Botanica SERBICA 43 (1): (2019) 73-83

Original Scientific Paper

Heavy metal tolerance of *Pontechium maculatum* (Boraginaceae) from several ultramafic localities in Serbia

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ABSTRACT: *Pontechium maculatum,* a facultative metallophyte, was collected from four ultramafic localities in Serbia and analysed in terms of micro- and macroelement accumulation. The aim of the study was to reveal trace element profiles and differences in uptake and translocation of heavy metals in populations growing under heavy metal stress. The concentrations of major and trace elements in soil samples (Ca, Mg, Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co, Cd) and in plant tissues (Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co, Cd) are presented. The results of our analysis indicate that *P. maculatum* efficiently absorbs Zn and Cr, while for most of the other elements accumulation levels fit in the range of values obtained for several other species from ultramafic localities on the Balkan Peninsula.

Keywords: Boraginaceae, *Echium russicum*, trace metal, Balkan Peninsula

Received: 27 September 2018 Revision accepted: 02 November 2018

UDC: 582.929.2:546.3(497.11+292.464)
DOI: https://doi.org/10.2298/BOTSERB1901073J

INTRODUCTION

Ultramafic bedrock and soils derived from them are known as inhospitable environments due to specific, often extreme physical and chemical characteristics. These soils are characterised byan unfavourable Ca/Mg ratio, nutrient limitation and high amounts of Fe, Ni, Cr and Co. In addition to these edaphic stressors, plants growing on ultramafic soils are exposed to drought, high temperature and intensive light (Freitas *et al.* 2004; Brady *et al.* 2005). Such conditions pose a challenge for the survival of species, and different strategies in overcoming those challenges have resulted in a whole series of specialised endemic taxa and plant communities. Plants that exclusively grow on ultramafic soils and have not been found on any other type of substrate are called serpentinicolous or serpentine-obligate plants. Generally, the ultramafic flora is richest in endemic metallophytes, with more than 1000 such taxa (Pollard *et al.* 2014). However, there are also serpentine-tolerant or serpentine-facultative plants, which survive on ultramafic substrates, but can also be found elsewhere (Reeves *et al.* 1996; Stevanović *et al.* 2003; Freitas *et al.* 2004). Still, due to the high energy cost of mechanisms related to survival on demanding substrates such as ultramafic ones, these species can be competitively weak on non-ultramafic bedrock and are found on ultramafic substrates more often than on other types of substrate (Wójcik *et al.* 2017). Both obligate and facultative serpentinophytes can respond in different ways to high heavy metal levels, either by excluding or by accumulating (hyperaccumulating) elevated metal concentrations. In this regard, two basic tolerance strategies can be distinguished: metal exclusion (metal avoidance or prevention of transloca-

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tion to above-ground tissues) and metal accumulation. Depending on accumulation levels, there are three types of accumulators: indicators, accumulators and hyperaccumulators. Indicators take up metals in concentrations that reflect their concentrations in soil. Accumulators and hyperaccumulators take up and translocate metals in concentrations higher than those in the soil (accumulators), sometimes in quantities that go beyond the hyperaccumulation threshold when grown in nature (hyperaccumulators) (Prasad & Freitas 2003). The proposed nominal threshold criteria (in mg kg^{-1}) are: 100 for Cd, Se and Tl, 300 for Cu, Co and Cr, 1000 for Ni, Pb and As, 3000 for Zn and 10000 for Mn. There are also some additional criteria for defining plant species as hyperaccumulators: 1) bioconcentration factor >1 (often >50); 2) translocation factor >1; and 3) extreme metal tolerance (van der Ent *et al.* 2013). It is estimated that 85–90% of hyperaccumulator species are obligate endemics to metalliferous soils, and the rest of the taxa (10–15%) are facultative and occur on metalliferous and non-metalliferous soils (Pollard *et al.* 2014). Among the c. 500 so far listed hyperaccumulator species, the majority belong to the family Brassicaceae, and for the most part (nearly 400 taxa) they hyperaccumulate Ni (Baker *et al.* 2000; Assunção *et al.* 2003; Pollard *et al.* 2014). Relatively abundant on ultramafic substrates are representatives of the family Boraginaceae, with a number of proven obligatory and facultative serpentinophytes (Vicić 2014). Particularly interesting are representatives of the tribe Lithospermeae, where complex paths of serpentine endemism have been confirmed, with obligatory serpentine endemism occurring within *Halacsya* Dörfl., *Paramoltkia* Greuter and *Onosma* L., whereas facultative serpentinophytes occur within *Alkanna* Tausch, *Arnebia* Forssk., *Echium* L. and *Neatostema* I. M. Johnst. (Cecchi & Selvi 2009; Coppi *et al.* 2014). Within the genus *Echium* L., the species *E. vulgare* L. has been the subject of particularly detailed study in terms of its physiological response to metalliferrous habitats (Dresler *et al.* 2014, 2017). These studies pointed to the accumulation of Zn and Pb in individuals growing in areas burdened with these elements (mine waste deposits). Besides *E. vulgare*, *E. plantagineum* showed accumulation of Zn in a mining site in Morocco (up to 571 mg kg^{-1}), but with a low accumulation factor (metal concentration in plant/metal concentration in soil <1) (Boularbah *et al.* 2006). An especially abundant representative of the family Boraginaceae on ultramafic substrates in Serbia is *Pontechium maculatum* (L.) Böhle & Hilger [synonyms *Echium maculatum* L., *E. rubrum* Jacq. (non Forssk.) and *E. russicum* J. F. Gmel.]. This biennial Pontic-Pannonian plant grows in dry grasslands, steppe meadows, vineyards in lowlands and hilly areas, and while predominantly inhabiting ultramafic areas, it can also be found on different alkaline substrates (limestone, dolomite, etc.) (Cincović & Kojić 1974).

