THE EFFECTS OF NUTRIENTS ON BIOMASS PRODUCTION OF HYPERACCUMULATOR PLANTS AS A POTENTIAL SOURCE OF ENERGY

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Metal-rich soils and mainly ultramafic soils (*i.e.* serpentine soils) that are widespread over the Balkans and especially in Albania are a major problem for the development of agriculture due to toxicity of trace metals and low concentrations of macronutrients. The recovery of metals from soils with hyperaccumulator plant crops as an alternative to traditional agriculture is a challenge for the rural development of these areas as well as for the local mining and metallurgical industry. The Ni hyperaccumulator *Alyssum murale* Waldst. & Kit., is widely present on ultramafic regions and industrial areas of Albania (Bani *et al.*, 2008). Our objective for the plant production is to reach plant crops obtained from native sites and with limited agronomic practices, to optimize their biomass and Ni content. The experiment consisted in testing the phytoextraction potential of *A. murale* in the serpentine site of Pojske (Eastern Albania). We have been studying several fertilizations practices which may affect the efficiency of Ni phytoextraction on greenhouse pots and 36-m^2 plots in natural conditions. In this experiment, the biomass yields in fertilized plots have progressively improved in comparisons with unfertilized plots respectively from 0.38 to 4.6 t ha^{-1}. So have phytoextracted Ni: until 55 kg ha^{-1}. Li et al. (2003b) have done some work to develop efficient and cost-effective methods of Ni recovery. Recovery of energy by biomass burning or pyrolysis could help make phytoextraction more cost-effective.

Introduction

Serpentine soils, developed upon ultramafic rocks, are widely distributed in different parts of the world (Brooks *1987*). These soils contain high concentrations of Mg and Fe, and also relatively high amounts of Ni, Cr and Co. Concentrations of N, P, K are usually low, and the Mg/Ca ratio is high (Proctor *1999*). Plants growing on serpentine soils often accumulate metallic elements, resulting in elevated tissue metal concentrations relative to plants on normal soils (Brooks, 1987). These species, termed hyperaccumulators, may contain large amounts of Co, Cr, Cu, Mn, Ni or Zn (Brooks, 1987). Hyperaccumulators of Co, Cr, Cu, Pb, or Ni have concentrations of 1000 ppm dry mass, whereas hyperaccumulators of Mn or Zn are defined as those containing 10 000 ppm dry mass (Baker and Brooks, 1989). Phytoextraction employs plants to transport and accumulate high quantities of metals from soil into the harvestable parts of roots and aboveground shoots (Chaney, 1983; Chaney et al., 1997). When phytoextraction is designed as an economic agriculture for the recovery of valuable metals in ultramafic areas in which soils naturally contain high concentrations of Ni, Co and Cr, has been successfully implemented (Li *et al.*, 2003), it is called "phytomining". Chaney (1983) proposed the concept of using plant hyperaccumulators for phytoextraction. Nicks and Chambers (1995) have proposed that commercial exploitation of the annual Californian hyperaccumulator *Streptanthus polygaloides* would produce about 100 kg/ha of Ni after moderate application of fertilizers. This time the price of nickel was \$7.65 kg^{-1} and the energy of combustion of the biomass turned into electricity yielded \$131 ha^{-1}. A later study by Robinson et al. (1997) in Italy using *Alyssum bertolonii* arrived at a conservative value of 72 kg Ni/ha containing 0.08% Ni. Chaney et al. (1998, 2000, 2005) and Li et al. (2003b) showed that *Alyssum murale* Waldst. & Kit. and *Alyssum corsicum* Duby can grow biomass containing 400 kg Ni ha^{-1}. This time, Ni metal was trading at more than \$40 kg^{-1}, so Ni phytomining has become a highly profitable agricultural technology (crop value = \$16 000 ha^{-1}) for Ni contaminated or mineralized soils. Li et al. (2003b) reported highly effective (32%) Ni recovery from *Alyssum* Ni hyperaccumulator shoots biomass ash. They suggested that biomass could be combusted immediately for its energy value and the plant ash stored until the world price improved. Serpentine soils cover 10% of the Albanian area and they extend towards NC & SE. These areas will continue to be habitat for Trans-regional endemics of Albanian serpentine (Stevanovic et al., 2003) and Ni-hyperaccumulators. *Alyssum murale* Waldst and Kit. (Brassicaceae) is a nickel-hyperaccumulator plant that is widespread in Albanian mineralized soils. For several years *A. murale* has been discussed and tested for phytoextraction of nickeliferous and contaminated soils. The aim of the experiment was to improve the agronomy of *Alyssum murale* for optimising extensive phytoextraction methods for phytomining that would be adapted in the Albanian context

