° 36’ 760” – 44° 04’ 34” N, 20° 42’ 046” – 20° 32’ 24” E (read by GPS Garmin-etrex, vista HCx). The maximum daily temperature during the summer and winter are 22°C and - 1°C, respectively. Average precipitation in this area is higher than 734 mm.

Fig. 1. Kamenica village

Soil - sampling and analysis

Six soil samples were collected for analysis from the level 1-10 cm of the depth and near roots of researched plants. This depth was selected since it corresponds to the major rooting zone of the herbs and small shrubs (Reeves et al., 2007). Soil samples were air-dried and sieved to 2 mm after stone pieces removal, and finally stored at 4 °C, until the analysis. Sub-samples of 10 g were ground to pass a 70-mesh sieve (<215 µm) and then oven-dried at 105 °C for 24h.

A further sub-sample of 3 g was transferred to a Kjeldahl digestion tube for extraction with 10 ml of concentrated HNO_3. Tubes were left at room temperature overnight and then were placed in a heating block. Each was covered with an air condenser and refluxed gently at 80 °C for 2 h. After cooling, the digests were filtered through a moistened Whatman No. 40 filter paper into a 50 ml volumetric flask. Flasks were then made up to the volume by distilled water. Analyses of Ca, Mg, Fe, Mn, Cu, Zn, Ni, Pb, Cd, Co and Cr were performed directly from the solution by inductively coupled plasma-mass atomic emission spectrometry (ICP-OES iCAP 6500). The detection limits of Ca, Mg, Fe, Mn, Cu, Zn, Ni, Pb, Cd, Co and Cr in soil were: 0.009, 0.007, 0.0056, 0.0065, 0.0076, 0.0051, 0.0059, 0.0089, 0.003, 0.0079 and 0.0092 $mgkg^{-1}$, respectively.

Plant material determination and metal analysis

The plant material was determined at the Department of Biology and Ecology, Faculty of Science in Kragujevac, by standard determination keys: Javorka and Csapody (Javorka et al., 1979), Flora of the Republic of Serbia (Josifović, 1991) and Flora Europaea (Tutin, 1964).

Identified plant material was elutriated in distilled water and then dried at room temperature. The drying in the dryer (Binder/Ed15053) followed during 24 hours, at 105ºC. After washing and drying on l05°C to constant mass, the plant material was measured on analytic scale (2 g of sample ± 0.01g). The samples then were transferred to Kjeldahl and perfused with 10 ml of concentrated HNO_3. Reaction mixture was heated by flame, until it became dry. The treatment was repeated until the clearing up of the solution was reached, and stopped for nitric vapors release. Samples were cooled then and the content in the Kjeldahl's dish was perfirsed by 6 ml of concentrated $HClO_4$ and then heated. The heating was stopped at solution volume of approximately 3 ml in Kjedahl's dish when solution has became clear and achromatic. The solution was cooled and distillated water was added. The content of

Kjeldahl's dish was filtered through a moistened Whatman No. 40 filter paper into 50 ml volumetric flask. This solution was used for determination of heavy metals in plant material.

Total of eleven metals were analyzed (Ca, Mg, Fe, Mn, Cu, Zn, Ni, Pb, Cd, Co, Cr) in eight plants (*Chrysopogon gryllus* (L.) Trin.; *Cotinus coggygria* Scop.; *Dorycnium pentaphyllum* Scop. subsp. *herbaceum* (Vill.) Rouy; *Melica ciliata* L.; *Potentilla argentea* L.; *Sanguisorba minor* Scop.; *Teucrium chamaedrys* L. i *Teucrium montanum* L. (Table 1).

Table 1.
Investigated plant species

Anacardiaceae	Fabaceae	Lamiaceae	Poaceae	Rosaceae
Cotinus coggygria Scop.	*Dorycnium pentaphyllum* Scop. subsp. *herbaceum* (Vill.) Rouy	*Teucrium chamaedrys* L.	*Chrysopogon gryllus* (L.) Trin.	*Potentilla argentea* L.
		Teucrium montanum L.	*Melica ciliata* L.	*Sanguisorba minor* Scop.