Considering that *P. maculatum* occurs on both metalliferous and non-metalliferous soils, although predominantly on metalliferous ones, it can be categorised as a pseudometallophyte (Baker *et al.* 2010), and we can assume that it possesses mechanisms for growing under heavy metal stress, which has not been studied yet. In order to provide further insight into ultramafic populations of *P. maculatum* and their response to environmental conditions in metalliferous habitats, we set out to determine: 1) concentrations of Ca, Mg, Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co, Cd in soil samples; 2) concentrations of Fe and trace metals in plant tissues; and 3) differences in heavy metal uptake and translocation toaboveground tissues.

MATERIAL & METHODS

Study area. The samples were collected from four sites in Serbia: Mts Kopaonik, Mokra Gora, Maljen and Zlatibor, all on ultramafic substrates (harzburgite in SP1-3 and serpentinite in SP4) (Filipović *et al.* 1967-1971; Group of authors 1970; Mojsilović *et al.* 1977; Olujić & Karović 1985). The precise locations of sample taking (latitude and longitude) as well as habitat characteristics (altitude, type of ultramafic rocks and bioclimatic characteristics) are presented in Fig. 1 and Table 1.

The sampling sites belong to two different climate types: Mts. Mokra Gora and Maljen are characterised by the mountain type of continental climate, while Mts. Kopaonik and Zlatibor have a moderate continental climate (Ducić & Milovanović 2005). More precise climate data for each location were extracted using DIVA-GIS 7.5 software from the WorldClim set of global climate layers at a resolution of 30 arc-seconds (~ 1 km^2). The same software was used for preparation of Fig. 1. Country codes correspond with ISO 3166/2 (1998).

Soil analysis. Soil samples (~500g) were taken from the rhizosphere of analysed plants, air-dried and afterwards sieved through 2-mm and 0.2-mm sieves for the purpose of different analyses.

Actual (pH_{H2O}) and exchangeable (pH_{KCl}) pH of the soil was measured in distilled water and in 1 M KCl (w:v, 1:2.5) (McKeague 1978). The percentage of organic matter was determined by dichromate digestion (FAO 1974). Available P_2O_5 and K_2O were measured in an AL solution of 0.1 M ammonium lactate and 0.4 M acetic acid (1:20, w:v; Egnér *et al.* 1960). The concentration of phosphate was measured by the molybdenum-blue method and content of K_2O with an atomic absorption spectrophotometer (Shimadzu AA 7000, Kyoto, Japan). Atomic absorption spectrophotometry was also used for determination of available concentrations of Ca and Mg in 1 M ammonium acetate (1:50, w:v; van Reeuwijk 2002). Potentially leachable (available) concentrations of Fe, Mn, Zn, Cu, Ni, Co, Cr, Pb and Cd were determined in

Table 1. Characterisation of *Pontechium maculatum* sampling sites.

