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**ARTICLE**

# Heavy metals tolerance on seed germination and growth of serpentine plant species *Alyssum murale* L.

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**ABSTRACT** *Alyssum murale* L. is a nickel hyperaccumulator herbaceous perennial species in a natural serpentine soil and adapted to arid, infertile soils. The objective of this study was to investigate how the seed germination, root, and hypocotyl growth of this plant species respond to exposure to different concentrations of heavy metals such as lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr) and manganese (Mn). The highest germination, even higher than in the control group, was observed at lower concentrations of Ni, Cd, Pb and Mn, while almost all higher concentrations of the metals decreased germination. Germination time was significantly slower (from about 6 to 10 days) for seeds treated with higher metal concentrations, especially Mn, Cd and Ni, and faster (from about 3 to 5 days) for seeds treated with lower concentrations. The strongest inhibitory effect on root and hypocotyl length occurred in treatments with different concentrations of Pb, Cd, and Mn, and seeds treated with lower concentrations of Ni showed a positive effect on root and hypocotyl growth. Seeds treated with Ni showed relatively high tolerance to this metal, presenting the potential for practical use in various fields of phytoremediation technology. **Acta Biol Szeged 66(2):116-124 (2022)**

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## Introduction

There are several serpentine soils around the world that have formed on ultramafic rocks, particularly on rocks that contain considerable amounts of minerals related to serpentine (Brooks 1987). High levels of Mg and Fe, as well as moderately high levels of Ni, Cr, and Co, are present in these soils. Compared to other soil types, manganese levels can also be higher. Due to the soils being both Ca-poor and Mg-rich, concentrations of N, P, and K are often low, while the Mg/Ca quotient is high (Proctor 1999). Serpentine soils are characterized by three features: poor plant productivity (small number of plants), high ratio of endemic plants, and special types of vegetation, which stand out from other neighboring environments (Hearth et al. 2014). Many aspects of the so-called serpentine syndrome have been discussed in a recent review (Kazakou et al. 2008).

According to data from different authors, plants are extremely tolerant to high concentrations of nickel in serpentine soils. Among the tolerant plants are several different species of the genus *Alyssum*, some of which can have high levels of heavy metals, especially nickel (about 10000 mg $kg^{-1}$), in their leaves and also have the ability to tolerate higher concentrations of nickel in soil (up to 3000 mg $kg^{-1}$) (Seregin and Kozhevnikova 2006; Bani et al. 2013).

The largest areas of serpentine soils in Europe are found in the Balkan Peninsula, including Kosovo and south-eastern Albania. In the territory of the Republic of Kosovo there are several serpentine areas, which are found mainly in the Northern, Central and Western parts (Jakovljević et al. 2011). The serpentine flora of the Balkans is characterized by a relatively high degree of endemism. Serpentine soils are crucial for the diversity of plant species and the unique flora; while often lacking in vegetation, they are extremely rich in rare and endemic species (Osmani et al. 2018).

A unique flora with a wealth of endemic species that are reportedly suited to toxic levels of heavy metals is supported by ultramafic soils. Because certain species of this flora actively take in and accumulate exceptionally high amounts of metal elements in their tissues, they have been classified as metal hyperaccumulators (Brooks 1998). Nickel hyperaccumulators have been used to examine the mechanisms of metal accumulation, tolerance, and detoxification.

According to studies by different authors (Baker et al. 2010; Krämer 2010; van der Ent et al., 2013), more than 400 plant species accumulate high levels of metals such as Cd, Cu, Co, Mn, Ni, and Zn. The highest known number of Ni hyperaccumulator plant species (50) in the Brassicaceae family are found in the genus Alyssum, several of which may accumulate up to 30 g $kg^{-1}$ Ni in dry leaf biomass (Baker and Brooks, 1989; Reeves and Adigüzel, 2008; van der Ent et al. 2015). *Alyssum murale* is a plant native to Mediterranean serpentine soils with high Ni concentrations, and it is recognized as both a Ni and Co hyperaccumulator (Tappero et al. 2007). Therefore, use of different *Alyssum* species in phytomining of Ni has been proposed and many field trials have been set up (Nkrumah et al. 2016). However, uncertainty persists regarding the evolutionary cause of metal hyperaccumulation. To explain how this phenomenon evolved, several theories have been put forth. Six main ideas were given by Boyd and Martens (1992): inadvertent uptake, metal tolerance, disposal from the plant body, drought resistance, pathogen–herbivore defense and interference with neighboring plants. Although Ni hyperaccumulation is a constitutive property for *Alyssum* species, it is not known whether they secrete organic and/or amino acids into the rhizosphere to solubilize Ni, or if they can make use of such acids within the soil to greatly facilitate uptake. According to other studies for *Alyssum* species, nickel is stored mainly in the leaves, and is particularly concentrated in vacuoles of epidermal cells and trichome pedicels (Gill et al. 2012). Morever, antioxidant increases in leaves of *A. montanum* from increased substrate Ni concentration is an enzymatic defense response involved in the protection of mesophyll tissues important for the maintenance of photosynthetic function, pointing to considerable heavy metal tolerance of the species (Ievinsh et al. 2020). Furthermore, with multi-metal stress under in vitro conditions, *A. montanum* clearly demonstrated fewer phytotoxic symptoms and morphogenetic response, growth tolerance and increased antioxidant defense (Muszyńska et al. 2018). Tolerance of physiological characteristics of *A. murale* to different concentrations of nickel, when grown in soil mixed with sewage sludge, suggested that nickel is not a stress factor as it does not affect the function of the photosynthetic machinery of this species (Sellami et al. 2012).

