THE HYPERACCUMULATOR PLANT *ALYSSUM MURALE* AS A POTENTIAL AGENT FOR PHYTOMINING OF NICKEL IN AN ALBANIAN SITE

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The plant of *Alyssum murale* (Waldst. & Kit.)

ABSTRACT

Serpentine soils, developed upon ultramafic rock, contain relatively high concentrations of heavy metals and are the preferred substrate for several adapted plants, especially those that accumulate Ni in their above-ground tissues. A serpentinized peridotite outcrop located in Pojske hosts a population of the Ni-hyperaccumulator species *Alyssum murale*. Recent work has been carried out on the site of Pojske to optimize agronomic practices which may affect the efficiency of Ni phytoextraction by native stands of *A. murale* on 18-m^2 plots in natural conditions (three replicates for each treatment). In soil fertility management studies, we have used 120 kg ha^{-1} NPK. Only two different fertilization regimes (fertilized vs. non-fertilized treatments) were tested in 2005. In 2006, we studied the effect of targeted herbicide treatment to control the main competing weed (*Chrysopogon gryllus*). The two treatments were crossed (herbicide + fertilization). We found that NPK application significantly increased shoot biomass yield, without reducing shoot Ni concentration. In weed control practices, the use of anti-monocots herbicide (Focus™ ultra) allowed for the full development of *A. murale* stands. In this two-year experiment, biomass yields in fertilized and herbicide treated plots have progressively improved: 2.6-3.7t ha^{-1}. So have phytoextracted Ni quantities: 22.6-29.5 kg ha^{-1}. Such crop management practice studies have improved phytoextraction efficiency and extensive phytomining could be promising in the Albanian context by domesticating already installed natural populations.

Key-words: Ultramafic soil, Nickel hyperaccumulator plant, Serpentine flora, Bioavailability, Phytomining

Përmbledhje

Tokat serpentine të formuara nga alterimi i shkëmbinjve ultramafike, përmbajnë përqëndrime të larta të metaleve të rënda dhe janë zona të preferuara për disa bimë, veçanërisht për ato që akumulojnë nikel në indet e tyre. Zona e Pojskës (Pogradec) me toka me prejardhje nga peridotite të serpentinizuara është e populluar nga hiperakumulatorja e nikelit, *Alyssum murale*. Ky studim u krye në zonën e Pojskës me qëllim optimizimin e praktikave agronomike të cilat mund të ndikojnë efektivitetin e fitoekstraktimit të nikelit nga popullimet e *A. murale* në parcela me sipërfaqe 18 m^2 në kushte natyrore (3 përsëritje për secilin trajtim). Në kuadrin e menaxhimit të pjellorisë së tokës, ne kemi përdorur 120 kg ha^{-1} NPK (Azot-Fosfor-Potas). Në 2005 u testuan dy rregjime plehrimi (trajtimi i plehruar dhe ai i paplehruar). Në vitin 2006, u studiua efekti i trajtimit me herbicide për kontrollin e barërave të këqija më konkurente si *Chrysopogon gryllus*. U ndërthurën dy trajtime (herbicid + plehrim). Nga rezultatet gjetëm se aplikimi i NPK-së rrit prodhimin e biomasës së pjesës mbitokësore të bimës, pa reduktuar përqendrimin e nikelit (Ni) në të. Në praktikat për kontrollin e barërave të këqija përdorimi i herbicidit anti-monokotiledon (Focus™ ultra), lejon zhvillim të plotë të popullimit natyral të *A. murale*. Në këto dy vite eksperimentimi, prodhimi i biomasës së *A. murale* në parcelat e trajtuara u përmirësua progresivisht deri në 2.6-3.7t ha^{-1}. Kështu, u fitoekstraktuan sasitë e Ni: 22.6-29.5 kg ha^{-1}. Studimi i praktikave të tilla menaxhuese përmirësoi efikasitetin e fitoekstraktimit dhe ky ekstraktim i nikelit me anë të *Alyssum murale* mund të jetë premtues në konteksin shqiptar duke përdorur popullime natyrale të kësaj bime.

