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REGULAR ARTICLE

In-situ phytoextraction of Ni by a native population of *Alyssum murale* on an ultramafic site (Albania)

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Abstract Ultramafic outcrops are widespread in Albania and host several Ni hyperaccumulators (e.g., *Alyssum murale* Waldst. & Kit.). A field experiment was conducted in Pojske (Eastern Albania), a large ultramafic area in which native *A. murale* was cultivated. The experiment consisted in testing the phytoextraction potential of already installed natural vegetation (including *A. murale*) on crop fields with or without suitable fertilisation. The area was divided into six 36-m^2 plots, three of which were fertilised in April 2005 with (NPK + S). The soil (Magnesic Hypereutric Vertisol) was fully described as well as the mineralogy of horizons and the localisation of Ni bearing phases (TEM-EDX and XRD). Ni availability was also characterised by Isotopic Exchange Kinetics (IEK). The flora was fully described on both fertilised and unfertilised plots and the plant composition (major and trace elements) and biomass (shoots) harvested individually were recorded.

The soil had mainly two Ni-bearing phases: high-Mg smectite (1.3% Ni) and serpentine (0.7% Ni), the first one being the source of available Ni. Ni availability was extremely high according to IEK and confirmed by Ni contents in *Trifolium nigriscens* Viv. reaching 1,442 mg kg^{-1} (A new hyperaccumulator?). Total biomass yields were 6.3 t ha^{-1} in fertilised plots and 3.2 t ha^{-1} in unfertilised plots with a highly significant effect: fertilisation increased dramatically the proportion of *A. murale* in the plots (2.6 t ha^{-1} vs. 0.2 t ha^{-1}). Ni content in the shoots of *A. murale* reached 9,129 mg kg^{-1} but metal concentration was not significantly affected by fertilisation. Phytoextracted Ni in total harvest reached 25 kg Ni ha^{-1} on the fertilised plots. It was significantly lower in unfertilised plots (3 kg Ni ha^{-1}). Extensive phytomining on such sites could be promising in the Albanian context by domesticating already installed natural populations with fertilisation.

Keywords In-situ phytoremediation · Hyperaccumulator plant · Nickel · Phytomining · Bioavailability · Serpentine

Introduction

Serpentine substrate covers large areas in Balkans, more than in any other part of Europe which could justify the development of phytomining activities as an alternative to local agriculture on such unproductive

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lands. Biodiversity in the area is high, with a great number of interesting local and regional endemics. More than 300 endemic taxa occur on serpentine in Balkans. The high number of endemics indicates the importance of serpentine habitats as centers for floristic differentiation and speciation. Serpentine areas in Balkans exist in large blocks or as small outcrops. Ultramafic substrates in Albania cover 30% of the areas and they extend towards NC & SE. Ultramafic outcrops host a rich vegetation (Shallari et al. 1998) including a certain number of endemics and subendemics (Stevanovic et al. 2003) such as trans-regional endemics (e.g., *Polygonum albanicum*, *Bornmuellera baldacii*, *Alyssum markgrafii* or *Viola dukadjinica*) or regional endemics (e.g., *Alyssum smolikanum*, *Viola albanica*, *Festucopsis serpentine*).

Hyperaccumulation is a mechanism that is believed to allow plants to survive on serpentine soils (Brady et al. 2005). Hyperaccumulators are defined as plants that contain in their tissue more than 1,000 mg kg^{-1} dry weight of Ni, Co, Cu, Cr, Pb, or more than 10,000 mg kg^{-1} dry weight of Zn, or Mn (Baker and Brooks 1989). Aside from metal tolerance, hyperaccumulation is thought to benefit the plant by means of allelopathy, defense against herbivores, or general pathogen resistance (Boyd and Jaffré, 2001; Boyd and Martens 1998; Davis et al. 2001). There are at least 400 known Ni hyperaccumulators. The Ni hyperaccumulator *Alyssum murale* Waldst. & Kit., is widely present on ultramafic regions and industrial areas of Albania. The need to manage the Ni polluted soils necessitates the study of the behaviour of this hyperaccumulator plant in conditions in situ. Previous studies clearly evidenced a great variability in phytoextraction potential by different Albanian populations of *A. murale* depending on the site of collection (Chardot et al. 2005; Massoura et al. 2004; Shallari et al. 1998). This variability probably results from the interrelationships between the ecophysiology of specific populations and the edaphic characteristics of their native site.

