

INFLUENCE OF Zn ON THE AVAILABILITY OF Cd AND Cu TO VACCINIUM SPECIES IN UNPOLLUTED AREAS – A CASE STUDY OF STARA PLANINA MT. (SERBIA)

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Abstract: This paper presents a study of Zn, Cu and Cd availability to three *Vaccinium* species (*V. myrtillus*, *V. uliginosum*, *V. vitis-idaea*) in the natural field conditions of Stara Planina Mt. (East Serbia). The *Vaccinium* species are often used for both consumption and production of different pharmaceutical products. It has been found that some species of this genus are tolerant to the high content of trace elements, which makes them highly recommended for many phytoremediation programs. The study was conducted at two localities (Babin Zub and Javor) whose dominant soil type is Dystric Leptosol. At both localities the soil was sampled from soil profiles in eight sites. At each sampling site the soil was sampled at depths of 0-5, 5-10, 10 -20 and 20 - 40 cm, and for each species the plant material studied was collected from a 10x10 m square plot. The air dried soils and plant material were milled and digested with *aqua regia*. The *aqua regia*-extractable content of trace elements was measured. The content of trace elements extractable with the DTPA chelat agent is defined as mobilisable metals. The contents of trace elements (Cd, Cu and Zn) in the extracts were determined by AAS. The contents of *aqua regia*-extractable trace elements in the studied soil were lower than the limit values for multifunctional use. The highest concentrations of Cd and Zn were measured in *V. uliginosum*, and of Cu in *V. myrtillus*. The correlation found between the content of trace elements in plants and trace elements in the soil is important for a better understanding of their interactions, as well as for the intensive use of *Vaccinium* species in food production in the investigated region of Serbia.

Keywords: Cd, Cu, Zn, availability, *V. myrtillus*, *V. uliginosum*, *V. vitis-idaea*

1. INTRODUCTION

Trace elements in soils are of both natural and anthropogenic origin (Adriano, 2001) and can be found in different soluble and particulate forms, which has an impact on their mobility and bioavailability (Yi et al., 2007). Many studies have shown that the adsorption of trace elements in the soil is highly dependent on the pH-value (Antoniadis et al., 2007), and this phenomenon is explained by different mechanisms (Basta & Tabatabai, 1992). The retention of trace elements is defined by the mechanisms of chemical immobilization in the surface soil layer, and further transport through the profile and their availability to plants depends on soil and geochemical processes typical for the specific vegetation and

edaphic conditions of a site. Activities between trace elements bound to soil components in various forms, change over time due to the effects of the process of pedogenesis. Trace elements are complexed with different compounds in the soil. Copper is mainly found in the residual and organic matter fractions. Cd is mainly bound to the carbonate fraction, while Zn is bound to Fe and Mn oxides. (Altaher, 2001. According to Sauv  (2000) organically bound Cd is a major component of soluble Cd in soil solutions. The use of the DTPA solution for determining Cd, Zn, and Cu mobile forms has been widely accepted (Lindsay & Norvell, 1978, Mbila et al., 2001; Flues, et al., 2004).

Species of the genus *Vaccinium* belong to the family Ericaceae (Flora Europaea). These species are

dwarf and evergreen shrubs. They are adapted to cold and moderately moistened habitats with acid and very poor soils in Northern and Central Europe (Timoshok, 2000). Three species of the genus *Vaccinium* have been described in the hilly and mountainous areas of Serbia, including *V. myrtillus*, *V. uliginosum* and *V. vitis-idaea* (Janković, 1972). *V. myrtillus* and *V. uliginosum* are dwarf shrubs and *V. vitis-idaea* is an evergreen shrub. Some studies have concluded that *V. vitis-idaea* can be used as a bioindicator in areas contaminated with copper, nickel and radioactive cesium (Pöykiö, et al., 2005, Barcan et al., 1998; Rodushkin et al. 1999; Levula et al., 2000).

According to the Rocky Mountain Research Station (2009), *V. uliginosum* is tolerant to the high content of trace elements in soils. The leaf tissue of this species can accumulate uranium, copper, lead, zinc, nickel and iron in large amounts with no apparent harm to the plant. This ability makes *V. uliginosum* highly suitable for use in certain phytoremediation programs.

