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The phytomining of nickel from industrial polluted site of Elbasan, Albania

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Abstract:

Large ex industrial areas in Albania could be suitable for phytomining using nickel hyperaccumulator Alyssum murale Waldst. & Kit. which grows in Albanian serpentine areas. We undertook a three-year field experiment in 2013-2016 on ex metallurgical industrial site in Elbasan. The following aspects were studied on 8 m² plots planted with Alyssum murale seeds from serpentine site of Prrenjas: (i) the effect of chemical fertilizer, tilling soil, irrigation, plant density on growth parameters (ii) Nickel yield per ha, and (iii) the reduction of Ni availability in soil after 3 years successive cropping of Alyssum murale.

The area was cleared in late summer 2013 and then ploughed and the soils characterized. 8 m² was planted with A. murale seeds at a density of 6-16 plants m^{-2} in September 2013. In three years (at the end of June), was harvested 6 m², to study biomass and Ni phytoextraction. In the plots treated with fertilizers and irrigated during the warm seasons, the biomass yield progressively improved

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from 0.6 to 1.1 kg m^{-2} and phytoextracted Ni increased from 304 to 853 mg Ni m^{-2}. While, in untreated plots the biomass and Ni phytoextraction varied respectively from 0.3 to 0.5 kg m^{-2} and from 193 mg Ni m^{-2} to 313 mg Ni m^{-2}. Nickel availability was 15 % lower after 3-years of plant harvested in our field experiment with Alyssum murale. Our study demonstrates that A. murale represents a source for remediation of metal polluted soil in industrial site.

Key words: ex industrial site, phytomining, heavy metal, phytoextraction, hyperaccumulator plants

INTRODUCTION

Phytoextraction is a developing technology that uses plants to accumulate elements from contaminated or mineralized soils and transport them to shoots, which may then be harvested to remove the elements from the field (Chaney et al. 2007). It is a type of phytoremediation, while the term “phytomining” has been applied to the latter case in which the economic value of the recovered metal is the primary motive. 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).

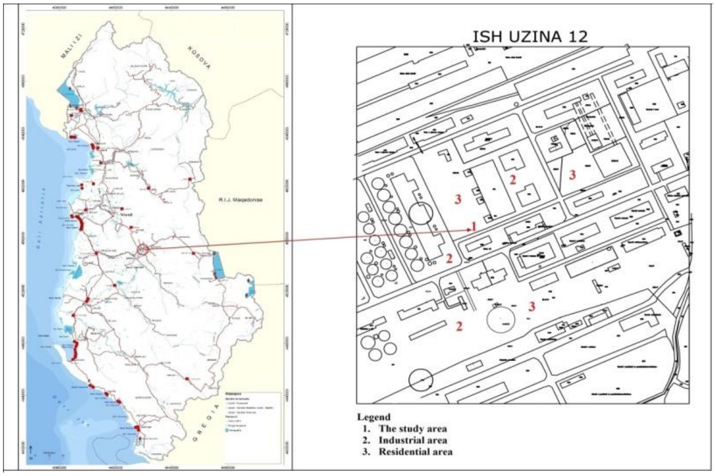
Brooks et al. (1977) first used the term hyperaccumulators to describe plants, which contain >1000 μg/g (0.1%), Nickel in their dried tissues. Hyperaccumulators are species capable of accumulating metals at levels 100-fold greater than those typically measured in shoots of the common non accumulator plants. Chaney et al. (2000, 2005) and Li et al. (2003) showed that *Alyssum murale* Waldst. & Kit. could accumulate Ni at concentrations > 20 000 mg Ni kg^{-1} shoot dry weight with no evidence of phytotoxicity when grown on serpentine soils with minimal addition of fertilizers. However, for a potential use in phytomining, we need to focus on “hypernickelophorous” species that can accumulate more than 10000 mg kg^{-1} (Chaney et al. 2007). In order to meet commercial phytoextraction requirements, Li et al. (2003) have continued to develop commercial technology using hyperaccumulator plant species (i.e., *Alyssum* species) in order to phytoextract nickel from contaminated and / or Ni-naturally rich soils. They showed that with a minimal addition of fertilizers, *Alyssum murale* Waldst. & Kit. could accumulate more than 20 000 mg Ni kg^{-1} in shoots when grown on serpentine soils (Li et al. 2003). Furthermore, *A. murale* with modern use of herbicides and other agricultural management practices, could reach a biomass production of 20 t ha^{-1} and the consequent phytoextraction of Ni can be up to 400 kg Ni ha^{-1} (Li et al. 2003). The largest number of Ni-hyperaccumulators is found in the *Brassicaceae* family in temperate climates, especially Mediterranean Europe and Turkey (Reeves and Adigüzel, 2008). The genus *Alyssum* (*Brassicaceae*) contains the greatest number of reported Ni hyperaccumulators, many of which can achieve 30 g kg^{-1} Ni in dry leaf biomass (Baker and Brooks 1989). The Balkans has the highest diversity in Ni hyperaccumulator plants in Europe and is home to the widespread plant *A. murale*, one of the most studied species worldwide for phytomining (e.g. Nkrumah et al. 2016). The Albanian flora contains a wide range of Balkan endemic taxa, including some serpentine-obligate (Stevanovi´c et al. 2003) among which, the most efficient Ni-accumulator individuals of the species *A. murale* (Bani et al. 2009; 2010). *A. murale* occurs widely on these ultramafic Vertisols (Bani et al. 2009) and is a spontaneous weed to other crops.