The main objective of this study is to compare the effects of different concentrations of heavy metals (Ni, Cd and Pb) on seed germination of the hyperaccumulator plant *A. murale*. The results of this study will provide more information and new achievements in the context of understanding heavy metal effects on seed germination and to better highlight the importance of hyperaccumulator species for environmental remediation purposes. There are a few studies of soil contamination by heavy metals in different regions in Kosovo, but until now, there is lack of research focusing on remediation purposes.

## Materials and methods

Seeds of *Alyssum murale* were collected in August of 2021 from native populations distributed on serpentine terrains in Golesh Massif, Kosovo. This serpentine massif is predominantly built up of ultramafic rocks, situated 15 km southwest of the capital city of Kosovo, Prishtina. This composition of soil with the presence of heavy metals supports a high number of plant taxa and a variety of serpentine endemism (Krasniqi et al. 2019). *A. murale* is a Ni-hyperaccumulator species growing in serpentine soil with high concentrations of Ni, Cr and Co (Bani et al. 2013; Bani et al. 2010; Bani et al. 2007). Seeds of this species were collected with their fruits from more than 30 plants and were extracted manually from the fruits. Seeds were stored in dark conditions at room temperature until the beginning of the experiments. Seeds were disinfected with 70% ethanol for three minutes and rinsed three times with sterile distilled water before treatments. For seed treatment we used different concentrations of heavy metals: $NiCl_2$, $CrCl_2$, $CdCl_2$, $PbCl_2$ and $MnCl_2$. All chemicals used throughout the experiments were of analytical grade and were obtained from Sigma-Aldrich. The necessary quantities of each heavy metal were calculated in order to obtain certain heavy metal concentrations in the standard solutions for seed treatment. Standard solutions were prepared in these concentrations: 500, 1000, 2000, 4000 and 8000 ppm for Ni; 100, 500, 1000, 1500 and 2000 ppm for Cr; 10, 20, 50, 100 and 200 ppm for Cd; 10, 20, 50, 100 and 200 ppm for Pb; and 500, 1000, 2000, 4000 and 8000 ppm for Mn. The range of concentrations was based on metal concentrations commonly encountered in serpentine soil, from lower to several-fold higher than concentrations measured in this soil. For each treatment, the experiment was conducted with three replicates of 50 seeds of *A. murale* which were germinated on top of double layered papers (ISTA 1996) with 5 ml of respective heavy metal concentration in 10 cm Petri dishes. Distilled water was used as a control. These Petri dishes were placed into sealed plastic bags to avoid moisture loss. The experiment of seed germination was conducted under controlled conditions in the vegetation room at 24 ± 1 °C and with a light regime of 16 h light (day)/8 h darkness (night).

Daily assessments and observations were taken throughout the entire germination phase. The germination percentage is an estimation of the viability of

seeds. Germination was considered to have occurred when the seminal roots were 2 mm long. The number of seeds germinated was counted daily till the end of the experiment (three consecutive days with a constant number of germinated seeds). The following germination parameters were recorded:
Final germination percentage (FGP):

$$FGP = (\text{number of germinated seeds}/\text{number of total seeds}) \times 100$$ (Gashi et al. 2019).

Mean Germination Time (MGT):

$$MGT = \Sigma Dn / \Sigma n,$$

where n is the number of seeds germinated on day D, and D is the number of days counted from the beginning of germination (Moradi et al. 2008).

Seed root and hypocotyl length were measured using stereomicroscope SMZ-161, Motic.

Metal tolerance of *A. murale* seedlings was estimated using root growth inhibition by different Ni, Cr, Cd, Pb and Mn concentrations, expressed as index of tolerance (IT) (Auda et al. 2004). Index of Tolerance was calculated according to the following equation: IT = root length + metal / root length without metal × 100

## Data analysis

The experiment was performed in a randomized design with three replicates. Differences between treatments were tested using SPSS 17 statistical program. Statistical variance analysis of all data was performed using one-way ANOVA, and mean comparison was performed with Duncan Multiple Range Test at the 5% level of significance.

