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# Assessment of *Alyssum murale* Waldst. & Kit. behavior on an infertile ultramafic area in Albania

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**Abstract:**

*Serpentine topsoil and subsoil were collected for this study from former Chromium and Ferro-nickel mining area located on an ultramafic outcrop in the north part of Albania in Kukes region. The objective of this study was to evaluate Alyssum murale Waldst. & Kit. behavior in infertile ultramafic areas and moreover to evaluate the influence of Ca on nickel uptake and biomass production of Alysum murale and Trifolium pratense under the same background of treatment with chemical fertilizers. A greenhouse pot experiment was undertaken in which A. murale was grown in two type of soil* *materials: the first topsoil of serpentine soil of Kukes (0-30 cm) noted K1 (Kukes1) and the second subsoil taken more than 2 m depth of first serpentine sites noted K2 (Kukes 2). Alyssum murale seeds, used in this study were collected in the same area. The plots were treated with N,P K, (100 mg kg$^{-1}$), and 50 mg CaO kg $^{-1}$soil (DM). Ten plants were left per pot and were harvested after seven months. This study showed significant differences between the subsoil and topsoil of ultramafic outcrop of Kukes. The addition of Ca to the topsoil and to the subsoil of Kukesi ultramafic material contributed to the growth of the biomass production of Alyssum murale and Trifolium pratense as well. Furthermore, the results show a very strong Ca influence Ni accumulation by Alyssum murale. The soil Ni uptake from the clover plants is not affected by soil material or the addition of Ca to the soil. The nature of soil materials and Ca affected the biomass and in Ni phytoextraction yield of Alyssum murale plants. Data showed that Alyssum murale could be an alternative to cover the infertile soils.*

**Key words:** serpentine, hyperaccumulator, *Alyssum murale*, topsoil, subsoil

## INTRODUCTION

Ultramafic bedrock are usually enriched in Mg, Fe, Mn, Cr, Ni, and Co, whereas the concentrations of Ca, N, P, K, Mo and B are typically low (Baker and Brooks, 1989; Brooks 1987; Proctor and Woodell, 1975). Albanian serpentine soils are developed in ultramafic rocks and have three main features: high levels of Ni, Cr and Co; rich in Mg and poor in Ca; and very low content of macronutrients (N, P, K) in soil (Shallari 1997; Shallari et al. 1998; Bani et al 2009, 2010, 2018a, b). Serpentine soils are difficult terrains for plant growth because of high Mg:Ca ratio, lack of macronutrients (especially P) and in some cases lack of water (Proctor and McGowan, 1976, Bani et al. 2009). Heavy metals hyperaccumulator plants represent species that are quite well adapted and/or have evaluated over terrains with high content of these elements (Çuni et al. 2015, 2016). Chaney (1983) was the first to propose the use of metal hyperaccumulator plant species for soil remediation, and introduced the concept of phytomining a technology that uses such plants to accumulate soil Ni into the plant shoots where they can be harvested and used as an alternative ore for Ni (Nkrumah et al. 2018). Local Ni hyperaccumulator species are recommended for phytomining because of their adaptation to local edaphic conditions (Bani et al. 2007; Bani et al. 2014). Hyperaccumulator species have been adapted to "specific" terrains by supporting limiting growth factors. In the case of serpentine soil, the limiting factors are the toxicity of Mg, the low content of nutrients and the lack of water (Bani et al. 2014). Baker AJM (1999) showed that some plant species can be used to cover the asbestos mine wastes area. Previous study of post-mining area shows that Ni hyperaccumulator *Alyssum murale* or other tolerant species can be grown sporadically and cover the infertile terrains such as Cr or Fe-Ni mining waste damps (Shallari et al. 1997; Osmani et al 2018). However, the mining waste or the sides of roads constructed through ultramafic formations have difficulty to be colonized by spontaneous flora (Sallaku et al. 2002; 2003). Such sites are subject to erosion and are considered to be high environmental risk for the spread of contamination to the water and the food chain (Figure 1).

**Figure 1 Views from Cr and Fe-Ni mining waste in Albania**

Many authors show that high content of Mg reduces the availability of Ca for the plants. This hypothesis has been supported over the years by a number of studies according to which the addition of Ca to the soil increases the possibility of growing plants in serpentine terrains. (Kruckeberg, 1984).

The objective of this study was to evaluate *Alyssum murale* Waldst. & Kit. behavior in infertile ultramafic areas moreover to evaluate the influence of Ca on nickel uptake and biomass production of *Alysum murale* and *Trifolium pratense* under the same background of treatment with chemical fertilizers.