Introduction

Ultramafic terrains occupy 1 % of the earth's land surface and are host to distinctive flora [5]. Ultramafic rocks and particularly serpentinites containing very high magnesium (18-24%) and high iron (6-9%) but very low Ca (1-4%) and aluminium (1-2%) [2]. The soils derived from ultramafic rocks, such as peridotites, dunites and serpentinites – termed serpentine soils – are also strongly influenced by the geochemistry and mineralogy of the parent material [2]. These soils share a number of chemical properties, including a high content of specific heavy metals (nickel, chromium and cobalt), a low Ca:Mg concentration ratio and low concentrations of macronutrients [5]. In general, the vegetation of serpentine soils is well differentiated with respect to the surrounding areas. Among the metals widely presents in ultramafic soils, Ni is probably the one that causes the most significant toxicity to non-adapted plants. Ni hyperaccumulation has been defined as the accumulation of at least 1000 mg kg^{-1} Ni in the dry biomass of plants grown on a natural substrate [6, 14]. Hyperaccumulation has become recognized as an unusual response and specific ecophysiological adaptation to the elevated metal concentrations generally found in soils derived from ultramafic areas. Magnesium-rich soils in Albania consist of about 10,000 ha out of 700,000 ha of total agricultural land [15]. One of many ophiolitic massifs called Shebeniku is located in the ophiolites of eastern Albania and it hosts several Ni hyperaccumulator species (e.g., *Alyssum murale* Waldst. & Kit.) [4]. *A. murale* can be used to profitably phytoextract Ni from ultramafic soils as an alternative to the traditional cropping in metal-toxic soils. Phytoextraction of Ni in ultramafic areas is called "phytomining" and has been successfully implemented in soils containing natural high concentrations of Ni, Co and Cr [12]. The objectives of this work were to assess: 1) the approximate yield of Ni; 2) the relation between Ni content in harvested plant parts and the status of Ni available in the soil; 3) the effect of fertilization and weed on biomass and Ni content of harvests; This is of particular interest nowadays since several potential applications of hyperaccumulators, such as phytoremediation and phytomining, are being tested.

Materials and methods

The experiment was carried out in Pojskë (700 m, Pogradec), with latitude of 40°59'55, 28" N and longitude of 20°38'03, 92" E, and with a Mediterranean climate characterized by annual rainfall averages of ~730 mm and a mean temperature of ~10°C. The experimental site was a colluvial downslope (10-15%) characterized by spontaneous native ultramafic vegetation. The soil profile was described and samples were taken from the horizons identified in the field (0-30 cm, 30-50 cm, 50-70 cm, 70-120 cm). Physical and chemical characteristics of the soil samples were determined by the Soil Analyses Laboratory of INRA Arras in France [1]. X-Ray diffraction (XRD) was run on the 50 µm fraction to determine mineralogy. Ni-bearing phases were performed with transmission electron microscopy and coupled with X-ray spectroscopy (TEM-EDX) techniques to identify the minerals and their elemental composition. Ni chemical availability in soil samples of surfaces was characterized by DTPA-TEA [11]. Concentration of Ni in soil extracts were determined by plasma emission spectrometry (ICP-OES).

A field experiment was conducted in 2005-2006. The experimental site was already covered by spontaneous native ultramafic vegetation in March 2005 (an abandoned cropped field). The experimental area in 2005 was divided into six 36-m^2 plots, three of which were fertilized in April with 120 kg ha^{-1} N, P, K (**(**NH_4NO3, K_2SO_4 and $Ca(H_2PO_4)_2$). In 2006, each experimental plot was divided into twelve 18-m^2 plots**,** 3 of which were only fertilized (the same regime of fertilization, FNH), 3 were fertilized and treated with anti-monocots herbicide (Focus™ ultra 33 mL in 3L water for 108 m^2) (FH), 3 were only treated with herbicide (NFH) and 3 were not treated (NFNH)**.** Plants were harvested each year on June 28th.

