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Which populations of *Alyssum murale* from southeastern Albania are more efficient in biomass production?

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Abstract:

Our previous study for the Ni hyperaccumulator A. murale helped us in the development of a better growing system for phytoextraction and the opportunity for the phytomining of nickel in Albanian ultramafic soils. In order to find the best populations in nickel phytoextraction and the best harvesting time it is important to

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investigate the characteristics of different populations of A. murale in southeastern Albania. As biomass of hyperaccumulator A. murale was the main contributor in Ni phytoextraction yield this study focuses on the variation of dimensions of plants and plants organs during flowering stages of A.murale, in order to evaluate when biomass production is at a maximum. In the three sites in southeastern Albania: Rajce, Pojske, Prrenjas we took samples at 3 plots for each site. At each site of A. murale we collected 25 specimen. Soil samples were also taken as composite samples collected randomly at different locations of plant sampling (in a 0.5m radius around the plants) for each site. We determined, the size of the plants at different time, then leaves, fruits and flowers for each population. Biomass production was found to be maximal at end of the flowering stage and at the beginning of fruiting stage for all populations, while maximal phytoextraction capacity was found in Pojska populations.

This study show variation in Ni concentration across populations in each of the plant's organs and concluded that the end of the flowering stage and beginning of fruiting stage is the best time for harvesting.

Key words: *Alyssum murale*, biomass production, flowering stage, plant size variation.

INTRODUCTION

Ultramafic (i.e. serpentine) substrates cover quite large areas in the Balkans, more than in other parts of Europe (Brooks, 1987; Tatić and Veljović, 1992). Those soils known to have abnormally high concentrations of potentially phytotoxic trace elements, including nickel

(Ni), cobalt (Co) and chromium (Cr), low calcium/magnesium (Ca/Mg) ratio and general nutrient deficiencies (Brooks 1987; Harrison and Rajakaruna 2011). In Albania where they are about 11% of the territory of the country which could justify the development of phytomining activities as an alternative to local agriculture on such

unproductive land Bani et al., 2007; 2015a). In this frame those soil are of great importance in the study of plant penology, ecology, evolution, metal accumulation used in phytoremediation, and phytomining practice (van der Ent et al. 2015). Nickel agromining operations use cultivated hyperaccumulator plants ('metal crops') on Ni rich (ultramafic) soils, followed by harvesting and incineration of the biomass, to produce a high-grade 'bio-ore' from which Ni metal or pure Ni salts are recovered (Nkrumah et al. 2016). Such technology could be part of a modern agricultural activity in Albania, where the most efficient Ni-accumulating wild species, *Alyssum murale* Waldst. & Kit, can be cropped on the large suitable ultramafic surfaces (Bani et al. 2013, 2015ab, 2018). This species occurs widely in Albania as a native weed in agricultural lands on ultramafic Vertisols and Cambisols and it is considered suitable for phytoextraction of heavy metals (Bani et al. 2015a, b), for agromining (Nkrumah et al. 2016), and for the restoration of metal-enriched sites degraded by mining activities (O'Dell and Claassen 2011).

The aim of this study was to investigate natural serpentine populations of *A. murale* in southeastern Albania: to compare the chemical compositions of the soil and to determine differences of growth characteristics between populations of southeastern Albania during the flowering period, and the effect of fertilizer in biomass production and consequently in phytoextraction yield.

MATERIAL AND METHODS

Sampling Sites

Pojska (Pogradec district) is in the east of Albania, on the western shore of lake Ohrid (40°59'55.28"N, 20°38'0.92"E) at an elevation of 700 m above sea level. It is a serpentine site with high contents of Fe, Ni, and Co silicates. Up to 2% Ni has been

recorded in the iron-type laterites of the Librazhd-Pogradec region (Anonymous 1992). In this area, there are also Cr mines. The soils at the collection site were a Magnesic Hypereutric Cambisol and a Cambic Hypermagnesic Hypereutric Vertisol located close to the shores of Lake Ohrid at the piedmont of ultramafic hills (Serpentinized Harzburgite) (Bani et al 2009)

The Prrenjas region is at an elevation of 450 m. The parent material is rich in Fe, Ni, Cr, and Co (Anonymous, 1992). Sampling was conducted on serpentine rock of Prrenjas (Leptic Hypermagnesic Cambisol; 41°04'13"N, 20°33'53"E) and also on the Domosdova field, which contains a soil developed from a colluvium of ultramafic and magnesite origin (Cambic Hypermagnesic Hypereutric Vertisol; 41°04'08"N, 20°33'11"E) (Bani et al 2009, 2015b).

