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## The natural plant colonization of ultramafic post-mining area of Përrenjas, Albania

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### ABSTRACT

A post Ferro-nickel mining land management needs to recognize the physical, chemical characteristics of soil and plants that grow naturally by tolerating the levels of heavy metals. The objective of this study were to: i) investigate and evaluate the natural process of plant colonization on abandoned ferro-nickel mining sites located on ultramafic substrate; to ii) understand the relationship between soil properties and species distribution and to iii) assess the potential of hyperaccumulator plants for the soil phytoremediation.

This area was divided into five 100 m<sup>2</sup> plots that were representatives of the variability of the site, based on localization, land use and total vegetative cover. For each plot, the physicochemical characteristics of the soils, the inventory of plant species and the accumulation of Ni by all plant taxa were determined. The soils in the ex-mining area of Përrenjas appeared highly polluted by Ni, Co and Cr compared to the natural levels of the surrounding ultramafic soils. The toxic levels of trace metals and the deficiency of organic matter appeared as major constraints for plant colonization. In total, 96 plant species belonging to 26 families in the studied mining sites of Përrenjas were recorded. Several plants show the ability to tolerate and accumulate metals and may be useful for phyto-stabilization. *Alyssum murale* was the best candidate for Ni phyto-extraction technologies, with concentrations above 1000 mg/kg Ni in plant tissues.

Keywords: ex-mining serpentine area; heavy metals; nickel tolerance; organic matter; plant colonization.

### INTRODUCTION

The industrial activities led to numerous adverse environmental changes, thus the normal development of the vegetation was prevented. Ultramafic substrates and their derived minerals additionally may impose particular

constraints to plant establishment because these soils contain high concentrations of Mg and Fe, and relatively high concentrations of Ni, Cr and Co, as well as deficient levels of major nutrients (i.e. K and P). Ex-mining ultramafic areas are often almost completely devoid

of vegetation due to toxic levels of trace metals and other unfavorable edaphic physico-chemical conditions (Echevarria and Morel, 2015). Some of the soil properties affecting plant growth include soil texture, organic matter, aggregate size, porosity, aeration and water holding capacity (Campbell et al., 1996).

The development of vegetation in metal contaminated areas depends on the metals mobility in soil and on the availability for plant (Simon, 1978). Previous studies have shown that seeds coming from surrounding areas through various natural factors (Chambers and Sidle, 1991; Marrs and Bradshaw, 1993) can overgrow on ex-mineral sites. Natural colonizers of operating sites or disused dumps possess adaptations to particular facets of environmental stress, especially metal toxicity and the lack of major nutrients.

Plants like metalliferous, develop different strategies for survival in harsh environmental conditions. Usually, non-metalliferous plants suffer from metal stress, which results in injury or death of the plant, whereas metalliferous plants survive and reproduce by strategies of avoidance or tolerance to stress of high metal concentrations (Antonovics et al., 1971; Baker, 1987). Plants growing on metal-loaded soils respond by exclusion, indication or accumulation of metals (Baker, 1981). Some of the plants make stable metal complexes in the root cells to prevent metal translocation from the roots to above-ground tissues (Ashraf et al., 2011). Plants that tolerate metals are capable to accumulate them in plant cells in different concentrations. Such plants colonize the greatest part of the metalliferous areas, associated in typical plant communities. The ability of plant populations to evolve metal tolerance is one of the most important characters that determines the structure, density and development of the vegetation in such areas (Simon, 1978). The abilities of plants to evolve tolerance against serpentine stress depends on their genetic resources and takes more time for plants growing on naturally metalliferous soils (Kuta et al., 2014). A number of plant species endemic to metalliferous soils have been found to accumulate metals to extraordinarily high levels (>1%) in contrast to normal concentrations in plants. Approximately 400 metal hyperaccumulators have been identified (Baker, 1995) and most of them (Ni hyperaccumulators) are found on ultramafic substrate. Recently, accumulators or hyperaccumulators plants are widely used in phytomining to decontaminate soils by phytoextraction.

Serpentine soils developed upon ultramafic rocks and in particular on rocks containing significant proportions of serpentine-related minerals, are widely distributed in different parts of the world (Brooks, 1987). Albania has a large proportion of its surface covered by serpentine and ultramafic soils, about 10% of the territory of the country.

It also has a high density of abandoned or active mining sites as well as metal smelters. Ferro-nickel mining was active in Përrenjas, southeast of Albania, an area where serpentine soils are also well represented. The presence of Ni hyperaccumulator plant, *Alyssum murale* Waldst. & Kit, in the serpentine area of Përrenjas was detected by Shallari et al. (2001).

The main goal of this study was to i) investigate and evaluate the natural process of plant colonization on abandoned ferro-nickel mining sites located on ultramafic substrate; to ii) understand the relationship between soil properties and species distribution, and to iii) assess the potential of hyperaccumulator plants for the soil phytoremediation.

## MATERIAL AND METHODS

### Study area

The study area is a former Ferro-nickel mining area located on an ultramafic outcrop in the east of Përrenjas (41°03'58.97" N, 20°32'21.34" E) at an elevation of 650 m. The climate in this hilly area is continental, with cold and wet winters, and hot dry short summer. The average annual temperature is 13.4 °C.

In this area, for 30 years, there have been mining activities and minerals being disposed. Since 1990, the intensive activity has been discontinued but since 1995, the zone was closed definitively. However, as a consequence of the mining activities the study area has some important problems; like landscape degradation, high concentrations of heavy metals, slope instability, visual negative impact for inhabitants, and for people that travel every day in "Via Egnatia" (50 m away from the study area). To evaluate the impact of mining the ex-industrial area was divided into five study plots around 100 m<sup>2</sup> each. This selection was done to represent differences in land use, relief, soils, plant diversity, and ecological conditions (Figure 1).

Plots 1, 2, and 3 are contaminated with mineral residues, but are also affected by erosion and deposition. Plots 2 and 3 are situated in the western part of the hill, whereas plot 1 is on the southwestern part on steep slope with scarce vegetation. In all the three plots ore deposits from ex-mining activities appear. Also, some sediment from the ultramafic hill runs off into the plot 3 periodically. In the same plot, the vegetation is denser compared to the first two plots. Plot 4 is also located in the western part of the hill, but the soil is mainly of serpentine origin, polluted by mining activities. Plot 5 is located in the southwestern flat terrain with serpentine soil.

### Soil sampling and analysis

For each of the studied plots pH, organic matter, soil texture, micronutrients (Ni, Fe, Cr, Zn, Pb, Cd, Cu, and Co) were determined.