## 2. MATERIAL AND METHODS

### The study area

The study site is a former Chromium and Ferro-nickel mining area located on an ultramafic outcrop in the north part of Albania in Kukes region. The region is part of the north Albanian mountain climatic region, with Mediterranean influence. The average temperature of January throughout the entire site reaches low values. Average temperature oscillate from a minimum -2° to -3°C (January) to maximum 19°-21°C (from June to August), and annual average between 9° and 12°C. The average annual precipitation ranges from 1300 up to 1600 mm, with rainfall occurring mainly from the end of October to the end of May (Bani et al. 2009).

About 600 kg soil material was taken *in Kuksi site*. The experiment was designed using two soil materials: the first top soil of serpentine soil of Kukes (0-30 cm) noted K1 (Kukes 1) and the second subsoil taken more than 2 m depth of first serpentine sites noted K2 (Kukes 2). *Alyssum murale* seeds are collected in the same area.

**Table. 1. Physico-chemical characteristics of the studied soils, Kukes-Albania**

| | Serpentine soil K1 | Underground material K2 |
|---|---|---|
| Clay < 2 µm (mg $g^{-1}$) | 230 | 122 |
| Silt : 2-20 µm (mg $g^{-1}$) | 145 | 56 |
| Silt : 20-50 µm (mg $g^{-1}$) | 112 | 44 |
| Sand : 20-200 µm (mg $g^{-1}$) | 314 | 450 |
| Sand : 200µm-2 mm (mg $g^{-1}$) | 199 | 328 |
| pH $H_2O$ (1:2.5 soil-water) | 7.89 | 8.42 |
| Organic matter (mg $g^{-1}$) | 12.3 | 3.4 |
| N total (mg $g^{-1}$) | 0.62 | 0.20 |
| P total (mg $kg^{-1}$)s | 142.1 | 90.7 |
| Mg (mg $kg^{-1}$) | 29307.3 | 28979.2 |
| K (mg $g^{-1}$) | 115.2 | 89.16 |
| Ca (mg $kg^{-1}$) | 948.0 | 3789.5 |
| Ni total (mg $kg^{-1}$) | 2017.37 | 1682.43 |

The data in the table 1 above show significant differences between the material obtained in the subsoil and topsoil of serpentine soil (0-30cm). The material taken from the depth varies in the texture characteristics with less clay and dominates the fractions of sand and gravel. The content of macroelements is lower in the material obtained from the depth as well as the ratio Mg: Ca in favor of Mg. The physico-chemical characteristics of K2 confirm that conditions for plant growth are difficult. Soil samples were air-dried and sieved to 5 mm and placed in plastic plots (6 kg of soil dry matter) over a granite stone drainage previously purified with diluted acid and distilled water.

**Experimental plots treatments**

The soil was placed to the field capacity for 24 hours with distilled water. The plots were treated with N 100 mg $kg^{-1}$, P 100 mg $kg^{-1}$and P 100 mg $kg^{-1}$ of dry soil by dissolving the fertilizers in water and using 0.5 litre of solution for each pot. In the same way we added 50 mg CaO kg $^{-1}$soil (DM). Seeds were distributed randomly using 20 seeds for each plots( in

*Alyssum murale* or *Trifolium pratense* pots) and ten seeds for each species in case of pots with both plants (*Alyssum murale* + *Trifolium pratense*) (Figure 2).


**Figure 2. Experimental plots treatments scheme**

Plants germinated after 10 days and 1 week later, for each plots we left 10 plants. The experiment was conducted during the October 20$^{th}$,2014 to June 4$^{th}$,2015 (7 months). The pots were irrigated throughout the period with distilled water. Treatments were performed in 5 replicates by adding the pots without plants. These 5-replicates are subject of any treatment (fertilization and irrigation) throughout the experiment time. The greenhouse conditions were about 20°C and air humidity around 60%. Plots were at 70% of the field capacity by adding distilled water as needed. The soil pH is determined in a soil/ water suspension (1:5) with a pH-meter model Sartorius PB-11 (EN ISO 10523: 2012 method). Samples of soil were taken for each pots in the, dried and a sample was prepared for analysis. The biomass obtained after seven months of vegetation from each pot was purified with distilled water and dried in the air, then weighed and an composite sample was taken for analysis. For each pot, average biomass production was calculated.

Samples were mineralized with a microwave digester (Ethos One Pro-24), where 0.3 g of soil or plants sample was digested by adding 8 ml HNO3 69% and 2 ml $H_2O_2$. Solutions were filtered and were adjusted to 50 ml with distilled water. Heavy metals were determined spectrochemically using Atomic absorption spectrophotometer (Nov AA-350). The concentration of Ni, Cr, Co, Ca and Mg is determined by AAS (atomic absorption spectrophotometry), model NovAA 350 and 400P (Agro-Environmental Science Laboratory, University of Agriculture of Tirana). The reference materials were analyzed in order to ensure the quality of the data. In plant samples was measured the amount of Ni accumulated by the plants.