In the serpentine sites of southeastern Albania, the climate data are respectively: for Prrenjas—mean January temperature -5.6 °C, mean July temperature -22.5 °C, and total annual rainfall - 744 mm; for Pojska—mean January temperature - 4.7 °C, mean July temperature - 22.3 °C, and total annual rainfall - 707 mm.

The third location was serpentine site of Rajce, and the sampling site was Fushe Rrajce (41°05 40'N, 20°34 32"E). This sampling site is at elevation 560m. For Rajce mean January temperature is 3.6 °C, mean July temperature -21.5 °C, and total annual rainfall 1300 mm.

Measurements

The investigation of morphological differences between *A. murale* populations was performed between 25 individuals per each site using 8 morphological characteristics. For each individual we measure the length and width of entire plant, basal leaves, flowers and fruits, It was performed twice during the flowering stage and once at beginning of fruiting stage. All measurements were done on dried plants.

Designing pot experiment for Ni phytoextraction by different populations of A. murale

Effect of fertilization on dry mass and nickel phytoextraction on this experiment was also tested for nine months in a well-ventilated unheated greenhouse pot experiment (with soil from Pojske, Prrenjas, Rajse) that was taken simultaneously in which *A murale* seedlings were treated with this regime of fertilization; N260:P105:K260 or 0.43 kg plant^{-1} pig manure. For fertilization treatment, four replicates of 5-kg pots were made. 3 plants were sown per pot. Pots were daily watered to 100% of the water holding capacity. After 6 months, one plant per pot was harvested and the growth parameters and nickel phytoextraction yield were measured (Bani et al. 2018 in press).

Soil and plant analyses

Nine 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. Plant samples were washed with distillated water, dried and ground to a fine powder. The total concentration of heavy metals was determined by plasma emission (ICP) spectrometry 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. The trace metals, Mg and Ca concentrations in the soil after acid-digestion were also measured by ICP-AES (in Laboratoire Sols et Environnement, Université de Lorraine-INRA, Nancy, France).

Data analyses

T-tests were used to test for significant differences in growth characteristics of *A. murale*- across years and date of collections. After that an analysis of variance (ANOVA–single

factor) was applied to determine whether growth characteristics of g varied between sites of collection.. ANOVA was applied for each characteristics measured in different sites for each date of collection. Post hoc analysis (Multiple Comparisons with Fisher's LSD) was performed to see if there were significant differences for each parameter between sites

RESULTS AND DISCUSSIONS

Elemental Concentrations in serpentine sites of Pojske, Prrenjas and Rrajce

The soils samples collected in three studied soils were characterized by high total concentrations of metals (such as Ni, Cr, Co) that are typical of ultramafic environments (Table 1, 1/1). The total Ni concentrations ranges from 2838 mg kg^{-1} (Pojske) to 3498 and 3765 mg kg^{-1} respectively in Prrenjas and Rajce in the rhizospheric soil of *A. murale* plants. Prrenjas and Pojska soils had high iron (Fe) concentrations (above 8%) as expected for soils of ultramafic origin (Bani et al. Prrenjas and Pojske soil are infertile with regards to total potassium (K) and phosphorus (P). The levels of those elements are lower in soils of Rajce, even in other ultramafic sites of Albania (Bani et al. 2014, 2018). The total Ca concentrations were low and also typical for an ultramafic soil, ranging from 0.25% to 0.65%, while total Mg concentrations were 3.7–7% in Pojske and Prrenjas and lower in Rajce (0.84%). The zinc (Zn) concentrations fell within the ranges for normal soils lying in the range from 84 to 116 mg kg^{-1} (Kabata-Pendias and Pendias 1984). The concentrations of Cu, S, Na and Pb were low. Manganese concentration ranges from1517 to 2248 mg kg-1 and was typical for Albanian serpentine soils (Bani et al.2009, 2014, Echevarria 2018).

Table 1.Total major elements in serpentine sites of Pojske, Prrenjas and Rrajce.