The use of nickel hyperaccumulator plant species for Nickel phytominig in Albanian ultramafic soil is a reality. Bani et al. (2015b) showed that, the phytoextraction potential of *A. murale* under different agronomic practices in Albanian vertisol can be 112 kg Ni ha^{-1}. *A. murale* Waldst. & Kit is the most efficient Ni hyperaccumulator plants in Albania (Bani et al. 2013; 2015a).

This study was designed to identify the Ni phytoextraction or phytomining potential of the hyperaccumulator *A. murale* Waldst. & Kit on industrial polluted site of Elbasan, Albania. The objectives were to i) investigate the effect of chemical fertilizer, tilling soil, irrigation, plant density on growth parameters; to ii) determine the Nickel yield for hectares and to iii) evaluate the reduction of Ni availability in soil after 3 years successive cropping of *Alyssum murale*.

MATERIAL AND METHOD

The metallurgical plant is located in Elbasan, in the centre of Albania, near the Shkumbin River, about 60-km southeast from Tirana. It is the largest plant in the country with a surface of 155 hectares and a treatment capacity of 800 thousand tons/year of iron-nickel and produced an estimated 44.8 tons of toxic dust. The main plants, which have been operating (1967-1990), are Nickel-Cobalt Plant (Ish-Uzina12), Metallurgy-Electrolysis Plant and Ferro-Chrome Plant (Shehu, 2009). After the '90s, the population growth and the migration from villages towards cities, have transformed a part of this industrial area in residential area, like which now is called Former Plant 12 (Ish-Uzina 12). This is the place with the highest risk of pollution and toxins, and where at least 11 hectares of soil is spotted by the ferrochrome wastes. As a result of industrial activity, this soil is contaminated with heavy metals (Shallari et al. 1998; Sallaku et al. 1999; Osmani et al. 2015). The study area is Former Plant 12 (Ish-Uzina 12), which is located 4 km far from the Elbasan city and 0.5 km from the Shkumbin River. The experiment was conducted for a 3 year period (2014-2016).



A) B)

Figure 1. A) The map of Albania with the location of the study area (Elbasan); B) Description of Ish-Uzina 12

Experimental design and agronomic techniques

Two types of soil are both planted with *A. murale* Waldst. & Kit seeds from serpentine site of Prrenjas, which have the same climatic conditions as Elbasan city (Krutaj et al. 1991). Seeds were collected in July 2013. The study area i8 m^2, divided into 2 plots by 4 m^2 each; one is treated with fertilizers DAP (Diammonium phosphate 16 % N and 46% P_2O_5) and Polysulphate (48% SO_3, 14% K_2O, 6% MgO and 17% CaO) and the other is kept in natural conditions (non-fertilized). The amount of chemical fertilizer used is 3 kg/100 m^2 DAP and 5 kg/100 m^2 polysulphate. After seed germination, in every 1 m^2 we had 8 plants. The agronomic practices that are carried out every year, for both types of soils, are presented below (table 1).

Table 1. The agronomic techniques for each plot during 2014-2016

Agronomic practices	Year 2014	2015	2016
Soil tilling	6 March	March, around plants	March, around plants
Soil fertilized	4 m² fertilized, 9 March with DAP and 25 March with Polysulphate	3 m² fertilized, 9 March with DAP and 26 March with Polysulphate	2 m² fertilized, 10 March with DAP and 27 March with Polysulphate
Soil non-fertilized	4 m² non-fertilized	3 m² non-fertilized	2 m² non-fertilized
Planted with seeds	12 March	-	-
Soil irrigation	once a week	once a week	once a week
Harvest	4 July, fertilized 1 m²	3 July, fertilized 1 m²	3 July, fertilized 2 m²
	4 July, non-fertilized 1 m²	3 July, non-fertilized 1 m²	plants didn't grow up

Soil analysis

In the Laboratory of Agro-environment and Ecology department, in Agricultural University of Tirana, Albania, were determined the physico-chemical characteristics of the soil.

