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Original scientific paper

BIOACCUMULATION, TRANSLOCATION AND PHYTOREMEDIATION BY ENDEMIC SERPENTINOPHYTE *Artemisia alba* TURRA.

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ABSTRACT. The aim of this study was to determine the concentrations of some metals (Ca, Mg, Fe, Mn, Zn, Ni, Cr) in the soil and in the endemic serpentinophyte *Artemisia alba* Turra. (*Artemisia lobelii* All.) on one serpentine site (Central Serbia), for determination the ability of this plant species in bioaccumulation, translocation and phytoremediation of researched metals. The concentrations of Ni and Cr in the investigated soil were above remediation values, the maximum allowable concentration, as well as of prescribed limit value of substances in the soil according to regulation of Republic of Serbia. Good translocation of Ca, Mn and Zn through the trees and leaves of the *A. alba* species, as well as the translocation and accumulation of Mg and Ni in the leaves of the investigated plant has been shown. We can consider *A. alba* as a potential candidate for phytoextraction and photoremediation of soils contaminated with Ca, Mg, Mn, Zn and Ni.

Key words: metals, plants, bioaccumulation, translocation, phytoremediation

INTRODUCTION

The term “serpentine” strictly speaking refers only to the serpentine group of minerals with the general formula $Mg_3Si_2O_5(OH)_4$ which are important constituents of weathered “ultramafic rocks”. Serpentines are rocks that from as a result of metamorphisam or metasomatism of primary magnesium-iron silicate minerals. Ultramafic (serpentine) soils represent a category of substrates derived from ultramafic bedrock and distributed around the world. Properties commonly shared among ultramafic soils include high Fe and Mg concentrations and low Al and Ca concentrations, relatively high concentrations of Cr, Co and Ni, high magnesium-to-calcium (Mg:Ca) quotients in the exchange complex and low concentrations of P and K (both total and extractable) (ECHEVARRIA, 2018). Serpentine substrates cover quite large areas in the Balkans, more than in other parts of Europe (BROOKS, 1987, TATIĆ and VELJOVIĆ, 1992). They exist as large blocks or as small outcrops separated from other geological formations, in Central Bosnia and Western and Central Serbia, and extend towards North, Central and South-Eastern parts of Albania and further to the serpentine formations in the regions of Epirus and Thessaly in Greece (BANI *et al.* 2010).

Some factors of the serpentine soil chemistry ("serpentine syndrome") make it inhospitable environment for many plants. The potential toxic Mg level, Ca/Mg imbalance, phytotoxic Ni level and deficiency P level of serpentine soils are primary reasons for serpentine soil infertility, which causes evolution of serpentine. Since the serpentine soil properties are disadvantageous for most plants, distinctive vegetation communities have evolved on serpentine soils (BROOKS, 1987). Species growing on these soils can be classified into two categories: serpentine-tolerant plants (serpentine-facultative plants) which withstand the serpentine conditions, but they are also found more widely, and often show better growth elsewhere and serpentine-endemic plants (serpentine-obligate plants) restricted exclusively to serpentine soil and are not found on other substrates (FREITAS *et al.*, 2004). The serpentine flora of the Balkan is characterized by a relatively high degree of endemism. According to STEVANOVIĆ *et al.* (2003) there are 335 Balkan endemic vascular plant taxa growing on serpentine, of which 123 are obligate serpentinophytes.

The aims of this study were to determine the content of some metals (Ca, Mg, Fe, Mn, Zn, Ni, Cr) in the soil and in the endemic serpentinophytes *Artemisia alba* Turra. (*Artemisia lobelii* All.) that grow on it, to determine the ability of the plant species in bioaccumulation, translocation and phytoremediation of investigated metals.

MATERIALS AND METHODS

Study site

The researched locality was located near the village of Kamenica in Central part of Serbia. This site presents a part of a much larger part of serpentine substrate, located in Western and Central Serbia, and extend towards North, Central and South-Eastern parts of Albania. The investigated site is at 352-410 m above sea level 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).