Phytoextraction employs metal hyperaccumulator plant species to transport high quantities of metals from soils into the harvestable parts of roots and aboveground shoots (Kumar et al. 1995, Chaney et al. 1997). Effective phytoextraxtion requires both plant genetic ability and the development of optimal agronomic management practices (Li et al. 2000). It has been well-documented that modifying soil fertility may affect the efficiency of phytoextraction of heavy metals such as Ni, Zn, Co, Cd with a single crop (Bennett et al. 1999; Li et al. 2003a; Kukier et al. 2004). But the effects of fertilisation are less predictable when dealing with a native vegetation cover. In the case of phytomining, the use of native flora (including local populations of hyperaccumulators) with limited agronomic practices (extensive phytoextraction) could be an alternative to intensively managed crops (Li et al. 2000) provided that Ni bioavailability in soils is high and not limiting over time and that hyperaccumulator cover is reasonably efficient. However, there is an evident need of fertilisation and phytoextraction yield improvement (Robinson et al. 1997) to achieve sufficient Ni extraction. Such extensive practices of phytoextraction could be more easily implemented in a country such as Albania (small surfaces, limited investment capacity of farmers).

This paper reports the first findings of an in situ experimental work aiming at studying the response of a native vegetation cover, including *A. murale*, to agronomic practices in the view of optimising the phytoextraction yield of Ni. The objectives were (i) to optimise extensive phytoextraction methods that would be adapted in the Albanian context and (ii) to understand the relationships between the ecophysiology and the mineral nutrition of stands of native *A. murale* and other species, and the specific properties of a soil which displays high Ni availability. The soil of the site was therefore carefully studied to identify the factors influencing plant growth and Ni uptake. Then, 36-m^2 experimental plots were designed to study the effect of mineral fertilisation on species distribution, specific biomass yield, mineral nutrition and Ni phytoextraction.

Materials and methods

Site and soil characterisation and experimental plot design

An in situ field experiment was conducted in Pojske (500 m) (Pogradec, East of Albania), a wide ultramafic area in which native *A. murale* populations were grown. The experiment was carried out in spring 2005. The experimental area was a colluvial downslope (10–15%) above the lake surrounded by

Table 1 Monthly climatic parameters at Pojske in 2005

Month	Max temperature (°C)	Min temperature (°C)	Average temperature (°C)	Rainfall (mm)	Potential evapotranspiration Penman (mm)
Jan	5.6	3.8	0.9	62.0	4.0
Feb	4.8	−5.3	−0.2	71.0	1.2
Mar	9.8	−1.5	4.1	77.4	16.5
Apr	14.6	2.5	8.6	33.9	34.5
May	21.4	7.4	14.4	55.1	57.2
Jun	24.0	9.5	16.8	30.2	67.2
Jul	26.8	11.9	19.4	19.5	77.2
Aug	27.0	9.8	18.4	13.5	73.2
Sep	22.3	7.5	14.9	47.3	59.2
Oct	16.4	3.2	9.8	61.4	40.0
Nov	11.3	2.3	6.8	80.4	27.0
Dec	7.7	−0.7	3.5	179.4	14.1

ultramafic hills. The parent material was colluvium of ultramafic and magnesite origin. Climatic data for Pojske in 2005 are given in Table 1. The soil profile was described and samples were taken from the three horizons identified in the field (0–20 cm, 20–35 cm, >50 cm). Analyses of Ni-bearing phases with transmission electron microscopy, coupled with X-ray spectroscopy (TEM-EDX) techniques, were performed to identify the minerals and their elemental composition. X-Ray diffraction (XRD) was run on the 50 μm fraction to determine mineralogy. Ni availability in different soil samples of surfaces was characterised by Isotopic Exchange Kinetics (IEK) (see complete details and procedure in Echevarria et al. 1998). DTPA-extractable Ni were determined using the method of Lindsay and Norvell (1978). Concentration of Ni in soil extracts were determined by plasma emission spectrometry (ICP).

The experimental site was already covered by spontaneous native ultramafic vegetation in March 2005 (an abandoned cropped field which had hardly received any fertiliser in the past). The experimental area was divided into six 36-m^2 plots, three of which were fertilised in April 2005 with the following regime: 120 kg ha^{-1} (of each of the following elements N, P, K and S) with NH_4NO_3, K_2SO_4 and $CaHPO_4$. The rest of the plots did not receive any fertiliser.

Soil surface samples (0–20 cm) were taken on each plot before fertilisation, during vegetation period (May) and after harvest (June), for full characterisation (including mineral fertility and Ni availability). Soil samples were air dried and sieved (2 mm) prior to analyses.