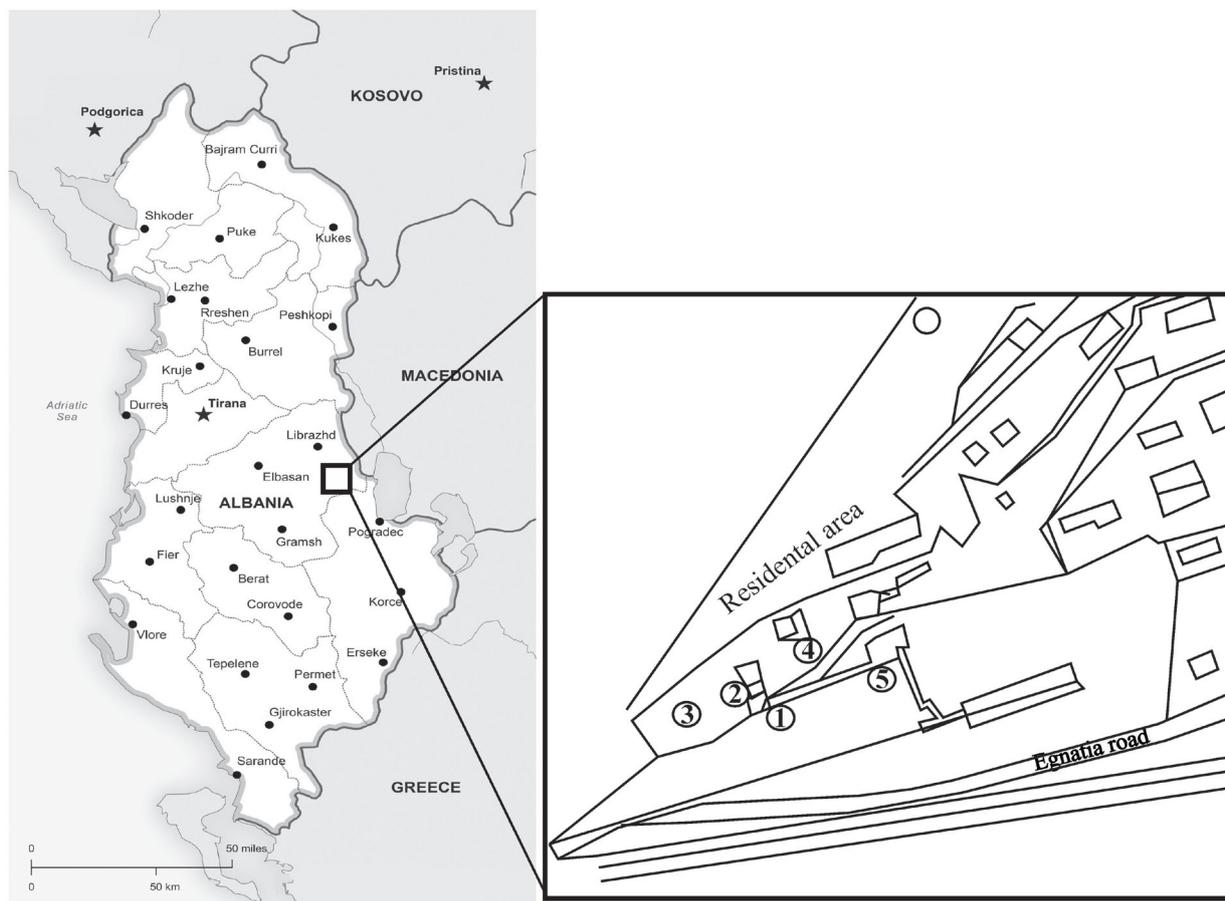


Figure 1. Map of Albania with the study area and the location of the five plots.

One to three soil samples per plot were taken from the upper horizon at a depth of 0-30 cm where possible. Soils samples were air-dried and processed in the laboratory. The determination of organic matter 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 (Bouyoucos, 1962). Total nitrogen (N) and phosphorus (P) were determined using Kjeldahl digestion method.

For determination of the trace, metals soil samples were mineralized with a microwave digester. Conditions for mineralization were 6 ml HCl, 2 ml HNO<sub>3</sub>, and 3 ml H<sub>2</sub>O<sub>2</sub> per 0.5 g soil. Nickel availability in soil samples was characterized by the DTPA-TEA, using the method of Lindsay and Norvell (1978). DTPA-extractable Ni (Ni<sub>DTPA</sub>) was chosen as an estimate of soil Ni chemical availability.

Total major (Ca, Mg, and Fe), trace elements (Co, Cr, Ni, Zn, Pb) and Ni in soil extracts were measured in digestion

solutions by atomic absorption spectrophotometry (AAS) in the Public Health Laboratory of Tirana, Albania. Exchangeable Ni, Ca and Mg (considered as the very labile and bioavailable fraction) in the different soil samples were determined by the Mehlich-3 method (Schröder et al., 2010). Soils Ca:Mg ratio were calculated. The soil pH (in water) was also measured.

#### Plant sampling and analysis

The floristic investigations were conducted from March to September 2014, in order to list plants growing in the selected plots in all vegetative seasons. Plant material was collected for determination in the Laboratory of Agro-environment and Ecology Department in Agricultural University of Tirana. The collected plants were determined following the Flora of Albania (Paparisto, 1988; Qosja, 1992, 1996; Vangjeli, 2000). The same literature was used to check plant life forms.

Plant samples (96 in total) from species found in plots were collected. Aerial plant biomass of randomly collected plants (3-5) from each species was analyzed for

Ni concentration. Additionally, Ca and Mg concentrations for Ni hyper accumulating species (in plots that appear) were measured. All plant samples were washed, dried and ground to a fine powder. Trace metal 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<sub>3</sub> and 2 ml of H<sub>2</sub>O<sub>2</sub>.

#### Data analysis

ANOVA single factor was applied to find out if there are statistically verified differences in the content of trace metals, total organic matter (TOM) and macronutrients between the five plots under study, where the plots are fixed and soil samples are collected randomly. To compare the main soils indicators in plots, multiple comparisons by Fisher's least significant difference (LSD) were applied. The used values for each plot were mean after three replications.

## RESULTS AND DISCUSSION

### Concentrations of chemical elements in the soil

The concentration of the trace metals (Ni, Fe, Cr, Zn, Co, Pb and Cu), soil macronutrient (N, P, K, Ca and Mg), pH, total organic carbon (TOC) and total organic matter (TOM) were measured for the selected sites. The results are shown in Table 1.