## Results and discussion

Serpentine soils are characterized by high concentrations of metals. According to Osmani et al (2018), in some serpentine soils in Kosovo the levels of metals such as Ni, Co and Cr ranged from 1500 to 1600 mg $kg^{-1}$ for Ni, 130 to 140 mg $kg^{-1}$ for Co, and 380 to 450 mg $kg^{-1}$ for Cr. Based on these results, our experiment used solutions with a range of metal concentrations commonly encountered in serpentine soil, from lower to several-fold higher concentrations than measured in this soil. The metal concentrations used in our experiment corresponded to the amounts of these metals, especially nickel, used for in vitro toxicity tests in the physiological investigations of *A. murale* by other authors (Pavlova et al. 2018; Sellami et al. 2012). A great importance of the metal concentrations we have used is also related to the heavy metal pollution of agricultural soil that comes from industry, especially that from the nickel smelter in Drenas, and the lead and zinc smelter in Mitrovica, Kosovo (Zogaj and Düring 2016; Gashi et al. 2020). Furthermore, lower concentrations of these metals than those we used had no effect on seed germination, while higher concentrations than those we used had a total inhibitory effect on *A. murale* seed germination (data not shown). According to Bani et al (2007) nickel content in the shoots of *A. murale* reached 9,129 mg $kg^{-1}$ but metal concentration was not significantly affected by fertilization. Several metals such as Ni, Co, Cr, Mn, and Fe of different serpentine plant species are accumulated in plant leaves and mostly in the seeds of these species (Amari et al. 2017; Meindl et al. 2014; Bani et al. 2010; Psaras and Manetas 2001). In line with this, it is reasonable to assume that these metals may have a role in the seed coat structure, which directly affects the seeds' resistance. They may also be active in metabolic pathways, functioning as an ionic activator for an enzyme during seed germination.

Results showed that almost all of the heavy metal treatments with higher concentrations (Ni, Cr, Cd, Pb and Mn) considerably affected the germination process and germination time of *A. murale* seeds (Table 1). In general, lower concentrations of heavy metals used for seed treatments had positive effects on the final germination percentage (FGP) and mean germination time (MGT) of seeds of this plant species. Germinability increased at concentration 500 ppm of Ni and Mn and 10, 20 and 50 ppm of Cd, compared to the control group of seeds. Among other results, the highest FGP (97.00%) was found at concentration 20 ppm of Cd and the lowest germination (12.00 %) at concentration 2000 ppm of Cr. The most effective inhibition of seed germination, significantly higher compared to other metals, was obtained with 500, 1000, 1500 and 2000 ppm Cr treatments (37.00; 31.00; 18.00 and 12.00%, respectively). Cadmium and chromium toxicity have been reported for some crop plants; seed germination showed highest sensitivity to Cd, even under lower concentrations (Baruah et al. 2019).

No significant differences in FGP were shown between the seeds of *A. murale* treated with different concentrations of Pb and the control group. There were significant differences between the seeds treated in some cases with higher concentrations of Ni and seeds from the control group. In this case, among other metals (Cr, Cd and Mn), higher concentrations of Ni used for treatment of seeds of *A. murale*, demonstrated that influenced germination process but not in high level of significance. This indicates that seeds of *A. murale* can tolerate a higher concentration of Ni. In similar results reported from other studies

**Table 1.** Final germination percentage (FGP) and mean germination time (MGT) of *A. murale* seeds treated with different concentrations (ppm) of Ni, Cr, Cd, Pb and Mn.

| | FGP | | | MGT | | |
|---|---|---|---|---|---|---|
| Treatments | Mean | St. Dev. | Range | Mean | St. Dev. | Range |
| Control | 93.00 $^{AB}$ | 1.00 | 92.00-94.00 | 3.36 $^{AB}$ | 0.45 | 3.32-3.41 |
| Ni (ppm) | | | | | | |
| 8000 | 76.00 $^{D}$ | 4.00 | 72.00-80.00 | 6.55 $^{J}$ | 0.24 | 6.31-6.80 |
| 4000 | 93.00 $^{AB}$ | 1.00 | 92.00-94.00 | 4.52 $^{G}$ | 0.01 | 4.52-4.53 |
| 2000 | 84.00 $^{C}$ | 2.00 | 82.00-86.00 | 3.82 $^{CD}$ | 0.17 | 3.65-4.00 |
| 1000 | 94.00 $^{AB}$ | 0.00 | 94.00-94.00 | 3.49 $^{AB}$ | 0.02 | 3.47-3.51 |
| 500 | 95.00 $^{AB}$ | 1.00 | 94.00-96.00 | 3.55 $^{BC}$ | 0.10 | 3.45-3.65 |
| Cr (ppm) | | | | | | |
| 2000 | 12.00 $^{H}$ | 0.00 | 12.00-12.00 | 4.16 $^{EF}$ | 0.16 | 4.00-4.33 |
| 1500 | 18.00 $^{G}$ | 2.00 | 16.00-20.00 | 3.94 $^{DE}$ | 0.06 | 3.88-4.00 |
| 1000 | 31.00 $^{EF}$ | 1.00 | 30.00-32.00 | 4.29 $^{FG}$ | 0.09 | 4.20-4.38 |
| 500 | 37.00 $^{E}$ | 1.00 | 36.00-38.00 | 3.94 $^{DE}$ | 0.55 | 3.89-4.00 |
| 100 | 93.00 $^{AB}$ | 1.00 | 92.00-94.00 | 4.25 $^{FG}$ | 0.10 | 4.15-4.35 |
| Cd (ppm) | | | | | | |
| 200 | 28.00 $^{F}$ | 0.00 | 28.00-28.00 | 7.39 $^{K}$ | 0.35 | 7.36-7.43 |
| 100 | 92.00 $^{AB}$ | 4.00 | 88.00-96.00 | 6.71 $^{J}$ | 0.17 | 6.54-6.89 |
| 50 | 95.00 $^{AB}$ | 1.00 | 94.00-96.00 | 5.04 $^{H}$ | 0.14 | 4.90-5.19 |
| 20 | 97.00 $^{A}$ | 1.00 | 96.00-98.00 | 3.39 $^{AB}$ | 0.06 | 3.33-3.45 |
| 10 | 96.00 $^{A}$ | 0.00 | 96.00-96.00 | 3.38 $^{AB}$ | 0.13 | 3.25-3.52 |
| Pb (ppm) | | | | | | |
| 200 | 94.00 $^{AB}$ | 0.00 | 94.00-94.00 | 3.48 $^{AB}$ | 0.01 | 3.47-3.49 |
| 100 | 92.00 $^{AB}$ | 2.00 | 90.00-94.00 | 3.35 $^{AB}$ | 0.04 | 3.31-3.40 |
| 50 | 90.00 $^{AC}$ | 4.00 | 86.00-94.00 | 3.35 $^{AB}$ | 0.16 | 3.19-3.51 |
| 20 | 93.00 $^{AB}$ | 1.00 | 92.00-94.00 | 3.44 $^{AB}$ | 0.05 | 3.39-3.49 |
| 10 | 94.00 $^{AB}$ | 0.00 | 94.00-94.00 | 3.18 $^{A}$ | 0.01 | 3.17-3.19 |
| Mn (ppm) | | | | | | |
| 8000 | 45.00 $^{E}$ | 6.00 | 42.00-48.00 | 9.59 $^{L}$ | 0.30 | 9.29-9.89 |
| 4000 | 88.00 $^{BC}$ | 6.65 | 84.00-96.00 | 7.57 $^{K}$ | 0.52 | 7.05-8.10 |
| 2000 | 91.00 $^{AB}$ | 1.00 | 90.00-92.00 | 5.38 $^{I}$ | 0.05 | 5.33-5.43 |
| 1000 | 94.00 $^{AB}$ | 2.00 | 92.00-96.00 | 4.21 $^{EF}$ | 0.13 | 4.08-4.35 |
| 500 | 95.00 $^{AB}$ | 1.00 | 94.00-96.00 | 3.60 $^{BC}$ | 0.20 | 3.40-3.81 |

