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PHYTOMINING AND AGROMINING CANDIDATE PLANTS OF HYPERACCUMULTIVE FLORA OF KOSOVO

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ABSTRACT

Phytomining technology employs hyperaccumulator plants to take up metal in harvestable plant biomass. Harvesting, drying and incineration of the biomass generates a high-grade bio-ore. We propose that “agromining” (a variant of phytomining) could provide local communities with an alternative type of agriculture on degraded lands; farming not for food crops, but for metals such as nickel (Ni). This type of operation is most efficient using perennial species that regenerate aboveground biomass rapidly after harvesting. This is a biennial investigation that involves the hyperaccumulative flora of Kosovo. As the present investigation aims to identify plant species for phytoremediation and phytomining purposes, *Odontarrhena muralis (synonym Alyssum murale)* and *Noccaea ochrolecum* which belong to Brassicaceae family were identified, because they are able to uptake more than 1000 mg kg^{-1} of Ni from the corresponding soil and accumulate that in their tissues.

Samples were collected from ten sampling sites across Kosovo. The results showed Ni levels varying from moderately high 1586 mg kg^{-1} to high (up to 7564 mg kg^{-1}) in all the *Odontarrhena muralis* samples, and Zn extracted in tissues of *Noccaea ochrolecum* resulted 798.7 and 5888 mg kg^{-1}. It has been proved that *Odontarrhena muralis* from serpentine soils of Kosova could be a candidate for agromining and phytomining.

Keywords: agromining, phytomining, Ni hyperaccumulation, ultramafic, Kosovo

1. INTRODUCTION

Phytomining is the phytoextraction and recovery of metals for commercial gain (Chaney *et al*., 2007; Tang *et al*., 2012). Ni hyperaccumulation has been defined as the accumulation of at least 1,000 mg kg^{-1} Ni in the dry biomass of plants grown on a natural substrate (Brooks *et al*., 1977).

However, for a potential use in phytomining, we need to focus on "hypernickelophorous" species that can accumulate more than 10,000 mg kg^{-1} (Chaney *et al*., 2007; Bani *et al*., 2014).

Ultramafic outcrops in Europe cover more than 10,000 km^2 and soils derived from this bedrock are generally characterized by low fertility (low total N and available K and P contents) and productivity, making them unattractive for agriculture (Bani *et al*., 2015a,b; Bani *et al*., 2019). In Albania, ultramafic outcrops cover 11% of the surface and the Mg-rich agricultural soil have been estimated to cover about 20907.4 ha of the about 313300 ha of total area of ultramafic substrates available in the country (Lekaj *et al*., 2019).

Serpentine soils cover 4.48 % of the territory of Kosovo from which 1.31 % (142.8 km^2) are soils developed on serpentinite and 3.16 % (344.32 km^2) are soils on harzburgite (peridotite).

The Ibri Valley in the North of Kosovo represents the largest ultramafic complex. Other ultramafic complexes are the Golesh Massif in Central Kosovo, and the southwestern Kosovo.

Several plant species that we know elsewhere that are Ni-hyperaccumulators (Bani *et al*., 2009; Bani *et al*., 2010; Bani *et al*., 2014) or that are suspected to be hyperaccumulators (i.e. Bornmuellera dieckii) could also be found in Kosovo (Stevanović *et al*., 2003; Salihaj *et al*., 2016; Salihaj *et al*., 2018) including *Odontarrhena muralis and Noccaea ochroleuca(Boiss and Heldr) (*Meyer F.K.. 1973). Salihaj *et al*., (2018) said that the ultramafic hyperaccumulators grown in Kosovo are able to accumulate extremely high concentrations of Ni, and sometimes Co, in their aerial biomass. It has been showed that we can apply phytomining, cultivating *O. chalcidica (syn. Alyssum murale)* plants that are able to accumulate trace metals from metal-rich soils and transport them to the shoots (>1%), which can then be harvested as a bio-ore to recover highly valuable metals such as Ni (Li *et al*., 2003; Bani *et al*., 2007; 2015a, b; van der Ent *et al*., 2015; Bani *et al*., 2019). It was proven to be an efficient opportunity in (Chaney *et al*., 2007; Bani *et al*., 2015a; b).