Materials and methods

Field experiment

The experiment was carried out in Pojske (500 m) (Pogradec), 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 ~ 707 mm and a mean temperature of ~ 10.6°C. The experimental site was a colluvial down slope (10–15%) characterized by spontaneous native ultramafic vegetation. The soil profile was described and samples were taken from the horizons identified in the field. Physico-chemical characteristics of the soil samples were determined by the Soil Analyses Laboratory of INRA Arras in France (AFNOR. 2004). Ni chemical availability in soil samples of surfaces was characterised by DTPA-TEA (Lindsay et al.1978). Concentration of Ni in soil extracts were determined by plasma emission spectrometry (ICP-OES). A field and greenhouse experiment was conducted in 2005-2008. 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 fertilisation) (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 in June 28th. In 2008 in the experimental site has been prepared the seedbed (tillage of the soil). Seeds collected when mature but before being dispersed were planted in 20 September. The planting method was direct seeding with hand. The experimental area in 2008 was divided into six 36-m^2 plots, three of which were fertilized in April with 100 kg ha^{-1} P, K and 50 kg ha^{-1} N and treated with anti-monocots herbicide (FH). We have used 50 kg ha^{-1} N two weeks later. During dry period in 18 may, 5 e 20 June, the treated experimental plots were irrigated by water that came from natural sources of this area. The flora of each plot was fully described, prior to harvest (with the help of European and Albanian Flora). The plant species were sampled at site (Table 4). The shoots of each specie were individually collected and biomass yields were recorded for each plot. Plant samples were taken, rinsed with deionised water and dried at 80°C for 24 h. Trace metal contents in shoots were analysed by plasma emission (ICP) spectrometry after 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.

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 air dried before the 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.

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 ((Proctor 1971). 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. Ni availability assessed by DTPA was high, reaching 130 mg kg^{-1}. The soil was suitable for phytomining (Bani at al 2007).

Table 1. Physico-chemical characteristics of the three horizons of the soil at experimental site

	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
Horizon	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	1420	2260	3440
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	1210	2110	3500

Natural levels of nickel in Alyssum murale

Natural concentration levels of Ni in *Alyssum murale* from Pogradec are given in Table 2. The average Ni content in shoots was about 0.9% at harvest time in 2005 in the fertilized plots, 0.8% in 2006 and 1.2% in 2008. It was almost exactly the same as recorded for others (Reeves personal communication). The mean Ni concentrations in plant were on average 2.3- 3.4 times that of the soil. Ni extracted by a crop of *Alyssum murale* (kg/ha) has changed depending on the development stage of plants (Perronnet et al., 2003). This has consequences on the time of harvest and the parts to be harvested. Since the Ni concentrations are higher in leaves (2%) and flowers (1.4%) than other plant organs, plants will have to be collected when they have more leaves and flower (Li et al. 2003b, Keller 2006) The mean Ni concentration in *A. murale* was higher in 2008, because plants were harvested at the beginning of flowering stage while in the 2 other years plants were harvested at the end of the flowering stage.