Fertilisation pot trial

A greenhouse pot experiment was undertaken simultaneously in which *A. murale* was treated with different combinations of nutrients to determine the optimal fertilisation regime on this soil and to orientate the fertilisation on the field. Four addition treatments of 0:0:0, 50:40:40, 100:80:80, 120:120:120 kg of N:P:K per ha (1 kg per ha corresponds to 0.33 mg kg^{-1}. The experiment was conducted in a well-ventilated unheated greenhouse. Soil from A_p horizon in Pojske similar to that of the experimental plot was sieved (2 mm) and airdried before experiment. For each fertilisation treatment, four replicates of 1-kg pots were made. Five plants were sown per pot. Pots were daily watered to 100% of the water holding capacity and cultivated for 3 months after germination. After 3 months, plants were harvested (cut at 1 cm above ground surface) and the plant samples were rinsed in deionised water before drying at 80°C for 24 h.

Plant identification and harvest on experimental plots

The flora of each plot was fully described in June 2005 prior to harvest (with the help of European Flora). About 12 plant species were sampled at site (Table 5) and the plant composition was carefully recorded. *Alyssum murale* Waldst. & Kit., *Chrysopogon gryllus* (L.) Trin., *Trifolium nigriscens* Viv. were the most frequent species in site, the other species were much rarer. The shoots of plants of the three dominant species were individually collected and biomass yields were carefully recorded for each plot. The rest of the biomass from other species was pooled together. Total root biomass (all species) was also sampled on 900-cm^2 areas located in the center of each plot. For each plot and species (1-m^2 surface in the centre of the plot), plant samples were taken, rinsed with deionised water and dried at 80°C for 24 h.

Plant sample mineralisation, elemental analyses and statistical analysis of data

Trace metal contents in plants samples were analysed 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, 2 ml H_2O_2. Solution were filtered and adjusted to 25 ml with 0.1 M HNO_3. Certified reference materials (V463 maize) were analysed in order to control the data quality. We have also double-checked the high concentrations with dilutions of some Alyssum samples.

Digestion of plants samples, to determine N contents in harvested biomass, was made in digestion tubes using 4 ml of 98% H_2SO_4, 1 g of potassium sulfate-catalyst mixture, 6 ml H_2O_2. Digestion tubes were heated in digestion block to 350°. After digestion, samples were diluted to 25 ml with deionised water. Total N was determined using Kjeldhal method. Analyses of variance and Newman–Keuls Test were used to test significance of the differences between treatments.

Results

Edaphic properties of the site

The parent material is strongly weathered serpentine mixed with colluvium. The pattern of pedogenesis is strongly related to climate. The main phase in the weathering process is recombination of Si and Mg to smectite clays at the base of slopes, where excess SiO_2 recrystallises to form quartz and excess Mg is precipitated. This phase occurs in dry conditions and leads to the formation of soils that are hypermagnesic, including vertisols dominated by smectites. Soils on down slopes are less shallow (>50 cm) than upslope. They are stony and have numerous fragments of weathering rock on the surface. The clay content is high, but there is still a substantial proportion of sand (Table 2). The soil had a neutral pH (ranging from 6.9 to 7.3) and a high CEC, which was dominated by Mg^{2+} ions: there was relatively a low concentration of Ca^{2+}. In the soil, total Ca concentration was 0.24–0.32%, whilst total Mg concentration was 6.7–8.3%. The Mg:Ca ratio was therefore extremely high: 29. According to FAO WRBSR (1998) the soil at the experimental site was classified as Magnesic (Hypermagnesic) Hypereutric Vertisol.

Localisation of Ni-bearing phases and Ni availability

XRD analyses of the three horizons revealed that chlorite, smectite and serpentine (undetermined type) were the three predominant minerals in the soil. TEM and EDX observations and analyses showed that the last two were both Ni-bearing phases (Fig. 1). It was also shown that serpentines were mainly of the antigorite or lizardite type with very scarce chrysotile particles. Chlorite and serpentine were the main primary minerals occurring in soils derived from ultramafic rocks and smectite was the main secondary phyllosilicate occuring in soil. Two types of smectites could be distinguished according to their Mg and Al content. The average Ni concentration in Mg-rich smectites was highest (1.3%). It was lower in serpentines (0.7%) and in Al-rich smectites (0.5%) (Decarreau et al. 1987) (Table 3). The highest Ni concentrations were observed in some particles of a Mg-rich and Al-poor smectite also rich in Mn (19.5%), thus reaching 4.9% Ni in weight (Fig. 1A).