The ultramafic soils of Kosovo are considered suitable for the cultivation of plants that could be used for phytomining and agromining purposes. It is worth mentioning that most of these areas are state property, and here the access is easier and more convenient.

The present investigation aims at identifying the hyperaccumulator plants in serpentine habitats, and the scanning for Ni hyperaccumulation along with their potential to be used for phytomining and agromining based on soil characteristics.

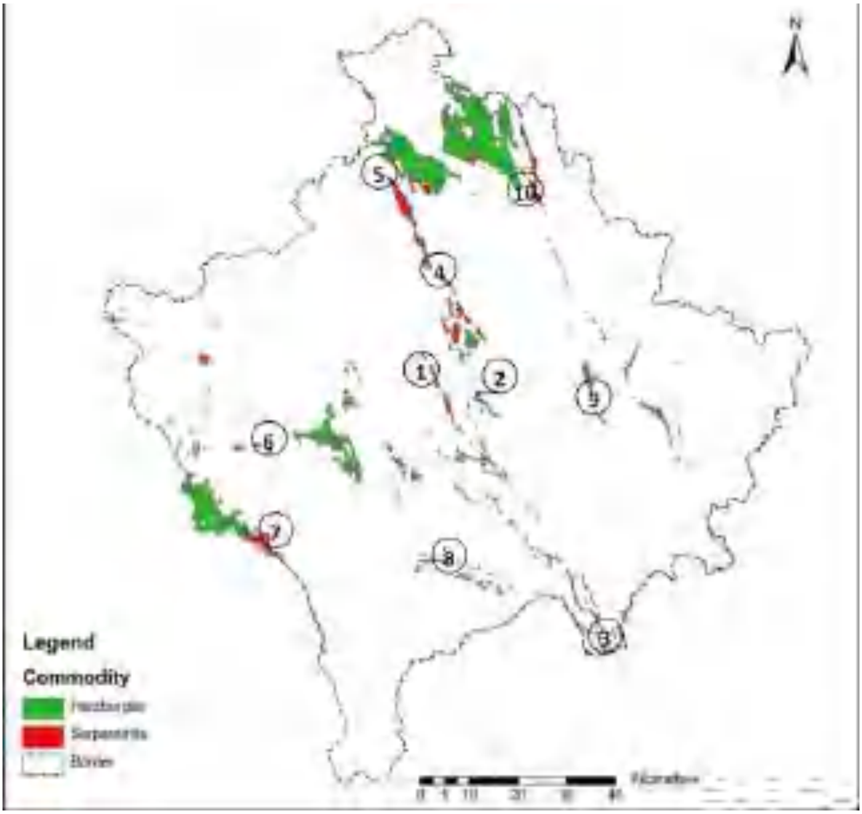

Fig. 1. The map of ultramafic soils of Kosovo.

2. MATERIALS AND METHODS

Sampling was carried out in ten sampling sites across Kosovo on June 2014. These areas are the most representative in terms of phytommining and agromining candidate plants. Twelve representative soil profiles from 10 different sites across the country were dug, and 27 horizon samples were collected. Regarding serpentine flora, at total of 162 plant species present in the ultramafic sites were collected. *Odontarrhena muralis* (syn. *Alyssum murale* Waldst. and Kit) and *Noccaea ochroleuca* (Boiss and Heldr.) Meyer F.K. 1973 (syn.*Thlaspi ochroleucum*) were the only potential candidate plant species for phytomining and agromining. In addition, the entire flora here located was identified, collected, air-dried, sieved to less than 2 mm and analysed for heavy metals concentrations and the calcium and magnesium content. Soil samples were collected from the rhizosphere area of the plants. The soil samples were air-dried ground and sieved to 2 mm. Once air-dried ground and sieved, the samples were transported in polyethylene bags to the laboratory to analyse the total Ca, Mg, Ni, and Zn, and the results showed that each site exhibited a high concentration of one or more heavy metals.

All soil and plant's samples were air-dried ground and sieved to 2 mm or less. Then they were mineralized with a microwave digester. Conditions for mineralization were 6 ml HCl, 2 ml HNO3, and 3 ml H2O2, per 0.5-g soil. Soils were air-dried and sieved to mm. Total major (N, P, K, Ca and Mg) and some of the trace elements (Ni, Zn,) were determined in mineralization solution by atomic absorption spectrophotometry. The Mechlich 3 method was applied for the extraction of Ni, Ca, and Mg bioavailability in different soil samples.