## RESULTS AND DISCUSSIONS

The biomass of plants in the studies pots was evaluated as the average of the whole biomass harvested in pots of every treatment.

Data based in dry biomass production of every treatment indicate a strong influence of Ca which was added to the soil. The addition of Ca to serpentine soil has increased the biomass production of *Alyssum murale* from 23.12 g to 42.57 g dry matter per plots. Also, the addition of Ca on the soil taken from subsoil has contributed to increase the biomass production of *Alyssum murale* from 13.54 g to 28.22 g DM per plots. The same Ca influence is observed in the growth of biomass of *Trifolium pratense* or *Alyssum murale* associated with *T. pratense* (figure 3).

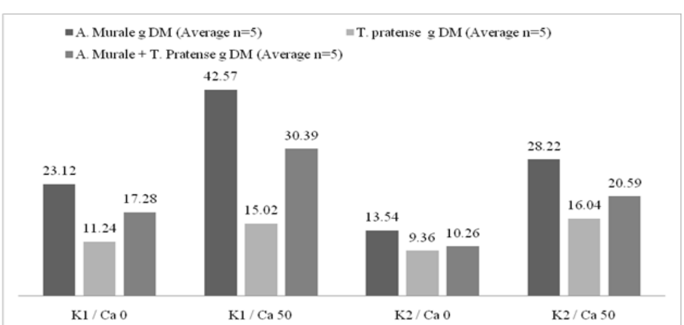


**Figure 3. Biomasses production of *A. murale* and *T. pratense* according to experimental treatments (g per pot) (average n=5).**

*Alyssum murale* accumulate high concentration of soil Ni in their biomass. The data showed that the highest concentration of Ni accumulates in treatments where Ca is added. Nickel concentration in *Alyssum murale* on serpentine soil without Ca was 13368 mg Ni $kg^{-1}$ DM while it was 15654 mg Ni $kg^{-1}$ DM in the case of serpentine soil where Ca is added (Figure 4).

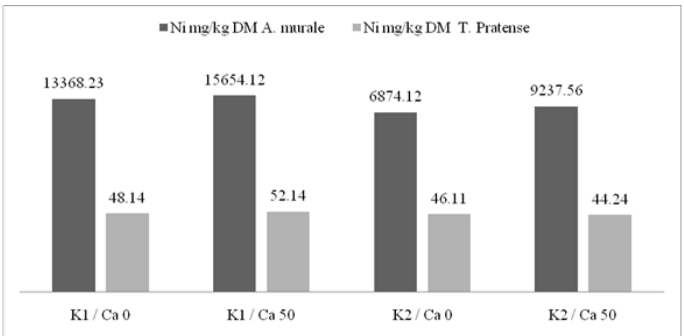


**Figure 4. The Ni concentration (mg $kg^{-1}$) in *A. murale* and *T. pratense* according to experimental treatments (average n=5).**

This indicates the very strong influence of Ca in the accumulation of Ni by the *Alyssum murale* plant.The same trend is observed in the case of plant cultivation on subsoil material (K2). Nickel concentration in *Alyssum murale* was 6874 mg $kg^{-1}$ DM in pots without Ca (added) while it was 9237 mg Ni $kg^{-1}$ DM in plots with Ca.The results showed that the accumulation of Ni from the clover plants is not affected by the nature of soil materials or the addition of Ca on the soil. The Ni concentration in *Trifolium pratense* biomass, ranges from 48-52 mg Ni $kg^{-1}$ in the case of serpentine soil and from 44-46 Ni $kg^{-1}$ DM for the subsoilterial. The Ni content in the red clover biomass is similar to the results reported by Shallari (1997) and Osmani et al (2017). Figure 5, shows the amount of Ni phytoextracted from the *Alyssum murale* for each plot.

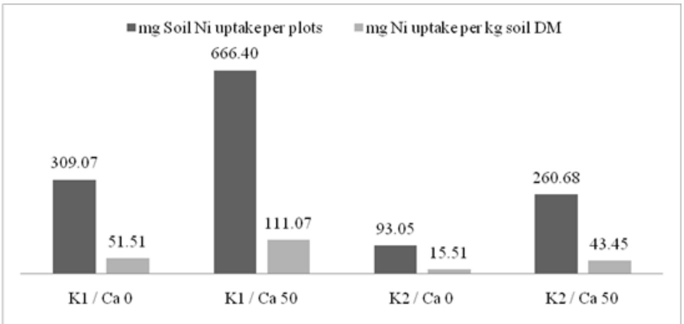


**Figure 5. Soil Ni phytoextracted by *Alyssum murale* (mg Ni per pot and mg Ni $kg^{-1}$soil)**

The type of soil materials and the addition of Ca affected the production of biomass and the Ni phytoextraction by *Alyssum murale*. The results showed that the addition of Ca to the top soil of serpentine soil has increased the amount of metal extracted for each plots from 309 to 666 mg per pot or from 51 to 111 mg Ni $kg^{-1}$of soil DM. In the case of extracted soil Ni from *Alyssum murale* the quantity increases from 15.5 to 43.4 mg Ni per kg of soil DM. These data prove that Ca has a very important role in increasing biomass but also in Ni phytoextraction of *A. murale*, confirming the fact that Ca /Mg ratio in soil is a limiting factor for the growth of plants in serpentine soils). 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 increase in biomass yield after fertilization (Bani et al 2007, 2018a).