Means in each column followed by the same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

with seeds of *A. murale* from Albanian serpentine soil, a significantly higher germination percentage of this species was found in seeds treated with lower concentrations of Ni compared to control group (Pavlova et al. 2010). Leon et al. (2005), in their studies with seeds of *Grevillea exul* var. *rubiginosa*, an endemic serpentine Proteaceae of New Caledonia, reported that the seed coat can reduce the amount of Ni entering the seed, and that a high level of Ni induced the mobilization of macronutrients. This feature of serpentine plants may also be the reason why the seeds of *A. murale* in our study may have tolerated high concentrations of nickel. Furthermore, Abu Auda et al. (2004) reported that seeds of *A. murale* growing in different metalliferous regions of North Greece showed differences in seed germination percentage and in plant tolerance index when grown in nutrient solutions of various Ni or Mn concentrations, probably indicating the adaptation of populations to increased Ni or Mn concentrations in the soil where they come from. By contrast, most plant species, especially many crops, show retarded seed germination and growth due to nickel toxicity and its effect on enzyme activities such as protease, amylase and ribonuclease (Ahmad and Ashraf 2011). According to other studies, lead (Pb) has a significant impact on the morphology and physiology of seeds. It prevents germination, root extension, seedling development, and other

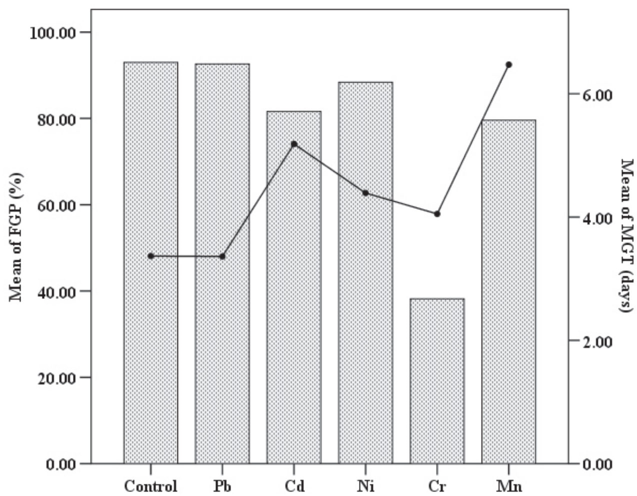


**Figure 1.** Average heavy metal concentration effects on Final germination percentage (FGP) and Mean germination time (MGT) of *A. murale* seeds.

physiological processes (Pourrut et al. 2011). Pb toxicity has been shown to inhibit the emergence of radicals through increased protein and carbohydrate content, affecting enzyme activity (Singh et al. 2011).