The flora of each plot was fully described in June, prior to harvest (with the help of European and Albanian Flora). The plant species were sampled at site (s 3 & 4). The shoots of each species were individually collected and biomass yields were recorded for each plot. The rest of the biomass was pooled together (other non-frequent species). For each plot and species, plant samples were taken, rinsed with deionized water and dried at 80°C for 24 h. Trace metal contents in shoots were analyzed by plasma emission (ICP) spectrometry after the digestion of plant samples in microwave oven. A 0.25-g DM plant aliquot was digested by adding 8 mL of 69% HNO_3, 2 mL H_2O_2. Solution were filtered and adjusted to 25 mL with 0.1 M HNO_3.

RESULTS AND DISCUSSION

SOIL CHARACTERISTICS

All soil characteristics confirmed its ultramafic nature: low concentration of Ca, K and P and elevated Ni, Cr, Co, Mn, Fe (Table 1). This soil showed high total Fe contents with values of 10 %. It had 6% Mg and a strong Ca deficiency (0.3 %). The Mg:Ca ratio was high (20 as total concentration and 7.4 as exchangeable cations), a range that is commonly reported in serpentine soil material (13). Potassium total contents in soils were low (0.4%).

According to FAO WRBSR (1998) the soil at the experimental site was classified as Magnesic (Hypermagnesic) Hypereutric Vertisol. XRD analyses of the three horizons revealed that smectite and serpentine (undetermined type) were the two predominant minerals in the soil. TEM and EDX observations and analyses showed that both were in the Ni-bearing phases (Table 2). Ni availability assessed by DTPA was high, reaching 130 mg kg^{-1}. Although soil pH was quite high (neutral to slightly alkaline), Ni availability in this soil was very high because it was associated with high charge phyllosilicates smectites (high charge phyllosilicates) and amorphous Fe oxides [10]. The soils were suitable for phytomining.

EFFECT OF FERTILIZATION ON SPECIES COMPOSITION AND BIOMASS PRODUCTION

A. murale, *C. gryllus* and *T. nigriscens* were the

	Particle size distribution			pH	CO	MO	N	P	C/N	CEC	Exchangeable cations				Total major elements						Total trace elements					
	Clay	Silt	Sand				total	Olsen			**Ca^{2+}**	**Mg^{2+}**	**K^{2+}**	**Na^{2+}**	Ca	Mg	Al	K	Na	Fe	Co	Cr	Mn	Cu	Zn	Ni
Horizon	%			H_2O	%					cmol+ kg^{-1}					%						mg kg^{-1}					
0 -30 cm	51.6	27.7	20.7	7.45	2.74	4.7	0.23	0.01	12	38.9	5.42	40.5	0.37	0.08	0.30	6.01	2.7	0.5	0.2	10	207	1600	2060	23	130	3150
30-50 cm	52.2	14.8	33	7.75	1.36	2.4	0.10	0.01	13	43.8	3.41	49.4	0.37	0.09	0.18	5.56	2.6	0.4	0.2	11	224	1610	2920	13	95	3050
50-70 cm	37.4	11.9	50.7	7.62	0.38	0.7	0.05	0.01	8.3	42.4	1.47	48.6	0.32	0.09	0.11	7.00	1.9	0.2	0.1	12	160	1490	1460	11	91	3300
70-120 cm	57.5	23.3	19.2	7.87	0.34	0.6	0.04	0.01	8.3	38.9	1.14	50.5	0.42	0.11	0.15	4.61	4.0	0.6	0.3	10	134	1170	1320	11	91	2070

Table 1. Physical-chemical characteristics of the four horizons of the soil at the experimental site of Pojskë (Albania)

	Ni-bearing phase	Ni concentration (%)
Bedrock	Serpentine [8]	0.30 ± 0.17
0-30 cm	Serpentine [8]	0.69 ± 0.28
	Al-rich smectites [3]	0.44 ± 0.22
	Mg = Al smectites[3]	1.00 ± 0.24
	Mg-rich, Al poor smectites [1]	1.87
30-50 cm	Serpentine [3]	0.60 ± 0.31
	Al rich smectites [6]	0.57 ± 0.17
	Mg = Al smectites [3]	0.72 ± 0.28
	Mg-rich, Al poor smectites [2]	3.12 ± 2.50
50-70 cm	Serpentine [4]	0.66 ± 0.12
	Al-rich smectites [1]	0.66
	Mg = Al smectites [4]	0.99 ± 1.00
	Mg-rich, Al poor smectites [3]	1.55 ± 0.54