Sample point	Locality	Coordinates	Altitude (m)	Annual mean temperature (°C)	Annual precipitation (mm)	Type of bedrock	Voucher number
SP1	Mt. Kopaonik (Treska)	43.25985 N 20.78522 E	1604	4.85	950	Harzburgite	BEOU-35314
SP2	Mt. Mokra Gora (Panjak)	43.74994 N 19.49358 E	879	8.95	970	Harzburgite	BEOU-40669
SP3	Mt. Maljen (Divčibare)	44.1207 N 20.01181 E	1050	7.09	901	Harzburgite	BEOU-40700
SP4	Mt. Zlatibor	43.61427 N 19.64827 E	1100	7.32	985	Serpentinite	BEOU-35286

0.05 M EDTA extracts of soil (S:L of 1:10, w:v; McGrath 1996). The total concentrations of Fe, Mn, Zn, Cu, Ni, Co, Cr, Pb and Cd were determined after digestion of soil samples in HCl and HNO_3 (ISO 11466 1995). For both total and available metal concentrations, an atomic absorption spectrophotometer was used. The metal concentrations in samples were determined by comparing their absorption values with those of known standards. All measurements were performed in triplicate.

Plant analysis. Samples of *Pontechium maculatum* (~10 individuals) were collected from each sampling point, separated into roots and shoots, thoroughly washed with tap and distilled water and air-dried.

Ground-up material was oven-dried at 105°C and afterwards digested using a modified wet procedure with a boiling mixture of HNO_3 and H_2SO_4 (ISO 6636/2 1981). Concentrations of P_2O_5 were determined by a modified version of the molybdenum blue method (Chen *et al.* 1956). Concentrations of K_2O, of Fe, Mn, Zn, Cu, Ni, Co, Cr, Pb and Cd were also determined with an atomic absorption spectrophotometer (Shimadzu AA 7000).

Data analysis. The obtained results were subjected to statistical analyses, and considering that the data were not normally distributed, non-parametric statistics was used. Metal concentrations at the sample sites were compared using non-parametric Kruskal-Wallis ANOVA. All statistical analyses were performed using the Statistica 7.0 for Windows work package (StatSoft 2004).

To estimate the heavy metal tolerance of *P. maculatum*, the biological concentration factor (BCF = root/soil), accumulation factor (AF = stem/soil) and translocation factor (TF = stem/root) were calculated.

RESULTS

Soil analysis. Chemical characteristics of the soil samples (active and exchangeable pH, % C, available Ca and Mg, total and available concentrations of Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co and Cd) are shown in Table 2. The pH values, both active and exchangeable, varied in all samples from acidic (pH_{KCl} at SP1 and SP4) to neutral (pH_{H2O} at SP2 and SP3). The organic content was found to be medium to high, with highest fertility in samples from Mts. Kopaonik (SP1) and Maljen (SP3). The Ca/Mg ratio was low, and varied between 0.32 (SP4) and 2.6 (SP3). The soil sample from Mt. Kopaonik (SP1) had the highest concentrations of Pb, both total and available, and total Cr. At the same time, this sample contained the lowest concentrations of total and available Fe, Mn

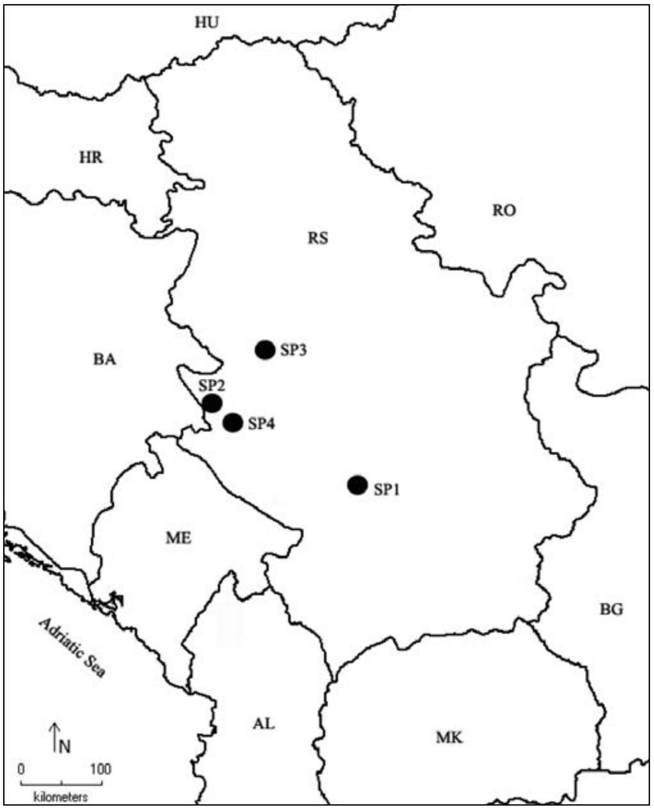

Fig. 1. Distribution of sampled *Pontechium maculatum* populations in Serbia.