The metal concentration in plant samples was determined by inductively coupled plasma-mass atomic emission spectrometry (ICP-OES iCAP 6500) at the Institute of Public Health Division of Hygiene and Medical Ecology in Kragujevac, directly from the obtained solution. The detection limits of Ca, Mg, Fe, Mn, Cu, Zn, Ni, Pb, Cd, Co and Cr for plant material were: 0.0087, 0.007, 0.0053, 0.0051, 0.0056, 0.0055, 0.006, 0.003, 0.0027, 0.0054 and 0.0053 $mgkg^{-1}$, respectively. The six replication of one samples were prepared for determination of metal concentrations in both soil and plant material. The mean values were then calculated. Bioconcentration factor (BCF) was calculated for each metal dividing the total metal content in the plant by the total metal content in soil. The content of metals in the soil and plants were expressed in $mgkg^{-1}$ of dry matter ($mgkg^{-1}$ d.m.).

RESULTS AND DISSCUSSION:

The results of metal content in the soil showed that the soil is richest in Mg (59603,5 mg/kg), but the poorest in Cd (1,4 mg/kg) (Table 2). The mean values of metal concentrations found in the soil are presented in descending order: Mg>Fe>Ca>Ni>Cr>Mn>Co>Zn>Pb>Cu>Cd. We found that concentrations of Ni, Cr and Cd were higher then the values required by legislation of Republic of Serbia for content of metals in the soil (Službeni glasnik RS, br. 23/94, Službeni glasnik RS, br. 88/2010, prilog 3). Ni and Cr are present in the soil in the concentrations that are higher than maximum allowed value, limiting value and remedition value, while concentration of Cd was higher than limiting value. Concentrations of Cd, Co, Cr and Ni were also higher then the average values for other locations according to the literature data (Toth et al., 2016). Concentrations of Ni and Cr were higher even than the limiting values suggested by EU legislation (EU Directive 86/278/EEC). Cd, Co, Cr and Ni were present in the concentrations that were several times higher then those found throughout the world (Kabata-Pendias, 2011) (Table 2).

Table 2.
The mean values of metal concentrations in the soil (mg/kg)

Ca	*Mg*	*Fe*	*Mn*	*Cu*	*Zn*	*Ni*	*Pb*	*Cd*	*Co*	*Cr*
1109,1±6,1	59603,5±321	35709,9±320,8	288,8±6,3	6,1±0,2	23,1±0,1	931,4±23,7	13,2±0,1	1,4±0,01	33,6±0,1	485,2±10,7

mean values of metal concentrations (n=6)±standart devistion

Different characteristics of the soil may serve as a preferred monitoring tool, since they insignificantly vary over the time, allowing consistent assessment of areal and temporal contamination (Keshav et al., 2011). The content of heavy metals in the soil depends on factors such as: specific ability of some plants to over-accumulate toxic heavy metals, different chemical and physical soil characteristics and on interactions among metals (Sústriková et al., 2004). Although the total heavy metal concentrations in plants may indicate the metal load in the plant, generally these data are not sufficient to elucidate the possible risks of metal toxicity for plants themselves. On the contrary, the low content and/or availability in the soil of microelements, may be sufficient to cause metal deficiency for plant metabolism. It is generally regarded that the bioavailability of heavy metals in the soil is closely related to their chemical specializationof metal rather than total concentration in soils. Heavy metals occur in different geochemical forms of the soil, presenting distinctive mobility, biological toxicity and chemical behaviour. It is essential to distinguish and quantify the various molecular and ionic species of metals, in order to evaluate both actual and potential environmental impact of contaminated soil on the plants. Direct determination of specific chemical forms is considered impractical, due to various binding phases that metals may undergo. The concentration of heavy metals in soil may indicate the increase over the time, because there is no activity to funnel out the soil and dilute the effects of pollution.

Table 3.
The mean values of metal concentrations (mg/kg) in plant material

	Chysogryll	*Cotincoggy*	*Dorycherba*	*Meliciliata*	*Potenargent*	*Sanquimin*	*Teucrcham*	*Teucmonta*
Ca	1098.8±6.4	6185.4±77.5	9402.1±41.7	1838.1±8.9	8750.4±20.6	13414.6±79.9	2507.5±19.1	4548.3±18.3
Mg	3756.7±26.6	2330.6±24.9	3693.7±15.7	4289.2±18.4	13145.0±46.4	10870.8±77.9	17005.4±61.5	10051.3±37.9
Fe	647.0±8.0	141.5±0.4	567.3±1.7	1146.1±15.2	2054.4±43.8	1790.0±7.6	1781.9±7.9	2434.6±41.8
Mn	27.7±0.3	28.3±0.1	45.2±0.1	44.0±0.3	91.2±0.9	57.9±0.8	149.7±1.9	78.5±0.6
Cu	1.7±0.02	4.6±0.03	2.8±0.02	1.4±0.006	3.9±0.02	4.2±0.01	2.9±0.03	3.8±0.05
Zn	26.7±0.1	17.8±0.1	10.2±0.07	15.5±0.1	42.1±0.1	19.5±0.08	12.3±0.06	22.9±0.04
Ni	26.7±0.2	9.3±0.05	49.7±0.4	41.2±0.4	147.9±0.5	128.9±0.6	145.7±0.6	117.4±0.3
Pb	0.4±0.01	0.4±0.05	0.3±0.006	1.2±0.02	1.1±0.01	0.3±0.03	3.1±0.02	3.0±0.01
Cd	0.06±0.003	0.02±0.002	0.04±0.001	0.08±0.003	0.3±0.003	0.1±0.003	0.2±0.003	0.2±0.002
Co	1.4±0.01	0.3±0.006	1.3±0.01	2.4±0.02	7.0±2.9	5.8±0.02	8.8±0.07	6.5±0.02
Cr	13.5±0.1	1.3±0.006	9.8±0.02	19.6±0.1	39.2±0.3	25.6±0.1	92.1±1.1	56.5±0.6