The aim of the paper is to determine the possible correlations between Cd, Cu and Zn uptake by *V. myrtillus*, *V. uliginosum* and *V. vitis-idaea* and Cd, Cu and Zn contents in the soil. A better understanding of these interactions could improve methods for growing these *Vaccinium* species and their proper use.

2. MATERIAL AND METHOD

2.1. Study area

Stara Planina Mt. is located in Eastern Serbia. The region of Stara Planina is a transitional zone between the temperate continental and mountain climate zones, with an annual precipitation ranging from around 600 mm to 700 mm. The vegetation of Stara Planina Mt., as a whole, is characterized by a diversity of forest, shrub, grassland and pasture communities, including the communities of peat bogs, and as such it represents one of the centers of floristic diversity of the Balkan Peninsula. A total of 147 endangered and endemic species have been recorded in this area (Mišić, et al., 1978., Randelović et al., 2002, Obratov-Petković et al., 2006).

Two localities, Babin Zub (N = 43°22'35 .7" E = 022 37'38 .3" (average altitude 1547 ± 4m) and Javor (N 43°23'07 .05 " E 22 ° 44'18 .18 ", average altitude 1361 ± 6 m) were chosen for investigation.

Pastures in the studied sites are occupied by the phytocoenosis of *Agrostietum vulgaris (capillaris) Pavl. 1955*.

Eight sampling sites were selected at both localities, and one soil profile was opened in each of them.

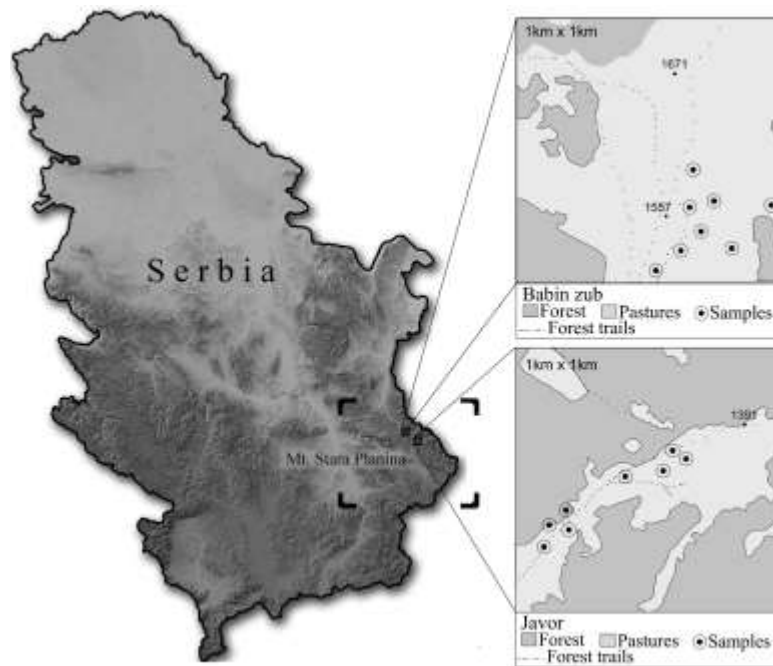


Figure 1. Study area

The soil samples were taken from the depths of 0-5 cm, 5 – 10 cm, 10-20 cm and 20 – 40cm, and for the purpose of complete soil characterization the samples from deeper layers were taken up to the parent material. After FAO (1985) classification, the soil types determined on pasture soils were Dystric Leptosol on sandstone, Dystric Leptosol on chlorite schists and Dystric Cambisol on chlorite schists. The dominant soil type is Dystric Leptosol on sandstone.

2.2. Soil analysis

The soil samples were air dried and sieved (<2mm). The measurement of the pH was performed in 0.1 M CaCl₂ using a 1:3 soil: solution ratio. The total organic carbon content was determined by the Tjurin method and the total nitrogen content was measured by the Kjeldahl macromethod.

The cation exchange capacity (CEC) was determined by extraction in 0.1 BaCl₂. The analyses of mechanical composition of the soil were performed by the pyrophosphate B method.