Table 2. %C, pH, available Ca and Mg, total and available concentrations of Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co and Cd (in mg kg^{-1}) in soil samples (given as mean values and standard deviation).

	SP1	SP2	SP3	SP4
% C	9.78±0.448	3.16±0.145	8.86±0.264	3.44±0.090
pH (H2O)	5.97±0	6.83±0	7±0	6.24±0
pH (KCl)	5.41±0	5.9±0	5.88±0	5.46±0
Ca (a)	2881±153.6	1463±133	3280±154	765±33
Mg (a)	1490±151	1885±115	1262±123	2417±231
Ca/Mg	1.93	0.78	2.6	0.32
Fe (t)	92407±2823	103442±498	101220±9690	102100±1612
Fe (a)	804±50.8	1038±17.7	1207±10.8	1279±7.78
Fe(a)/Fe(t) (%)	0.87	1.00	1.19	1.25
Mn (t)	1461±111	1540±26.5	1912±26.8	2240±28.7
Mn (a)	721±24.6	878±14.3	929±4.98	1092±2.17
Mn(a)/Mn(t) (%)	49.35	57.01	48.59	48.75
Ni (t)	1081±81.8	2110±21.1	1183±21.9	1694±31.3
Ni (a)	292±10.4	382±8.46	277±6.84	509±8.02
Ni(a)/Ni(t) (%)	27.01	18.10	23.42	30.05
Pb (t)	137±6.30	34.2±0.239	86.3±3.59	39.2±1.55
Pb (a)	102±1.63	8.51±0.350	47.4±0.392	14.9±0.143
Pb(a)/(Pb(t) (%)	74.45	24.88	54.92	38.01
Cr (t)	1380±87.2	666±16.5	963±14.3	1203±18.6
Cr (a)	4.06±0.081	2.06±0.131	4.89±0.110	4.63±0.256
Cr(a)/Cr(t) (%)	0.29	0.31	0.51	0.38
Zn (t)	62±0.996	43.2±0.320	71.2±1.26	47.7±1.36
Zn (a)	23.6±1.23	7.66±0.798	30.9±0.358	9.25±1.16
Zn(a)/Zn(t) (%)	38.06	17.73	43.40	19.39
Cu (t)	10.7±3	17.9±0.365	12.6±0.461	16.4±0.506
Cu (a)	7.25±0.157	6.21±0.170	6.55±0.206	7.93±0.286
Cu(a)/Cu(t) (%)	67.76	34.69	51.98	48.35
Co (t)	92.8±3.64	111±14.4	143±4.59	147±3.57
Co (a)	59.6±0.397	76±0.773	85.1±0.690	112±1.12
Co(a)/Co(t) (%)	64.22	68.47	59.51	76.19
Cd (t)	0.98±0.036	0.893±0.021	1.24±0.025	0.837±0.012
Cd (a)	0.903±0.021	0.33±0.01	1.01±0.021	0.383±0.015
Cd(a)/Cd(t) (%)	92.14	36.95	81.45	45.76

and Co, as well as total Ni and Co. In the sample from Mt. Mokra Gora (SP2), the highest total concentrations of Fe, Ni and Cu were encountered, but the lowest concentrations of total and available Zn, Cr and Pb, and the lowest concentrations of available Cd and Cu.

The highest content of Ca and highest content of both total and available Zn and Cd were determined in the sample from Mt. Maljen (SP3), which also showed the lowest content of available Mg and Ni. The sample from Mt. Zlatibor (SP4) contained the highest concentrations of total and available Mn and Co, as well as the highest concentrations of available Mg, Fe, Ni and Cu. The lowest contents of Ca and total Cd were determined in this sample. The ratio of available to total metal concentrations varied significantly. The highest values were calculated for Cd (92.14 at SP1), with significant values determined also for Co, Pb and Cu. The lowest ratios were calculated for Cr and Fe.

According to the results of Kruskal-Wallis ANOVA (Table 3), there were significant differences among soil samples in almost all parameters, except pH and total Fe concentration.

