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Original Research

Interactive Effects of Ni, Cr, Co, Ca, and Mg in Seeds Germination Test: Implications for Plant Growth in Ultramafic Soils

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Abstract

Weathering of peridotites and serpentinites leads to the formation of ultramafic soils characterized by several peculiar properties such as low Ca/Mg ratios and elevated concentrations of Ni, Cr, and Co. The aim of our study was to investigate the effects of Ca^{2+} and Mg^{2+} on seed germination in the environment that mimic ultramafic and non-ultramafic soils. We hypothesized that Ca and Mg alleviate toxicity of Ni, Cr, and Co in relation to seed germination and root length. Regardless of concentration, metal salts inhibited seed germination and root growth for almost all studied plant species compared to control. An increase in germination was observed in treatments containing high concentrations of Ni^{2+} and Co^{2+} with the addition of Ca^{2+} and Mg^{2+}. Roots of plants had greater length in high Ni^{2+} and Co^{2+}-treatments with Ca^{2+} and Mg^{2+} in relation to metal treatments without the addition of Ca^{2+} and Mg^{2+}. In low-content metal treatments with Ca^{2+} and Mg^{2+}, roots had similar or smaller lengths compared to metal treatments. Our results demonstrate that Ca^{2+} and Mg^{2+} alleviate toxicity of Ni^{2+} and Co^{2+} during seed germination and root growth under conditions simulating ultramafic soils. Therefore, in soils derived from ultramafic rocks enriched in Ca, an increase in germination and root growth is expected as opposed to soils occurring on Ca-poor ultramafic rocks. In non-ultramafic soils containing relatively low amounts of Ni, Cr, and Co, the roles of Ca and Mg are negligible.

Keywords: *Brassica napus*, *Vicia sativa*, excluders, serpentinites, bioassays

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Introduction

Metal contamination of soils is an important problem due to a possible negative response in living organisms. Most of the scientific research concerns metallic elements coming from anthropogenic sources such as mining, smelting, power plants, transport, industry, and landfills [1-8]. Nevertheless, the geogenic origin of metals related to volcanic activity and weathering of rocks anomalously rich in metals should not be neglected. A relevant geogenic source of metals is weathering of ultramafic rocks, which leaves a footprint in the soil as well as plant chemistry. Ultramafic rocks include igneous peridotite and its metamorphic analog – serpentinite – which forms as a result of a metamorphic process called serpentinization. Both parent rocks are characterized by relatively low content of silica (<45%) and Ca (<4%), and high content of Mg (18-24%; [9-18]). Furthermore, peridotites and serpentinites contain elevated amounts of Ni, Cr, and Co. On the other hand, mineral composition among them is variable. The primary minerals in peridotites are olivine (forsterite Mg_2SiO_4), clinopyroxene (diopside – $CaMg(Si_2O_6)$), and orthopyroxene (enstatite $Mg_2(Si_2O_6)$). Serpentinite is mostly composed of serpentine-group minerals $Mg_6[(OH)_8Si_4O_{10}]$ with relics of primary phases derived from protolith. Soils derived from ultramafic rocks are called ultramafic soils (formerly serpentine soils) regardless of the type of parent rock (reviewed in [11]). The peculiarity of ultramafic soils results from the Ca deficiency and excess of Mg (Ca/Mg ratio<1). Furthermore, these soils contain elevated amounts of Ni, Cr, and Co with deficiency of N, P, K. For example, Ni content in some ultramafic soils ranges from 1300 mg kg^{-1} up to 7000 mg kg^{-1} [13, 19-23]. Climate is an important soil-forming factor that controls the behavior of elements in soils. In a temperate climate, Mg-silicates from peridotites and serpentinites undergo incomplete hydrolysis, therefore Mg is still observed within soil profiles (soils are represented by Leptosols and Cambisols [11]). On the contrary, in a tropical climate complete hydrolysis is observed in peridotites and Mg is leached down the profiles, hence Fe-oxides are accumulated in the upper part of profiles whereas secondary Mg-silicates are at the bottom. Peridotite-derived soils in a tropical climate are classified as Ferralsols.

