

RESEARCH ARTICLE



Variation in nickel accumulation in organs of *Alyssum murale* from serpentine site of Albania

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Abstract

Environmental factors, such as climate and chemical land composition, are a primary force in shaping the distribution of plant species. While many plant species can be found growing in a variety of habitats, some species become entirely restricted to a particular soil type. Serpentine soils, that constitute 10% of the Albanian surface, provide one of the most remarkable examples of plant adaptation to atypical soils. Albania has a very rich nickel hyperaccumulator flora which could serve as candidates to be used in phytoextraction and phytomining. Phenological studies give contribution for obtaining adequate information on the way to the appropriate time of collection of the hyperaccumulator plants. The metal accumulation amounts vary based on the plant organs, and as well as by the stages of its biological cycle. During our work we observed the variation in nickel absorption in the organs of *Alyssum murale* in a typical Albanian Vertisols; Prenjas. Samples of soils and plants were collected during the flowering period of *A. murale* plant. In serpentine soils of Prenjas the available nickel was 24 mg kg⁻¹. Depending on climate, macronutrient and heavy metals in soils the heavy metal concentration in stems, leaves and flowers in Prenjas samples of *A. murale* were respectively 4666, 15855, 12302 mg kg⁻¹. Leaf and flower were the plant organs with the highest concentration of nickel. So this population is promising for the phytomining processes.

Keywords: phenology; Nickel hyperaccumulators; Heavy metals; *Alyssum* species; Plant organs

1. Introduction

Serpentine soils, that constitute 10% of the Albanian surface, provide one of the most remarkable examples of plant adaptation to atypical soils. Serpentine soils contain high levels of heavy metals such as Ni, Cr, Co, Fe, also they are rich in Mg. Some of these are toxic for plants. Serpentine soils often are deficient in essential plant nutrients such as nitrogen, potassium, and phosphorus, calcium and their physical conditions are also inhospitable for many plants [4]. Environmental factors, such as climate and chemical land composition, are a primary force in shaping the distribution of plant species. The metal accumulation amounts vary based on the plant organ, but also by the plant during the Phenological development stages of its biological cycle.

While many plant species can be found growing in a variety of habitats, some species become entirely restricted to a particular soil type. Albania has a very rich nickel hyperaccumulator flora which could

serve as candidate to be used in phytoextraction and phytomining [1, 2, 3].

Plant phenology involves the stage, duration and abundance of current biological phenomena, including reproductive events such as flowering, fruiting, seed dispersal and germination. Phenological studies contribute in obtaining the adequate information on the way to the appropriate time of collection of hyperaccumulator plants collection. Hyperaccumulation of Ni, i.e., accumulation in aerial parts up to concentrations above 1000 mg kg⁻¹ on a dry-matter (DM) basis [5, 12] was first discovered in *Alyssum bertolonii* in Italy [11]. The aim of this study is to determine the most effective populations of nickel hyperaccumulator plants in the southeast of our country, as one of the possible areas to apply phytomining. Our objectives were; phenological recognition of characteristics in *Alyssum murale* populations depend of edaphic conditions;

Dynamic evaluation of metal absorption by natural populations of *Alyssum murale* plants in Prenjas in different phenological phases;

Evaluation of the relationship between metal availability in the soil and nickel accumulation potential of *A. murale* populations.

2. Material and Methods

2.1 Study Area

The studied area is situated on the southeastern part of Albania, in Prrenjas which is a typical serpentine soil, mainly in areas populated by *A. murale* individuals.

The site at Prrenjas surrounds the city of Prrenjas at an elevation of 600 m. The parent material is rich in Fe, Ni, Cr, and Co. Sampling was conducted on serpentine field Domosdova field, which contains a soil developed from a colluvium of ultramafic and magnesite origin (Cambic Hypermagnesian Hypereutric Vertisol; 41°04'08"N, 20°33'11"E). These areas were chosen and surveyed because they host a significant presence of the hyperaccumulators in these southeastern regions [2, 3].

In the above mentioned areas we have taken 3-5 specimens (the whole plant) as plant samples. Soil samples were also taken as compound samples collected randomly at the different precise locations of plant sampling (in a 0.5-m radius area around the plants). Soil was collected from the upper horizon at a depth of approximately 20 cm (when possible) or less, air-dried, and sieved to 2-mm.

2.2 Identification of the phenological stages for each site

We have monitored the plant development in 20 different individuals as well as observe the phenophase for each periodic monitoring, which was in two first weeks of each month, beginning from January and ending in December. Parameters that we measured for each phenological stage are the leaf length (at the top and base), as well as the length of

the plant, that helps us to determine the most appropriate harvesting time of plants.

The measurements were during the phenological stages; W-vegetation, O-bloom, Fl-flowering and Fr-fruitification [7].

