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Nickel accumulation by *Alyssum murale* in serpentine sites of Prrenjas and Rajce, Albania

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Abstract

In Albania, ultramafic outcrops cover about 10% of the surface and the Mg-rich arable have been estimated to cover about 14,889 ha out of about 700,000 ha of total agricultural land available in the country. The purpose of this paper was to understand the potential of Ni hyperaccumulation of Alyssum murale Waldst and Kit. (syn. Odontarrhena chalcidica) populations from Prrenjas and Rajce region in close relation with the characteristics of their soil environments.

This study focused on the variation in Ni concentration across the different phenological stages, populations and organs of A. murale, in order to evaluate when Ni concentration is at a maximum. At both sites (Prrenjas and Rajce) we collected 25 individuals of A. murale in different phenological stage. Soil samples were also taken as composite samples collected randomly at different precise locations of plant sampling for each site. We determined the nickel concentration in entire plants and in separate organs of the plant for each population at different time of collection. We also determined the available fraction of

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the nickel in the corresponding soil. Nickel concentration was found to be maximal at end of the flowering time and beginning of fruiting stage for all populations, also it was higher in soil where the available nickel was higher. This study shows that A. murale from Prrenjas and Rajce displayed the best Ni-efficiency for use in phytomining and suggested that, the end of the flowering time and beginning of fruiting time is the best time for harvesting.

Key words: *Alyssum murale*, *Odontarrhena chalcidica,* hyperaccumulator, flowering stage, serpentine, plants organs.

1. INTRODUCTION

The definition of hyperaccumulation was provided by Reeves (1992) for Ni: "a hyperaccumulator of Ni is a plant in which a Ni concentration of at least 1000 mg/kg has been recorded in the dry matter of any above-ground tissue in at least one specimen growing in its natural habitat." In fact the most hyperaccumulators are endemic to Ni-rich serpentine soils. Serpentine soils share a number of chemical particularities including a low calcium-to-magnesium ratio with Ca at significantly lower concentrations than in other soils in surrounding areas. They contain elevated levels of heavy metals, such as Fe, Mn, Ni, Cr and Co, which could induce toxicity for most of the plants. Serpentine soils are often deficient in essential plant nutrients such as N, K and P (Brooks 1987, Proctor and Woodell 1975). Nickel hyperaccumulators comprise the great majority of the current reports of hyperaccumulation, and metal hyperaccumulators have recently gained considerable interest because of their potential use in phytoremediation and pytomining (Li et al., 2003; Chaney et al., 2005; Bani et al 2007, 2015 ab, Kidd et al 2018a).

The Balkan has the highest diversity in Ni hyperaccumulator plants in Europe in which *A. murale* is one of the most studied species for phytomining (e.g. Nkrumah et al. 2016 Bani et al 20015a,b, 2018a).

On serpentine soils, in particular in southeast of Albania, there can frequently exist a nearly monospecific community of a Ni hyperaccumulator, e.g. Alyssum spp. Prrenjas and Rajce are the most representative serpentine sites in southeast of Albania where the serpentine soil cover 10-28% of the territory and soil are suitable for profitable phytomining (Bani et al 2015b, Lekaj et al 2017). In those sites natural populations of *Alyssom murale* grew naturally. *Alyssum murale* (*Odontarrhena* genus) is a Ni-hyperaccumulating flowering plant from the family Brassicaceae that occurs widely in the eastern Mediterranean region. The taxonomy of the Albanian hyperaccumulating species of the *Odontarrhena* genus was revised and it revealed the presence of the tetraploid *O. chalcidica* (Janka) Španiel, Al-Shehbaz, D.A.German & Marhold (Cecchi et al. 2018). *Alyssum murale* (syn. *Odontarrhena chalcidica*) is a perennial species which can be found on ultramafic Vertisols and Cambisols of Albania and is a spontaneous weed in other crops. 10 years of field study allowed optimization of the growth of efficient populations of *A.murale* for use in phytomining (Bani et al. 2007, 2009, 2010, 2015a, and 2018a).

The main goal of this study was to determine Ni concentration across the different phenological stages (from leaf development (main shoot) to development of fruit, populations and organs of *Alyssum murale* in close relation with the characteristics of their soil environments.