As a consequence of nutrient deficiency (especially Ca), excess Mg and elevated amounts of metals, the serpentine syndrome is observed in plant communities [24-26]. The serpentine syndrome is reflected in low plant productivity along with stunted vegetation in comparison to adjacent non-ultramafic sites. Nevertheless, plant growing in ultramafic soils for a long period of time are getting used to unfavorable conditions and tend to adapt to edaphic stress [27]. Therefore, some plant species in ultramafic ecosystems evolved into endemites (e.g., Ni-hyperaccumulators). Nickel hyperaccumulators are plant species containing at least 1000 mg kg^{-1} Ni in dry matter of aboveground tissues [28, 29]. The high content of Ni in leaves and high biomass of some hyperaccumulators allow us to use these plants in phytoremediation technologies and agromining [15, 30-37].

Recent years have seen increasing concern about the influence and type of ultramafic rock on soils and plants. In a tropical climate, Van der Ent et al. [38] have shown that Ferralsols do not differ significantly from adjacent non-ultramafic soils. On the contrary, serpentinite-derived Leptosols and Cambisols have inherited chemical properties of parent rock, hence edaphic stress for plants has been observed in outcroppings of fully-serpentinized peridotites. In other studies, Kierczak et al. [39] demonstrated that the mobility of Ni in ultramafic soils developed under temperate climate depends on the type of ultramafic parent material. The authors have shown that nickel is more mobile in soils derived from peridotites and/or partially serpentinized peridotites compared to those occurring on serpentinites, which has been explained by the susceptibility of minerals to weathering. Olivine as a major constituent in peridotites is more susceptible to weathering than serpentines being major phases in serpentinites. Under atemperate climate, Pędziwiatr et al. [40] have demonstrated that the accumulation and translocation of Ni in excluder plant species can be controlled by Ca in soils from Ca-bearing minerals and Mg from easily susceptible to weathering olivine. Calcium and Mg compete with Ni, hence aboveground parts of plants growing in peridotite-derived soil have a lower content of Ni compared to these from erpentinite-derived soil.

Seeds are responsible for plant reproduction and dispersion in the ecosystem. Furthermore, seeds are the first in contact with soil and/or soil solution. The peculiarity of ultramafic soils, (e.g., metal enrichment, low Ca to Mg ratio, low nutrient content) can be a factor that modifies the rate of germination. Metals change the germination by their toxicity *sensu lato* and by the inhibition of water uptake [41]. For example, nickel affects seed germination due to changes in protease and ribonuclease activity, whereas an increase of RNA is observed [41, 42]. Nevertheless, the effect of metals on seeds depends on the permeability of seed coats (their morphology) that represent a defensive barrier against, i.e., edaphic stress. The defense strategies in seedlings are observed also at the biochemical stage. In *Oryza sativa*, an increase of ascorbate (antioxidant) was observed against reactive oxygen species induced by Ni [43]. Therefore, studies of seed ability to germinate in natural metal-enriched environments can deliver interesting data that may be useful for the management of contaminated sites.

The aim of our study is to investigate the effect of Ca and Mg on seed germination under conditions that mimic ultramafic and non-ultramafic sites (based on the experiments with high- and low-concentration metals (Ni, Cr, Co) salt solutions respectively). We hypothesize that Ca and Mg that can be delivered into the ultramafic soil from Ca-bearing minerals and olivine, promote seeds germination and roots growth. We also expect that Ca and Mg play the same role in non-ultramafic soils with relatively low content of metals.

Materials and Methods

Selected Plant Species

Five plant species from three families were chosen for our experiment. *Raphanus sativus* L. and *Brassica napus* L. belong to Brassicaceae. Fabaceae is represented by *Trifolium pretense* L. and *Vicia sativa* L. *Triticum aestivum* L. is from Poaceae. Model species were selected due to relative large seeds and fast growth. It is worth noting that species from Brassicaceae, Fabaceae and Poaceae are widely distributed in ultramafic soils in Poland [44, 45]. Furthermore, the Organization for Economic Co-operation and Development (OECD) recommended these species for testing chemicals [46]. It also should be emphasized that approximately 20% of all known Ni-hyperaccumulators belong to Brassicaceae [47].