Soil and plant sampling and analysis

One serpentine locality was surveyed to collect endemic obligate serpentinophyte *Artemisia alba* Turra. *(Artemisia lobelii* All.*)* together with their associated soils. Six soil samples were collected from 1 to 10 cm depth, near roots of researched plant. This depth corresponds to the major rooting zone of the herbs and small shrubs (REEVES *et al.*, 2007). Soil samples were initially air-dried and stone pieces were removed, sieved to 2 mm, and stored at 4 °C until 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 (Binder/Ed15053).

The determination of plant material was performed in the laboratory of the Institute of Biology and Ecology, Faculty of Science in Kragujevac, with the help of standard keys for determination: Javorka and Csapody (JAVORKA and CSAPODY, 1979), Flora of the Republic of Serbia (JOSIFOVIĆ, 1991) and Flora Europaea (TUTIN, 1976). Voucher specimens (17483) are deposited in Herbarium of the Institute of Botany and Botanical garden "Jevremovac", University of Belgrade (BEOU). *Artemisia alba* is classified into Kingdom: Plantae, Subkingdom: Tracheobionta, Superdivision: Spermatophyta, Division: *Magnoliophyta*, Class: Magnoliopsida, Subclass: Asteridae, Order: Asterales, Family: Asteraceae, Subfamily:

Asteroideae, Tribe: Anthemideae, Subtribe: Artemisiinae, Genus: Artemisia L., species: *Artemisia alba* Turra. (VALLES *et al.*, 2003).

Identified plant material was prepared for further analysis (BRANKOVIĆ *et al.*, 2015). In order to determine the total metal content in soil and plant material, the samples were prepared by digestion with nitric acid and hydrogen peroxide according to EPA 3050 b (US EPA, 1997) in relation to HNO_3:H_2O_2 = 5:1; relationship soil pattern/digestion mixture was 1:12. In order to check the accuracy of the applied method, blank tests and standard reference materials were used: MEES-3 (trace elements in soils) and LGC7173, for plant material. The values obtained ranged in the range of ± 5% of the certified values.

Seven metals (Ca, Mg, Mn, Fe, Zn, Ni, Cr) were analyzed in soil and plant material (root, stem, leaf, whole plant). The metal concentrations in plant and soil samples were determined by inductively coupled plasma-mass atomic emission spectrometry (Perkin Elmer 3300), directly from the solution at the Institute of Chemistry, Faculty of Science in Kragujevac. The six replication of soil and plant material were determined, also mean values and standard deviation were calculated. Also, different factors as indicators of ability of the plant species in bioaccumulation, translocation and phytoremediation of researched metals were calculated on way presented in next table (Table 1).

Table 1. Formulas for calculating the factors for bioaccumulation, translocation, enrichment and apsorption coefficient

FACTOR	FORMULAS	Elements of formula
Bioaccumulation factor (BF) (GHOSH and SING, 2005)	$BF = C_{root} / C_{soil}$	C_{root} - the metal concentration in the plant root C_{soil} - the metal concentration in the soil
Translocation factor (TF) (GUPTA *et al.* 2008)	$TF_{stem} = C_{stem} / C_{root}$ $TF_{leaf} = C_{leaf} / C_{root}$	C_{stem} - the metal concentration in the plant stem
Enrichment factor (EF) (GHOSH and SING, 2005)	$EF_{stem} = C_{stem} / C_{soil}$ $EF_{leaf} = C_{leaf} / C_{soil}$	C_{leaf} - the metal concentration in the plant leaf
Apsorption coefficient (AC) (KABATA-PENDIAS, 2001)	$AC = C_{plant} / C_{soil}$	C_{plant} - the metal concentration in the whole plant

Also, the ratio between metal concentration of aboveground and underground organs as well as leaf and stem metal concentration were determined. The content of metals in soil and plant materials were expressed in mg kg^{-1} of dry matter (mg kg^{-1} d.m.).

RESULTS

The mean concentrations of Ca, Mg, Mn, Fe, Zn, Ni and Cr (mgkg^{-1} d. m.) in soil and species *A. alba* are shown in Table 2.

Generally, results of our study showed that the mean concentrations of investigated metals were far higher in the soil samples than those calculated for the same metals in plant samples (except for Ca).