mean values of metal concentrations (n=6)±standart devistion

Content of a metal in plant material dependeds on both facts – type of a metal and genetic plant predisposition to accept a certain metal (Table 3). The mean values of metal concentrations found in the plants are presented in descending order: Mg>Ca>Fe>Ni>Mn>Cr>Zn>Co>Cu>Pb>Cd. All metals were present in lower concentrations in the plants than in the soil, with exception of Ca and Zn. High concentrations of Mg, Mn, Pb, Co, Cr were detected in *T. chamaedrys* (17005.4 mgMg/kg; 149.7 mgMn/kg; 3.1 mgPb/kg, 8.8 mgCo/kg, 92.1 mgCr/kg respectively). Species *P. argentea* accumulated the most of Zn, Ni and Cd (42.1 mgZn/kg; 147.9 mgNi/kg; 0.3 mgCd/kg respectively). Species *C. coggygria* accumulated the most of Cu (4.6 mg/kg), while species *T. montanum* the most of Fe (2434.6 mg/kg). Content of Ca found in the plants varied between 1098.8 – 13414.6 mg/kg. The plant that accumulated the most of Ca was *S. minor*. The lowest metal concentration in all investigated species was the one of Cd (0,02 - 0,3 mg/kg), while the most of Cd was accumulated by *P. argentea.*

The metal content in both soil and plant material depends on different factors. Metal content in the soil may influenced by the type of the soil and type of the metal, but also by climate and geological base of the soil. Since different plant species possesses different physiological needs and ability for metal accumulation, the metal content in the plant will be mostly dependent on genetic predisposition of the species as well as on type of the metal and its bioavailability in the soil.

Our results showed that investigated plants found in the village Kamenica contained more of Ni, Cd, Co and Cr than other plants in Serbia (Kastori, 1993). The literature data indicate that critical concentrations in plant tissue for Fe may reach 50 mg/kg, for Mn 10 -25 mg/kg, for Ni are lower than 0.1 mg/kg, for Cu 2 -5 mg/kg, and for Zn 10-20 mg/kg. The sufficient level in plant for Fe, Mn, Ni, Cu and Zn literature lists values of 50-500 mg/kg, 20-300 mg/kg, 0.01-10 mg/kg, 5-30 mg/kg and 20-150 mg/kg, respectively (Hooda, 2010). The results of this study showed that all plant species except *C. coggygria* accumulated Fe and Ni in the concentrations that are higher than sufficient level, while Mn and Zn higher than critical values. Since some authors consider that accumulating plants for Cr and Ni may be considered as such if the threshold of 50 mgCrkg i 100 mgNikg has been reached (Boyd, 2011), our results indicate that both Teucrium species may be considered as accumulating plants of Cr and Ni, while *S. minor* and *P. argentea* may be considered accumulating plants of Ni.

The literature data shows that specific characteristics of serpentine soil are often low availability of Ca in relation to Mg and high content of potentially toxic elements (Fe, Ni, Cr, Co, Mn, Cu) (Shallari et al., 1998; Adriano, 2001; Kabata-Pendias, 2011). These conditions of serpentine geological base and substrate formed on it, crucially influence metal assimilation and accumulation in the plants.

Biological absorption coefficient is the relation of metal concentrations in the plants to the metal concentrations of the soil (Kabata-Pendias, 2011). High value of biological absorption coefficient of certain plants indicate their possible use in phyto-extraction, especially if its value is higher than 2 (Pandy et al., 2010). Content of Ca in all our investigated species was higher then Ca found in the soil (Table 4). Biological absorption coefficient for *Ch. gryllus* and *P. argentea* was higher than one for metal Zn.