The Ni content in the *Trifolium pratense* plants is in low levels and remains unaffected by the addition of Ca to the soil. On the figure 6 data are presented in (µg / DM). Differences are the product of Ca's influence on biomass produced by the clover plant. The Ni concentration in *Alyssum murale* was measured in both cases, alone or associated with the red clover. It was higher for both soils materials in the case when *A. murale* was associated with *T. pratense* and in the pots where we added Ca (Figure 6).

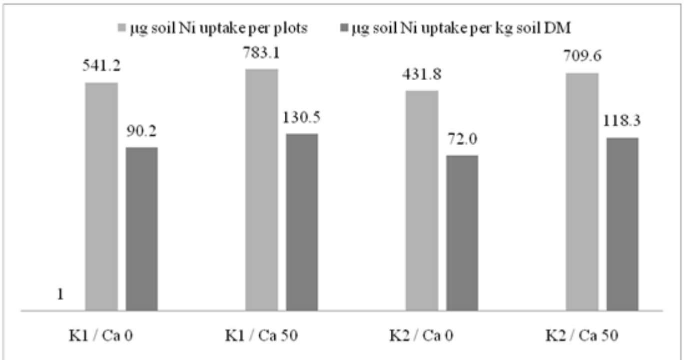


**Figure 6. Soil Ni phytoextracted (µg per pot and µg per kg soil) by *Trifolium pratense***

The biomass obtained from the pots when *Trifolium pratense* was associated by *Alyssum murale*, was higher than when it was alone. The results showed that Ni content in the plants of *Alyssum* is also influenced by the addition of Ca to the soil K1 and K2. But in all cases, the amount of Ni accumulated by *Alyssum* is higher in the biomass of plants associated with clover (Figure 7). This fact can be explained by the possible impact of the clover rhizosphere environment on soil Ni availability (Shallari et al. 2001).

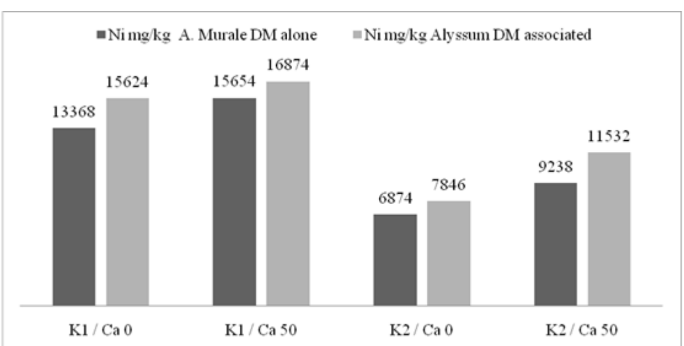


**Figure 7. Ni concentration (mgkg$^{-1}$) in *Alysum murale* plants for both treatments; alone and associated**

The presence of red clover has helped the Ni movement in a higher flow to the soil solution. *Trifolium pratense* influence may be explained by the tendency to lower soil pH or other mechanisms that need to be studied in the future.

## CONCLUSIONS

The addition of Ca to the topsoil of serpentine soil (K1) or to the subsoil material (K2) in Kukesi serpentine soil has contributed to the growth of the biomass yield of *Alyssum murale* and *Trifolium pratense* as well. Furthermore, the results showed a very strong Ca influence on the accumulation of Ni by hyperaccumulator plant, *Alyssum murale*. The soil Ni uptake from the clover plants is not affected by soil material or the addition of Ca to the soil. The Ni content in red clover biomass was similar to the results reported by other authors. The nature of soil materials and soil Ca affected the biomass and Ni phytoextraction yield of *Alyssum murale*. The concentration of Ni by *Alyssum* is higher in plants associated with clover. The presence of red clover affects to Ni bioavailability of the soil. Ca added to the soil has a very important role in increasing biomass but also in uptake of soil metal from *A. murale* plants, confirming the fact that the high Mg/Ca ratio in soil is a limiting factor for the growth of plants in serpentine soils. Data showed that *Alyssum murale* could be an alternative to revegetate the infertile soils where, the possibilities for growing other species are small.

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