The BF, an index of ability of the plant to accumulate a metal, with respect to its concentration in the soil substrate is shown in Table 3. The content of Ca in root of *A. alba* was higher than its content in soil (BF>1 for Ca).

The EF (ratio of a metal concentration in a plant aboveground part to its concentration in soil) higher than 1 for Ca was found at stem and leaf, and higher than 1 for Zn at leaf of investigated plant species.

Table 2. The mean concentrations of Ca, Mg, Mn, Fe, Zn, Ni and Cr (mg kg^{-1} d. m.) in soil and in *Artemisia alba* plant parts.

METAL	*SOIL*	*PLANT*			
		root	*stem*	*leaf*	*whole plant*
Ca	1109.08±6.1	5068.83±54.01	3401.33±434.95	7341.33±7.28	5270.50±1678.54
Mg	59603.59±312.0	2460.50±17.00	3382.17±12.72	8922.83±17.19	4921.83±2936.83
Mn	288.86±6.4	19.38±0.39	10.68±0.48	28.22±0.61	19.43±7.38
Fe	35709.91±320.9	214.00±2.19	415.67±1.21	318.83±1.17	316.17±84.75
Zn	23.12±0.2	11.53±0.30	18.43±0.34	28.60±0.51	19.52±7.22
Ni	931.49±23.7	32.93±0.69	35.48±0.50	93.52±0.75	53.98±28.80
Cr	485.24±10.7	5.42±0.31	2.75±0.15	9.18±0.15	5.78±2.72

the mean value (n=6) ± standard deviation

Table 3. Bioaccumulation factor (BF), enrichment factor (EF) and apsorption coefficient (AC) of *Artemisia alba*

METAL	*BF*	*EF stem*	*EFleaf*	*AC*
Ca	4.57	3.07	6.62	4.75
Mg	0.04	0.06	0.15	0.08
Mn	0.07	0.04	0.10	0.07
Fe	0.01	0.01	0.01	0.01
Zn	0.50	0.80	1.24	0.84
Ni	0.04	0.04	0.10	0.06
Cr	0.01	0.01	0.02	0.01

In our study, the value of AC (also known as the plant uptake factor) was between 0.01-4.75 (Table 3), where almost all the metals showed the AC<1 (except for Ca), with the minimum value for Fe and Cr (AC was 0.01). The BF for Ca was with the maximum value (4.75).

The TF or mobilization ratio was assessed for determination of the relative translocation of metals from underground organs to aboveground organs (stem, leaf) of plant species (Table 4). The TF>1 at stem of species *A. alba* was found for Ca, Mn and Zn. Also, TF>1 for Mg, Mn, Zn and Ni was established at investigated species at leaf.

Table 4. The translocation factor (TF) and ratios of the investigated metals content (leaf / stem and aboveground organs / underground organs) of *Artemisia alba.*

METAL	TFstem	TFleaf	leaf/stem	above/under
Ca	8.05	0.13	0.02	0.09
Mg	0.75	1.01	1.35	0.01
Mn	1.22	1.56	1.28	0.06
Fe	0.55	0.53	0.97	0.01
Zn	1.14	1.68	1.47	0.07
Ni	0.72	1.08	1.51	0.04
Cr	0.50	0.48	0.97	0.06

DISCUSSION

Serpentine soils containing high levels of potentially phytotoxic elements such as Ni, Cr, Co and sometimes Mn and/or Cu, present inhospitable environment for many plant species. Besides, they have low availability of Ca relative to Mg and deficiency of some essential macronutrients such as P, N and K, that make them stressful for plant growth.

We found that the serpentine soil of mountain Goč contained 1109.08 mg Ca kg^{-1}, while species *A. alba* contained 5270.50 mg Ca kg^{-1}. The results of our study agree with some previous the reports (ROBINSON *et al.,* 1997, REEVES *et al.,* 2007), although there are some literature data about the adequate concentration of Ca in plant tissue from 5 g kg^{-1} (SHALLARI *et al.,* 1998).