The mean germination time (MGT) was clearly different among metals and their concentrations in almost all treatments (Table 1). The germination process of the *A. murale* seed started after 3 days of incubation and ended 10 days after. Based on this, due to the extreme conditions where this plant grows in natural conditions, we can assume that this fast germination time is related to mechanisms that have developed to adapt to these environmental conditions. There are rare plant species that have such a high rate of seed germination. Almost all concentrations of Pb and lower concentrations of Cd used for seed treatments had significant effects on mean germination time, from 3 to 3.5 days to start the germination process. On the other hand, germination time was significantly slower in seed treatments with higher concentrations of metals, especially with Mn, Cd and Ni (from ~ 6 to 10 days). Based on what we emphasized above, it seems that there is a relationship between the time of seed germination and the percentage of final germination, because exactly in the same concentrations when FGP was higher, the MGT was the lowest. It has been reported that cadmium (Cd) delays germination, induces membrane damage, and impairs food reserve mobilization by increased cotyledon/embryo ratios of total soluble sugars, glucose, fructose and amino acids (Rahoui et al. 2010). A similar treatment has been shown to block alpha-amylase and invertase activity and decrease the percentage of germination, embryo growth, and biomass dispersion (Sethy and Ghosh 2013). Other authors reported that seeds of *A. murale* treated with a higher concentration of copper had decreased germination and seedling growth and the velocity of seedling (Brînză et al. 2018).

Furthermore, the results of average of heavy metal concentrations in seed treatment showed that stronger effects on germination percentage (FGP) were observed when seeds were treated with Cr (Figure 1). It seems the toxicity level of chromium is many times higher compared to other metals and inhibition of the percentage of germinability was more than 60%. The ranking according to level of seed germination inhibition of this species by metal is as follows: Cr>Mn>Cd>Ni>Pb. On other hand, the germination time (MGT) was significantly slower in seed treatments with Mn and fastest in control and Pb treatments. Similar results of higher concentration effects of heavy metals have been reported by other authors. Siddiqui et al. (2012) reported that seed germination percentage of *B. rapa* var. *turnip* decreased significantly when heavy metal concentrations increased. However, Cr and Pb were less toxic as compared to Cd on seed germination and the resulting toxicity order of the selected heavy metals remained the same (Cd > Cr > Pb). Leon et al. (2005), in their studies with seeds of *Grevillea exul* var. *rubiginosa*, an endemic serpentine Proteaceae of New Caledonia, reported that the seed coat was able to reduce the amount of Ni entering the seed, and that a high level of Ni induced the mobilization of macronutrients.

The results of root and hypocotyl length of *A. murale* seedlings are presented in Table 2. In general, the length of root from seeds treated with almost all concentrations of heavy metals significantly decreased, except with 500 ppm and 1000 ppm Ni (24.00 and 21.10 mm; respectively), compared to the control group (24.20 mm). In this case, results showed that length of root from seeds treated with different Ni concentrations showed a slight statistical effect compared to other metals. The most inhibitory effects on root growth were obtained after treatment with different concentrations of Pb (from 1.30 to 2.20 mm), Cd (from 1.00 to 8.50 mm) and Mn (from 1.25 to 4.40 mm). Similar results were obtained also for hypocotyl lengths. For seeds treated with lower concentrations of Ni, hypocotyl lengths were higher (28.70 and 26.20 mm) compared to other metals and the control group (25.30 mm). After seed treatments with other higher concentrations of Ni the hypocotyl lengths showed no or slight effects. For seeds exposed to lower concentrations of Cr and Cd, the hypocotyl lengths decreased and reached a 50% inhibition level. Hypocotyl growth was more sensitive to higher concentrations of Cr and Cd, and to all concentrations of Pb and Mn. Furthermore, the results of seed treatment with average heavy metal concentrations showed more marked effects on the root and hypocotyl growth

**Table 2.** Root length, hypocotyl length, and metal index of tolerance (IT) of *A. murale* seedlings treated with different concentrations (ppm) of Ni, Cr, Cd, Pb and Mn.

| | **Root length (mm)** | | **Hypocotyl length (mm)** | | **Index of Tolerance (%)** | |
|---|---|---|---|---|---|---|
| Treatments | Mean | St. Dev. | Mean | St. Dev. | Mean | St. Dev. |
| Control | 24.20 A | 5.60 | 25.30 A | 3.33 | | |
| Ni (ppm) | | | | | | |
| 8000 | 9.50 D | 2.50 | 20.00 B | 2.82 | 39.20 D | 6.40 |
| 4000 | 15.00 C | 5.70 | 20.80 B | 3.08 | 62.00 C | 8.60 |
| 2000 | 18.70 B | 3.90 | 24.80 A | 3.96 | 77.30 B | 8.10 |
| 1000 | 21.10 B | 7.10 | 28.70 A | 4.54 | 87.20 B | 5.20 |
| 500 | 24.00 A | 5.00 | 26.20 A | 3.73 | 99.20 A | 7.20 |
| Cr (ppm) | | | | | | |
| 2000 | 1.80 E | 0.20 | 1.20 H | 0.10 | 7.40 EF | 1.20 |
| 1500 | 2.80 E | 0.40 | 2.30 H | 0.10 | 11.60 EF | 2.30 |
| 1000 | 9.10 D | 6.30 | 7.30 EF | 1.70 | 37.60 D | 7.60 |
| 500 | 13.50 C | 4.70 | 10.80 D | 2.29 | 55.80 C | 4.70 |
| 100 | 20.60 B | 4.20 | 19.60 B | 2.67 | 85.10 B | 5.30 |
| Cd (ppm) | | | | | | |
| 200 | 1.00 E | 0.20 | 1.30 H | 0.10 | 4.10 F | 0.60 |
| 100 | 1.80 E | 0.40 | 2.20 H | 0.20 | 7.40 EF | 0.80 |
| 50 | 2.00 E | 0.20 | 5.40 FG | 1.50 | 8.30 EF | 0.60 |
| 20 | 2.70 E | 1.10 | 11.60 D | 0.96 | 11.20 EF | 1.10 |
| 10 | 8.50 D | 2.12 | 17.50 C | 2.32 | 35.10 D | 2.60 |
| Pb (ppm) | | | | | | |
| 200 | 1.30 E | 0.10 | 1.20 H | 0.10 | 5.40 EF | 1.60 |
| 100 | 1.50 E | 0.10 | 1.50 H | 0.10 | 6.20 EF | 1.20 |
| 50 | 1.50 E | 0.10 | 1.50 H | 0.10 | 6.20 EF | 1.20 |
| 20 | 2.00 E | 0.20 | 1.00 H | 0.10 | 8.30 EF | 1.80 |
| 10 | 2.20 E | 0.40 | 5.20 G | 2.04 | 9.10 EF | 2.10 |
| Mn (ppm) | | | | | | |
| 8000 | 1.25 E | 0.10 | 1.30 H | 0.10 | 5.40 EF | 0.60 |
| 4000 | 1.80 E | 0.20 | 1.80 H | 0.20 | 5.40 EF | 0.40 |
| 2000 | 1.80 E | 0.20 | 4.78 G | 1.14 | 7.40 EF | 0.80 |
| 1000 | 2.30 E | 0.30 | 6.60 GH | 2.42 | 9.50 EF | 1.50 |
| 500 | 4.40 E | 2.30 | 8.00 E | 1.14 | 18.20 E | 2.60 |