The plots have similar pH ranging from 8.44 to 8.64. The same nature of pH suggests a similarity to the mineralogical composition, the nature of the organic matter as well as the chemical composition of the colloidal complex. Plots 1, 2 and 3 are similar in metal concentrations and also in organic matter content, but plot 3 displays minor changes due to its position in the landscape (ground material depositions eroded by the nearby hills). Plots 4 and 5 have also mineral and organic similarities (Fe concentration as a geological indicator) but plot 4 has a higher concentration of Ni, Cr, Co, Cu and Pb as a result of contamination during mining activities. The concentration of Zn is at the normal level of ultramafic soil since it is not associated with high levels of mineral materials of ultramafic origin.

Based on the results obtained it is observed that the ranking of the total organic carbon (TOC) and total organic matter (TOM) percentage were in following order Plot 5>Plot 4>Plot 3>Plot 2>Plot 1. The percentages of TOC (2.92%) and TOM (5.03%) were the highest in plot 5 and the lowest in plot 1 (0.17% and 0.3%, respectively). The values of total organic matter in soil samples indicate initial stages of pedogenesis in plots 1, 2, 3, and more advanced stages in plots 4 and 5. The nitrogen (N) concentration in the plots is similar to that of the organic matter. The level of nutrients, organic matter, N, P in plots 4 and 5 were at normal levels for the Përrenjas serpentine

Table 1. The pH, heavy metals, macronutrients, cation exchange capacity (CEC), total organic carbon (TOC) and total organic matter (TOM) in soil of ex-industrial area of Përrenjas. Values with the same letters indicate no significant difference between CEC, TOC, TOM and concentration of elements of study plots and inverse for values with different letter (ANOVA one way, Multiple Comparisons with Fisher's LSD at the P<0.05 level).

|       | Unit                | Plot 1        | Plot 2        | Plot 3        | Plot 4        | Plot 5        |
|-------|---------------------|---------------|---------------|---------------|---------------|---------------|
| pH    |                     | 8.44          | 8.46          | 8.53          | 8.61          | 8.64          |
| CEC   | cmol/kg             | 17.01 ± 1.4a  | 17.04 ± 0.8a  | 19.0 ± 1a     | 28.23 ± 1b    | 40.6 ± 0.7c   |
| TOC   | %                   | 0.17 ± 0.03a  | 0.19 ± 0.02a  | 0.25 ± 0.02a  | 2.79 ± 0.2b   | 2.92 ± 0.1b   |
| TOM   | %                   | 0.29 ± 0.01a  | 0.33 ± 0.02a  | 0.43 ± 0.01a  | 4.79 ± 0.1b   | 5.03 ± 0.2b   |
| Ni    | mg kg <sup>-1</sup> | 5072 ± 111a   | 4435 ± 79b    | 5684 ± 93c    | 1833 ± 74d    | 1317 ± 2.3e   |
| Fe    | mg kg <sup>-1</sup> | 30867 ± 419a  | 29309 ± 343b  | 29729 ± 501b  | 25103 ± 208d  | 25632 ± 355d  |
| Cr    | mg kg <sup>-1</sup> | 384 ± 48 a    | 353 ± 57a     | 496 ± 62b     | 103 ± 35c     | 93 ± 25c      |
| Zn    | mg kg <sup>-1</sup> | 82.3 ± 9.9a   | 86.6 ± 14a    | 216.3 ± 22b   | 96.4 ± 11a    | 151.2 ± 13c   |
| Cu    | mg kg <sup>-1</sup> | 22.3 ± 2.6a   | 19.5 ± 0.8b   | 18.1 ± 1.2b   | 15.2 ± 0.4d   | 13.3 ± 0.6d   |
| Co    | mg kg <sup>-1</sup> | 401 ± 37a     | 321 ± 55b     | 480 ± 53c     | 139 ± 6.7d    | 126 ± 13d     |
| Pb    | mg kg <sup>-1</sup> | 23.3 ± 0.6a   | 22.4 ± 1.1a   | 25.6 ± 1.4b   | 19.8 ± 0.6c   | 17.1 ± 0.8d   |
| N     | mg kg <sup>-1</sup> | 339 ± 20a     | 583 ± 30b     | 862 ± 36c     | 1150 ± 17d    | 1472 ± 61e    |
| P     | mg kg <sup>-1</sup> | 183 ± 13a     | 238 ± 21b     | 210 ± 23a     | 337 ± 12c     | 453 ± 21d     |
| Ca    | %                   | 0.37 ± 0.003a | 0.35 ± 0.002b | 0.31 ± 0.002c | 0.46 ± 0.002d | 0.51 ± 0.002e |
| Mg    | %                   | 4.73 ± 0.03a  | 4.07 ± 0.02b  | 4.62 ± 0.008c | 2.12 ± 0.01 d | 2.10 ± 0.01d  |
| Mg/Ca |                     | 12.7          | 11.6          | 14.9          | 4.6           | 4.11          |

zone (Bani et al., 2009). Nitrogen level was lower in plots 1, 2, 3 than in plots 4 and 5. Phosphorus concentration was low in all the studied plots, which is normal for ultramafic soils (Bani et al., 2014). The highest amount of organic matter and nutrients in plots 4 and 5 can be related to the denser plant cover in these plots. This nutrient improves the soil structure thereby increasing water penetration and providing a more favorable soil environment for growth of plant roots and soil microorganisms (Schoonover et al., 2015). The concentration varied from 2.1% to 4.3 % Mg, which corresponds to serpentine soil materials and ultramafic originated residues. The concentration of Mg is higher in the area contaminated with mineral residues than on serpentine soil (Table 1). This can be explained by the fact that ferromagnetic minerals are very rich in Mg (Shallari et al., 1998; Bani et al., 2014). The Ca concentration varied from 0.31 to 0.5% with a strong deficiency in plots 1, 2 and 3 (reaching less than 0.4%). Accordingly Mg:Ca ratio varied from 4.11 to 14.9 a range that is reported in ultramafic materials (Proctor, 1971; Shallari et al., 1998; Bani et al., 2014). The plant species growing on this former mining area must be physiologically adapted to cope with the high Mg/Ca ratio and Ca deficiency.

The Ni concentration in the plots varies from 1317 to 5684 mg kg<sup>-1</sup>. In plots 1, 2, and 3 the concentration is higher than in plot 4, which was less affected by mining activity. In plot 5 the Ni concentration is lower than 1317 mg kg<sup>-1</sup>, similar to the serpentine soil of Përrenjas region (Shallari et al., 2001; Bani et al., 2009). The available Ni,

called Ni<sub>DTPA</sub>, varied from 4 to 8 mg kg<sup>-1</sup> DW in mining site and from 11 to 14 mg kg<sup>-1</sup> in serpentine soil. Although it only reflects the potential pool of available Ni (Chaney, 2008) it allows relative comparison of the soils. It has also been correlated to isotopically labile Ni (Echevarria et al., 1998). We used this measurement as an indicator of the relative variability of Ni availability of the different plots in this study.