Table 4.
Relation of found concentrations in plant material and the soil

Plant/soil	*Chysogryll*	*Cotincoggy*	*Dorycherba*	*Meliciliata*	*Potenargent*	*Sanquimin*	*Teucrcham*	*Teucmonta*
Ca	*1.00*	*5.58*	*8.48*	*1.66*	*7.89*	*12.09*	*2.26*	*4.10*
Mg	0.06	0.049	0.06	0.07	0.22	0.18	0.29	0.17
Fe	0.02	0.004	0.02	0.03	0.06	0.05	0.05	0.07
Mn	0.09	0.09	0.16	0.15	0.32	0.20	0.52	0.27
Cu	0.27	0.76	0.45	0.23	0.65	0.68	0.48	0.63
Zn	*1.16*	0.77	0.44	0.67	*1.82*	0.84	0.53	0.99
Ni	0.03	0.01	0.05	0.04	0.16	0.14	0.16	0.13
Pb	0.03	0.03	0.03	0.09	0.08	0.02	0.23	0.23
Cd	0.04	0.02	0.03	0.05	0.19	0.09	0.11	0.12
Co	0.04	0.01	0.04	0.07	0.21	0.17	0.26	0.19
Cr	0.03	0.003	0.02	0.04	0.08	0.05	0.19	0.12

The determination of metals in both soil and plants is important for monitoring environmental contamination. The content of elements in the soil is dependent on natural and anthropogenic sources in the local ecosystems. Plants that are "miners" of the Earth's crust, have potential to absorb different elements from soil to the different extent. Some elements are essential, because they are necessary for completing life-cycle of different plants. Therefore, some plant species are able to accumulate high concentrations of toxic metals, up to the levels which exceed metal concentrations in the soil. Some plants have the ability to accumulate heavy metals which are essential for their growth and development (Mg, Fe, Mn, Zn, Cu, Mo and Ni). There are also certain plants that have the ability to accumulate heavy metals that have no biological function (Cd, Cr, Pb, Co, Ag, Se and Hg) (Memon et al., 2001).

It is considered that a live organism reflect environmental pollution if its ability to take up elements is proportional to concentration of a polutant in the environment (Ravera et al., 2003). However, this is not case for the plants which have low capacity to differentiate elements, and which are in turn, accumulated independently from the organism's physiological needs. Therefore, such metal plant uptake, does not follow plant physiological level of the metal. Instead, the metal level is regulated by plant organism itself *via* physiological mechanisms. Our study exhibited that different heavy metals reached different concentrations in investigated plant species. Metal uptake by plants depended on the bioavailability of the metals in the soil, which itself was a function of metal retention time and interactions with other elements and substances in the soil.

Species *T. chamaedrys* contained the highest concentrations of five elements (Mg, Mn, Pb, Co, Cr), while *P. argentea* contained the highest concentrations of Zn, Ni and Cd. Both investigated Lamiaceae species (*T. montanum* and *T. chamae*drys) and Rosaceae species (*S. minor* and *P. argentea*) showed ability for phytoaccumulation of Ni, and therefore they can be considered accumulating plants of Ni, while *Teucrium* species can be considered accumulating plants of Cr. Values of biological absorption coefficient indicate for *Ch. gryllus* and *P. argentea* call for consideration of these plants for phyto-remediation in the case of Zn loaded soil.

CONCLUSION:

Our results showed that concentrations of Ni and Cr were higher than maximum allowed value, limiting value and remedition value than it is allowed according to the local state regulations, while concentration of Cd was higher than limiting value. There was more of Ni and Cr even by EU regulation, while concentrations of Cd, Co, Cr and Ni were multiple times higher than mean values found in other parts of the world. We conclude that serpentine soil, having specific metal content, influenced higher metal content in the investigated species. The content of the specific metal was in relation with the plant ability to assimilate and accumulate it. *T. chamaedrys* contained highest concentrations of Mg, Mn, Pb, Co, and Cr, while *P. argentea* contained the most of Zn, Ni and Cd. *S. minor*, *C. coggygria* and *T. montanum* accumulated the among other metals the most Ca, Cu, and Fe, respectively. Since *T. montanum* and T. *chamaedrys* accumulated Ni and Cr, and species *S. minor* and *P. argentea* accumulated Ni, they may be considered accumulating plants for these metals. Signicant values biological absorption coefficientindicate that species *Ch. gryllus* and *P. argentea* could be possible plant candidates in phyto-remediation of the Zn polluted soil.

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