The milled soil (3g) was digested with *aqua regia* under reflux for 2 hours with water-cooled condensers on a digestion block using the standard procedure (International Organization for Standardization ISO 11466:1005 Soil quality, 1995). The digest was cooled, filtered and diluted with 0.5 M HNO₃.

The available Zn, Cu and Cd contents of soils were determined by extraction in the DTPA (a mixture of 0.005 mol l⁻¹ DTPA, 0.01 mol l⁻¹ CaCl₂, 0.1 mol l⁻¹ triethanolamine (TEA) with the pH adjusted to 7.3 and with a 1 mol l⁻¹ HCl solution).

The contents of Zn, Cu and Cd were determined by flame atomic absorption spectrophotometry (AAS) using a Thermo MSeries AA instrument (USA). The referent soil sample for trace elements was determined for the verification of results (ERM-CC141 loam soil, Institute for Reference Materials and Measurements from Belgium).

2.3. Plant material analysis

The investigated species (*V. myrtillus*, *V. uliginosum* and *V. vitis-idaea*) were randomly sampled from 10 x10 m square plots. The collected plant material of each studied species represents one sample. The vegetation samples were air dried and milled into powder. The Zn, Cu and Cd contents were determined using the same procedure as the one

described for determining *aqua regia*-extractable trace elements in soil samples.

2.4. Statistical analysis

The statistical analyses were performed using the SPSS (version) 16 software. The effects of treatments on all variables were tested by ANOVA. The significance of their correlations was analyzed via the Pearson correlation matrix (SPSS, 2007).

3. RESULTS

3.1. Characteristics of study soils

The soils at the investigated localities on Stara Planina Mt. have been studied in Serbia by several studies conducted for different purposes (Vrbničanin, et al., 2004, Belanović, et al., 2003, Belanović & Košanin, 2005; Ristić, et al., 2012). Dystric Leptosol is the most common soil type in certain parts, and due to terrain configuration Dystric Cambisol can also be observed, while highly skeletal Leptosol is common on the ridges. According to its texture Dystric Leptosol belongs to the loam textural class. In terms of chemical properties (Table 1) Dystric Leptosol is characterized by a very strong acid to moderately acid reaction and low base-saturation. Humus content in the surface layer is high, and the decline in the percentage of humus is uniform with depth.

3.2. Zinc, copper and cadmium concentrations in the soil

The average values of the *aqua regia*-extractable content of Zn, Cu and Cd, as well as the contents of these elements extracted by the DTPA chelate agent are shown in table 2.

Within each depth increment, means with the same letter are not significantly different (P<0.05) The Zn and Cu contents extracted in *aqua regia* are uniform at all soil profile depths (Table 2). The contents of Cd extracted in *aqua regia* decrease with depth, up to the depth of 10 cm, which is followed by an increase (Table 2). However, the contents of DTPA-extractable trace elements decrease with depth, and the highest contents of all investigated microelements were measured in the 0 - 5 cm layer.

The content of DTPA-extracted Zn statistically significantly decreased (p <0.05) at the depth of 5 - 10cm compared to the 0 - 5 cm layer, as well as

relative to the 20 - 40 cm layer. The content of DTPA-extracted Cu is statistically significantly decreased up to the depth of 10 - 20 cm, and the layers below reveal no statistically significant differences. The content of DTPA-extracted Cd is statistically significantly different in the 0 - 5 cm layer compared to the other investigated depths (Table 2).

In the mineral layers up to 40 cm, which are characterized by the largest mass of the root system,

correlation was found between *aqua regia*-extractable and DTPA-extractable trace elements (Table 3). A significant correlation was found between the *aqua regia*-extracted and DTPA-extracted copper and zinc contents in the average sample at all depths. On the other hand, correlation between *aqua regia*-extractable and DTPA-extractable forms of Cd was not found (Table 3). However, there was a significant correlation between DTPA-soluble Cu and Zn ($r = 0.816^{**}$), Cu