3. RESULTS AND DISCUSSIONS

Soil characteristics

All the sampling sites were characterized by high levels of heavy metals exhibiting typical properties of an ultramafic environment.

Total Ni availability varied from 872 and 3298 mg kg^{-1}, whereas bioavailable Ni is found at the range of 57.2 mg kg^{-1} and 125 mg kg^{-1}, respectively. There are some correlations between the bioavailable Ni in soil with Ni level found in *Odontarrhena muralis* such as sample site Golesh #2 where bioavailable Ni in soil (**table 1.**) corresponds with hyperaccumulated Ni in this plant (**table 2.**) Regarding the Ca content in serpentine soil, results showed that the bioavailable Ca concentration is very low compared to Mg, which results in a negative Ca/Mg ratio in most sampling sites. Regarding the N, P and K content, most of the soil samples are characterized by low level of these nutrients, which is a general characteristic of ultramafic soils (Whittaker 1954; Brooks 1987). Fertilization of native *A. murale* with NPK promoted shoot biomass yields without affecting Ni concentration in shoot, resulting in increased Ni removal (Bani *et al.*, 2007). In fertilized plots the biomass yields progressively improved from 2.6 to 6.0 t ha^{-1}, and Ni removal increased from 22.6 to 69 kg ha^{-1} (Bani *et al.*, 2007; 2009).

The low content of available phosphorus in serpentine soils might be connected to the high affinity of soluble phosphates to serpentine (Brooks *et al.*, 1987).

Potassium content is in Table 3 reported. As it could be noted the potassium content is very low. Moreover, the content of bioavailable macro elements such as Ca, K is also very low. It varies from 1028 to 3480, and from 52.2 to 471.7 for Ca and K, respectively. This is typical of ultramafic soils.

Levels of Mg and Fe proved that serpentine soils are lithological weathered products which consist predominantly of ferromagnesian silicate minerals.

Ni content in hyperaccumulative plants

Odontarrhena muralis reported the highest Ni concentration. This plant species is dominant in all the serpentine sites. The Ni uptake varied from 1585-7564 mgkg-1, which is lower than Ni concentration found in the same species grown in the serpentine soils of Albania (19100 mgkg-1). Here, the edaphic characteristics (topographic characteristics) of these sampling sites might be the source of low levels of Ni concentration rates. As the sites are very steep, nutrients specifically nitrogen leach from runoff waters. Consequently, Ni uptake by hyperaccumulator plants has significantly reduced.

On the other hand, Ni concentration found in *Noccaea* grown Kosovo varies between 799 mg kg^{-1} and 5888 mg kg^{-1}, which is higher than Ni concentration found in *Noccaea* grown in Albania (1360 mg kg^{-1}), or in Bulgaria (3400 mg kg^{-1}).

Basically Ca/Mg ratio to Nickel hyperaccumulator plants is greater than one (>1), which is contradictory to serpentine associated soils. This ratio is between 2 and 21 for *Odontarrhena muralis,* while for *Noccaea* is between 2 and 10 (**Table 2**.). Positive Ca/Mg ratio has also been observed for the *O. chalcidica* grown in serpentine soils of Albania.

Table 1 Zn, Ni, Ca and Mg concentrations in soil and Ca/Mg Ratio

	Total trace elements			Exchangeable elements		
Sample point	Zn	Ni mgkg^{-1}	**Ni**	**Ca** mgkg^{-1}	**Mg**	**Ca/Mg Ratio**
Site 1 Kishnareke P#1	31.8±0.9	2248±104.4	110±12	3480±74	4239±121	0.82
Site 1 Kishnareke P#2	35.6±2.7	2275±85.2	101±6.6	1082±40	3912±98	0.28
Site 2 Golesh P#1	31.8±2.6	872±18.2	115±5.4	1150±52	3784±120	0.30
Site 2 Golesh P#2	42.3±2.7	1347±23	125±3.4	1869±42	3331±116	0.56
Site 3 Rezhance	51.4±1.4	3298±13.0	68±6.4	1028±41	3645±68	0.28
Site 4 Vejshtine	32.7±2.7	1543±123	57.2±3.2	3061±68	1289±48	2.38
Site 5 Çaber	39±5.3	2235±47	40.8±3.5	1164±52	1548±78	0.75
Site 6 Radoniq H-A	31.7±1.7	2234±51.6	101±13	1711±62	1004±32	1.70
Site 7 Q. Prushit H-A	38.9±4.1	2272±85.2	90.7±5.8	2289±94	1782±44	1.28
Site 8 Mushtisht H-A	34.8±2.9	2428±84.4	68.7±8.2	1235±75	2680±115	0.46
Site 9 Badovc H-A	56.4±2.1	1049±98.3	67.4±7.6	2323±78	1928±65	1.21
Site 10 Kaqandoll H-A	33.7±1.7	1837±66.5	62.9±6.3	1300±110	1315±42	0.99