Plant analysis. The concentrations of Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co and Cd in plant (root and shoot) tissues of *P. maculatum* from four ultramafic localities in Serbia are presented in Table 4. The concentrations of iron in all plant tissues were high, higher than 1000 mg kg^{-1}, with the exception of its content in roots at SP4, and in shoots at SP2 and SP3. The shoot concentration of Zn at SP4 was higher than 100 mg kg^{-1}, while in all other samples both shoot and root concentrations were significantly lower. According to the results of Kruskal-Wallis ANOVA (not shown), significant differences were determined among plant samples in Mn, Ni and Zn concentrations in roots and shoots, in root concentrations of Cu and in shoot concentrations of Fe, Pb and Cr.

Accumulation and translocation. Table 5 presents values of the bioconcentration factor (BCF), accumulation factor (AF) and translocation factor (TF) for the analysed macro- and trace elements. High values of BCF (up to 4.23) were found for Cr , Zn, Cu and Fe, but not in all samples. Values of AF higher than 1 (up to 12.66) were detected for Zn, Cr and Fe in two samples. A TF value higher than 1 (up to 10.56) was found for Zn in all samples and for Fe, Mn, Cr and Cu in one or two samples.

Table 3. Results of Kruskal-Wallis test for concentrations of %C, pH, available Ca and Mg, and total and available concentrations of Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co and Cd in soil samples. Variables with P < 0.05 are indicated in boldface.

	H	df	P
% C	10.3846	3	**0.0156**
pH (H2O)	3.0000	3	0.3916
pH (KCl)	3.0000	3	0.3916
Ca (a)	10.4578	3	**0.0151**
Mg (a)	10.3846	3	**0.0156**
Fe (t)	4.9487	3	0.1756
Fe (a)	10.3846	3	**0.0156**
Mn (t)	9.4615	3	**0.0237**
Mn (a)	10.3846	3	**0.0156**
Ni (t)	9.9744	3	**0.0188**
Ni (a)	10.3846	3	**0.0156**
Pb (t)	10.3846	3	**0.0156**
Pb (a)	10.3846	3	**0.0156**
Cr (t)	10.3846	3	**0.0156**
Cr (a)	9.7006	3	**0.0213**
Zn (t)	10.3846	3	**0.0156**
Zn (a)	10.0094	3	**0.0185**
Cu (t)	9.4615	3	**0.0237**
Cu (a)	9.9744	3	**0.0188**
Co (t)	9.0513	3	**0.0286**
Co (a)	10.3846	3	**0.0156**
Cd (t)	10.4211	3	**0.0153**
Cd (a)	10.3846	3	**0.0156**

DISCUSSION

Soil analysis. One of the most important factors controlling metal solubility in soil is pH (Kashem & Singh 2001). In our soil samples, pH values are in agreement with the mean value determined for ultramafic soils (6.8 according to Brooks 1987). According to Belić *et al.* (2014), the analysed soils are characterised as slightly acidic (in samples from Mts. Kopaonik and Zlatibor) and neutral (samples from Mts. Mokra Gora and Maljen). The Ca/Mg ratio is generally low, especially at SP2 and SP4, but still not as extreme as found in samples from Albania (0.02) by Bani *et al.* (2010). A low Ca/Mg ratio is a common ultramafic characteristic and it additionally exacerbates soil infertility, primarily as a result of low nutrient content (Asemaneh *et al.* 2007). This unfavourable ratio is mainly due to high Mg concentrations, especially in comparison with non-ultramafic

Table 4. Concentrations of Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co and Cd (in mg kg^{-1}) in roots and shoots of four samples of *Pontechium maculatum*, given as means and standard deviations.

	SP1	SP2	SP3	SP4
Fe roots	1080±193	2595±374	1156±325	889±111
Fe shoots	1293±106	683±54.3	737±23.6	2811±346
Mn roots	69.3±9.24	42.8±4.52	38.7±2.21	29.8±1.20
Mn shoots	40.8±3.37	18.2±0.321	22.6±0.762	34.3±0.03
Ni roots	35.3±5.81	82.6±5.70	30.8±2.7	46.8±1.85
Ni shoots	17±2.26	17.3±0.359	12.5±1.90	24±0.909
Pb roots	<0.1	<0.1	<0.1	<0.1
Pb shoots	<0.1	<0.1	<0.1	1.38±0
Cr roots	11.7±1.44	8.71±0.894	7.43±2.17	9.62±0.719
Cr shoots	11.1±0.362	5.62±1.00	7.52±0.438	11.8±2.14
Zn roots	34.1±3.36	16.8±0.813	21.6±0.973	11.1±0.898
Zn shoots	35.6±9.16	83±10.6	44.6±8.1	117±3.72
Cu roots	11.4±1.17	18.6±3.25	17.8±2.28	2.38±0.319
Cu shoots	3.97±0.283	5.09±0.017	4.37±0.370	4.15±0.584
Co roots	<0.1	<0.1	<0.1	<0.1
Co shoots	3.54±0.911	3.77±0.737	2.84±0.709	4.94±0.701
Cd roots	<0.1	<0.1	<0.1	<0.1
Cd shoots	<0.1	<0.1	<0.1	<0.1