The high values of Ni in the plots could be explained by the fact that we are dealing with a former Ferro-nickel mining area, where processed material originates from the ultramafic area of Pogradec and Përrenjas (nickel mining) rich in Ni, Co and Cr (Bani et al., 2009). Ni<sub>DTPA</sub> found in our study was in accordance with previous study in this region (Shallari et al., 2001; Bani et al., 2015).

The plots 1, 2, and 3 which were most affected by mining activity, were characterized by elevated levels of Cr and Co typical for ultramafic materials (Table 1). Zinc concentrations fell within the ranges for normal soils lying in the range 82.3-151 mg kg<sup>-1</sup> except samples from plot 3 (216 mg kg<sup>-1</sup>). The highest Zn concentration, most probably is a result of anthropogenic pollution, although this element can sometimes be found at high concentration in serpentine soil (Bani et al., 2009). Soil concentrations of Pb and Cu were within the normal range.

The difference on the textural classes for study plots that influences the organic matter content and consequently the development of vegetation are presented on the Figure 2. As it can be seen, the first, the second and the third plot have the same textural class (sandy loam), while

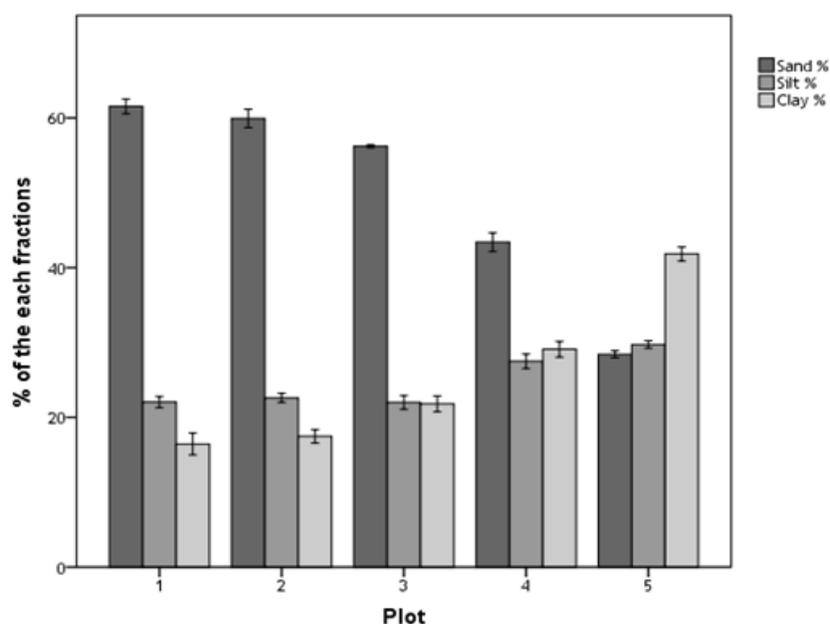


Figure 2. Particle size distribution in the studied plots.

for the fourth plot it is sandy clay loam and for the fifth plot - clay. The rank of plots according to clay percentage is Plot 5>Plot 4>Plot 3>Plot 2>Plot 1. As the organic matter (OM) and clay level increase from plot 1 to 4 or 5, it shows that there are differences between study plots as a result of pollution caused by mining activity. Plots 4 and 5 have similar OM, but have textural changes that are associated with changes in the physical properties of the soil, and consequently changes in the vegetation. Cation exchange capacity (CEC) was higher in plot 4 and 5 that is in the range of Albanian serpentine soil (Bani et al., 2014). Differences in the texture of plot 4 and 5 can be explained by the impact of sandy deposits.

The ANOVA results showed that there were statistically significant difference ( $P<0.05$ ) for trace metals, TOM, and Mg between studied plots (Table 1). There are statistically significant differences between plots 1, 2 and 3 with plots 4 and 5 from one hand, and also between plots 4 and 5 for Ni, Cr, Co, Mg, TOM, CEC from the other, showing the differences between serpentine soil and contaminated by mining activities plots (Table 1).

#### Edaphic factors and plant distribution

As a result of this study, 96 species and 24 families of vascular plants were found (Table 2). The most numerous family in all mining plots was Asteraceae (29.3% of all found species). The percentage of the representatives of other families was as follow: Lamiaceae (8.1%), Fabaceae (7.1%), Scrophulariaceae (7.1%), Boraginaceae (5.1%), Caryophyllaceae (6.1%), Brassicaceae (6.1%), Poaceae (3%), etc. These families were reported to occur on serpentine dumping grounds both in Poland and in Western Europe (Koszelnik-Leszek, 2013). The serpentine adapted species were the most competitive which contributed to the colonization of the studied area.

In the first plot, only 16 plant species were found (Table 2). The lower number of species is probably a result of recently initiated process of natural colonization and poor in nutrient elements soil. The plant species found in this plot belong to seven different families. Most of the representatives of Asteraceae are plants distributed in rocky heavy metal rich areas, also found in other places in the world (Vincent et al., 2008).

*Plantago lanceolata* L. was a species found only in this plot. This species appears in a wide variety of environments, with chemical and physical characteristics of the soils described by Troelstra (1992). *Plantago lanceolata* is generally confined to areas of either low metal concentration or high organic matter, or both, and therefore, low effective toxicity (Antonovics, 1972). Its appearance only in this plot is possibly by chance, as the soil, metal concentrations for most of the studied metals (Ni, Fe, Cr, Co) were high while the macronutrients, total

organic carbon and total organic matter were very low. It is an indifferent pseudo-metallophyte living regularly on contaminated soils but shows neither abundance nor particular vitality (Antonovics et al., 1971). Although metal tolerance was demonstrated by the serpentine seedlings of *P. lanceolata* (Pavlova, 2017) the low Ni concentration in plant tissues measured ( $13 \text{ mg kg}^{-1}$ ) shows that this species does not tolerate this metal. *Plantago lanceolata* was found to be tolerant to Pb and Zn (Simon, 1978), elements that normally in serpentine soils are identified in low concentrations. Plants that avoid abiotic stress can successfully colonize substrates containing increased loads of heavy metals. Such plants growing on soils with a naturally elevated concentration of heavy metals need more time to develop tolerance (Kuta et al., 2014).