MATERIAL AND METHODS

Sampling Sites

Sampling was conducted on serpentine site of Prrenjas (Beginning of Domosdova field; 41°04'N, 20°33` E) on ultramafic outcrops whose characteristics (topography and climate) have been described previously (Bani *et al*. 2007, 2009, Xhaferri et al 2018). Plots were located at an altitude of 450 m in a continental internal climatic zone with Mediterranean influences and with annual rainfall averages of ~700 mm, and a mean annual temperature of 10.6 ° C. Spontaneous native ultramafic vegetation at the sites was abundant and, in particular, many individuals of *A. murale* were widespread on the fields.

Also samples were collected on ultramafic outcrops of Rajce, in Fushe Rrajce (41°05'N, 20°34`E) that have been described previously (Bani *et al*. 2007, 2009, Xhaferri et al 2018). Sampling sites were located at an altitude of 560-600 m with annual rainfall averages of 1300 mm and a mean January temperature 3.6 °C, mean July temperature -21.5 °C.

Soil and plant analyses

Six composite soil samples were analyzed; three composite soil samples per each site at depth 0-20 cm were collected. The samples were collected nearby *A. murale* plants. Nickel availability in the samples collected in the surface horizon of the serpentine soils of Prrenjas and Rajce was determined using a diethylene triamine pentaacetic acid (DTPA)–triethanolamine (TEA) extractant (0.005M DTPA with 0.01M CaCl2 and 0.1M TEA) at pH 7.3. One gram of soil was mixed with 10mL DTPA–TEA solution, the mixture was shaken for 2 h, and then the suspension was centrifuged at 5000*g* for 20 min, and filtered through a 0.2-mm pore-size cellulose nitrate filter (SARTORIUS, Goettingen, Germany; Lindsay and Norvell

1978; Echevarria *et al.* 1998, 2006; Massoura *et al.* 2004, Bani et al 2015b). All extractions were performed in Triplicate.

Concentration of Ni and others elements in soil extracts was measured by ICP-AES (Laboratoire Sols et Environnement, Université de Lorraine-INRA, Nancy, France).

Plant samples were washed with distillated water, dried and ground to a fine powder. Trace metal concentrations in shoots and leaves, roots, flowers and whole plants were analyzed by plasma emission (ICP) spectrometry (Laboratoire Sols et Environnement, Université de Lorraine-INRA, Nancy, France) after digestion of plant samples in microwaves. A 0.25 g DM plant aliquot was digested by adding 8 ml of 69% HNO_3 and 2 mL of H_2O_2. Solutions were filtered and adjusted to 25 mL with 0.1 M HNO_3. Soil samples were air dried, sieved to pass a 2 mm nylon mesh and ground.

RESULTS AND DISCUSSIONS

Heavy metals availability along the serpentine soils of Prrenjas and Rajce, Albania

Since the total concentration of trace elements in soils does not show how strongly the element is bound to the surface of the soil constituents (Manceau et al., 1996) we used DTPA-extractable metals as the indicator of the relative variability of Ni availability of the soils in 2 typical serpentine sites in South-east of Albania (Table 1).

The mean value of DTPA-extracted Ni in soils of Prrenjas was 18.49 mg kg^{-1} while it was 56.4 mg kg^{-1} in serpentine soils of Rajce. In those sites the available nickel was in accordance with other previous studied in Domosdova field in Prrenjas (Bani et al 2009, 2015b). The pH values of both sites were slightly alkaline (7.04 in the Rajce soils and 7.46 in Prrenjas soils. Ni chemical availability in Rajce soils was higher than in Prrenjas soils. In this case, the direct effect of the higher elevation and rainfall intensity affecting Ni availability.

The concentration of DTPA-extracted Mg was high in both serpentine sites varied from 1066.6 mg kg^{-1} (soils of Rajce) to 1261.8 mg kg^{-1}(soil of Prrenjas). Our studies showed us that the soils of Rajce are poorer in macro and micronutrients than soil of Prrenjas (Xhaferri et al 2018). The differences in availability of elements in studies soils and its subsequent transfer to plants depend mainly on its mineralogical origin and therefore on the local pedogenetic properties (Kabata-Pendias, 1993, Massoura et al. 2006) which result from climatic conditions and weathering history of the soil as well as on soil characteristics (Baker and Walker, 1989).

Table 1. Soil elements availability assessed by DTPA-TEA extraction in serpentine soils from Prrenjas and Rajce (ND= not detected).