Ni availability as assessed by IEK can be described with the intensity factor (i.e., Ni concentration in solution) on one hand and the labile pools in the solid phase that are distributed according to their average time of exchange with the soil solution (i.e., E_t). Both are important for the definition of the possibility of root absorption of Ni by plants. Both intensity and labile pools were extremely high in this soil (Table 4). On average on the plots prior to fertilisation, C_{Ni} values were 0.25 mg l^{-1} and instantaneously

Table 2 Physico-chemical characteristics of the three horizons of the soil at experimental site

Horizon	Particle size distribution (g kg^{-1})			pH (water)	Organic C	C/N	CEC (Metson)	Exchangeable cations				Total major elements			Total trace elements			
	Clay	Silt	Sand					Ca^{2+}	Mg^{2+}	K^+	Na^+	Ca	Mg	Fe	Co	Cr	Mn	Ni
	g kg^{-1}				g kg^{-1}			cmol + kg^{-1}				g kg^{-1}			Mg kg^{-1}			
A_P (0–25 cm)	447	239	314	6.9	27	10	39.2	5.39	41.3	0.51	0.07	2.9	72	98	215	1,420	2,260	3,440
B_S (35–50 cm)	532	167	301	7.3	33	15	42.5	5.56	55.8	0.39	1.36	ND	ND	ND	ND	ND	ND	ND
BC	438	139	423	7.5	35	8.7	43.3	1.63	51.4	0.4	0.12	1.2	79	118	224	1,210	2,110	3,500

ND: not determined

exchangeable Ni ($E_{0-1\ min}$) was higher than 100 mg kg^{-1}. Medium term labile pools were also high and all the parameters showed an extremely high and well-buffered Ni availability in this soil (Echevarria et al. 2006). The DTPA-extractable Ni has confirmed the results of IEK, The amount of Ni extracted by DTPA roughly corresponds to the same amount (Fig. 2) as the exchangeable Ni ($E_{0-1\ min}$). DTPA-extractable Ni was higher after plant harvest than during plant growth but this was only a trend with no significant difference.

Effect of fertilisation on biomass production by *A. murale* and the other dominating species

A. murale (Brassicaceae), *C. gryllus* (Poaceae) and *T. nigriscens* (Fabaceae) were the most frequent species in this site but other species were reported on the plots although their contribution to biomass production was negligible (Table 5). The overall vegetation responded dramatically to fertilisation by doubling the biomass yield. The results showed that in fertilised plots we obtained a total biomass of 6.3 t ha^{-1} (dry weight) whereas in unfertilised plots it was only of 3.2 t ha^{-1} (Table 6) thus showing a highly significant difference ($P < 0.01$) between fertilised and unfertilised plots. However, the contribution of each of the species varied according to fertilisation. In unfertilised plots, *C. gryllus* accounted for most of the biomass whereas, in fertilised plots, *A. murale* was the main contributor. *A. murale* biomass abundance was dramatically increased from 6.1% to 40.7% whereas C. gryllus biomass abundance decreased from 77.5% to 54.5%. T. nigriscens dramatically decreased in abundance in fertilised plots (16.3–4.8%). In greenhouse experiment we found that the increase in biomass production ($P < 0.01$) was affected by fertilisation and shoot biomass yield did not affect shoot Ni concentration, and therefore total amount of phytoextracted Ni was increased. The significant differences were between unfertilised pots and fertilised pots, but there were no significant differences among fertilised pots.

Effect of fertilisation on Ni concentration in plant tissues and on phytoextraction yield

The chemical analyses of plant tissue showed the differences between three dominant species of