For each plot, every year, one to three soil samples were taken from the upper horizon at a depth of 0-30 cm when possible. Soils samples were air-dried and processed in the laboratory. The determination of total organic matter (TOM) and total organic carbon (TOC) was performed by the "Wet Combustion" method (Allison, 1965). The definition of the soil texture and the content of the organic matter were performed at the Laboratory of the Agricultural University of Tirana. The textural class of all soil samples was determined based on the triangle textural (Particle Size Analysis with Hydrometer Method, Bouyoucos, 1962) and soil pH (in water) was also measured. Total nitrogen (N) and phosphorus (P) were determined using Kjeldahl digestion method. Total-N and Total-P ware analyzed using digestion Kjedahl (Kruis, 2010). 0.3 g soil with 2.5 mL H_2SO_4 - Se mixture, was put for 2 hours in an oven at 100°C, then 1 ml aliquots of H_2O_2 was added and afterwards was put 2 hours in the preheated block at 330°C. The digest is diluted with about 15 ml of water, and about five pumice grains was added, boiled and after cooling made up to

50 ml in a volumetric flask. It was mixed well, and then it was let to settle the particles for 24 hours, before analysis. Total nitrogen (N) and phosphorus (P) were determined using spectrophotometer 6600 UV-VIS.

For the determination of nickel and the total major (Ca, Mg, and K), soil samples were mineralized with a microwave digester. Conditions for mineralization were 6 ml HCl, 2 ml HNO_3, and 3 ml H_2O_2, per 0.5 g soil. The final solution was filtered and made up to 25 ml with deionized water. The availability of Ni in soils was measured using a DTPA–TEA extraction (0.005 M DTPA with 0.01 M $CaCl_2$ and 0.1 M triethanolamine (TEA) at pH 7.3. A ratio of 1 g soil: 10 mL DTPA-TEA solution was shaken for 2 h, and then the suspension was centrifuged at 5,000 g for 20 min, filtered through a 0.2 µm pore size cellulose nitrate filter (SARTORIUS) (Echevarria et al. 1998). All extractions were performed in triplicate. Ni concentrations and the total major (Ca, Mg, and K) in the soil extracts were determined spectrochemically using Atomic absorption spectrophotometer (Nov AA-350).

Plant analysis

Measuring growth indicators and harvest

For each plot we have determined the number of growth plant. In 2016, the length of plants was measured with millimetre paper.

Every year, 1m^2 from each plot is harvested. In 2016, we have harvested 2m^2 from fertilized plots, because the plants in the untreated plot died. After the harvest, the plants were weighed to determine the fresh biomass and after being dried in natural conditions, they were weighed again for dry weight.

All plant samples were washed, dried and ground to a fine powder. Nickel concentrations in plants were determined by plasma emission (ICP) spectrometry after microwave digestion of plant samples. A 0.25-g DM plant aliquot was digested by adding 8 ml of 69% HNO_3 and 2 ml of H_2O_2. The final solution was filtered and made up to 25 ml with deionized water. The nickel concentration was measured in digestion solutions by atomic absorption spectrophotometry (AAS).

Nickel phytoextraction

The efficiency of phytoextraction depends on the level of contamination in soil and the amount of metals accumulated by plants. Metal phytoextraction is determined by two main factors which should have high values: biomass production and heavy metals bio concentration degree (Mc Grath and Zhao, 2003). The biomass (dried) was weighed in each plot, in order to calculate the nickel phytoextraction yield, as the product of plant biomass (B) with the concentration of nickel in the cultivated hyperaccumulator plant (C_P) (mg kg^{-1}).