Table 1 - Average properties and standard deviation of Dystric Leptosol

Layer		Layer			
Parameter		0 – 5 cm	5 – 10 cm	10 – 20 cm	20 – 40 cm
pH	Average±Sd	4,81±0,09	4,81±0,06	4,88±0,08	4,91±0,10
	Range	4,63-4,92	4,73-4,90	4,80-5,02	4,82-5,12
CEC cmolkg ⁻¹	Average±Sd	24,21±4,92	26,09±5,42	24,18±5,84	25,84±4,41
	Range	18,06-34,8	17,98-33,83	17,30-36,69	19,3-34,67
%C	Average±Sd	8,56±1,72	5,62±1,08	4,26±1,16	2,98±1,02
	Range	5,79-11,96	4,50-7,90	3,07-6,92	1,46-4,88
%N	Average±Sd	0,83±0,18	0,62±0,18	0,54±0,16	0,41±0,13
	Range	0,63-1,16	0,42-0,93	0,34-0,84	0,23-0,58
% clay	Average±Sd	13,61±2,58	14,05±2,23	13,49±3,20	15,19±1,71
	Range	10,10-19,10	12,2-17,9	8,10-19,70	12,00-17,30

Table 2 - Average content of Zn, Cu and Cd and standard deviation in study soils

Layer		Layer			
Parameter		0 – 5 cm	5 – 10 cm	10 – 20 cm	20 – 40 cm
Zn _{AR} mg.kg ⁻¹	Average±Sd	88,90±16,8	85,77±13,6	81,59±13,6	81,21±11,4
	Range	69,49-139,01	69,48-115,5	62,99-111,98	63,5-107,48
Zn _{DTPA} mg.kg ⁻¹	Average±Sd	6,28 a ±2,59	2,32 b ±1,05	1,09 bc ±0,53	0,58 c ±0,23
	Range	1,98-13,08	0,88-5,31	0,46-2,30	0,32-1,16
Cu _{AR} mg.kg ⁻¹	Average±Sd	30,11±6,59	27,46±9,69	26,28±10,1	26,21±10,3
	Range	22,5-45,99	15,0-45,50	11,0-45,99	12,5-46,49
Cu _{DTPA} mg.kg ⁻¹	Average±Sd	1,66 a ±0,37	1,02 b ±0,25	0,77 c ±0,24	0,61 c ±0,21
	Range	0,96-2,46	0,66-1,58	0,36-1,32	0,32-1,06
Cd _{AR} mg.kg ⁻¹	Average±Sd	0,98±0,45	0,89±0,50	0,88±0,39	0,96±0,46
	Range	0,45-2,18	0,40-2,19	0,40-1,72	0,45-2,19
Cd _{DTPA} mg.kg ⁻¹	Average±Sd	0,11 a ±0,084	0,044 b ±0,035	0,027 b ±0,03	0,014 b ±0,01
	Range	0,012-0,32	0,0065-0,149	0,003-0,12	0,003-0,035

Table 3 - Correlation coefficients of the soil content of trace elements extracted in *aqua regia* and in DTPA

	Zn _{AR}	Zn _{DTPA}	Cu _{AR}	Cu _{DTPA}	Cd _{AR}	Cd _{DTPA}
Zn _{AR}	1.000					
Zn _{DTPA}	0.355 ^{**}	1.000				
Cu _{AR}	0.689 ^{**}	0.129	1.000			
Cu _{DTPA}	0.451 ^{**}	0.816 ^{**}	0.464 ^{**}	1.000		
Cd _{AR}	0.436 ^{**}	-0.014	0.573 ^{**}	0.263 [*]	1.000	
Cd _{DTPA}	0.479 ^{**}	0.694 ^{**}	0.327 ^{**}	0.661 ^{**}	0.119	1.000

Note: Pearson's correlation coefficients for pairs of variables are given

**Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level.

and Cd ($r = 0.661^{**}$) and DTPA-soluble Zn and Cd ($r = 0.694^{**}$) (Fig. 2). The *aqua regia*-extractable forms of the elements studied also show significant correlations, including the correlation between Cu_{ar} and Zn_{ar} ($r=0.689^{**}$) or Cu_{ar} and Cd_{ar} ($r=0.573^{**}$), as well as between Zn_{ar} and Cd_{ar} ($r=0.479^{**}$).