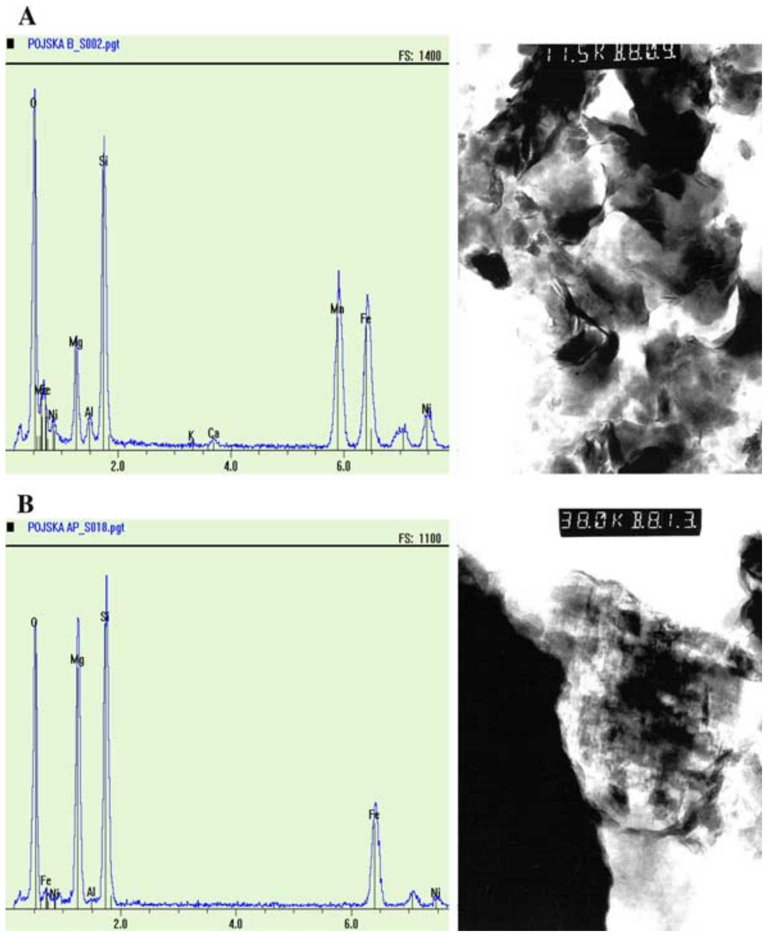

Fig. 1 **A** & **B**: TEM pictures (right) and EDX spectra (left) of typical Mg-rich smectite (**A**) and non-chrysotile serpentine (**B**) found in the two lower soil horizons of the Magnesic (Hyperamagnesic) Hypereutric Vertisol at experimental site in Pojske (Albania)

Table 3 Ni concentrations in the main Ni-bearing minerals of the soil (all three horizons) at experimental site. Results are given as mean ± standard deviation

	Ni concentration (%)
Al-rich smectites (n = 10)	0.54 ± 0.18
Mg-rich smectites (n = 19)	1.30 ± 1.04
Serpentines (n = 16)	0.73 ± 0.34

Table 4 Ni available compartments in surface soils (averaged sample) prior to culture as assessed by Isotopic Exchange Kinetics Methods (IEK)

Total soil Ni (mg kg^{-1})	3,440
C_{Ni} (Water soluble Ni in mg l^{-1})	0.251
$E_{0\text{–}1\ min}$ (Ni exchangeable within 1 min in mg kg^{-1})	124
$E_{1\ min\text{–}24\ h}$ (Ni exchangeable between 1 min and 24 h in mg kg^{-1})	119
$E_{24\ h\text{–}3\ mo}$ (Ni exchangeable between 24 h and 3 mo in mg kg^{-1})	119
$E_{3\ mo\text{–}1\ yr}$ (Ni exchangeable between 3 mo and 1 yr in mg kg^{-1})	46

experimental site. There were differences for nitrogen concentration. The mean N concentration in *A. murale* was 0.85% in unfertilised plots and 1% in fertilised plots. *C. gryllus* had the lowest value 0.60%, whilst *T. nigriscens* had the highest value 1.5%. Potassium concentrations in *A. murale* shoots were 1.5% (previous study on Pojske plants collected close to the site).

Ca concentrations in shoots were about 0.6% for *A. murale* whilst for *C. gryllus* the value was 0.24% and for *T. nigriscens* it was 0.86% (Table 7). For Mg, the mean concentration for *A. murale* was the lowest (0.28%) in fertilised plots, for *C. gryllus* it reached 0.52% and the highest value was found in *T. nigriscens* (0.81%). *A. murale* showed a different uptake pattern in response to the high concentration of Mg

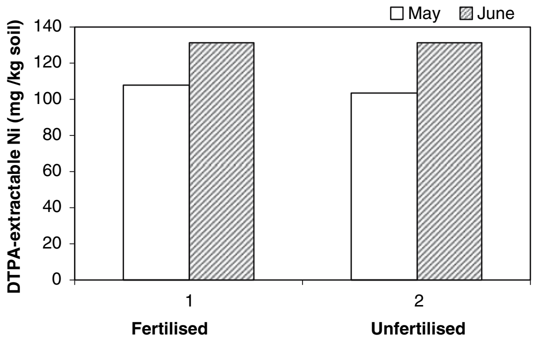

Fig. 2 Evolution of the DTPA-extractable Ni during culture in surface soil samples of the fertilised and unfertilised plots. There were no significant differences between fertilised and unfertilised soils and between May and June extractions (ANOVA, $P = 0.05$)

Table 5 List of plant species collected on the experimental plots in late June 2005 prior to harvest

Species	Family
Dominant species	
Alyssum murale Waldst. et Kit	Poaceae
Chrysopogon gryllus (L.) Trin.	Brassicaceae
Trifolium nigriscens Viv.	Fabacaeae
Accompaning species	
Cichorium intybus L.	Compositae
Lotus corniculatus L.	Fabacaeae
Aegilops geniculata Roth.	Poaceae
Viola arvensis Murray	Violaceae
Petrorhagia prolifera L.	Caryophyllaceae
Vicia villosa Roth.	Fabacaeae
Dasypyrum villosum (L.) P. Condargy	Poaceae
Centaurea solstitialis L.	Asteraceae
Lolium perenne L.	Poaceae

and the low concentration of Ca in soils than the two other species. It had a much lower Mg:Ca ratio in shoots than *C. gryllus* and *T. nigriscens*. So *A. murale* had a Mg:Ca quotient much lower than 1, the highest being 2.2 for *C. gryllus*. Fertilisation tended to decrease this ratio in all three species.