2.3 Soil and plant analyses

Trace metal and macroelements (Mg, Ca, Fe) concentrations in shoots and leaves of *Alyssum murale* were analyzed 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% HNO₃ and 2 mL of H₂O₂. Solutions were filtered and adjusted to 25 mL with 0.1 M HNO₃. Soil pH was determined in a soil/water (1:2.5) suspension with a pH-meter. Nickel availability in different soil samples was characterized by DTPATEA. DTPA-extractable Ni was determined using the method of [9]. Concentration of Ni in soil extracts was determined by plasma emission spectrometry (ICP-OES).

3. Results and Discussion

It is important to know the size of the plant because the biomass has a direct effect in Ni phyto extraction yield. Table 1. shows us that in the middle of the blooming time, we notice that whole plants and leaves have bigger size for the populations of Prrenjas. This is in accordance with [1], for *Alyssum murale* grown naturally in Pojska site, Pogradec.

The chosen area is typically serpentine soils. Ultramafic rock and derived soils (serpentine) are characterized by: Ca deficiency, low Ca/Mg ratio, high concentrations of Fe, Mg, Ni, Co, Cr and low concentrations of plant nutrients (P.N.K). The highest level of Fe in *A. murale* is found in the site 1 (97920 mg/kg). The Ca concentration is less than 4000 mg kg⁻¹ while Mg concentration is very high up to 71440 mg kg⁻¹.

Table 1. The size of the whole plants and leaves of *Alyssum murale* plants- Prrenjas taken on during flower time.

| Areas Prrenjas | The plant length | | Leaf/base | | Flower | | Fruit | |
|-------------------|------------------|----|-----------|-----|--------|-----|-------|-----|
| | (cm) | | (cm) | | (cm) | | (cm) | |
| | L | W | L | W | L | W | L | W |
| 22.05.2016 | 45 | 20 | 1.3 | 0.4 | 2 | 0.3 | - | - |
| 12.06.2016 | 55 | 36 | 3.8 | 0.5 | 1 | 0.9 | 0.3 | 0.3 |

Table 2. Total major elements trace in Perrenjas mg kg⁻¹

| Area | Al | Ca | Fe | K | Mg | Mn | Na | P | S |
|-----------|-------|------|-------|--------|-------|--------|-------|-------|-------|
| PERRENJAS | | | | | | | | | |
| Site 1 | 17686 | 3654 | 97920 | 1977 | 69320 | 1515,6 | 82,4 | 183,2 | 143 |
| Site 2 | 17312 | 3332 | 96200 | 1938,6 | 70800 | 1492,2 | 79 | 176,6 | 136,6 |
| Site 3 | 17574 | 3656 | 96680 | 2002 | 71440 | 1543,6 | 101,4 | 185,2 | 149,4 |

Table 3. Total trace elements (mg kg⁻¹) in Pprenjas site.

| Area | As | B | Co | Cr | Cu | Ni | Pb | Zn |
|-----------|------|------|-------|-------|-------|------|------|-------|
| Perrenjas | | | | | | | | |
| Site 1 | 1.67 | 9.54 | 136.2 | 601.5 | 16.25 | 3504 | 5.28 | 72.76 |
| Site 2 | 1.77 | 9.3 | 168.1 | 605.1 | 65.48 | 3462 | 4.75 | 178.3 |
| Site 3 | 1.82 | 9.16 | 137.6 | 559.9 | 16.77 | 3516 | 5.48 | 72.86 |

Table 4. Metal availability (DTPA_{nickel} mgkg⁻¹) in serpentine soils of Pprenjas.

| | Cu | Fe | Mg | Mn | Ni | Pb | Zn |
|-----------|------|-------|---------|------|-------|------|------|
| PERRENJAS | | | | | | | |
| Site 1 | 0.70 | 16.37 | 1068.60 | 6.03 | 56.74 | 0.09 | 0.45 |
| Site 2 | 0.74 | 16.67 | 1087.40 | 6.54 | 57.54 | 0.10 | 0.40 |
| Site 3 | 0.71 | 16.10 | 1044.00 | 6.09 | 55.02 | 0.10 | 0.37 |

Table 5. Ni, Ca, Mg concentration in *A. murale* organs in different time during flowering phases.

| Perrenjas | | Ni mg/kg | Ca mg/kg | Mg mg/kg |
|------------|---------|----------|----------|----------|
| 22.05.2016 | Roots | 1905 | 3556 | 2.746 |
| | leaves | 12302 | 48466 | 3.937 |
| | flowers | 12945 | 26568 | 7.357 |
| | shoots | 4977 | 7007 | 1563 |
| | Plant | 6357 | 30240 | 4.563 |
| 12.06.2016 | Root | 2258 | 3280 | 4.098. |
| | leaves | 15855 | 21920 | 4.517 6 |
| | flowers | 13302 | 26568 | 7.357 |
| | shoots | 4668 | 6024 | 2.558. |
| | Plant | 7401 | 20218 | 5.038 |

Distributions of Ni hyperaccumulators depend on the biology of the species and edaphic factors [4, 9]. Ni concentration is very high (3462-3504 mg kg⁻¹), typical for serpentine soils. This finding is also in accordance with [2]. Also Cr and Co concentration is high. Co concentration varied from 136.2 to 168.1 mg kg⁻¹. Cr concentration in our Pprenjas sites varied from 559 to 605.1 mg kg⁻¹.