	Ca	Fe	K	Mg	Mn	Na	P	S	Ca/Mg
Sites	mg kg^{-1}								
Pojske	6500±436	88200±2540	1721±115	37973±1161	2248±1153	73±2.2	194±6.7	157±6.2	0.17
Prrenjas	3547±186	96933±887	1972±31	70520±1087	1517±25	87±12	181±4.5	143±6.4	0.05
Rajce	259±32	28300±4014	303±12	844±17	1680±12	34±0.7	53±1.8	667±12	0.3

Table 1/1Total trace elements in serpentine sites of Pojske, Prrenjas and Rrajce.

	Co	Cr	Cu	Ni	Pb	Zn
Sites	mg kg^{-1}					
Pojske	183±81	507±56	17±1.5	2838±231	10±4	84±9.6
Prrenjas	147±18	387±29	32±28	3498±28	5.2±0.3	107±60
Rajce	156±21	555±38	34±27	3765±14	10±4.9	116±52

Table.2 The size of the whole plants and organs of *A. murale* collected during flowering stage and beginning of fruiting stage in fields of Pojske, Prrenjas and Rajce. Abbreviations A=Absent. Values with different letters indicate significant difference between growth parameters of Alyssum murale and inverse for values with te same letter (ANOVA one way, Multiple Comparisons with Fisher LSD at *P*<0.05).

	Plants		Leaves/base		Flowers		Fruits	
Collection site and date	Height	Width	Length	Width	Length	Width	Length	Width
22.05.2016 (flowering stage)	Cm							
Pojske	38±8a	21±3.8a	1.6±0.2a	0.6± 0.2a	0.29±048a	0.2±0.08a	A	A
Prrenjas	41.9±5.8b	18.6±3.3b	1.5±0.44a	0.5±0.15a	0.3±0.05b	0.2±0.03a	A	A
Rajce	32.4± 5.6c	14.6±2.4c	0.8±0.2c	0.4±9.2b	0.22±0.03a	0.30±0.05a	A	A
22.05.2017 (flowering stage)	Cm							
Pojske	35±3.3a	34±4.5a	2.2±0.3a	0.3±0.07a	0.29±0.2a	0.2±0.08a	A	A
Prrenjas	40±2.8b	30±5.2b	1.3±01b	0.3±0.04a	0.5±0.04b	0.6±0.04b	A	A
Rajce	30±5.4c	16±4.4c	0.8±0.2c	0.15±0.1b	0.42±0.03c	0.44±0.1c	A	A
12.06.2016 (beginning of fruiting stage)	Cm							
Pojske	41±5.4a	28.6±3a	1.8±0.3a	1.2±0.2a	0.4±0.15a	0.28±0.06a	0.41±0.07a	0.4±0.07a
Prrenjas	52.6±7b	24.85±3b	1.6±0.3b	0.7±0.18b	0. 57±0.1b	0.6±0. 17b	0.4±0.12a	0.34±00.8b
Rajce	38.2±4.6c	17±3.5c	1.3±0.2c	1±0.20c	0. 47±0.01a	0.37±0.07c	0.26±0.08b	0.19±0.07c
12.06.2017 (beginning of fruiting stage)	Cm							
Pojske	45±5.5a	46.±9a	2.8±0.4a	0.46±0.5a	0.37±0.24a	0.27±0.17a	0.49±0.5a	0.33±0.07a
Prrenjas	51.2±8b	37±10b	2.5±0.7a	0.4±0.11a	0.73±0.16b	0.68±0.11b	0.3±0.04b	0.24±0.06b
Rajce	39.1±9c	21±8.8c	1.3±0.3b	0.3±0.08b	0.62±0.1c	0.62±0.07b	0.3±0.02b	0.23±0.05b

Table 3. Significant differences for the growth parameters of constituent organs of *Alyssum murale*- between years for each date of collection in each site according to t-tests for equality of means