The mean Ni content in the above ground part of *A. murale* was 9,129 mg Ni kg^{-1} for fertilised plots and 8,483 mg Ni kg^{-1} for unfertilised plots. The fertilisation increased slightly the Ni concentration in plant tissue but with no significant effect. The Ni concentrations in above ground parts of *A. murale* were on average 2.9 times higher than in the soil. This ratio (i.e., transfer factor) shows an indication of optimal hyperaccumulation conditions due to high availability of Ni. The higher concentration of Ni was found in above ground parts of plants than roots, inverse was found for Mn and Fe. It was observed that the concentration of Ni in *C. gryllus* and *T. nigriscens* tissue was extremely high (>1,000 mg kg^{-1} in some plots for *T. nigriscens*) for such plants that are not considered as Ni-hyperaccumulators. The Cr contents in these two plants was reasonably low (between 6 mg kg^{-1} and 9 mg kg^{-1}). The hypothesis of soil particle contamination of plant aerial parts was therefore discarded.

There were strong differences in Ni phytoextraction yield (Table 6) between fertilised plots and unfertilised plots. The total phytoextraction yield (Sum of phytoextraction by the three species) was 24.9 kg Ni ha^{-1} in the fertilised plots, whilst it was 3.1 kg Ni ha^{-1} in unfertilised plots with a highly significant difference ($P < 0.001$). The contribution of *A. murale* was 22.6 kg ha^{-1} (91%) in fertilised plots and that of *C. gryllus* was 2.2 kg ha^{-1} (8.7%). In unfertilised plots, the low total phytoextraction yield was recorded with a much lower contribution of A. murale (54%). The relative and net increase in biomass production of *A. murale* was the main reason for increase of phytoextraction yield since the Ni concentration in shoots was not significantly affected by fertilisation.

The phytoextraction yield in pots in greenhouse experiment was 9.9 mg Ni/pot in the unfertilised treatment, 17.2 mg Ni/pot in the 50:40:40 kg ha^{-1} NPK treatment, 19.9 mg Ni/pot in 100:80:80 kg ha^{-1}NPK treatment, and 19.0 mg Ni/ pot in the 120:120:120 kg ha^{-1} NPK treatment (Table 8).

Discussion

Soil characteristics and Ni availability to *Alyssum murale*

The serpentine soil in Pojske is a clayey and Mg-saturated soil that contains elevated levels of heavy metals, such as Mn, Ni, Cr, Co that are typical from ultramafic environments. The three soil horizons are characterised by extremely high magnesium-to-calcium ratios which are toxic to unadapted plant species. In general, the Mg:Ca ratio observed in ultramafic soils was between 2.5 and 47 (Proctor 1971). Soil mineralogy was simple and homogenous

Table 6 Biomass production and phytoextraction yield (per ha) of the three main species grown on fertilised and unfertilised plots. Values for each species within a plot are given as mean values ± standard deviation. For the sum of biomass and phytoextraction yield (i.e., 'Total'), different letters indicate statistical difference at the $P < 0.01$ level for biomass and at the $P < 0.001$ for phytoextraction yield (ANOVA and Newman–Keuls Test)

Species	Plots	Biomass (t ha^{-1})	Ni phytoextraction yield (kg ha^{-1})
Alyssum murale	Fertilised	2.56 ± 0.70	22.60 ± 0.44
Chrysopogon gryllus		3.43 ± 0.35	2.17 ± 0.15
Trifolium nigriscens		0.30 ± 0.20	0.17 ± 0.12
Total		6.30 a	24.93 a
Alyssum murale	Unfertilised	0.20 ± 0.44	1.67 ± 0.12
Chrysopogon gryllus		2.53 ± 0.32	0.83 ± 0.49
Trifolium nigriscens		0.53 ± 0.12	0.60 ± 0.30
Total		3.27 b	3.10 b

through the soil profile. Although soil pH was quite high (neutral to slightly alkaline), Ni availability in this soil was very high and therefore mainly influenced by the Ni-bearing minerals. This is in agreement with a previous study which showed that Ni availability in the soil is generally much higher when Ni is associated with poorly crystallised Fe oxides or high charge phyllosilicates such as smectites despite of high pH values (Massoura et al. 2006). We suggest in the present case that the availability of Ni from primary clay minerals (e.g., serpentines) was low and attributed to the presence of Ni in the crystal lattice. Subsequently, the Ni in secondary clay minerals (smectites) was probably sorbed onto the mineral surfaces or located on internal exchangeable sites, and therefore, its availability was very high. This soil is then highly suitable for profitable phytoextraction (e.g., phytomining) as it will supply over the long