Sampling Sites	Cu	Fe	Mg	Mn	Ni	Co	Cr	Pb	Zn	K	P
Prrenjas	mg kg^{-1}										
Site 1	1.27	22.62	1251.2	2.79	17.43	0.13	0.12	1.38	0.41	ND	0.36
Site 2	1.2	24.46	1274.2	2.96	18.82	0.14	0.12	1.97	0.54	ND	0.7
Site 3	1.22	24.1	1260.2	3.07	19.22	0.11	0.12	1.47	0.55	ND	0.69
Mean	1.23	23.72	1261.8	2.94	18.49	0.13	0.12	1.61	0.5	-	0.58
SD	0.036	0.97	11.59	0.141	0.93	0.02	0	0.32	0.07	-	0.19
Rrajce											
Site 1	0.696	16.372	1068.6	6.028	56.74	0.21	0.06	0.09	0.446	ND	ND
Site 2	0.738	16.674	1087.4	6.538	57.54	0.19	0.06	0.1	0.396	ND	ND
Site 3	0.712	16.104	1044	6.094	55.02	0.18	0.06	0.1	0.372	ND	ND
Mean	0.71	16.3	1066.6	6.22	56.4	0.19	0.06	0.1	0.405	-	-
SD	0.02	0.28	21.7	0.27	1.28	0.02	0	0.01	0.03	-	-

Uptake of elements by Alyssum murale plants

Analysis of metals performed on roots, leaves, stems and entire plant, showed different plant responses to the presence of available elements in soils collected at the sites of Prrenjas and Rajce (Table 2, 3). The highest Ni values in plants were recorded for *A. murale* which was harvested in 12.6.2016, but Ni concentration in this species was highly dependent on the site of collection. The highest Ni concentration in entire plant of *A. murale* was observed in populations of Rajce (11964 mg kg^{-1}), while in *A. murale* from Prrenjas, harvested in the same date, it was lower (9401 mg kg^{-1}). The Ni concentration in leaves (17523 mg kg^{-1}) and flower (13795 mg kg^{-1}) of *A. murale* form Rajce was higher than leaves (15855 mg kg^{-1}) and flower (12302 mg kg^{-1}) of *A. murale* from Prrenjas and it was higher for plant harvested in 12.6.2016. The higher Ni concentration in plant from Rajce can be explain with the fact that in serpentine soils of Rajce the available nickel was higher than in serpentine soil of Prrenjas. Although the nickel available on the soil of Rajce and Prrenjas was lower than that of the Pojske (Bani et al 2014), the concentration of the Nickel in entire *A. murale* plants, and in separate organs of the plant is comparable to that of the *A. murale* populations from Pojske.

Calcium concentrations in entire plants, leaves and flower for both sites were very high despite the low Ca levels in the soil of Rajce and Prrenjas (Xhaferri et al 2018). We found high Ca values for *A. murale* Prrenjas collected in 12.6.2016 (20218 mg kg^{-1}) and *A. murale Rajce* (19997). The Ca concentration in leaves and flower was higher in the early phenological stage and lower in the roots. This observation has been reiterated by others (Lombini et al. 1998; Shallari et al. 1998, Bani et al 2009, 2010, 2015a). The mean Mg concentration for the *Alyssum murale* was in the range 0.3-0.5%. It was higher in plants from Prrenjas where the soil available Mg was higher. The mean Fe concentrations were higher in roots than in whole plants and other organs. The mean P and K concentrations for the *Alyssum murale* in Rajce and Prrenjas sites was in the range from 1678 to 1961 mg kg^{-1} (P) and from11716 to13357 mg kg^{-1} (K) respectively.

Table 2. Total concentrations of Ca, Mg, Fe, P, K, (mg kg^{-1}), in *Alyssum murale* plants collected in serpentine sites of Prrenjas and Rajce (the value in table is a mean of 3 replications).