Growth parameters	Pojske						Prrenjas						Rajce					
	data 22.05			data 12.06			data 22.05			data 12.06			data 22.05			data 12.06		
	T	df	P value	T	Df	P value	t	df	P value	t	Df	P value	T	df	P value	t	df	P value
Plants_height	-1.72	31	0.09	2.47	48	0.017	6.05	34	< 0.001	-.52	38	0.606	-7.3	48	< 0.001	.477	33	0.637
Plants_width	12.4	48	< 0.001	8.51	28	< 0.001	14.6	35	< 0.001	4.2	25	< 0.001	-1.8	48	0.078	1.73	31	0.092
Leaves _length	8.04	48	< 0.001	1.49	28	0.146	9.72	48	< 0.001	5.5	33	< 0.001	-5.3	36	< 0.001	-.566	41	0.574
Leaves_ width	-9.23	31	< 0.001	-6.62	48	< 0.001	-3.14	29	0.004	-6.8	48	< 0.001	-2.3	48	0.023	-19.0	38	< 0.001
Flowers_length	-3.98	48	< 0.001	-1.70	38	0.096	26.2	43	< 0.001	3.4	48	0.001	8.1	48	< 0.001	4.92	48	< 0.001
Flowers_width	1.20	42	0.23	-.625	30	0.536	21.3	48	< 0.001	1.1	41	0.295	22.1	48	< 0.001	12.4	48	< 0.001
Fruits_length	-	-	-	.146	26	0.885	-	-	-	-5.6	31	< 0.001	-	-	-	2.15	26	< 0.001
Fruits_width	-	-	-	-6.61	48	< 0.001	-	-	-	-5.5	44	< 0.001	-	-	-	5.38	48	< 0.001

Table 4. Significant differences for the growth parameters of constituent organs of *Alyssum murale*- between 2 different dates of collection in each site (2017) according to t-tests for equality of means

Growth parameters	Projske			Prrenjas			Rajce		
	t	Df	P-value	t	Df	P-value	T	df	P-value
Plants_height	-7.72	39	< 0.001	-5.13	27	0.001	-3.9	36	0.001
Plants_width	-6.05	29	< 0.001	-2.20	29	0.035	-2.1	36	0.045
Leaves_base_length	.590	27	0.56	-7.64	30	0.001	-2.4	41	0.020
Leaves_base_width	-1.51	48	0.13	-5.65	33.	0.001	-2.4	48	0.020
Flowers_length	-2.63	24	0.01	-6.21	28.	0.001	-8.0	48	< 0.001
Flowers_width	-1.31	29	0.20	-3.60	31	0.001	-10	37	< 0.001

Characterization of *A. murale* growth parameters and biomass production

The ranges for growth parameters values across the 2 different harvesting times of *A. murale* are presented in Table 2. The results show effects of the phenological status on populations of *A. murale* and their growth parameters, consequently this can also affect the biomass production. The mean height (Table 2) of *A. murale* collected in field ranged from 32.6 to 41.9 and from 30 to 40 cm respectively in May 2016 and 2017 (beginning flowering stage) while they ranged from 38.2 to 52.6 and from39 to 51.2 cm in June 2017 (beginning of fruiting stage).The height of plant was higher in plant collected in Prrenjas and Pojske. The width of plants was higher in *A murale* plants that belong populations of Pojske (21-28. 6cm for 2016 and 34-46 cm in 2017) while that in populations of Rajce it was lower for at both collected times; respectively 14.6, 17 cm for 2016 and 16, 21cm for 2017. Also the dimenssions of leaves and fruits are higher in Pojska site and lower in Prrenjas. The plants that belong to Prrenjas and Rajce had the higher length and width of flower than those from Pojke.

ANOVA showed significant differences of growth parameters of *A. murale* between different sites. Multiple Comparisons with Fisher`s LSD demonstrated that there were significant differences of height, and width for the plant between sites ($P < 0.05$) (Table 3). Also ANOVA showed significant differences of other growth parameters between

different sites of collection for each harvesting time except for; leaves parameters in Pojske and Prrenjas (dt 22.05), flowers width in three sites (dt 22.05), height of leaf in Prrenjas and Pojske, width of flower and fruit dimensions in Prrenjas and Rajce (dt 12.06 2017).

The T-test for Equality of Means used to assess the growth parameters of A. murale between years for 2 harvesting times in three sites of collection showed significant variations for most of the growth parameters except for width of leaves, flowers and fruits in Pojske (dt12.06), width of flowers in Pojske (dt22.05). height of plants and flowers in Prrenjas (dt12.06) and height of plants and flowers in Rajce (12.06), (Table 3).

The T-test for Equality of Means showed significant variations for growth parameters between 2 harvesting times for each site of study in 2017 expect leaves parameters and flower's width in Pojske (Table 4).