[n]: number of particles analysed (EDX)

Table 2. Identification of Ni-bearing phases and associated Ni content (%Wt) in the bedrock and horizons of the soil determined by TEM-EDX

most frequent species in this site but other species were reported on the plots as well, although their contribution to biomass production was negligible (Table 3). According to what was expected on such soils with low K and P availability the overall vegetation responded dramatically to fertilization by doubling the biomass yield. However, the contribution of each species varied according to fertilization. In unfertilized plots, *C. gryllus* accounted for most of the biomass, whereas in fertilized plots, *A. murale* was the main contributor. *A. murale* biomass abundance increased dramatically from 6.1% to 40.7% whereas *C. gryllus* biomass abundance decreased from 77.5% to 54.5%. *T. nigriscens* decreased in fertilized plots (16.3-4.8%). The Ni phytoextraction yield was 22.6 kg Ni ha^{-1} in the fertilized plots compared to 1.67 kg Ni ha^{-1} in unfertilized plots. This difference was highly significant ($P < 0.01$). *A. murale* was the main contributor in total phytoextraction yield (Table 4). In 2006, when the herbicide treatments were included to allow for the full development of *A. murale* we obtained a biomass of *A murale* of 3.7 t ha^{-1} (dry weight) in FH plots while the biomass in NFNH plots was only of 0.25 t ha^{-1}. The herbicide treatment seemed to efficiently control the population of *C. gryllus*. The biomass production of *A murale, C. gryllus* and *T. nigriscens* in fertilized plots increased respectively 14.8-, 2.0- and 2.4-fold in comparison to the unfertilized plots. The biomass production of *C. gryllus* and *T. nigriscens* decreased in 2006 since we harvested before maturity of *T. nigriscens (*Therophyte) in untreated plots (2005) and the harvested buds of *C. gryllus* (Hemicryptophyte) on the experimental site were exposed to low temperatures in winter. The yield of Ni phytoextraction in 2006 was 29.5 kg Ni ha^{-1} in the fertilized and herbicide treated plot compared to 2 kg Ni ha^{-1} in the unfertilized with no herbicide plots. These differences were highly significant ($P < 0.01$).

Year	Species	Plots	Biomass (t ha^{-1})	Ni yield (kg ha^{-1})
2005	*A. murale*	F	2.56 ± 0.70 a	22.6 ± 4.4 a
	Ch. gryllus		3.43 ± 0.35	2.17 ± 0.15
	T. nigriscens		0.30 ± 0.20	0.17 ± 0.12
	Total		6.30	24.93
	A. murale	NF	0.20 ± 0.44 b	1.67 ± 0.12 b
	Ch. gryllus		2.53 ± 0.32	0.83 ± 0.49
	T. nigriscens		0.53 ± 0.12	0.60 ± 0.30
	Total		3.27	3.10
2006	*A. murale*	FH	3.70 ± 1.06 a	29.5 ± 8.6 a
	T. nigriscens		0.075 ± 0.04	0.06 ± 0.04
	Other		0.38 ± 0.16	0.31 ± 0.13
	Total		4.15	29.87
	A. murale	FNH	2.16 ± 1.42 a	18.4 ± 11.4 a
	Ch.. gryllus		0.97 ± 0.45	0.81 ± 0.43
	T. nigriscens		0.025 ± 0.014	0.0059 ± 0.004
	Total		3.15	19.1
	A. murale	NFH	1.1 ± 0.3b	8.9 ± 4.5b
	T. nigriscens		0.039 ± 0.024	0.013 ± 0003
	Other		0.19 ± 0.06	0.08 ± 0.014
	Total		1.33	8.99
	A. murale	NFNH	0.25 ± 0.05 c	2.00 ± 0.34 c
	Ch.. gryllus		0.47 ± 0.16	0.17 ± 0.011
	T. nigriscens		0.031 ± 0.026	0.0021 ± 0.0007
	Total		0.75	2.17

Table 3. Biomass production and phytoextraction yield of the main species grown for each treatment. Values for each species within a plot are given as mean values ± standard deviation. For the biomass and phytoextraction yield of A murale, different letters indicate statistical difference.