Means in each column followed by the same letters are not significantly different at P 0.05 by one-way ANOVA with Duncan's multiple range tests.

of *A. murale* seedlings when they were treated with Cd, Pb and Mn (Figure 2). On the other hand, the mean Ni concentration effects on root and hypocotyl elongation were less compared to other metals and the control group of seedlings. Based on this, we can also presume that *A. murale*, as a serpentine Ni hyperaccumulator, is adapted to resist nickel-rich soils even in very high concentrations. Similar results for the effects of higher concentrations of metals on root and hypocotyl lengths of seeds of *A. murale* from Albania were reported by Pavlova et al. (2010); root elongation decreased approximately 50% compared to the controls when a concentration of 1 mM Ni was applied. Other studies reported similar results; seed germination of *Sarcocornia perennis* and *S. fruticosa* was not affected by any metal concentration, but seedling growth, mainly radicle length, was reduced by increasing metal concentrations (Sanjosé et al. 2022).

The maximum metal index of tolerance was found on seeds treated with different concentrations of Ni compared to other metals (Table 2). Moreover, the index of tolerance slightly decreased in the highest Ni concentration (from 39.20 to 87.20 %) and lower concentrations had no significant effects (99.20 %). The index of tolerance was more than 50% at lower concentrations of Cr. Depending on treatments with other metals such as Pb, Mn and Cd, the index of tolerance was in the range from 5.40 % (Pb

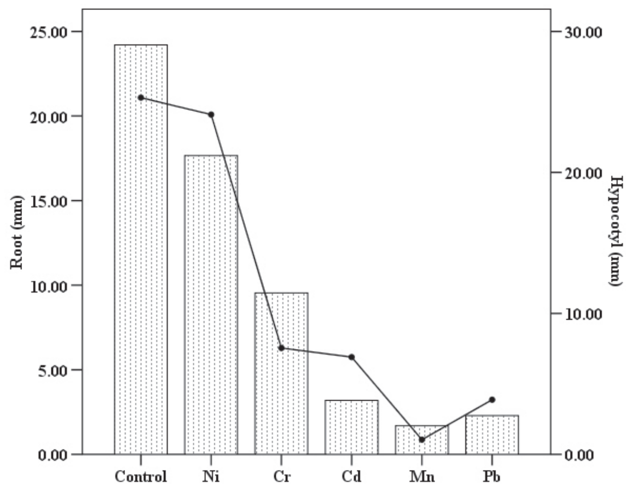


**Figure 2.** Average heavy metal concentration effects on root and hypocotyl length of *A. murale* seedlings.

200 ppm) to 18.20 % (Mn 500 ppm). Higher concentrations of metals, especially Pb and Mn, caused abnormalities in the seedlings of this species, including chlorosis, necrosis, and coiling of the hypocotyl and root. Similar to results from other studies, *Alyssum* species survived in the soil with higher Ni and Mn concentrations and tolerated these metal concentrations for a long time (Broadhurst and Chaney 2016; Muszyńska et al. 2018). Based on the results of our research it appears that *A. murale* seeds can tolerate high concentrations of some metals during germination, but they suffer damage during growth from these metals, except nickel. Abu Auda et al. (2004) observed negative correlations of seed germination and tolerance index of *A. murale* from Greece in relation to increasing Ni and Mn concentrations in the nutrient solution, probably attributed to the toxic function of the above metals.