soils. Due to the ferro-magnesium nature of ultramafic rocks, high Fe content is expected (Reeves 1992). However, in our samples these concentrations are even higher than those found by numerous authors in ultramafic substrates of the Balkan Peninsula (Pavlova & Karadjova 2013; Jakovljević *et al.* 2015; Đurović *et al.* 2016; Matko-Stamenković *et al.* 2017; Tomović *et al.* 2018).

The amounts of manganese and nickel correspond with those found on the same type of substrata (Reeves *et al.* 2009; Bani *et al.* 2010; Tumi *et al.* 2012; Tomović *et al.* 2018), particularly regarding their total concentrations. However, the content of available Mn in our soil samples was considerable higher, while that of available Ni was in the range of the values obtained in similar studies, with somewhat higher concentrations in the sample from Mt. Zlatibor. Similarly elevated values from the same area were also determined by Tomović *et al.* (2018) in their analysis of hyperaccumulation in three *Armeria* species from Serbia. The obtained Zn concentrations were in the range of soil samples under *Silene* taxa and several species from the family Brassicaceae (Tomović *et al.* 2013; Đurović *et al.* 2016). As in the case of many trace metals, the key factor in determining Zn solubility and mobility is pH: they increase with decrease of pH (Meers *et al.* 2006). Elevated Cr concentrations represent a constitutive ultramafic feature, and the obtained amounts are in the range of values reported by several authors in samples from Serbia and Bosnia & Herzegovina (Tomović *et al.* 2013, 2018; Matko-Stamenković *et al.* 2017). At the same time, these concentrations are lower than those determined by Bani *et al.* (2010), also in countries on the Balkan Peninsula (Albania, Greece and Bulgaria). In view of the highly limited availability of Cr, it is not surprising that its available concentrations are considerably lower (availability of 0.3–0.5%). Similar values were observed by Tumi *et al.* (2012), but there are also different results indicating a percentage of availability of up to 10 (Đurović *et al.* 2016). Total and available Cu concentrations were quite uniform among soil samples, and most of them were close to those obtained by Jakovljević *et al.* (2015) and Tomović *et al.* (2018). Concentrations of Co, Cd and Pb were also similar to those obtained by numerous investigators in studies of ultramafic substrates on the Balkan Peninsula (Bani *et al.* 2010; Tumi *et al.* 2012; Tomović *et al.* 2013, 2018; Đurović *et al.* 2016). Somewhat higher Pb values were found in the samples from Mts. Kopaonik and Maljen, as was previously recorded for these areas in several other studies (Tumi *et al.* 2012; Tomović *et al.* 2013; Đurović *et al.* 2016).

Table 5. Accumulation potential (BCF, AF and TF) for samples representing four populations of *Pontechium maculatum*.

BCF	Fe	Mn	Ni	Pb	Cr	Zn	Cu	Co	Cd
SP1	**1.34**	0.10	0.12	0.00	**2.88**	**1.44**	**1.58**	0.00	0.00
SP2	**2.50**	0.05	0.22	0.00	**4.23**	**2.20**	**3.00**	0.00	0.00
SP3	0.96	0.04	0.11	0.00	**1.52**	0.70	**2.71**	0.00	0.00
SP4	0.70	0.03	0.09	0.00	**2.08**	**1.20**	0.30	0.00	0.00
AF	Fe	Mn	Ni	Pb	Cr	Zn	Cu	Co	Cd
SP1	**1.61**	0.06	0.06	0.00	**2.73**	**1.51**	0.55	0.06	0.00
SP2	0.66	0.02	0.05	0.00	**2.73**	**<u>10.84</u>**	0.82	0.05	0.00
SP3	0.61	0.02	0.05	0.00	**1.54**	**1.44**	0.67	0.03	0.00
SP4	**2.20**	0.03	0.05	0.09	**2.54**	**<u>12.66</u>**	0.52	0.04	0.00
TF	Fe	Mn	Ni	Pb	Cr	Zn	Cu	Co	Cd
SP1	**1.20**	0.59	0.48	0.00	0.95	**1.04**	0.35	0.00	0.00
SP2	0.26	0.43	0.21	0.00	0.64	**4.94**	0.27	0.00	0.00
SP3	0.64	0.59	0.41	0.00	**1.01**	**2.06**	0.25	0.00	0.00
SP4	**3.16**	**1.15**	0.51	0.00	**1.22**	**<u>10.56</u>**	**1.74**	0.00	0.00