Table 7 Concentration of Ni, Mn, Cr Fe, Ca and Mg in harvested parts of plants on fertilised and unfertilised plots. Results are given as mean values ± standard deviation as there were no significant differences between fertilised and unfertilised plots (ANOVA)

Species	Plots	Ni (mg kg^{-1})	Mn (mg kg^{-1})	Cr (mg kg^{-1})	Fe (%)	Ca (%)	Mg (%)
Alyssum murale	Fertilised	9,129 ± 1,275	10 ± 2	–	0.04 ± 0.04	0.60 ± 0.13	0.28 ± 0.07
Chrysopogon gryllus		648 ± 50	52 ± 25	8.7 ± 0.4	0.19 ± 0.09	0.24 ± 0.03	0.52 ± 0.08
Trifolium nigriscens		727 ± 497	22 ± 4	8.3 ± 0.6	0.11 ± 0.05	0.86 ± 0.12	0.67 ± 0.18
Roots (all species)		1,132 ± 73	29 ± 1	ND	0.13 ± 0.06	0.31 ± 0.04	0.24 ± 0.06
Alyssum murale	Unfertilised	8,483 ± 477	5.5 ± 2.2	–	0.02 ± 0.02	0.57 ± 0.16	0.49 ± 0.14
Chrysopogon gryllus		337 ± 148	35.0 ± 17.4	7.5 ± 9.0	0.18 ± 0.08	0.17 ± 0.05	0.44 ± 0.12
Trifolium nigriscens		910 ± 585	24.7 ± 8.5	6.4 ± 3.1	0.11 ± 0.05	0.68 ± 0.05	0.81 ± 0.11
Roots (all species)		896 ± 199	35.0 ± 10.0	ND	0.21 ± 0.03	0.37 ± 0.02	0.30 ± 0.10

Table 8 Biomass production, Ni content in shoots of *A. murale* and phytoextraction yield as affected by fertilisation level in pot experiments with the surface soil from Pojske. Values with the same letter indicate no significant difference (ANOVA, Newman–Keuls test at the $P = 0.05$ level)

Fertilisation treatment (N:P:K)	Biomass yield (g kg^{-1})	Ni concentration (mg kg^{-1})	Ni phytoextracted (mg kg^{-1})
0:0:0	0.87 c	11,385 a	9.9 b
50:40:40	2.04 b	8,454 ab	17.2 a
100:80:80	2.95 a	6,772 b	19.9 a
120:120:120	3.04 a	6,261 b	19.0 a

term large and accessible quantities of labile Ni with a high buffer capacity (Echevarria et al. 2006; Li et al. 2000).

The presence of high Ca concentrations in soils may inhibit both Ni and Co hyperaccumulation by *Alyssum.* Acceptable Ca concentrations in soil were reported to range from 0 to such a value that exchangeable soil Ca is less than 20% of exchangeable soil Mg. (Chaney et al. 1998). In our soil, the Ca:Mg ratio was close to 13% in the surface horizon and therefore matched these recommended values. For phytomining with *A. murale*, the optimal pH was defined within the following range: 4.5–6.2; and preferably between 5.2 and 6.2. In a previous study, it was shown that pH favoured Ni phytoextraction yield by *A. murale* on an industrially contaminated soil (Kukier et al. 2004). But this effect was not observed during the same study on a serpentine soil in which the dominant Ni-bearing phases seemed to be Fe oxides. The phases that bear available Ni in our soil (i.e., high-Mg smectites) are different from that previous serpentine soil, and are not believed to strongly modify their Ni retention capacity with pH. Moreover, the pH in the soil is neutral and the *A. murale* population used here is native to this soil. We therefore believed that soil conditions were optimal for Ni phytoextraction with *A. murale*.

Effect of fertilisation on species abundance and biomass production

Alyssum murale, *C. gryllus* and *T. nigriscens* were the most frequent spontaneous species in this site. According to what was expected on such soils with low potassium and phosphorus availability, the overall vegetation responded dramatically to fertilisation by doubling the biomass yield. Unexpectedly, the contribution of each of the species varied according to fertilisation and *A. murale* was highly favoured by fertilisation compared to the two other species. It is obvious that N fertilisation tended to decrease the biomass production of the *Leguminosa* in this competition context, however, the decrease of abundance of the *Poacea* was a surprise. In this experimental study of phytoextraction with natural hyperaccumulator stands, the next step in improving hyperaccumulator biomass would be to apply selective herbicides to control the population of *C. gryllus*. This practice has been implemented in 2006 and the plots have now all been subdivided to include a new treatment with an anti-monocots herbicide.