In *A murale* plants under fertilization treatments we noticed an increase in the number of stems where the highest were in Pojske and Rajce with pig manure fertilization reaching 40 stems per plants (Table 5). The number of branches in stems in the case of organic fertilizer treatment was about 2-4 times higher than in unfertilized pots; consequently the average fresh weight and dry weight of plant was higher. We also noted differences in length of the plants ranged from 29, 35 and 28 cm in unfertilized pots to than 44, 50 and 35 cm in fertilized plots of *A. murale* respectively in Pojske, Prrenjas and Rajce. The organic fertilizers increased the average fresh weight of *A. murale* in the order 194 (Rajce) < 153 (Pojske) < 90 (Prrenjas) and consequently the dry weight (Table 5). The *A. murale* plant of Rajce populations where the soil was abundant on nutrients was better responded on organic fertilizer than *A. murale* of Pojske and Prrenjas. The fresh and dry weight, the number of shoots, the height of the plants, then the length of the branches and the weight of the root of *A. murale* plants that were under

mineral and organic fertilizer changed significantly compared to unfertilized plots. This study confirmed the previous findings that serpentine soil characterized by low nutrient availability, have a strong positive effect on the biomass production of Ni hyperaccumulating plant of *Alyssum murale* if fertilizer is utilized and consequently on Nickel phytoextraction yield (Chiarucci et al. 1995, Li et al., 2003, Bani et al., 2015a, 2018a; Kidd et al., 2015).

Table: 5. Growth parameters of *Alyssum murale* and phytoextraction yield as affected by fertilization in pot experiments with the surface soil from Rajce, Prrenjas and Pojskë. The values in the table are means of three repetitions. Abbreviations F =fertilized, NF= non fertilized

Origin of plants	Treatment	Steams	Branches	Height Plant	Leaf dimensions	Fresh mass	Dry Mass	Length of branch	Root weight	Nickel	Ni phytoextracted
		No	No	cm	cm	gr	Gr	Cm	gr	g kg^{-1}	mg/pot
Rajce	F	7	40	35	2.8X0.5	194	71	24	64	5.6	397
	NF	6	10	28	1.7X0.4	22	11	14	32	7.5	82.5
Pojske	F	5	40	44	2.4x0.6	153	62	29	31	6.42	399
	NF	5	20	29	1.8x0.3	24.2	9	24	25	9.4	84.6
Prrenjas	F	5	20	50	2x0.35	90	40	24	17.35	6.3	252
	NF	4	11	35	1.7x0.5	22	18	14	13	8.55	119.7

Ni concentration and phytoextraction yields

The ranges for all Ni concentration values across the different populations of *A.murale* in plot experiment are presented in Table 5. Nickel concentration in *A. murale* plants was in the following order Pojske Pots > Prrenjas Pöts> Rajce Pots for plants of both unfertilized and fertilized plots, but it was higher in first ones. We can explain that with dilution effect due to the higher biomass production in fertilized plots (case of close plot experiment with limited soil content) (Bani et al. 2007, Bani et al 2009b, Bani et al 2018 in press). Since the harvesting occurred in the same day we noticed the differences in Nickel concentration and biomass production of different populations. The ranges for phytoextracted Ni values across the three populations of *A. murale* are also presented in Table 3. The pytoextraction yield was higher in fertilized plots of *A murale* in order Pojke Pots >Rajce Pots> Prrenjas Pots, while in

unfertilized plots it was in the order: Prrenjas Pots > Pojske Pots> Rajce Pots. The population of Rajce with lower nutrient level was responding better the fertilization. As was showed in previous study the use of organic fertilizers improving soil quality consequently increases the biomass and Ni phytoextracted yield (Bani et al. 2018 in press).

Conclusions

From the data presented in previous section it is evident that there were differences in soil characteristics and growth parameters of *A. murale* populations from three most remarkable serpentine sites in southeast of Albania. Soil of serpentine site of Rajce was abundant in nutrients (Ca P, K) while in soil of Prrenjas the ratio Ca/Mg was very low. This study told us differences in growth parameters values across the 2 different harvesting times between three populations. The measurement of plants collected in field and in plot's experiment told us that all growth parameters were higher in second week of June (12 June) and that can be the best time for harvesting of *A murale* in southeastern Albania. The different organs of *A. murale* populations showed variation in their sizes, mostly in height and width of plant, number of branches that are the main contributors in fresh and dry biomass production. This study told us that *A. murale* from Pojska had higher biomass production and nickel phytoextraction yield whereas *A. murale* from Rajce was responded better in organic fertilizer and was a good candidate for phytoextraction.

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