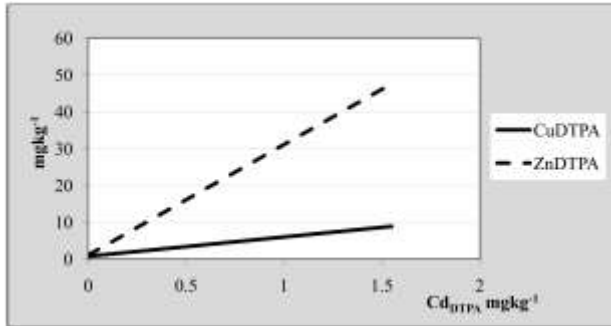


Figure 2. Correlation between content of DTPA extractable Cd and DTPA-soluble Cu and Zn in the soil.

3.3. Zinc, copper and cadmium concentrations in leaves and stems of the *Vaccinum* species

The average values of Zn, Cu and Cd in the aboveground parts of the *Vaccinum* species are shown in table 4. The content of some microelements examined in the studied *Vaccinum* species is statistically significantly different. The highest Cd content that was on average 10 times higher than in the other two species was measured in *V. uliginosum*. There was a statistically significant difference between the Cd content in *V. uliginosum* and the Cd content in *V. vitis-idaea* and *V. myrtillus*. As regards the content of copper and zinc a statistically significant difference ($p < 0.05$) was found among the three species studied. The highest content of zinc was found in *V. uliginosum* and the highest copper content in *V. myrtillus*.

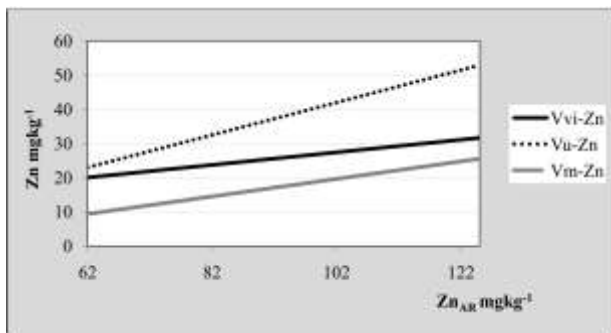


Figure 3. Correlation between content of Zn in *Vaccinum* species and content of *aqua regia*-extractable Zn in the soil

A statistically significant positive correlation was found between the content of *aqua regia*-

extracted Zn in the soil, and the Zn content in *V. myrtillus* ($r = 0.693^{**}$), *V. vitis-idaea* ($r = 0.505^{*}$) and *V. uliginosum* ($r = 0.508^{*}$) plant material (Fig. 3, Table 5).

On the other hand, the content of *aqua regia*-extracted Cu in the soil shows a negative correlation with the determined content of this element in *V. myrtillus* ($r = -0.581^{*}$) and *V. vitis-idaea* ($r = -0.545^{*}$), whereas the correlation with the Cu content in *V. uliginosum* ($r = 0.802^{**}$) is positive (Fig. 4, Table 5).

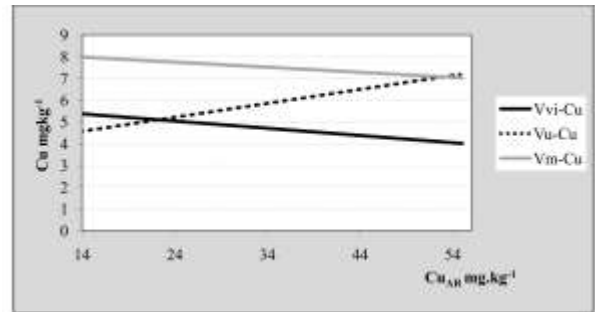


Figure 4. Correlation between content of Cu in *Vaccinum* species and content of *aqua regia*-extractable Cu in the soil

The correlations between the content of *aqua regia* extracted Zn in the soil and the Cu contents in *V. Myrtillus* ($r = -0.568^{*}$), *V. vitis-idaea* ($r = -0.547^{*}$), and *V. uliginosum* ($r = 0.749^{**}$) are significant (Fig. 5, Table 5).