Twenty species were found in the second plot and some of them were common also to other plots, as well. The plant taxa distributed in this plot were about 28% of the plants found in plot 5, which was identified as the richest in species. Their distribution was patchy and open area prevailed in plots 1 and 2. The representatives of Asteraceae were 65% of all the plants distributed in plot 2. The increased number of species in this plot in comparison to plot 1 could be related to a slight improvement of soil properties (an increase of OM, CEC) and decreased concentrations of Ni and Co as well. *Medicago lupulina* L. just happened to colonize the second plot. Its presence in this plot can be explained by the fact that this plot is closest to the area where it is commonly presented. Previously, *M. lupulina* was found to increase colonization by arbuscular mycorrhizal fungi (AMF) and phosphorus and micronutrient uptake (Turmel et al., 2011). Taking into consideration the fact that arbuscular mycorrhizal fungi reduces the toxicity of heavy metals to such degree that mycorrhizal plants can colonize a soil toxic for non-mycorrhizal individuals (Shaw, 1990) it is quite possible that this factor is important for the distribution of the species in the plot.

Ernst (1990) explains the low number of immigrants in metalliferous sites with the low potential of most plant species to evolve metal resistance. Although *M. lupulina* has low potential of metal translocation to the aboveground parts (Amer et al., 2013), Ni concentration ( $198 \text{ mg kg}^{-1}$ ) found in the aerial plant parts demonstrates good tolerance of the species to the elevated soil Ni.

The total number of species found in plot 3 was 22. In comparison to the previous two plots, 7 new species and one new family (Brassicaceae) were found. One of the characteristic species for the metalliferous soils in the Mediterranean area, the Ni hyperaccumulator *Alyssum murale*, appears here. This species occupies a considerable part of the plot area and this is probably related to dissemination of the seeds from the upper part

Table 2. List of plants from the studied plots and Ni concentration in plant (mg kg<sup>-1</sup>).

| No                    | Taxa   | Life form | Plot 1 | Plot 2 | Plot 3 | Plot 4 | Plot 5 | Ni (mg kg <sup>-1</sup> ) |
|-----------------------|--|-----------|--------|--------|--------|--------|--------|---------------------------|
| <i>AMARANTHACEAE</i>  |  |           |        |        |        |        |        |                           |
| 1                     | <i>Amaranthus albus</i> L.                     | T         |        |        |        |        | x      | 41                        |
| 2                     | <i>A. retroflexus</i> L.                       | T         |        |        |        |        | x      | 36                        |
| 3                     | <i>A. spinosus</i> L.                          | T         |        |        |        |        | x      | 29                        |
| <i>APIACEAE</i>       |  |           |        |        |        |        |        |                           |
| 4                     | <i>Eryngium campestre</i> L.                   | H         |        |        |        |        | x      | 21                        |
| 5                     | <i>E. creticum</i> Lam.                        | H         |        |        |        |        | x      | 28                        |
| 6                     | <i>Tordylium apulum</i> L.                     | T         |        |        |        | x      | x      | 21                        |
| <i>ASTERACEAE</i>     |  |           |        |        |        |        |        |                           |
| 7                     | <i>Arctium lappa</i> L.                        | H         |        |        |        | x      |        | 15                        |
| 8                     | <i>Artemisia absinthium</i> L.                 | Ch        | x      | x      | x      | x      | x      | 23                        |
| 9                     | <i>A. scoparia</i> Waldst. & Kitam.            | H         |        |        |        | x      |        | 32                        |
| 10                    | <i>A. vulgaris</i> L.                          | H         | x      | x      | x      | x      | x      | 11                        |
| 11                    | <i>Carduus acanthoides</i> L.                  | H         | x      | x      |        |        | x      | 15                        |
| 12                    | <i>C. pycnocephalus</i> L.                     | H/T       | x      | x      |        |        | x      | 16                        |
| 13                    | <i>Carlina acaulis</i> L.                      | H         | x      | x      |        |        |        | 28                        |
| 14                    | <i>Centaurea affinis</i> Friv.                 | H         |        | x      | x      | x      | x      | 29                        |
| 15                    | <i>C. alba</i> L.                              | H         |        | x      | x      | x      | x      | 25                        |
| 16                    | <i>C. nigra</i> L.                             | H         |        | x      | x      | x      | x      | 23                        |
| 17                    | <i>C. sonchifolia</i> L.                       | H         |        | x      | x      | x      | x      | 34                        |
| 18                    | <i>Cichorium intybus</i> L.                    | H         |        | x      |        |        | x      | 56                        |
| 19                    | <i>Cirsium arvense</i> (L.) Scop.              | G         |        | x      | x      | x      | x      | 13                        |
| 20                    | <i>C. boujartii</i> (Piller&Mitterp.) Sch.Bip. | H         |        | x      | x      | x      | x      | 21                        |
| 21                    | <i>C. creticum</i> (Lam.) d'Urv.               | H         |        | x      | x      | x      | x      | 22                        |
| 22                    | <i>Conyza canadensis</i> L.                    | T         | x      |        |        |        | x      | 19                        |
| 23                    | <i>Crepis vesicaria</i> L.                     | T/H       |        |        |        | x      | x      | 42                        |
| 24                    | <i>Dittrichia graveolens</i> (L.) Greuter      | T         |        |        |        |        | x      | 41                        |
| 25                    | <i>D. viscosa</i> (L.) Greuter                 | H         | x      | x      | x      | x      | x      | 35                        |
| 26                    | <i>Eupatorium cannabinum</i> L.                | H         |        |        |        |        | x      | 63                        |
| 27                    | <i>Matricaria trichophylla</i> (Boiss.) Boiss. | H         |        |        |        | x      | x      | 42                        |
| 28                    | <i>Sonchus asper</i> (L.) Hill.                | T/H       |        |        |        | x      | x      | 25                        |
| 29                    | <i>S. crepioides</i> (Poir.) Sm.               | H         |        |        |        | x      |        | 33                        |
| 30                    | <i>S. arvensis</i> L.                          | H         | x      |        |        | x      | x      | 63                        |
| 31                    | <i>Taraxacum officinale</i> L.                 | H         |        |        |        |        | x      | 67                        |
| 32                    | <i>Tussilago farfara</i> L.                    | G         |        |        |        |        | x      | 49                        |
| <i>BORAGINACEAE</i>   |  |           |        |        |        |        |        |                           |
| 33                    | <i>Anchusa officinalis</i> L.                  | H         |        |        |        | x      | x      | 68                        |
| 34                    | <i>Cynoglossum officinale</i> L.               | H         | x      | x      | x      | x      | x      | 236                       |
| 35                    | <i>Echium italicum</i> L.                      | H         |        |        | x      | x      | x      | 84                        |
| 36                    | <i>E. vulgare</i> L.                           | H         |        |        | x      | x      | x      | 67                        |
| 37                    | <i>Heliotropium europaeum</i> L.               | T         |        | x      |        | x      |        | 75                        |
| <i>BRASSICACEAE</i>   |  |           |        |        |        |        |        |                           |
| 38                    | <i>Aethionema saxatile</i> (L.) Br.            | Ch        |        |        |        | x      |        | 119                       |
| 39                    | <i>Alyssoides sinuate</i> Medik.               | H         |        |        |        |        | x      | 220                       |
| 40                    | <i>A. utriculata</i> (L.) Medik.               | Ch        |        |        |        |        | x      | 193                       |
| 41                    | <i>Alyssum murale</i> Waldst. & Kit.           | Ch        |        |        | x      | x      | x      | 1012-2508                 |
| 42                    | <i>Capsella bursa-pastoris</i> L.              | T         |        |        |        | x      | x      | 30                        |
| 43                    | <i>Nasturtium officinale</i> R. Br.            | H         |        |        |        |        | x      | 176                       |
| 44                    | <i>Sisymbrium officinale</i> (L.) Scop.        | T         |        |        |        | x      | x      | 107                       |
| <i>CAPRIFOLIACEAE</i> |  |           |        |        |        |        |        |                           |
| 45                    | <i>Sambucus ebulus</i> L.                      | G         |        |        |        |        | x      | 8                         |