Generally, serpentinite rocks contain very high Mg (18–24%) and high Fe (6–9%) but low Ca (1–4%) and Al (1–2%) (ALEXANDER 2004). Magnesium was recognized as an essential macronutrient involved in many enzyme activities and the structural stabilization of tissues. It is particularly important to plants with 75% of leaf Mg involved in protein synthesis and 15-20% of total Mg associated with chlorophyll pigments (WANIL *et al.* 2016). In our study, the magnesium concentration in investigated soil was 59603.59 mg kg^{-1}, while species *A. alba* contained 4921.83 mg Mg kg^{-1} that is more than concentrations reported for 17 species belonging Artemisia order (ASHRAF et al., 2010). Mg availability by plants depends mainly on chemical and physical characteristics of soil, so some factors can lead to lower Mg availability, such as acidic soils with low cation exchange capacity, aluminium toxicity, heat stress, droughty soil and high levels of competing elements (K, Ca, NH_4, Na).

Some the limiting factors that make ultramafic soils unfavourable substrates for plant growth are the low Ca:Mg quotients (due to low Ca and high Mg) and high heavy metal concentrations, especially Ni (BRADY *et al.,* 2005). Our results showed low Ca:Mg ratio (0.019). Similar results were described by many authors (ROBINSON *et al.,* 1997; SHALLARI *et al.,* 1998). Ability of the serpentine-tolerant species to absorb quantities of Ca without taking up excessive quantities of Mg allows them to survive on soils with depleted levels of Ca. There are some suggestions that the plant ability to maintain high leaf Ca:Mg by selective translocation of Ca and/or inhibited transport of Mg from roots is a key evolutionary change needed for survival on serpentine soils (O'DELL *et al.,* 2006).

The total content of Mn in the soil mainly originates from the parent substrate, but also by anthropogenic way (wastewater and sludge, steel industry, fertilizers). Most serpentine soils contain high levels of Mn in the form of oxides. The natural level of Mn in the soil varies widely from 10 to 9000 mg kg^{-1}, and the estimated mean value of this metal in the world's land is 437 mg kg^{-1} (KABATA-PENDIAS, 2011). According to ADRIANO (2001), regular Mn content for most of soil types ranges from 500-1000 mg kg^{-1}. However, our results presented concentration of 288.86 mg Mn kg^{-1} in soil samples. Manganese ions activate numerous enzymes in plants cells, as well it is involved in the process of decomposition of water molecules with releasing the oxygen (PRASAD and FREITAS, 1999). The manganese has a range between 20 and 300 mg kg^{-1} in most plants, while its level may be as high as 1500 mg kg^{-1}, without harm to some plant (PAIS and JONES, 2000). Therefore, comparing our data with the previous cited, we could say that *A. alba* was contained lower concentration of Mn (19.43 mg kg^{-1}). It could be explained by antagonism in uptake between Fe and Mn, and with very high negative correlation in content of Ca and Mn between plant and soil. Also, some literature data indicate that normal concentrations of Mn in plant tissues are 10-25 mg kg^{-1}, while the surplus values are in the range of 20-300 mg kg^{-1} (HOODA, 2010).

Iron originates from primary and secondary minerals. Iron reserves in the soil are mostly of inorganic nature, so the total iron content is usually between 0.5 and 4.0%. Some authors point out that the mean Fe concentration in the soil is 20000-40000 mg kg^{-1} (KABATA-PENDIAS, 2011), while for others the total content of this metal in the soil ranges from 7000-55000 mg kg^{-1} (NAGAJYOTI and LEE, 2010). The iron concentration in investigated soil was

35709.91 mg kg^{-1}. Our results agree with earlier findings that serpentine soils contain high amounts of iron (REEVES *et al.*, 2007, BECH *et al.*, 2008). Iron is an essential element for plants and plays an important role in numerous biological processes (photosynthesis, chloroplast construction, chlorophyll biosynthesis, redox system, etc.). The plants adopt iron in the form of ions Fe^{2+}, Fe^{3+} and in the form of chelates. Adoption of iron as well as its mobility in plants is middling to poor, since even 80-90% of iron is firmly bound. However, in this study, the concentrations of Fe in investigated plant (316.17 mg kg^{-1}) is in accordance with previous cited.