## Conclusions

Results of this research with seeds of the nickel hyperaccumulator *A. murale*, tested in laboratory conditions for the effect of heavy metals (Ni, Cr, Cd, Pb, and Mn) on seed germination, mean germination time and root and hypocotyl length, showed that higher concentrations of these metals considerably affected the germination and growth process. The results also suggest that lower concentrations of metals, especially nickel, used in the seed germination process can present remarkable positive effects on this plant species, both on seed germination percentage and speed of germination, as well as root and hypocotyl growth. Seeds treated with Ni showed relatively high tolerance to this metal, presenting the potential for possible practical use in various fields of phytoremediation technology. However, further studies are needed to give more insight into the effects of combinations of metals and to explore the possibility for cultivation and eventual use in industrial areas, which are characterized by high soil pollution with heavy metals.

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## References

Abu Auda M, Symeonidis L, Babalonas D (2004) Mineral composition and seed germination of *Alyssum murale* populations under Ni and Mn stress. J Biol Res 2(IKEEART-2017-266):75-83.

Ahmad MS, Ashraf M (2011) Essential roles and hazardous effects of nickel in plants. Rev Environ Contam Toxicol 214:12567.

Amari T, Ghnaya T, Abdelly C (2017) Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. S Afr J Bot 111:99-110.

Auda M, Symeonisis L, Babolonas D (2004) Mineral composition and seed germination *of Alyssum murale* populations under Ni and Mn stress. J Biol Res 2:75-83.

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metalic elements—A review of their distribution, ecology and phytochemistry. Biorecovery 1:81-126.

Baker AJM, Ernst WHO, Van Der Ent A, Malaisse F, Ginocchio R (2010) Metallophytes: the unique biological resource its ecology, and conservational status in Europe, central Africa and Latin America. In Ecology of Industrial Pollution. Batty LC and Hallberg KB, Eds. Cambridge, UK: University Press, 7-40.

Bani A, Echevarria G, Sulçe S, Mullaj A, Morel JL (2007) In-situ phytoextraction of nickel by native population of *A. murale* on ultramafic site in Albania. Plant Soil 293:79-89.

Bani A, Imeri A, Echevarria G, Pavlova D, Reeves RD, Morel JL, Sulçe S (2013) Nickel hyperaccumulation in the serpentine flora of Albania. Fresenius Environ Bull 22(6):1792-1801.

Bani A, Pavlova D, Echevarria G, Mullaj A, Reeves RD, Morel JL, Sulçe S (2010) Nickel hyperaccumulation by the species of *Alyssum* and *Thlaspi* (Brassicaceae) from the ultramafic soils of the Balkans. Bot Serb 34(1):3-14.

Baruah N, Mondal SC, Farooq M, Gogoi N (2019) Influence of heavy metals on seed germination and seedling growth of wheat, pea, and tomato. Water Air Soil Pollut 230:1-15.

Boyd RS, Martens SN (1992) The raison d'etre for metal hyperaccumulation by plants. In: Baker AJM, Proctor J, Reeves RD, eds. The vegetation of Ultramafic (serpentine) soils. Andover, UK: Intercept, 279–289.

Brînză M, Chelariu EL, Aiordăchioaiei A, Draghia L (2018) Copper effect on seed germination and plant sprouting of *Alyssum murale* species. Sci Papers Series B Hortic 62:571-574.

Broadhurst CL, Chaney RL (2016) Growth and metal accumulation of an *Alyssum murale* nickel hyperaccumulator ecotype co-cropped with *Alyssum montanum* and perennial ryegrass in serpentine soil. Front Plant Sci 7:1-9.

Brooks RR (1987) In Dudley TR (Ed). Serpentine and its vegetation. Dioscorides, Portland, OR, USA.

Brooks RR (1998) Phytochemistry of hyperaccumulators. In Brooks RR (Ed). Plants that Hyperaccumulate Heavy metals. CAB International, New York, USA.

Gashi B, Osmani M, Aliu S (2019) Breaking seed dormancy of *Tulipa scardica* Bornm. and *Tulipa kosovarica* Kit tan, Shuka & Krasniqi by pre-chilling, plant growth regulators and some chemical treatments. Acta Agric Slov 113(2):203-210.

Gashi B, Osmani M, Aliu S, Zogaj M, Kastrati F (2020) Risk assessment of heavy metal toxicity by sensitive biomarker δ-aminolevulinic acid dehydratase (ALA-D) for onion plants cultivated in polluted areas in Kosovo. J Environ Sci Health, Part B 55(5):462-469.

Gill SS, Anjum NA, Ahmad I, Thangavel P, Sridevi G, Pacheco M, Pereira ME (2012) Metal hyperaccumulation and tolerance in *Alyssum*, *Arabidopsis* and *Thlaspi*: An overview. The plant family Brassicaceae: contribution towards phytoremediation. 99-137.

Herath I, Kumarathilaka P, Navaratne A, Rajkaruna N, Viyhanage M (2014) Immobilization and phytotoxicity reduction of heavy metals in serpentine soil using biochar. J Soil Sediment 15(1):126-138.

Ievinsh G, Andersone-Ozola U, Samsone I (2020) *Alyssum montanum* subsp. *gmelinii*, a rare plant species from coastal sand dunes, as a potential Ni accumulator: comparison with *Alyssum murale*. Environ Exp Biol 18:107-115.

ISTA (1996) Rules for Seed Testing. International Seed Testing Association. Seed Sci Technol. 24 Suppl. Zürich, Switzerland.