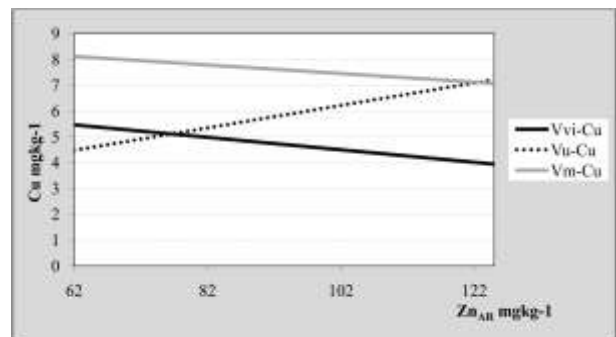


Figure 5. Correlation between content of Cu in *Vaccinum* species and content of *aqua regia*-extractable Zn in the soil

The Zn content in plants significantly influenced the decrease in the Cu content in *V. myrtillus* and *V. vitis-idaea*, and the increase in the Cu content in *V. uliginosum* (Table 5).

The content of Cd in the soil was negatively correlated with the content of Cd in *V. vitis-idaea*, whereas its correlation with the content of Cd in *V. uliginosum* was positive. In the case of *V. myrtillus* there was no significant correlation between the Cd

content in the soil and in the plant. A statistically significant positive correlation was found between the content of Cd in *V. uliginosum* and the contents of *aqua regia*-extracted Zn ($r = 0.875^{**}$), Cu ($r = 0.885^{**}$) and Cd ($r = 0.629^{**}$).

4. DISCUSSION

Depending on the soil characteristics and origin, trace elements are bound to soil components in different chemical forms (Aydinalp & Marinova, 2003, Cakmak et al., 2010, Finžgar et al., 2007). If pollutants are brought into the soil through anthropogenic influence, they are mainly bound to exchangeable chemical forms, and they are most often available to plants (Lăcătușu & Lăcătușu, 2008). Soluble, exchangeable and chelated elements in soils represent the labile fraction available to plants (Maiz et al., 2000; Takáč, et al., 2009, Galfati, et al., 2011), and these labile fractions should be complemented with the total amount of trace elements. The average content of microelements in the studied soils is slightly higher than the average values found in Serbia, i.e. Zn = 48mg/kg, Cu = 27 mg.kg⁻¹, and Cd = 0.805 mg.kg⁻¹ (Mrvic, et al., 2009). However, according to Kadović & Knežević (2002), the median values of

the studied elements of forest soils in Serbia are: 70.8 - Zn, 32.2 - Cu and 0.73 - Cd. In the studied soils Zn values are the highest relative to the average values, but all three elements are in the range of the natural dose rates (Legislative Decree, Republic of Serbia, 2010). The geological relationship between Cu and Zn has been confirmed many times (Adriano, 2001; Kabata Pendias & Pendias, 2000), as well as the geological relationship between Zn and Cd. Therefore, it is not uncommon that these forms are correlated. It is natural that *aqua regia*-extractable forms of these elements have the highest impact on their DTPA-soluble forms. However, such correlation concerning Zn contents was found only if the samples were separated by different layers in the profile, while no statistical correlations were found for Cd contents. This lack of correlation may be explained by the mutual impacts of the elements in the competition of Cu and Zn ions in the adsorptive soil complex, whereas Cd gets substituted due to the weakest bond (Alloway, 1995; Adriano, 2001). This explains a statistically significant correlation of available Cd forms with the total Zn content ($r = 0.636^{**}$) and the available forms of Cu ($r = 0.655^{**}$) and Zn ($r = 0.773^{**}$) (Fig. 2, Table 3).

Table 4 - Average content of Cd and Zn and standard deviation in the leaves and stems of the *Vaccinium* species

Element		Zn	Cu	Cd
Species		mg.kg ⁻¹		
<i>V. myrtillus</i>	Average±Sd	14,94 c ±4,87	7,76 c ±0,38	0,028 a ±0,02
	Range	8,27-20,34	7,38-8,47	0,001-0,063
<i>V. uliginosum</i>	Average±Sd	33,35 b ±12,3	5,43 b ±0,76	0,52 b ±0,09
	Range	25,7-59,4	4,51-6,87	0,41-0,69
<i>V. vitis-idaea</i>	Average±Sd	24,04 a ±4,80	4,94 a ±0,58	0,068 a ±0,01
	Range	18,1-32,52	3,87-5,67	0,046-0,083