*Values higher than one are indicated in boldface, values higher than 10 are indicated in boldface and underlined.

Plant analysis. Although high, the Fe concentrations in root and shoot samples of *Pontechium maculatum* fall in the range of concentrations obtained in other plant samples from ultramafic substrates on the Balkan Peninsula (Đurović *et al.* 2016; Tomović *et al.* 2018). The samples from Mts. Kopaonik and Zlatibor indicate possible accumulation, with values of content higher in shoots, whereas in the other two samples, from Mts. Mokra Gora and Maljen, there is the reverse situation, an indication of heavy metal exclusion. Despite considerably higher concentrations of available Mn in soil, the amounts of Mn in plant tissues are far from the hyperaccumulating threshold, and only the values obtained for the sample from Mt. Zlatibor indicate possible accumulation. Although the bulk of serpentinophytes accumulate Ni in plant tissues (as hyperaccumulators or excluders), a certain number of species have a nickel-avoiding strategy. They take up Ni in concentrations significantly lower than available ones in the soil, and our results indicate that *P. maculatum* is one of them. Absorption of Ni can be reduced by Zn, as could be the case in the samples from Mts. Kopaonik and Maljen. According to Deng *et al.* (2014), this is most likely a result of competition in the root uptake process, where Zn(II) strongly inhibits the Ni(II) influx due to shared transporting systems. Exclusion of Fe, Mn and Ni was also recorded in *Halacsya sendtneri* (Boiss.) Dörfl., another representative of the tribe Lithospermae (Vicić 2014).

Root concentrations of Zn are similar to those obtained in various serpentinophytes on the Balkan Peninsula whereas shoot concentrations, although far from hyperaccumulation thresholds, are significantly higher than those in roots. Elevated uptake and translocation of Zn to leaves was previously recorded for *H. sendtneri* (Vicić 2014). Even higher concentrations were recorded in *E. vulgare* growing on waste deposits rich in Zn (Wójcik *et al.* 2014). The levels of Cr are similar in the analysed root and shoot samples. Generally, concentrations of Cr in shoots are rarely higher than 5 mg kg^{-1} (Kabata-Pendias 2011), probably because of predominance of the insolubile $Cr(III)^+$ form in the soil. However, shoot concentrations in all four of our samples are higher, even up to 11 mg kg^{-1}, exceeding the threshold of critical leaf concentrations (Zayed & Terry 2003). Contrary to the case of Cr, Cu concentrations in the analysed plant samples are not very uniform, but significant variations--detected not only among different ultramafic species, but also in samples of the same species—have been recorded previously (Tumi *et al.* 2012; Tomović *et al.* 2013, 2018; Đurović *et al.* 2016). Although *H. sendtneri* showed elevated uptake and translocation of Cu to leaves (Vicić 2014), in our study that was the case only with the sample from Mt. Zlatibor. Regarding the concentrations of Pb, although its content in the soil is within or even higher than the range of values obtained at other ultramafic locations, the levels of Pb in plant tissues, except those of shoots in the sample from Mt. Zlatibor, were under the detection limit. According to Blaylock *et al.* (1997), in soil with pH between 5.5 and 7.5, only a small amount of Pb is available to plants, even if they possess a genetic capacity for accumulation. In addition, Pb is often bound to colloidal or organic material in the soil, resulting in its reduced uptake by roots (Sharma & Dubey 2005). Quite to the contrary, at high Pb concentrations in the soil, *E. vulgare* accumulates more than 200 mg kg^{-1}, indicating the existence of a positive correlation between the concentration in the soil and that in plants (Wójcik *et al.* 2014). The concentrations of Cd are generally low in plant samples, and in *P. maculatum* this content is even lower than the values obtained in serpentinophytes of the Balkan Peninsula (Jakovljević *et al.* 2015; Đurović *et al.* 2016; Matko-Stamenković *et al.* 2017; Tomović *et al.* 2018). Unlike *P. maculatum*, *E. vulgare* efficiently takes up Cd from the soil, with the tissue concentration positively dependent on Cd content in the soil and higher content in the roots than in the shoots (Dresler *et al.* 2014).