Species	Family	Biological form	Lifespan
Alyssum murale Waldst. et Kit	Brassicaceae	H	perennial
Chrysopogon gryllus L. Trin	Poaceae	H	Perennial
Trifolium nigriscens Viv.	Fabacaeae	Th	Annual
Lolium perenne L.	Poaceae	H	Perennial
Aegilops geniculata Roth.	Poaceae	Th	Annual
Dasypyrum villosum L. P. Cond.	Poaceae	Th	Biannual
Poa trivialis L.	Poaceae	H	Perennial
Centaura solstitialis L.	Asteraceae	H	Biannual
*Minuartia hybrida*L.	Caryophyllaceae	Th	Perennial
Lotus corniculatus L.	Fabacaeae	H	Perennial
Consolida regalis S.F.Gray	Ranunculaceae	Th	Annual
Plantago lanceolata L.	Plantaginaceae	H	Perennial
Petrorhagia prolifera L.	Caryophyllaceae	Th	Perennial
Tragopogon pratesis L.	Asteraceae	H	Annual
Bromus racemosus L.	Poaceae	H	Annual
Vicia villosa Roth.	Fabacaeae	Th	Biennial

H: Hemicryptophyte
TH: Therophyte

Table 4. List of plant species collected on the experimental plots of Pojskë (Albania)

The relative and net increase in biomass production of *A. murale* were the main reason for the increase of phytoextraction yield since the Ni concentration in shoots was not significantly affected by the fertilization and herbicide treatments. In two years of experiment, species showed an increasing pattern in total biomass production in response to fertilization with significant differences ($p < 0.05$) for *A. murale* only. *A. murale* was also the life-form showing the highest increase after fertilization ($p < 0.05$), and therefore a more competitive specie in terms of ecological adaptation. This experiment showed that nutritional stress represents an important limiting factor for the existence of many species and plant productivity which is in accordance with the findings of Chiarucci (7) in serpentine vegetation of Tuscany.

PLANT RESPONSES TO SERPENTINE

The chemical analyses performed on plant bulk shoots showed different plant responses to the presence of trace metals and nutrients in soil of the experimental site (Table 5 & 6). Mg mean concentration for *A murale* was lower than Ca concentration while the inverse occurred in others species. The Mg: Ca ratio in *A. murale* was therefore lower than 1. This confirms the ability of this plant to accumulate Ca and its positive response to Ca fertilization [9].

Among the species in the experimental site Ni hyperaccumulation was observed only in *A murale.* The average Ni content in shoots was about 0.9% at harvest time in 2005 in the fertilized plots and 0.8% in 2006. The Ni concentrations in shoots of *A. murale* were more than two times higher than in the soil. This ratio indicated for optimal hyperaccumulation conditions although the bioavailability of Ni is high. No particular trend regarding Fe and Mn accumulation was observed despite reports of Mn accumulation in *Alyssum* leaves in other studies. Cobalt concentration in shoots tissues varied from 0.29 to 4.9 mg kg^{-1}. Some species in our site showed that they were able to colonize those soils in the presence of other tolerant populations. Ni content in these other species varied from 187 to 670 mg kg^{-1}, whilst plants from non-serpentinitic substrata usually contain very low Ni concentrations (0.5 ± 10 mg kg^{-1}) [14]. *Centaurea solsticialis* was accumulating at very high levels of Ni for non-accumulating species.