+ Within each *Vaccinium* species, means with the same letter are not significantly different (p<0.05)

Table 5 - Correlation coefficients for each pair of trace elements in the leaves and stems of *Vaccinium* species and soil content of trace elements

	Cu _{AR}	Cu _{DTPA}	Zn _{AR}	Zn _{DTPA}	Cd _{AR}	Cd _{DTPA}	Vvi-Zn	Vu-Zn	Vm-Zn	Vvi-Cu	Vu-Cu	Vm-Cu	Vvi-Cd	Vu-Cd	Vm-Cd
Vvi-Zn	0.650**	0.246	0.505*	0.113	0.425	0.472	1.000								
Vu-Zn	0.596*	0.361	0.508*	0.211	0.358	0.636**	0.803**	1.000							
Vm-Zn	0.807**	0.582*	0.693**	-0.047	0.645**	0.338	0.487	0.510*	1.000						
Vvi-Cu	-0.545**	-0.336	-0.547**	-0.385	-0.175	-0.502*	-0.632**	-0.727**	-0.191	1.000					
Vu-Cu	0.802**	0.455	0.749**	0.230	0.366	0.571*	0.674**	0.680**	0.702**	-0.626**	1.000				
Vm-Cu	-0.581*	-0.370	-0.568*	-0.040	-0.449	-0.212	-0.582*	-0.327	-0.602*	0.232	-0.423	1.000			
Vvi-Cd	-0.681**	-0.214	-0.621*	-0.139	-0.384	-0.382	-0.595*	-0.552*	-0.483	0.575*	-0.900**	0.236	1.000		
Vu-Cd	0.885**	0.587*	0.875**	0.164	0.629**	0.478	0.609*	0.524*	0.897**	-0.413	0.838**	-0.656**	-0.698**	1.000	
Vm-Cd	0.005	0.004	-0.190	-0.344	0.234	-0.264	-0.218	0.152	0.250	0.171	-0.038	0.253	-0.028	-0.035	1.000

Note: Pearsons correlation coefficients for pairs of variables are given

**Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level.

Vvi - *V. vitis-idaea*; Vu - *V. uliginosum*; Vm - *V. myrtillus*.

The availability of trace elements in the profile decreases with depth (Table 2). This is likely to be influenced by the correlations between these elements, but it can also be caused by an advanced degree of weathering in the upper horizons, which is especially characteristic of Zn (Kabata-Pendias & Pendias, 2000, Adriano, 2001). Also, the fact that undeveloped land on mountain slopes is characterized by a severe process of erosion should be taken into account. There is no common accumulation of available forms in the deeper horizons, which is typical of unpolluted soils.

According to the de Vries & Bakker (1996) criteria, the Cd content in the studied vegetative plant parts is within the limits for *V. myrtillus* and *V. vitis-idaea*, whereas in the case of *V. uliginosum* the values exceed the upper limit at both localities. According to the Kabata-Pendias & Pendias (2000) criteria the contents of Cd, Cu and Zn in the plant material are within the limits. Also, the measured values in the aboveground shoots of *Vaccinium* species fall within the limit values measured in uncontaminated soils and the optimal values (Mróz & Demczuk, 2010; Levy et al., 1999), except in the case of Zn, whose content is slightly higher, but well below toxic values. The measured content of Cd and Zn in the leaves and stems of plants is the highest in *V. uliginosum*, while the highest Cu values were measured in *V. myrtillus*. On the basis of the average values, it can be observed that the Cd content in *V. uliginosum* is statistically significantly different from its content in *V. myrtillus* and *V. vitis-idaea* (Table 4). The mean values of the Zn and Cu contents are statistically significantly different among the three studied species of the genus *Vaccinium*.

The uptake of Zn and Cd in plant material and their mutual relationship is very complex, changeable and dependent on a number of factors. Many experiments have recorded the antagonism between these two elements in aboveground plant parts (Oliver et al., 1994, Schmidt et al., 1992, Moustakas, 2011). On the other hand, the addition of Zn up to a certain concentration may have an impact on the decrease of Cd contents or Cd accumulation (Haghiri, 1974). Different plant species respond differently to the relationship between zinc and cadmium, i.e. to their synergism or antagonism.