$$\eta = B \times C_P$$

RESULT AND DISCUSSION

Soil characteristics, concentrations of nutrients and Ni in soil

The pH, total organic carbon (TOC) and total organic matter (TOM) from the soil of our study were measured and the results are shown in Table 1. Effects of TOM on physical parameters and nutrient dynamics and their impact have been reported by several authors (Fageria, 1992). The TOM helps to maintain good aggregation and increase water holding capacity and exchangeable K, Ca, and Mg. It also reduces P fixation, leaching of nutrients. Textural group is silty-loam according to textural triangle. The reported characteristics show us a low-medium concentration of nutrients in the soil (Table 2,3) i.e. low concentrations of N, and P, medium level of Ca, Mg and K and elevated Ni, Cr and Fe compared with other agricultural soils of Albania. Total Ni reached was 700 mg kg^{-1} in the surface horizon and total Cr and Fe were assessed as 525 mg kg^{-1} and 6% respectively. As a result of the industrial activity, iron-nickel plant and other plants around, the soil is contaminated with both elements. The intervention value for Nickel when remedial action is necessary is 210 mg kg^{-1} (Denneman and Robberse, 1990). Although sources of pollution in the study area are minerals of ultramafic rocks, the total Ca:Mg ratio was over 1 (1.2), which is different from that in ultramafic soils.

The potassium and nitrogen total concentration in these soils were in medium value, respectively 0.8% and 1.6%. The phosphorus concentration was also quite low (411-572 mg kg^{-1}) in soil surface of the study sites and pH was alkaline.

Table 2. Soil characteristics of Ish-Uzina 12 Results are given as mean value (n = 3).

pH	TOC (%)	TOM (%)	Particle size distribution			Fe	Ni	Cr
			Sand (%)	Clay (%)	Silt (%)	%	mg kg^{-1}	
7.9	0.84	1.45	24.7	21.5	53.8	6	700	525

Based on the classification of Albania soils, the soils in Elbasan are brown soil (Pumo et al. 1990). They are distinguished by the small percentage of humus 2-3%, have low concentration of nitrogen (N) and phosphorus (P), and are rich with potassium (K).

Table 3. The macronutrients in the soil and the recommended limits (mg kg^{-1})

Year	Soil	Total-N	Total-P	K	Ca	Mg
2014	Fertilized	1817	609	10945	16588	11198
	Non-fertilized	1656	572	8189	13957	10916
2015	Fertilized	1934	732	11631	17863	12178
	Non-fertilized	1601	431	8252	13093	10880
2016	Fertilized	2048	776	12917	18538	12906
	Non-fertilized	1583	411	8234	12576	10693

According to Epstein (1972), these values are below the recommended level for plant growth. Potassium (K) (10334-19917 mg kg^{-1}), calcium (Ca) (12576-20538 mg kg^{-1}) and magnesium (Mg) (10693-12906 mg kg^{-1}) are within the limit of nutrient requirements; the use of fertilizers also has an impact on their value growth.

The amount of macronutrients was small in these soils because they have been industrial soil. About 20 years ago, these soils have returned to agricultural soil and more attention has been paid to apply agronomic practices to improve soil productivity.

Characterization of *A. murale* growth parameters

Agronomic techniques, tilling soil and irrigation, have helped the plants growth in both plots. In fertilized plots, as a result of fertilizers the concentration of macronutrients increased, consequently affecting the plant growth. Nutrients have improved the soil structure by increasing water penetration and providing a more favourable soil environment for growth of plant roots and soil microorganisms. The number of plants, in fertilized plots ranged from 10-14 plant / m^2, while in natural condition (non-fertilized) plots the number of plants ranged from 6-8 plant / m^2. In 2016, the lack of nutrients and available nickel caused the death of *Alyssum murale* plants in non-fertilized plots (Bani et al. 2009), while the length of plants in those fertilized plots ranged from 33.7-38 cm.

Non-fertilized 2015 Fertilized 2015 Fertilized 2016

Figure 2. ***Alysum murale*** **in fertilized and Non-fertilized plots**

Biomass, Ni concentration in plant and Ni yields

According to what was expected on such soils with low nutrients concentration the overall vegetation responded positively to fertilization, increasing the biomass yield (Bani et al. 2007; 2015a, b, 2018).

During the years, in the soil treated with fertilizers the plant biomass is higher than in non –fertilized plots. The use of fertilizers has influenced the growth of *A. murale*. The biomass of *A. murale* is represented by the whole biomass harvested in each of 1 m^2 plots. It variations depended on the treatment use and the passing of years as showed in Table 4. The biomass of *A. murale* was about 4 times higher in 2015 in fertilized plots compared with non fertilized plots in the first year of experiment.