The fertilisation treatment seemed to control quite efficiently the population of *T. nigriscens* and less that of *C. gryllus*. In terms of ecological adaptation, *A. murale* is therefore a more competitive species than the two others. However, anti-monocots herbicide treatments were included in the plots in 2006 to allow for the full development of *A. murale* (unpublished data). Soil management practices (fertilisation herbicide treatment) have been applied from others in serpentine and Ni contaminated soil to improve Ni phytoextraction. Li et al. 2003b in developing a commercial technology using hyperaccumulator plant species (*A. murale*) to phytoextract Ni reached a biomass of 20 t ha^{-1}. In our experiment (extensive technology), in which we stimulated natural vegetation stands with fertilisation (and later with herbicide treatment), *A. murale* biomass reached 3.7 t ha^{-1} and was therefore much lower than with intensive management (Li et al. 2003b) but we need more references on several years before making reliable comparisons with other in-situ experimental data.

Plant nutrition and Ni uptake by *A. murale*

Walker et al. (1954) surmised that serpentine-tolerant species survive on soils with depleted levels of Ca because they are still able to absorb quantities of Ca without taking up excessive quantities of Mg. The Ca concentration in plant tissue of *A. murale* was higher than Mg concentration, whilst in the two other species it was inverse. This confirms the ability of *A. murale* to accumulate Ca and its positive response to Ca fertilisation (Kukier et al. 2004). Fertilisation seems to improve plant uptake of Ca and to limit excess uptake of Mg for all species. This is probably a reason for the extreme increase in biomass yield after fertilisation. It was observed that the concentration of Fe and Mn in above ground parts of *A. murale* were lower in comparisons with two others species. In analysing tissues of a number of serpentine endemics from Zimbabwe, Brooks and Yang (1984) found the concentration of Mg in plant tissue to be inversely proportional to the concentration of other nutrients: Fe, Co, Mn. These data suggest that the uptake of Mg comes at a cost to the plant so that the uptake of other element nutrients is forfeited. So, Brooks and Yang (1984) proposed that the heightened level of Mg in serpentine soils and its antagonistic behaviour toward other elements could be the most important factor in serpentine syndrome.

The low Cr concentration in *T. nigriscens* and *C. gryllus* and trace quantities of this element in *A. murale* confirmed Brooks (1987) findings, that serpentine plants without exception contain only trace quantities of Cr. The low Cr content in *T. nigriscens* and *C. gryllus* and the high Ni:Cr ratio in plant tissues (much higher than the soil Ni:Cr ratio) confirmed the root uptake origin of Ni in these plants. Ni uptake by the two non-hyperaccumulator species was therefore extremely and surprisingly high. In fact, *T. nigriscens* should be added to the list of Ni hyperaccumulators with a maximal concentration of 1,442 mg kg^{-1} observed in this in-situ experiment. However, it is clear from the bioavailability data that these two species in other conditions could display much lower Ni concentrations in their tissues as opposed to a truly Ni hyperaccumulator such as *A. murale*. Nevertheless, *C. gryllus* contributed roughly to 10% of the Ni phytoextraction yield in the fertilised plots. Ni concentrations in *A. murale* were high, but not excessively, with regard to the high level of Ni availability in this soil. The latter species is genetically highly variable as it is widespread all over ultramafics in the Balkans and Turkey. The Albanian populations of *A. murale* collected in situ varied from 1,508 to 8,463 mg kg^{-1} (Shallari et al. 1998) except in one site in Prrenjas (Roger Reeves, personal communication). For phytoextraction purposes, the reasons that determine the level concentration of Ni in the total biomass harvested on the field should be investigated.

Effect of fertilisation on Ni phytoextracion yield and perspectives for future research

Alyssum murale was evidenced with great phytoextraction potential in situations where native vegetation stand is enhanced with simple low-cost agronomic actions (fertilisation). The fertilisation nearly doubled the total biomass harvested but dramatically increased by 10- to 15-fold the phytoextraction yield. Shoot Ni in field-grown plants reached 1% and was not affected or diluted by fertilisation. Phytoextracted Ni in harvested biomass reached 28 kg Ni ha^{-1} in one of the three fertilised plots with an average of 25 kg Ni ha^{-1}. With a price of Ni at 30 USD per kg, a reasonable income per ha would be 750 USD, provided that all the Ni contained in the biomass is recovered. This is why we started investigating the performance of low-cost phytoextraction with limited agronomic actions adapted to the Albanian context to see if extensive phytoextraction was feasible as an alternative to intensively managed phytoextraction (Li et al. 2003b). The first answer after one year of experiment is positive, however, we need to better understand the relationships between fertilisation and species distribution to clearly define pest control and its consequences on phytoextraction yield by stands of *A. murale* alone. Also, we need to improve the level of Ni concentration in the shoots of *A. murale* shoots through agronomic practices and possibly to compare it with sown accessions of *A. murale*.

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