In the case of the three *Vaccinium* species studied, it was shown that only the zinc content in plants was correlated with the *aqua regia*-extracted content of zinc in the soil. However, antagonism was observed in *V. vitis-idaea* (Table 5), i.e. zinc had an impact on the decrease in plant cadmium content.

An entirely opposite effect was observed in *V.*

uliginosum. The content of *aqua regia*-extracted cadmium in the soil had an impact on the increase of cadmium in the plant, unlike in the case of the other two species. Also, the increase in the Zn contents, both in the soil and in *V. uliginosum* plants, had a positive effect on the accumulation of cadmium in aboveground plant parts. The relationship between zinc and cadmium can be explained by the coincidence of different transporters of these elements in plants, and their different behavior depends on the species (Korshunova et al., 1999; Clemens, 2001).

Rhizosphere chemistry affects the contents of certain elements in the studied plants (Dong et al., 2007), and their availability to plants is regulated by the presence of microorganisms and mycorrhiza. The plants that are in symbiosis with ericoid mycorrhiza may colonize the soil with a low nutrient content, as well as the acidic soils with a high content of trace elements (Read, 1991). Bradley, et al. (1982) concluded that there are great differences in both the development of aboveground biomass and the content of Cu and Zn in *Vaccinium macrocarpon* plants depending on whether the plants were developed with or without the mycorrhiza. The mycorrhizal plants had significantly lower contents of trace elements in shoots, but higher contents in roots (Bradley et al., 1982). Cu-Zn interactions are commonly observed (Graham, 1981; Rinkis, 1972). These metals are apparently absorbed by the same mechanism and therefore each of them may competitively inhibit the root adsorption of the other. A significant negative effect of Zn on the uptake of Cu was observed in two species - *V. vitis-idaea* and *V. myrtillus* (Fig. 5). On the basis of the coefficient of correlation between the total copper and zinc contents in the soil and the copper content in plants, marked antagonism was found between these two elements. It should be noted that *V. myrtillus* has the highest copper content in aboveground plant parts.

5. CONCLUSIONS

This study presents the accumulation of Zn, Cu and Cd in the leaves and stems of three *Vaccinium* species under the natural conditions prevailing in the unpolluted soil of Stara Planina Mt. The availability of trace elements to plants depends on soil properties, the biology and specificity of species, and the origin and interactions of elements.

Different accumulations of trace elements were measured in aboveground plant parts of *V. myrtillus*, *V. uliginosum* and *V. vitis-idaea*. The measured contents of Cd and Zn were the highest in

V. uliginosum, whereas the highest values of Cu were measured in *V. myrtillus*. The analysis of variance revealed that the Cd content in *V. uliginosum* significantly differs from its content in *V. myrtillus* and *V. vitis-idaea*, and significant differences in the contents of Zn and Cu were found between the studied species of the genus *Vaccinium*. An increase in the zinc contents in both the soil and *V. uliginosum* plants had a positive effect on the accumulation of cadmium in aboveground plant parts. Marked antagonism of Zn to Cu uptake was observed in two species - *V. vitis-idaea* and *V. myrtillus*. On the basis of the coefficient of correlation between the total copper and total zinc contents in the soil with the copper content in plants, marked antagonism was found between these two elements.

On the basis of all analyzed correlations, it can be concluded that *V. uliginosum* has the highest affinity for mobilizable Cd and Cu and as such can be used as a remediation plant species, whereas the other two species studied can be grown on soils with elevated Cd levels, without a high risk of accumulation of this element.

7. ACKNOWLEDGEMENTS

This paper was realized as a part of two projects: "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (43007) and "Impact of soil quality and irrigation water quality on agricultural production and environmental protection" (37006) financed by the Ministry of Education and Science of the Republic of Serbia for the period 2011-2014.

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Received at: 04. 10. 2012

Revised at: 06. 02. 2012

Accepted for publication at 11. 05. 2013

Published online at: 06. 06. 2013