Jakovljević K, Lakušić D, Vukojičić S, Tomović G, Šinžar-Sekulić J, Stevanović V (2011) Richness and diversity of Pontic flora on serpentine of Serbia. Cent Eur J Biol 6(2):260-274.

Kazakou E, Dimitrakopoulos PG, Baker AJM, Reeves RD, Troumbis AY (2008) Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. Biol Rev 83(4):495-508.

Krämer U (2010) Metal hyperaccumulation in plants. Annu Rev Plant Biol 61:517-534.

Krasniqi E, Berisha N, Millaku F, Rexhepi F (2019) Contribution to the knowledge on the flora of Mt Golesh, central Kosovo. Nat Croat 28(2):423-440.

Leon V, Rabier J, Notonier R, Barthelemy R, Moreau X, Bouraïma-Madjèbi S, Viano J, Pineau R (2005) Effects of three nickel salts on germinating seeds of *Grevillea exul* var. *rubiginosa*, an endemic serpentine proteaceae. Ann Bot 95(4):609-618.

Meindl GA, Bain DJ, Ashman TL (2014) Nickel accumulation in leaves, floral organs and rewards varies by serpentine soil affinity. AoB Plants 6:1-9.

Moradi DP, Sharif-Zadeh F, Janmohammadi M (2008) Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). J Agri Biol Sci 3(3):22-25.

Muszyńska E, Labudda M, Różańska E, Hanus-Fajerska E, Znojek E (2018) Heavy metal tolerance in contrasting ecotypes of *Alyssum montanum*. Ecotoxicol Environ Saf 161:305-317.

Nkrumah PN, Baker AJM, Chaney RL, Erskine PD, Guillame E, Morel JL (2016) Current status and challenges in developing nickel phytomining: an agronomic perspective. Environ Pollut 406:55-69.

Osmani M, Tuna M, Elezaj IR (2018) Concentration of some metals in soil and plant organs and their biochemical profiles in *Tulipa luanica, T. kosovarica* and *T. albanica* native plant species. Physiol Mol Biol Plants 24(6):1117-1126.

Pavlova D, Vila D, Vila K, Bani A, Xhaferri B (2018) Effect of nickel on seed germination of Alyssum species with potential for phytomiming in Albania. Fresenius Environ Bull 27(3):1345-1352.

Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E (2011) Lead uptake, toxicity, and detoxification in plants. Rev Environ Contam Toxicol 213:113-136.

Proctor J (1999) Toxins, nutrient shortages and droughts: the serpentine challenge. Trends Ecol Evol 14:334-335.

Psaras GK, Manetas Y (2001) Nickel localization in seeds of the metal hyperaccumulator *Thlaspi pindicum* Hausskn. Ann Bot 88(3):513-516.

Rahoui S, Chaoui A, El Ferjani EJ (2010) Membrane damage and solute leakage from germinating pea seed under cadmium stress. Hazard Mater 178:1128-1131.

Reeves RJ, Adigüzel N (2008) The nickel hyperaccumulating plants of the serpentines of Turkey and adjacent areas: a review with new data. Turk J Biol 32:143-153.

Sanjosé I, Muñoz-Rodríguez AF, Ruiz F, Navarro F, Sánchez-Gullón E, Nieva FJ, Castillo JM (2022) Metal effects on germination and seedling development in closely-related halophyte species inhabiting different elevations along the intertidal gradient. Mar Pollut Bull 175:113375.

Seregin IV, Kozhevnikova AD (2011) Histochemical methods for detection of heavy metals and strontium in the tissues of higher plants. Russ J Plant Physiol 58:721-727.

Sethy SK, Ghosh S (2013) Effect of heavy metals on germination of seeds. J Nat Sci Biol Med 4(2):272-275.

Siddiqui MM, Abbasi BH, Ahmad N, Ali M, Mahmood T (2014) Toxic effects of heavy metals (Cd, Cr and Pb) on seed germination and growth and DPPH-scavenging activity in *Brassica rapa* var. turnip. Toxicol Ind Health 30(3):238-249.

Singh HP, Kaur G, Batish DR, Kohli RK (2011) Lead (Pb)-inhibited radicle emergence in *Brassica campestris* involves alterations in starch-metabolizing enzymes. Biol Trace Elem Res 144:1295-301.

Tappero R, Peltier E, Grafe M, Heidel K, Ginder-Vogel M, Livi KJT (2007) Hyperaccumulator *Alyssum murale* relies on a different metal storage mechanism for cobalt than for nickel. New Phytol 175:641-654.

van der Ent A, Baker AJM, Reeves RD, Chaney R L (2015) Agromining: farming for metals in the future? Environ Sci Technol 49:4773-4780.

van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H (2013) Hyperaccumulators of metal and metalloidtrace elements: facts and fiction. Plant Soil 362:319-334.

Zhang L, Angle JS, Chaney R (2007) Do high-nickel leaves shed by the nickel hyperaccumulator *Alyssum murale* inhibit seed germination of competing plants? New Phytol 173(3):509-516.

Zogaj M and Düring RA (2016) Plant uptake of metals, transfer factors and prediction model for two contaminated regions of Kosovo. J Plant Nutri Soil Sci 179(5):630-640.