The zinc is distributed evenly in the Earth's crust. The main sources of soil pollution with Zn are mines and iron foundries, the use of waste sludges, composted materials, pesticides and fertilizers. The most important land component that contributes to the adsorption of Zn is the colloidal soil fraction (minerals of clay, organic matter, hydrated metal oxides). In the soil, Zn is unevenly distributed in respect of its content with an interval of 10-300 mg kg^{-1} and an average value of 50 mg kg^{-1} (PAVLOVIC, 2016). According to other authors, the mean value of Zn content in soil of the world is 64 mg kg^{-1}, while regular Zn content for most soil types ranges from 1-800 mg kg^{-1} (KABATA-PENDIAS,2011). Therefore, comparing our data with the findings of some researches we could say that zinc content in analyzed soils (23.12 mg kg^{-1}) is in accordance with previous the findings. The Zn content in plant samples were 19.52 mg kg^{-1}. According to KABATA-PENDIAS (2011), Zn concentrations in plant tissues are in the range of 150-200 mg kg^{-1}, while the surplus values in the leaves range from 27-150 mg kg^{-1}.

The primary source of nickel in soil is the consumption of volcanic rock (rich in ferro-magnesium minerals and sulphates, also rich in Ni), but it comes also into the soil by anthropogenic activity (industry, coal and oil combustion, waste sludges, fertilizers). In our study the serpentine soils of the mountain Goč contented 931.49 mg Ni kg^{-1} d.m. Therefore, our data agree with previous the reports (SHALLARI *et al.*, 1998; REEVES *et al.*, 2007). The Ni as an activator of the enzyme urease allows plants to utilize nitrogen from urea. There are some opinions that as Ni^{2+} is absorbed by roots, a diffusion depletion zone is formed in the rhizosphere and Ni^{2+} is released from solid or soluble bound forms to maintain soil solution equilibria (FELLET *et al.*, 2009). According to some studies the normal plants and crop species generally contain 1-5 mg Ni kg^{-1} (BROOKS, 1987; CHANEY *et al.*, 2008.) and suffer from significant phytotoxicity below 100 mg Ni kg^{-1}. In our study the Ni content in *A. alba* was higher than normal values in plants (53.98 mg kg^{-1}). These observations agree with those obtained by other authors (ZAYED and TERRY, 2003) who found that Ca:Mg quotient is a relatively important factor in Ni uptake. There are some reports about interactions between Ca, Mg and Ni in plants. So, in plants, Ni^{2+} may competitively inhibit the uptake of divalent cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} that can lead to characteristic plant chlorosis symptom and consequently reduced efficiency of photosynthesis (TAPPERO *et al.*, 2007).

The origin of Cr in soil is due to its presence in the parent substrate. Also, a large part of Cr flows into the soil anthropogenically (agricultural material, atmospheric deposits, sludge, industry). According to some authors, the average content of Cr in the earth's soil is 40 mg kg^{-1} (ADRIANO, 2001), while others report that this content is 54 mg kg^{-1} (KABATA-PENDIAS, 2001). The results of this study indicate that the content of Cr in the test soil has exceeded the literature data (485.24 mg Cr kg^{-1}). The chromium is the contaminant with highest total contents in soils, but that only showed an average extractability of 0.008%, and some authors (ZAYED and TERRY, 2003) have found that nearly all the soil Cr was in a more resistant fraction (less soluble forms). The high Cr concentrations in the serpentine soils often are in the form of chromite, an unalterable mineral, and so Cr remains not bioavailable. In most of the soils, Cr is accessible in low concentrations, so that it is present in plants in concentrations 0.2-0.4 mg kg^{-1} (KASTORI, 1993; KASTORI *et al.*, 1997). According to REEVES and BAKER (2000), the normal values for Cr in plants are in range of 0.2-5 mg kg^{-1}. However, our findings were showed Cr concentration in the investigated plant samples of 5.78 mg Cr kg^{-1} that exceeded the concentration in the literary data. The concentrations of Ni and Cr in the investigated soil were above their the maximum allowable concentration in the soil, as well as limit and remediation values, according to regulation of Republic of Serbia (ANONYMOUS, 1997; ANONYMOUS, 2010). Also, the Ni concentration was above the limit value for the given metal in the soil according to the European Union Directive (EU Directive 86/278/EEC, 1986).