DTPA-extractable Ni (Ni DTPA) was chosen as an estimate of soil Ni chemical availability. Although it only reflects the potential pool of available Ni [1] it allows relative comparison of soils. The amounts of DTPA-extracted Ni were higher for the soils of southeastern Albania. The available level of nickel in the area of Pprenjas is on the average 56 mg/kg. Perrenjas Site 2 (57.54 mg kg⁻¹), Perrenjas-

Site 1 (56.74 mg kg⁻¹). The available Mg- is higher than Ca. The high level of Ni in our soils and its subsequent transfer to plants depend mainly on its mineralogical origin and as well as on the local pedogenetic properties which result from climatic conditions and weathering history of the soil.

It is important to know Ni in the plant because more Ni in plant, more Ni phytoextraction yield. In terms of plant organs, the highest concentration of nickel results in leaves and flowers. Analysis of metals performed on plant bulk shoots (i.e. stems, leaves and flowers/ fruits) and on leaf material showed different plant responses to the presence of Ni, Ca, Mg in soils collected at the different sites of Prrenjas. The highest level of Ni in *A. murale* is found in flowers (13302 mg/kg). If we compare the data of the two different moments, we can say that the level of Ni in the plant and in its organs is higher on June 12, which seems to be the best time to harvest plant.

4. Conclusions

Serpentine soils in the southeast of Albania, Prrenjas soil is suitable for phytomining, because the level of nickel available is higher, and also exist nickel hyperaccumulator plants.

The populations of Prrenjas are more effective in terms of nickel up take due to the high concentration of Ni in plants in comparisons of nickel availability in soil.

The populations of Prrenjas result in bigger plant sizes. The second week of June seems to be the most appropriate time for the harvest since the plant sizes are bigger and the concentration of nickel is higher. Leaf and flower are plant organs where the concentration of nickel is higher. Both populations are promising for phytomining of Nickel.

5. References

- Bani A, Echevarria G, Sulçe S, Mullaj A, Morel J.L: **In- situ phytoextraction of Ni by native populations of *A. murale* on an ultramafic site in Albania.** Plant Soil. 2007, 293, 79–89
- Bani A, Echevarria G, Mullaj A, Reeves R.D, Morel J.L, Sulçe S: **Ni hyperaccumulation by *Brassicaceae* in serpentine soils of Albania and NW Greece.** Northeastern Naturalist 16 (Special Issue 5). 2009, 385–404
- Bani A, Echevarria G, Morel, J.L, Sulçe S: **Improving the Agronomy of *Alyssum murale* for Extensive Phytomining: A Five-Year Field Study.** International Journal of Phytoremediation. 2015, 17:117-127 .
- Brooks R: **Serpentine and its Vegetation (A Multidisciplinary Approach).** Dioscorides Press, Portland, Oregon. 1987
- Brooks R, Lee J, Reeves R.D, Jaffré T: **Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants.** Journal of Geochemical Exploration. 1977, 7:49–57.
- Echevarria G, Massoura S.T, Sterckeman T, Becquer T, Schwartz and Morel J.L: **Assessment and control of the bioavailability of nickel in soils.** Environmental Toxicology and Chemistry. 2006, 25, 643-651.
- Gjeta, E.: **Study on flora, vegetation and natural habitats diversity in middle flow of Shkumbini river basin (Tiranë).** PhD Dissertation. 2014, 142.
- Kabata-Pendias A: **Behavioural properties of traces metals in soils, Applied Geochemistry.** 1993, 2, 3–9.
- Lindsay W.L, Norvell W.A: **Development of DTPA soil test for zinc, iron, manganese, and copper.** Soil Science Society of America Journal 1978, 42, 421–428
- Massoura S.T, Echevarria G, Becquer T, Ghanbaja J, Leclerc- Cessac E, Morel J.L: **Nickel bearing phases and availability in natural and anthropogenic soils.** Geoderma. 2006, 136, 28–37.
- Minguzzi C, Vergnano O: **Il contenuto di nichel nelle ceneri di *Alyssum bertolonii*** Desv Atti Soc. Tosc di Sci Nat , Mem. Serie A. 1948, 55: 49-77.
- Reeves R.D: **The hyperaccumulation of nickel by serpentine plants. . 253–277**