High calcium content in these hyperaccumulator plants is due to the unusual ability of these plants to accumulate high Ca concentration in their tissues, even from the soils with low Ca/Mg ratio which is typical property of serpentines soils. Consequently, these plants can lower Ca deficiency stress for themselves.

Table 2. Hyperaccumulators candidates for phytomining and agromining

Nr	**Species**		**Zn**	**Ni**	**Ca**	**Mg**	
		Sample Site		mgkg^{-1}			Ca/Mg Ratio
1	*Odontarrhena muralis (1)*	1	87.8	7010	34700	1640	21
2	*Odontarrhena muralis (2)*	1	58.9	7280	16490	2730	6
3	*Odontarrhena muralis (1)*	2	73.4	7564	23720	1860	13
4	*Odontarrhena muralis (2)*	2	59.2	6871	11580	1820	6
5	*Noccaea ochroleuca (1)*	2	425	4303	12840	1400	9
6	*Noccaea ochroleuca(2)*	2	512	5888	8770	900	10
7	*Odontarrhena muralis*	3	35.1	3066	13055	1138	11
8	*Odontarrhena muralis*	4	143	4039	5136	2343	2
9	*Odontarrhena muralis*	5	30.8	1586	20313	6252	3
10	*Odontarrhena muralis*	6	70.2	2980	15625	2343	7
11	*Noccaea ochroleuca*	6	593	799	14844	1772	8
12	*Odontarrhena muralis*	7	19.8	2742	5131	524	10
13	*Odontarrhena muralis*	8	34.8	7117	10493	2658	4
14	*Odontarrhena muralis*	9	22.1	2446	2905	1165	2
15	*Noccaea ochroleuca*	9	177	5862	6548	7859	1
16	*Odontarrhena muralis*	10	73.6	2764	16861	3609	5

Table 3. Macronutrients concentrations in soil

Site		**mg kg^{-1} DM**			**% DM**	**% DM**
	N	**P**	**K**	**Ca**	**Mg**	**Fe**
Vejshtine HA	440	105	104.7	3709	17.90	4.87
Vejshtine HB	320	91	88.6	5422	18.76	5.06
Çaber HA	510	120	471.7	1837	17.77	5.15
Çaber HB	470	89	140.9	728	19.20	4.50

Çaber HC	670	108	52.2	1072	19.29	3.65
Radoniq HA	1020	370	382.6	1845	16.00	3.93
Radoniq HB	980	215	149.5	629	19.37	2.43

4. CONCLUSIONS

The serpentine soils samples collected from the 10 different sites showed high Ni, Zn, Mg and Fe concentration. The high concentration of available Mg and Fe, and low to moderately high available Ca concentration make the soils be more or less of typical ultramafic content.

Presence of hyperaccumulator plants is so diverse. *Odontarrhena muralis* and *Noccaea* are dominant species, but *Noccaea* could be occasionally found in serpentine areas. Results report about a close relationship between the Ni amount in soil and Ni uptake in plants. The highest concentration of Ni in *Odontarrhena muralis* is exactly where the bioavailable Ni is at its spike. The ratio between the concentration of bioavailable Ni in the hyperaccumulative plant and the corresponding soil is 60:1.

These plants species can be used for phytomining and agromining purposes, when applying some additional measures to improve the edaphic conditions and fertilizers such as NPK and moderate amounts of Ca to soil not only to support the growth of plants, but also to decrease the soil pH, hence increasing the metal bioavailability (Bani *et. al*., 2008)

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