N.D=Not detected (Ni<2 mg kg<sup>-1</sup>)

H = hemicryptophytes, T = therophytes, G = geophytes, Ch = chamaephytes, Ph = phanerophytes, NPh = nanophanerophite

Table 2. ... Continued

| No                     | Taxa                                | Life form | Plot 1 | Plot 2 | Plot 3 | Plot 4 | Plot 5 | Ni (mg kg <sup>-1</sup> ) |
|------------------------|-------------------------------------|-----------|--------|--------|--------|--------|--------|---------------------------|
| <i>CARYOPHYLLACEAE</i> |                                     |           |        |        |        |        |        |                           |
| 46                     | <i>Dianthus sylvestris</i> Wulfen   | H         |        |        |        |        | x      | 148                       |
| 47                     | <i>Herniaria glabra</i> L.          | T/H       |        | x      |        | x      | x      | 113                       |
| 48                     | <i>Lychnis coronaria</i> Desr.      | H         |        |        |        | x      | x      | 115                       |
| 49                     | <i>L. divaricata</i> Rchb.          | H         |        |        |        | x      | x      | 134                       |
| 50                     | <i>L. flos-cuculi</i> L.            | H         |        |        |        | x      | x      | 122                       |
| 51                     | <i>Stellaria media</i> L.           | T         |        |        | x      | x      |        | 105                       |
| <i>CHENOPODIACEAE</i>  |                                     |           |        |        |        |        |        |                           |
| 52                     | <i>Chenopodium album</i> L.         | T         |        |        |        |        | x      | 116                       |
| <i>CONVOLVULACEAE</i>  |                                     |           |        |        |        |        |        |                           |
| 53                     | <i>Convolvulus arvensis</i> L.      | G         |        | x      |        | x      | x      | 121                       |
| 54                     | <i>C. althaeoides</i> L.            | H         |        |        |        | x      |        | 56                        |
| <i>EUPHORBIACEAE</i>   |                                     |           |        |        |        |        |        |                           |
| 55                     | <i>Euphorbia helioscopia</i> L.     | T         |        |        |        | x      |        | 156                       |
| 56                     | <i>E. heterophylla</i> L.           | T         |        |        |        | x      | x      | 101                       |
| <i>FABACEAE</i>        |                                     |           |        |        |        |        |        |                           |
| 57                     | <i>Gleditsia triacanthos</i> L.     | Ph        |        |        |        | x      | x      | 137                       |
| 58                     | <i>Medicago lupulina</i> L.         | H         |        | x      |        |        |        | 198                       |
| 59                     | <i>M. minima</i> (L.) L.            | T         |        |        |        | x      |        | 109                       |
| 60                     | <i>M. orbicularis</i> (L.) Bartal.  | T         |        |        |        | x      | x      | 114                       |
| 61                     | <i>Melilotus albus</i> Medik.       | T         |        |        |        | x      |        | 182                       |
| 62                     | <i>M. officinalis</i> (L.) Pall.    | H         | x      |        | x      | x      |        | 105                       |
| 63                     | <i>Trifolium campestre</i> Schreb.  | T         |        |        |        |        | x      | 135                       |
| <i>GERANIACEAE</i>     |                                     |           |        |        |        |        |        |                           |
| 64                     | <i>Erodiumcicutarium</i> (L.) L'Her | T         |        |        |        | x      | x      | 109                       |
| 65                     | <i>Geranium molle</i> L.            | T/H       |        |        |        | x      | x      | 54                        |
| <i>JUGLANDACEAE</i>    |                                     |           |        |        |        |        |        |                           |
| 66                     | <i>Juglans regia</i> L.             | Ph        |        |        |        |        | x      | N.D                       |
| <i>LAMIACEAE</i>       |                                     |           |        |        |        |        |        |                           |
| 67                     | <i>Marrubium vulgare</i> L.         | H         |        |        |        |        | x      | 35                        |
| 68                     | <i>Mentha aquatic</i> L.            | H         |        |        |        |        | x      | 53                        |
| 69                     | <i>M. longifolia</i> (L.) L.        | H         |        |        |        |        | x      | 61                        |
| 70                     | <i>M. pulegium</i> L.               | H         | x      |        |        |        | x      | 10                        |
| 71                     | <i>Salvia ringens</i> Sm.           | H         |        |        |        | x      |        | 58                        |
| 72                     | <i>S. verbenaca</i> L.              | H         |        |        |        | x      |        | 38                        |
| 73                     | <i>S. verticillata</i> L.           | H         |        |        |        | x      |        | 28                        |
| 74                     | <i>Thymus pulegioides</i> L.        | H         |        |        | x      |        |        | 39                        |
| <i>MALVACEAE</i>       |                                     |           |        |        |        |        |        |                           |
| 75                     | <i>Malva sylvestris</i> L.          | H         |        |        |        | x      | x      | 45                        |
| <i>MORACEAE</i>        |                                     |           |        |        |        |        |        |                           |
| 76                     | <i>Ficus carica</i> L.              | Ph        |        |        |        |        | x      | N.D                       |
| <i>PAPAVERACEAE</i>    |                                     |           |        |        |        |        |        |                           |
| 77                     | <i>Papaver rhoeas</i> L.            | T         |        |        |        | x      |        | 32                        |
| <i>PLANTAGINACEAE</i>  |                                     |           |        |        |        |        |        |                           |
| 78                     | <i>Plantago lanceolata</i> L.       | H         | x      |        |        |        |        | 13                        |
| 79                     | <i>P. media</i> L.                  | H         |        |        | x      |        | x      | 64                        |
| <i>POACEAE</i>         |                                     |           |        |        |        |        |        |                           |
| 80                     | <i>Cynodon dactylon</i> (L.) Pers.  | G/H       | x      |        |        | x      | x      | 251                       |
| 81                     | <i>Hordeum murinum</i> L.           | T         |        |        |        | x      | x      | 115                       |
| 82                     | <i>Poa nemoralis</i> L.             | H         |        |        |        | x      | x      | 102                       |