Effect of fertilization on species composition and biomass production

A. murale, *C. gryllus* and *T. nigriscens* 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 4). 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 specie varied according to fertilization, 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 fertilised plots (16.3– 4.8%). The Ni phytoextraction yield was 22.6 kg Ni ha^{-1} in the fertilized plots, while it was 1.67 kg Ni ha^{-1} in unfertilized plots. This difference was highly significant ($P < 0.01$). In 2006 when the herbicide treatments were included to allow 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,* in fertilized plots increased 14.8-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 harvest of the experimental site exposed the buds of *C. gryllus* (Hemicryptophyte) 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 plots compared to 2 kg Ni ha^{-1} in the unfertilized with no herbicide plots. These differences were highly significant ($P < 0.01$). The phytoextraction yield in pots in greenhouse experiment was 9.9 mg Ni/pot in the unfertilized 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 3).

As showed in the table, fertilizations, irrigation, herbicide treatment, increased the biomass production of *A murale* in 2008. So in treatment plots the biomass production was 4.6 t and Ni phytoextraction yield was 55 kg Ni ha^{-1}, whilst they were 0.38 t and 4.28 kg Ni ha^{-1} in untreated plots with a highly significant difference ($p < 0.05$). The relative and net increase in biomass production of *A. murale* was 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. This experiment showed that nutritional stress represents an important limiting factor for the plant productivity which is in accordance with the findings of Chiarucci (7) in serpentine vegetation of Tuscany.

Table 2. Biomass production and phytoextraction yield (per ha) of the three main species grown on fertilized and unfertilized plots. Values are given as mean values ± standard deviation.

years	Plots	Biomass	Concentrations of N in plant	Ni yield
		(t ha^{-1})	(mg kg^{-1})	(kg ha^{-1})
2005	F	2.56 ± 0.70a	9129 ±1275	22.60 ± 0.44a
	NF	0.20 ± 0.44b	8483 ± 477	1.67 ± 0.12b
2006	FH	3.7 ± 1.06a	7887 ± 446	29.5 ± 8.6a
	FNH	2.16 ±1.42a	8680 ± 617	18.35 ± 11.4a
	NFH	1.1±0.3b	7826 ± 1347	8.9 ± 4.5b
	NFNH	0.25 ± 0.05c	7210 ± 1004	2 ± 0.34c
2008	F	4.64 ±1a	12040 ±3301	55 ±11a
	NF	0.38 ±0.02b	11420 ±2197	4.28 ± 0.76b

Table 3. 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})
0:00:00	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

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

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

Biomass energy

We found a link between phytoextraction and the production of biomass energy crops and this has been made for the last 20 years (Baker, Revees, and McGrath, 1991; Goransson and Philippot, 1994). So the economic potential of our technology for phytoextraction of Ni can be estimated as follows:

- 55 kg Ni ha^{-1} and \$30 kg^{-1} Ni (Ni commercial value in the production time)
- rental costs are \$150 ha^{-1} and production costs are \$ 390 ha^{-1}
- 20 % of Ni value to cover cost of metal recovery.

The value of Ni in phytomining biomass can be \$780 ha^{-1} and methods to recover and market this metal are needed. One alternative to valorise phytoextracted metals could be incineration (Li et al. 2003b) or co-incineration of hyperaccumulator plants along with municipal waste (Keller *et al.*, 2005) Another profit that might be adopted would be to recover some of the energy released during incineration of the biomass (17,500 kJ/kg for cellulose material).

Conclusion

The soil on the site of Pojske (Pogradec) was clearly suitable for phytoextraction because it has highly available Ni. The experiment in serpentine vegetation showed that fertilization could enhance total plant cover. 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 productivity. *Alyssum murale* was observed with great phytoextraction potential in situations where native vegetation stand is enhanced with simple low-cost agronomic interventions. Fertilization increased more than 10 fold the biomass of *A. murale* produced. All these results clearly suggest that on such soils with high availability of Ni and where *A. murale* grows naturally, it is possible to develop an extensive phytomining activity by managing native stands through agronomic practices. We think that the recovery of metals from soils with hyperaccumulator plant crops as an alternative to traditional agriculture is a challenge for the rural development of these areas as well as for the local mining and metallurgical industry.

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