CONCLUSION

The soil on the site of Pojska was clearly suitable for phytoextraction. Ni-bearing phases in the

Species	Plots	Ni (mg kg^{-1})	Mn(mg kg^{-1})	Co(mg kg^{-1})	Fe (%)	Ca (%)	Mg (%)
Alyssum murale	FH	7887 ± 446	9.3 ± 1.8	2.1 ± 0.52	74 ± 30	0.49 ± 0.03	0.3 ± 0.01
Trifolium nigriscens		558 ± 528	18 ± 2.7	0.9 ± 0.34	327 ± 218	0.91 ± 0.07	0.9 ± 0.07
Alyssum murale	FNH	8680 ± 617	12 ± 3.7	2.6 ± 0.21	220 ± 180	0.64 ± 0.11	0.3 ± 0.05
Chrysopogon gryllus		812 ± 251	28 ± 15	2.1 ± 2.2	513 ± 313	0.3 ± 0.03	0.38 ± 0.06
Trifolium nigriscens		257 ± 196	15 ± 4.4	0.8 ± 0.01	182 ± 141	0.8 ± 0.14	1.1 ± 0.34
Alyssum murale	NFH	7826 ± 1347	9.9 ± 6.2	2.2 ± 1.14	263 ± 202	0.54 ± 0.1	0.36 ± 0.09
Trifolium nigriscens		468 ± 278	26 ± 17	2.1 ± 2.2	363 ± 62	0.88 ± 0.04	1.16 ± 0.18
Alyssum murale	NFNH	7210 ± 1004	7.9 ± 0.1	1.9 ± 0.01	145 ± 12	0.55 ± 0.02	0.36 ± 0.06
Chrysopogon gryllus		402 ± 141	28 ± 3.3	1.2 ± 0.22	803 ± 287	0.23 ± 0.032	0.32 ± 0.06
Trifolium nigriscens		90 ± 50	16 ± 1.5	0.7 ± 0.1	252 ± 1.4	0.77 ± 0.19	0.97 ± 0.06

Table 5. Mean concentrations ± standard deviations of Ni, Mn, Co, Fe, Ca and Mg in three dominant species collected on the experimental site of Pojskë in 2006

Species	Ni	Co	Fe	Ca	Mg	Mn	Mg/Ca
	mg kg^{-1}						
Lolium perenne	255	0.54	515	2314	4409	20	1.9
Aegilops geniculata	6.2	0.09	225	1200	2212	16	1.8
Dasypyrum villosum	15	0.28	172	1115	2640	20	2.6
Poa trivialis	229	0.31	1105	2268	2872	19	1.3
Centaurea solstitialis	643	1.43	1590	5505	6724	22	1.2
Minuartia hybrida	670	1.57	543	5298	8703	29	1.6
Lotus corniculatus	404	0.70	1075	4667	7780	25	1.6
Consolida regalis	31.8	0.49	142	4627	10969	15	2.4
Plantago lanceolata	69.4	0.35	408	3457	6973	8	2.0
Petrorhagia prolifera	208	1.60	1314	4895	8584	17	1.7
Tropogon pratesis	25.6	4.30	525	2755	7636	7	2.7
Bromus racemosus	60.4	5.80	271	2148	2794	20	1.3
Vicia villosa	140	0.96	534	5850	7565	12	1.3

Table 6. Concentrations of Ni, Co, Fe, Mn, Ca and Mg in harvested plant parts of species collected on experimental site of Pojskë

soil were mostly sources of highly available Ni. The experiment in serpentine vegetation showed that fertilization could enhance total plant cover and thus community production with slight changes in species richness and composition. Although these communities are mostly composed of different species, only hyperaccumulator plants are able to maintain a higher abundance under fertilization, whilst other species show a decrease of abundance. Fertilization, harvest and herbicide treatments affect plant community structure and productivity. *Alyssum murale* has a great phytoextraction potential in situations where native vegetation stand is enhanced by simple low-cost agronomic interventions. Fertilization treatment not only increased by more than 10 fold the biomass of *A. murale* production, but it also slightly increased the concentration of Ni in the harvested plant parts. All these results clearly suggest that on such soils with high availability of Ni and where *A. murale* grows naturally, it is possible do develop an extensive phytomining activity by managing native stands through agronomic practices.

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