The biomass production of metal hyperaccumulators depends on productivity of the soil, harvesting time, climatic conditions. The biomass is negatively correlated with Ni concentration in *A. murale* in fertilized plots the biomass is higher while the nickel concentration is lower than in natural condition plots. Fertilizers have influenced the increase of the biomass and so we had the dilution of Ni concentration in plant tissues, as a result of biomass growth. Nickel concentrations in plants (Table 4) were higher at 2016 in fertilized plots (769±50 mg kg^{-1}), as a result of better development of plants and the accumulation of Ni during plant growth. As it has been shown in previous studies, *A. murale* can hyperaccumulate up to 1% nickel. *A. murale* in this study could not accumulate Nickel more than 1000 mg kg^{-1} nickel, since the available nickel content on the soil is very small and also the Ca percentage on the soil is high. This finding is in accordance with previous study that showed in *A. murale*, at least, where appears to be an inverse relationship between the Ni uptake and the Ca concentration in the soil (Bani et al. 2010).

Table 4. Plant biomass, nickel concentration in plant and Ni phytoextraction for plot

Year	Soil	Plant biomass (kg)	Ni concentaration in plant (mg kg^{-1})	Ni yield mg Ni m^{-2}	kg Ni ha^{-1}
2014	Fertilized	0.66	452	298.3	2.98
	Non-fertilized	0.33	587	193	1.9
2015	Fertilized	0.81	615	499	4.99
	Non-fertilized	0.45	691	311	3.1
2016	Fertilized	1.11	769	853	8.5
	Non-fertilized	-	-	-	-

There were marked differences in Ni phytoextraction yield between fertilized plots and non-fertilized plots. In 2006, the Ni phytoextraction yield was 8.5 kg Ni ha^{-1} in the fertilized treated plot, compared to 3.1 kg Ni ha^{-1} in the non-fertilized plots. In 2016, the relative and net increase in biomass production of *A. murale* was the main reason for increase of phytoextraction yield.

Considering the biomass production and Ni accumulation, *A. murale* could be a potential candidate for phytoextraction of Ni in metal contamination site in ex industrial site.

Evolution of DTPA extractable Nickel in three years of experiment with *A. Murale*

For each surface horizon of the contaminated soil, chemical availability of Ni were measured and monitored from 2014 to 2016 in composite surface samples of each plot.

The Nickel concentration in soils polluted by anthropogenic activities (mainly ultramafic minerals) had low concentration of available Ni as previously was showed. This soil is poor in smectite, rich in high-Ni goethite and slightly alkaline (Massoura et al. 2006).

The amount of the available Nickel in the soil, called Ni $_{DTPA}$, significantly decreased with time of cultivation and treatments. DTPA-extractable Ni in the soil was lower after the harvest, mainly in the plots treatments with fertilizers. In non-fertilized plots, it decreased from 3.8 to 2.9 mg kg^{-1} and in fertilized from 3.8 to 2.2 mg kg^{-1}.

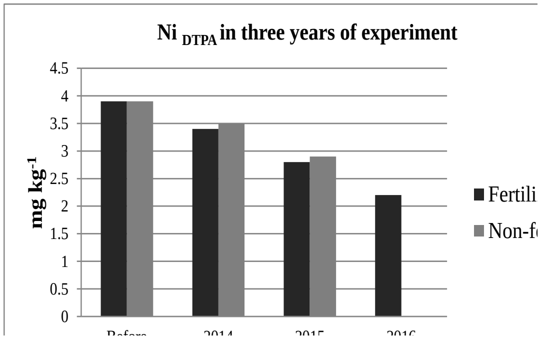


Figure 3. DTPA extractable Nickel in three years of experiment with *A. Murale*

Since the reduction of DTPA Ni after *A. murale* cultivation occurred, these results suggest that *A. murale* takes up Ni from a pool of soil Ni that can be partly quantified using DTPA. By reducing the DTPA-extractable pool of Ni in the soil after successive culture of *A. murale* it was limited the contamination potential of those waste that came from metallurgical factory. This demonstrates the potential of *A. murale* to accumulate nickel and remediate the soil.

CONCLUSION

The low concentration of available nickel in soil and the high content of calcium compared to the serpentine soils where *A. murale* grows naturally limits the accumulation of nickel. Tilling of the soil, adequate fertilization and appropriate plant densities are more important for developing efficient phytomining approaches. The use of fertilizer has influenced the increase of nutrients in the soil, which are essentials for plant growth. This will help in the growth of plant biomass and in the Ni phytoextraction. Nickel availability was 15 % lower after three years successful harvest of *Alyssum murale*. Consequently, *A. murale* represents a candidate for remediation of ex-industrial site, heavy metal polluted, as it is able to extract wide range of Nickel and to take up it in their upper part.

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