N.D=Not detected (Ni<2 mg kg<sup>-1</sup>)

H = hemicryptophytes, T = therophytes, G = geophytes, Ch = chamaephytes, Ph = phanerophytes, NPh = nanophanerophite

Table 2. ... Continued

| No                      | Taxa                           | Life form | Plot 1 | Plot 2 | Plot 3 | Plot 4 | Plot 5 | Ni (mg kg <sup>-1</sup> ) |
|-------------------------|--------------------------------|-----------|--------|--------|--------|--------|--------|---------------------------|
| <i>POLYGONACEAE</i>     |                                |           |        |        |        |        |        |                           |
| 83                      | <i>Polygonum aviculare</i> L.  | T         |        |        |        | x      | x      | 74                        |
| 84                      | <i>P. persicaria</i> L.        | T         |        |        |        | x      |        | 163                       |
| 85                      | <i>Rumex crispus</i> L.        | H         |        |        |        |        | x      | 21                        |
| 86                      | <i>R. pulcher</i> L.           | H         |        |        |        |        | x      | 19                        |
| <i>ROSACEAE</i>         |                                |           |        |        |        |        |        |                           |
| 87                      | <i>Rubus ulmifolius</i> Schott | NPh       |        |        |        | x      | x      | 63                        |
| <i>SCROPHULARIACEAE</i> |                                |           |        |        |        |        |        |                           |
| 88                      | <i>Gratiola officinalis</i> L. | H         |        |        |        |        | x      | 57                        |
| 89                      | <i>Linaria vulgaris</i> Mill.  | H         |        |        |        |        | x      | 71                        |
| 90                      | <i>Scrophularia nodosa</i> L.  | H         | x      |        | x      | x      |        | 59                        |
| 91                      | <i>S. canina</i> L.            | H         | x      |        | x      | x      |        | 61                        |
| 92                      | <i>Verbascum blattaria</i> L.  | T/H       | x      | x      | x      | x      | x      | 31                        |
| 93                      | <i>V. densiflorum</i> Bertol.  | H         |        |        | x      |        |        | 51                        |
| 94                      | <i>V. phlomoides</i> L.        | H         |        |        |        | x      |        | 21                        |
| <i>SOLANACEAE</i>       |                                |           |        |        |        |        |        |                           |
| 95                      | <i>Solanum nigrum</i> L.       | T         |        |        |        |        | x      | 45                        |
| <i>VERBENACEAE</i>      |                                |           |        |        |        |        |        |                           |
| 96                      | <i>Verbena officinalis</i> L.  | H         |        |        |        |        | x      | 25                        |

N.D=Not detected (Ni<2 mg kg<sup>-1</sup>)

H = hemicryptophytes, T = therophytes, G = geophytes, Ch = chamaephytes, Ph = phanerophytes, NPh = nanophanerophite

of the area down to the plot area (Bani et al., 2009). The perennial species *Thymus pulegioides* L., and *Verbascum densiflorum* Bertol. were also recorded in this plot. *Thymus pulegioides* is native to temperate parts of Europe. It grows in alkaline or neutral soil on hills, rocky outcrops, gravels, sandy places, waste ground and roadsides (Bani et al., 2009). *Verbascum densiflorum* is a plant that grows in rocky soils also rich in trace metals. These species, as most of the plants found in this plot, do not demonstrate tolerance to high Ni concentrations in the soil of the plot. The number of plant species growing on the first three plots was low because of Ca:Mg imbalances and Ca deficiency. Plant species not adapted to Ca deficiency have growth problems and a stunted growth form as well as poor root and inflorescence development because Ca is essential for cell elongation and division (Havlin et al., 1999).

In plot 4 a lesser impact of mining activity was observed than in the three other plots and this was expressed by the improvement of the soil properties like higher values of OM, Ca:Mg ratio, N and P concentrations and CEC, resulting in a higher number of species present. Thus, 58 species from 16 families were recorded here. In this plot more species that require a substrate with more nutrients were found. Most of the species were perennials, but phanerophytes (trees) were also recorded. The appearance of tree species like *Gleditsia triacanthos* L. is characteristic for rich soils. This species is not native to the Albanian flora but it is highly invasive and widely

distributed in Europe. It is typical for gravel and loamy soils (Petrova et al., 2013). Species typical for moderately poor and poor soils like *Cichorium intybus* L. and *Conyza canadensis* L. were also recorded.

In plot 5, the plant diversity was higher than in the other plots and typical for serpentine soils. Here 72 species from 23 families were found. Some species, characteristics for the serpentine areas on the Balkans were found in this plot and the previous plots as well. Such species are *Alyssum murale*, *Aethionema saxatile* (L.) Br., *Alyssoides sinuata* Medik., *A. utriculata* (L.) Medik., etc. Although ferns like *Notholaena maranthae* (L.) Desv. and *Asplenium cuneifolium* Viv. are typical for the serpentines in Europe (Brooks, 1987) such species in the studied area were not found. This fact could be explained by the lack of suitable ecological niches and soil properties. Similarly to plot 4 the soil in plot 5 was rich in macronutrients and the levels of toxic metals were not so high. In addition, the species *Amaranthus retroflexus* L., typical for very rich soils was spotted here. This species as well as *Amaranthus albus* L., *Artemisia vulgaris* L., *Conyza canadensis* L., *Tussilago farfara* L., *Echium vulgare* L., *Capsella bursa-pastoris* L., *Stellaria media* L., *Gleditsia triacanthos*, *Chenopodium album*, *Convolvulus arvensis* L., *Medicago lupulina*, *Melilotus officinalis* (L.) Pall., *Plantago lanceolata*, *Hordeum murinum* L., and *Polygonum aviculare* L. found in the studied area are part of the alien flora of Europe (Petrova et al., 2013) with excellent colonizing abilities

and distribution on different soil types.