		Ca		Mg		Fe		P		K	
	mg kg^{-1}										
Sampling dates	plant/organs	Prrenjas	Rajce	Prrenjas	Rajce	Prrenjas	Rajce	Prrenjas	Rajce	Prrenjas	Rajce
15.04.2016	Roots	2922	3510	2885	2675	6026	6502	2184	2343	9664	12560
	Leaves	34551	35971	6389	5700	1262	2600	3670	2540	11578	7363
	Flowers	A	A	A	A	A	A				
	Stems	7716	7365	2576	2500	913	1130	1817	2900	14081	4572
	Entire Plant	24240	21599	4563	4069	1873	2099	2706	2490	13039	7363
22.05.2016	Roots	3556	6362	2746	2839	1130	1430	1991	2450	9292	11900
	Leaves	48466	27702	3937	3767	326	762	1392	1177	11240	6824
	Flowers	26568	19415	6357	3367	331	313	2418	2341	12020	8990
	Stems	7007	9452	1563	1384	686	245	857	755	11495	19050
	Entire Plant	30240	11680	4323	2610	615	462	2570	1719	12200	10890
12.6.2016	Roots	3280	7234	4098	2234	1700	1070	2120	2263	10705	9184
	Leaves	26920	26799	4517	2900	700	239	1877	1676	13769	14330
	Flowers	21568	18963	7357	3240	290	302	2467	2143	14560	15340
	Stems	6024	3227	2558	2444	714	777	2960	2832	10119	10560
	Entire Plant	20218	19997	5038	3386	901	878	1961	1678	13357	11716

Table 3. Total concentrations of heavy metals (mg kg^{-1}) in *Alyssum murale* plants collected in serpentine sites of Prrenjas and Rajce (the value in table is a mean of 3 replications).

		Ni		Mn		Co		Cr		Zn	
	mg kg^{-1}										
Sampling dates	Plant/organs	Prrenjas	Rajce	Prrenjas	Rajce	Prrenjas	Rajce	Prrenjas	Rajce	Prrenjas	Rajce
15.04.2016	Roots	5839	3980	84	67	2.1	2	1.6	1.8	81	25
	Leaves	73301	6316	65	103	21	23	13	17	94	149
	Flowers	A	A	A	A	A	A	A	A	A	A
	Stems	4977	3427	24	32	0.5	4	0.9	8	45	26
	Entire Plant	6107	5418	82	43	23	13	15	15	63	121
22.05.2016	Roots	1905	3713	30	51	4.2	10	6.4	5.5	71	93
	Leaves	12302	13523	72	45	15.5	25	2.3	4	78	120
	Flowers	12945	11279	127	31	18	20	2	1.4	105	78
	Stems	4977	5922	23	13	4.6	4.9	4.9	4.3	63	103
	Entire Plant	6357	7730	57	24.9	11	20	4.2	3.2	42	51
12.6.2016	Roots	2258	4366	33	24	4.3	2.3	4.4	1.2	52	66
	Leaves	15855	17523	67	24	14	4.4	8.3	1.7	69	76
	Flowers	12302	13795	36	29	14	15	9.6	8.9	110	98
	Stems	4668	5463	23	22	3	1.9	2.9	4.6	41	93
	Entire Plant	9401	11964	45	24	13	9.2	8.8	7.4	82	89

Manganese concentrations in the plants ranged from 82 to 45 mg kg^{-1} in entire plant of *A. murale* Prrenjas, collected on 15.04.2016 and 12.6.2016 respectively and from 43 to 24 mg kg^{-1} in entire species of *A. murale* Rajce collected on 15.04.2016 and 12.6.2016 respectively. Concentrations of Cr in all the investigated plant's organs were very low for both sites. Cobalt concentrations rarely reach 10 mg kg^{-1} in plants on normal soils, and even on serpentine this level is not often exceeded. In this study, the concentration of Co in both sites in entire plants varied from < 9 up to 23 mg kg^{-1}. The Zn concentration in entire plants varied from 82 mg kg^{-1} in Prrenjas to 89 mg kg^{-1} in Rajce during flowering stage and beginning of fruiting stage being in accordance with previous study and being lower than *A. murale* in Pojske (Bani et al 2009, 2015a)

CONCLUSIONS

Ni availability was higher in serpentine soils of Rajce while the available Mg was higher in serpentine soil of Prrenjas. This study showed us the relationships of available elements in soil and their uptake by *Alyssum murale*. The highest level of Ni in entire plant of *A. murale* was found in the populations of Rajce for both harvesting times; 22/05 and 12/06 while the concentration of Mg was higher in *A. murale* that belongs to populations of Prrenjas. For both populations the tendency of Ni accumulation goes in the order: leaves > flower >entire plant. If we compare the data of the three different collection times, we can say that the level of Ni in *A. murale* plant and in its organs for both sites is higher on 12/06, which seems to be the best time for harvesting. Our study shows that at the initial stage of flowering there was a lower concentration of Ni than in the later flowering stage and beginning of fruiting stage. *A. murale* from Prrenjas and Rajce strongly accumulates Ni despite being in the presence of limited available pools (DTPA-extractable Ni) in soil and displayed the best Ni-efficiency for use in phytomining.

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