The current study showed different heavy metal concentration in investigated plant species, depending on kind of metal and organ. In soil samples metal concentrations had order: Mg>Fe>Ca>Ni>Cr>Mn>Zn, and in the plant Ca>Mg>Fe>Ni>Zn>Mn>Cr. The Ca:Mg ratio in soil samples was 0.019 and in plant samples 1.07. Also, in the leaves of species *A. alba*, the highest content of almost all investigated metals (except Fe) was determined, while the stem of this species contained the highest Fe. It has been shown that the roots of the *A. alba* species have better accumulation of the examined metals than the aboveground organs, and that the leaves have a higher accumulation capacity of Mg, Mn, Zn and Ni than the stem of the studied plant. Metal uptake by plants depends on the bioavailability of the metal in soils, which in turn depends on the retention time of the metal, as well as the interaction with other elements and substances. Also, metal phytoavailability depends on the form of the element in soil and on the considered plant species. However, it was found that the metal uptake does not necessarily correlate with metal content in the soil, probably because of diverse metal uptake mechanisms and to some disparities in their transport properties, resulting in differences in the metal concentrations in plants (GREGER, 2004).

Tolerance of plants to metals can be constitutive tolerance, acquired during phylogenesis or induced tolerance that represents stressful adaptation, response to adverse environmental conditions. Depending on location where metal tolerance mechanisms work, we can split them into external (apoplastic) and internal (simplastic). The external mechanism of plant tolerance to metals is based on the binding of metals in the cell wall (Zn, Cu, Pb), secretion of organic acids and chelates over the root into the external environment, the establishment of pH and redox barriers and others. The internal mechanisms of plant tolerance to metals is based on the creation of a complex of metals with proteins (metallothioneins), peptides (phytohelatins), organic acids (malic acid, citric acid), accumulation in vacuoles (compartmentation in the form of soluble and insoluble complexes) after their entry into the cell. Sequestration of metal in tissues or cellular compartments (vacuole), the translocation of excess metals into older leaves prior to their decay can be a mechanism of the tolerance of less sensitive plants to metals.

CONCLUSIONS

This study was carried out on endemic obligate serpentinophyte *Artemisia alba* Turra., which lives on serpentine area in the Central part of Serbia. The aim of this research was to determine the its capacity for bioaccumulation, translocation and phytoremediation of some metals (Ca, Mg, Mn, Fe, Zn, Ni and Cr). The concentrations of Ni and Cr in the investigated soil were above their maximum allowable concentration in the soil, also above their limit and remediation values in the soil, according to regulation of Republic of Serbia. Also, the Ni concentration was above the limit value for the given metal in the soil according to the EU Directive (1986). In soil samples metal concentrations had order: Mg>Fe>Ca>Ni>Cr>Mn>Zn and in the plant Ca>Mg>Fe>Ni>Zn>Mn>Cr. The results of our study showed that the concentrations of all examined metals were higher in soil than in plant (excepting Ca). The general trend of metal accumulation in soil was: Mg>Fe>Ca>Ni>Cr>Mn>Zn. On the other hand, *A. alba* has different ability for metal accumulation (Ca>Mg>Fe>Ni>Zn>Mn>Cr). Almost all the metals showed the BCF<1 (except for Ca). Our study exhibited different heavy metal concentration in investigated plant species, depending on kind of metal and plant organ.

The metal uptake does not necessarily correlate with metal content in the soil. The results of this study indicate the good absorption of Ca and its translocation through the stem to the leaves of the studied plant. Good translocation of Mn and Zn through stem and leaves of the species *A. alba* was also demonstrated, as well as the translocation and accumulation of Mg and Ni in the leaves of the investigated plant.

Based on the values of BF and TF, the species *A. alba* can be considered a potential candidate for the phytoextraction of soil contaminated with Ca, Mg, Mn, Zn and Ni. It has also been found that the leaves of the studied species can be used in the phytoremediation of soil loaded with Zn.

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