The analysis of the life forms of plants gives reason to conclude that in all plots hemicryptophytes (herbaceous perennials, which produce perennating buds at the soil surface) occurred to be dominant, as they constitute 60.4% of the plant taxa found (Table 2). The second most numerous group was that of the therophytes (26%) (annual plants that complete their life cycle in a short period when conditions are favourable and survive harsh conditions as seeds). Phanerophytes were the weakest presented group. Although annuals are plants that occupy more easily new habitats, metalliferous soils with high metal concentrations are not often colonized by annual plant species and the reason for this according to Ernst (1990) is that this “life history demands a too intensive metabolism”. One of frequently appearing annuals is *Polygonum aviculare*, which is more competitive in comparison with other annual or annual/biennial weed species like *Chenopodium album* and *Stellaria media*. The high number of perennials in the studied plots can be related to their ability to produce vegetative propagation organs. Most of the vegetative reproducing species were characteristic for plots 4 and 5 where higher percentage of organic matter, macronutrients and low concentrations of toxic metals characterized the soils. Ernst (1990) considers facilitation of metalliferous sites by perennial plants possible if metal toxicity can be decreased by the formation of less toxic and/or less plant-available metal complexes or by a dilution of metal concentrations. Some of the more frequently found perennials such as *Plantago media*, *Taraxacum officinale*, and *Tussilago farfara* are known to tolerate compacted and poorly aerated soils as well (Pavlova and Georgieva, 2015).

#### Nickel concentration in plants

Nearly all species were analyzed for Ni concentration in their aboveground parts. Data for these elements are shown in Table 3. The number of the tolerant to Ni species was different for the studied plots. The lowest number was found in plot 1 (3 species) and the highest was in plots 4 and 5 where 22 species were recorded per plot. The elevation of the number of the Ni tolerant species is not related to the soil Ni concentrations and depends on

the accumulation abilities of the species.

Most of the plants on Përrenjas ex-mining area and serpentine plots showed slightly elevated Ni concentrations in comparison to those on other soil types, about 12-251 mg kg<sup>-1</sup>, rather than 0.5-10 mg kg<sup>-1</sup>. In some species, the Ni concentration was elevated whilst samples of the non-serpentine substrate contain the expected very low Ni concentrations (Reeves, 1992). The highest Ni concentrations were found in local indicator species: *Cynodon dactylon* (L.) Pers. (251 mg kg<sup>-1</sup> DW), *Cynoglossum officinale* L. (236 mg kg<sup>-1</sup>), *Alyssoides sinuata* (220 mg kg<sup>-1</sup>), *A. utriculata* (193 mg kg<sup>-1</sup>), *Medicago lupulina* (198 mg kg<sup>-1</sup>), *Melilotus albus* Medik. (182 mg kg<sup>-1</sup>), *Polygonum persicaria* L. (163 mg kg<sup>-1</sup>), and *Euphorbia helioscopia* L. (156 mg kg<sup>-1</sup>). We confirm Brooks (1987) and Konstantinou and Babalonas (1996) that plants from families like Caryophyllaceae, Polygonaceae, Poaceae, Fabaceae have potential to grow in the hostile edaphic environment of serpentine soils and accumulate Ni in their tissues.

The highest Ni values in plants were recorded for *A. murale*, which is a native serpentine plant and Ni hyperaccumulator species, but Ni concentration in this species was highly dependent on the site of collection (Bani et al., 2013). Analysis of metals performed on plant bulk shoots showed different plant responses to the presence of Ni, Ca, Mg in soils collected at plots 3, 4 and 5 (Table 3). The highest Ni concentration in plants were recorded for *A. murale*, in plot 5 and lowest in plot 3. The concentration of Ni in *A. murale* in the study was lower than found by Bani et al. (2009) at other sites of Përrenjas region, but similar to the specimens sampled (i.e. 1508 mg kg<sup>-1</sup>) in the same post-mining site by Shallari et al. (1998). The ratio of Ni concentration in plant shoots to its concentration in soil was variable. It was 0.17, 0.66, and 1.9 respectively in plots 3, 4 and 5. This ratio represented the transfer factor since the soil was sampled around the roots of plant species (Kumar et al., 1995; Reeves, 2006). This ratio can be interpreted as an indication of hyper accumulation conditions depending on the availability of Ni in the soil and the accumulation potential of the plant. Different values of this factor show that Ni is less available in polluted by mining activity soils than in serpentine ones.

Table 3. Concentration of Ni, Ca, and Mg in Ni hyperaccumulator *Alyssum murale* from ex-mining and serpentine site of Përrenjas, Albania. Results are given as mean values of three replicates ± standard deviation.

| Species          | Sampling plot | Ni                  | Ca       | Mg       |
|------------------|---------------|---------------------|----------|----------|
|                  |               | mg kg <sup>-1</sup> |          |          |
| <i>A. murale</i> | Plot 3        | 1012±56             | 3500±134 | 600±144  |
| <i>A. murale</i> | Plot 4        | 1254±122            | 4800±65  | 1000±137 |
| <i>A. murale</i> | Plot 5        | 2508±88             | 5800±124 | 2244±234 |

## CONCLUSIONS

The soil samples collected at the former mining site of Përrenjas showed changes in the content of organic matter and textural composition compared to the surrounding native serpentine soils and also contained higher levels of metals typical for ultramafic environments e.g. Ni, Cr and Co, Mg, Fe.

As a result of the contamination from the Ferro-nickel mining activity conducted in the past, the study area underwent changes of soil texture, CEC, OM, compared to neighboring serpentine soils. The plants in the contaminated area were adapted to the physical and chemical properties of the soil. The most numerous represented plant families on the plots affected by mine heaps were Asteraceae (29.3%), Fabaceae (7.1%), Lamiaceae (8.1%) and Brassicaceae (6.1%). The serpentine flora is characterized by plant diversity and the most common species are the perennial grasses (hemicryptophytes). Among the species in the study area, 28.3% of the taxa are known to tolerate high levels of Ni in their growing substrate. Most of Ni tolerant species were distributed in plots 4 and 5. The Ni hyperaccumulator *A. murale* was present in both mining and serpentine sites, up taking different concentrations of Ni. In the mining area, the fraction of available Ni was lower than in the serpentine area. Phytoextraction in the former mining area of Përrenjas can lead to the depletion of the labile pool of Ni and also to the improvement of the physical properties of the soil, which would result in the total revegetation of the zone in a similar way as the neighboring ultramafic area.

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