Accumulation and translocation. The results of our analysis indicate that *P. maculatum* efficiently takes up and translocates Zn and Cr to above-ground tissues. Especially efficient are Zn uptake from soil to shoots and transfer from roots to shoots in the samples from Mts. Mokra Gora and Zlatibor. However, the tendency of Zn accumulation by shoots (leaves) is common. This translocation to the leaves could be a detoxification mechanism that acts through leaf fall, considering the several-fold higher concentrations in senescencing leaves compared to those in green ones, as was previously observed in *Armeria maritima* subsp. *halleri* and *Echium vulgare* (Baker 1981; Dahmani-Muller *et al.* 2000; Szarek-Łukaszewska *et al.* 2004). However, no clear correlation between Zn absorption and soil pH values was observed, as was also the case with Cr. Values of the bioconcentration factor were highest in the sample with the lowest content of available Cr. Antagonism between the BCF of Cr and Cr concentration in the soil was also found in analysis of agricultural soil in China (Huang *et al.* 2007). Additionally, our results confirm the previously established standpoint that the solubility and mobility of chromium are mainly determined by soil pH (Chang *et al.* 2014).

For some elements, like Ni and Mn, although their soil concentrations were in the range of values obtained for ultramafic localities (Reeves *et al.* 2009; Bani *et al.* 2010; Tumi *et al.* 2012; Tomović *et al.* 2018), their amounts in plant tissues were significantly lower, with BCF and AF values close to zero. Most likely, this is a result of interaction with other elements. While absorption of Ni can be reduced by Zn (Deng *et al.* 2014), Fe-Mn antagonism is widely known in soils with significant amounts of available Mn and a slightly acidic or acidic reaction (Kabata-Pendias 2011). However, this can also be due to avoidance or prevention of uptake of the metals in question into plant tissues, considering that they can be toxic if accumulated in higher excess (Liu *et al.* 2010).

CONCLUSION

The trace element profile of *P. maculatum*, with some variations, fits in the range of values obtained for several other species from ultramafic substrates of the Balkan Peninsula. The results of determining BCF, AF and TF values indicate strong uptake and translocation of Zn, Cr and (to some extent) Fe, whereas for certain other elements (like Mn, Ni and especially Pb) there is a strong tendency of avoidance, mostly due to interaction of elements, colloidal binding or pH values inadequate for uptake. Bearing in mind the fact that the values of Zn and Cr accumulation are below hyperaccumulation levels, and considering the non-uptake of Mn and Ni, we are able to conclude that *P. maculatum* is not suitable for phytoextraction and cannot be recommended for this process.

Acknowledgements – The Ministry of Education, Science and Technological Development of the Republic of Serbia supported this research through Grant 173030 for the project "Plant biodiversity of Serbia and the Balkans – assessment, sustainable use and protection".

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Botanica SERBICA

REZIME

Tolerancija na teške metale kod *Pontechium maculatum* (Boraginaceae) sa nekoliko ultramafitskih lokaliteta u Srbiji

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Pontechium maculatum, fakultativna metalofita, sakupljena je sa 4 ultramafitska lokaliteta u Srbiji i analizirana u smislu akumulacije mikro- i makroelemenata. Cilj rada je bio utvrđivanje profila teških metala i razlika u njihovom usvajanju i translokaciji kod populacija koje rastu u uslovima stresa izazvanog ultramafitskom podlogom. Prikazane su koncentracije makro- i mikroelemenata (Ca, Mg, Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co, Cd) u uzorcima zemljišta i u biljnim tkivima (Fe, Mn, Ni, Pb, Cr, Zn, Cu, Co, Cd). Rezultati analiza pokazuju da *P. maculatum* efikasno usvaja Zn i Cr, dok se nivoi većine drugih elemenata nalaze u opsegu vrednosti nekoliko drugih vrsta sa ultramafita Balkanskog poluostrva.

Ključne reči: Boraginaceae, *Echium russicum*, teški metali, Balkansko poluostrvo