Germination Test

The tolerance of plant species to Ni^{2+}, Cr^{3+}, and Co^{2+} was measured by means of a germination and root elongation test that is simple, fast, and easy to perform [48–50]. Seeds were purchased in a commercial seed market. Prior to the germination test, seeds were surface sterilized in 5% NaClO (CHEMPUR®) for 4 min in order to prevent fungal growth. In the next step, seeds were washed three times with MiliQ H_2O. Afterward, seeds were sown to filter-lined plastic Petri dishes (d = 10 cm) soaked with 10 cm^3 of a solution containing metals (described below). For each seed treatment, three independent replicates (each containing 30 seeds) were prepared. Therefore, the number of seeds (n) for one treatment was 90. The total number (N) of seeds for all plant species used in this study was 14 400. After sowing, Petri dishes were covered with lids and kept in darkness at an ambient temperature of 25±1ºC for 72 h following the protocols described in other studies [51-53].

The effect of Ca^{2+} and Mg^{2+} on Ni^{2+}, Cr^{3+}, and Co^{2+} toxicity was analyzed based on a metal salt solution in chloride form. This form of metal salts ensures higher solubility of $CaCl_2$ and $MgCl_2$ in ultrapure water as compared to sulfate form. Furthermore, the chloride form of Ni^{2+} was used in some studies [54, 55]. The used salts of metals ($NiCl_2$, $CrCl_3$, and $CoCl_2$) were analytical grade (CHEMPUR). We used a trivalent form of Cr because Kierczak [22] demonstrated calorimetrically that Cr^{6+} content is generally below the detection limit in Polish ultramafic soils. The amounts of metal salts used for preparing solutions at desired metal concentrations were calculated and the solutions were prepared. The first set of treatments included the following concentrations: (a) Ni^{2+} 200 mg dm^{-3}, (b) Ni^{2+} 200 mg dm^{-3} + Ca^{2+} 200 mg dm^{-3}, (c) Ni^{2+} 200 mg dm^{-3} + Mg^{2+} 200 mg dm^{-3}, and (d) Ni^{2+} 200 mg dm^{-3} + Ca^{2+} 200 mg dm^{-3}+ Mg^{2+} 200 mg dm^{-3}. In other studies, metal concentrations in solution were from 100 mg dm^{-3} up to 500 mg dm^{-3} Ni^{2+} [51, 54]. Moreover, we tested the effect of the addition of Ca^{2+} 200 mg dm^{-3} and Mg^{2+} 200 mg dm^{-3} alone. For Cr^{3+} and Co^{2+} we also used concentrations at 200 mg dm^{-3}. These concentrations simulate Ni, Cr, and Co-rich ultramafic soils. The concentration of Cr^{3+} (200 mg dm^{-3}) may be overestimated because the mobility of this element is very low in ultramafic soils due to the resistance of Cr-bearing minerals (spinels) to weathering [22, 23, 56, 57]. Nevertheless, increased HNO_3-extractability of Cr (from 500 mg kg^{-1} up to 1390 mg kg^{-1}) in ultramafic sites on the island of Unst was observed [58]. In studies at Niquelândia (Brazil), the slow weathering of spinels was demonstrated [59]. Furthermore, we decided to use a similar concentration as applied in the case of other elements in order to eliminate the competition effect stemming from different concentration ranges. In addition, it is important to stress that laboratory simulations do not ideally reflect real environmental conditions, but these experimental simulations are rather used for elucidating the mechanisms and processes (mostly related to the different stages of plant development) that may go on in the true ultramafic soil solution. Additionally, knowledge about chemical forms of Ni in ultramafic soils is rather poor – even in New Caledonia [54]. Therefore, we used another set of treatments containing relatively low concentrations of metals (25 mg dm^{-3} with or without Ca^{2+} and Mg^{2+} at concentrations of 100 mg dm^{-3}) that may reflect average metal content in soils globally since Ni average content in World soils was estimated at 29 mg kg^{-1} [60], and in the case of Cr low mobility of this element in ultramafic soils. Furthermore, each experiment was accompanied by the ultrapure water control treatment (n = 30, N = 90 in 10 cm^3 of MiliQ H_2O). The pH values of solutions measured by means of an ELMETRON pH-meter are given in Figs 1-6.

After 72 h the seedlings were carefully transferred onto scanner glass (Canon LiDE 120) and scanned at 600 dpi resolution. The image of seedlings was processed in ImageJ freeware software that was able to measure root length [61]. Only roots were measured (using a freehand line tool) because underground parts of plants are first to contact metals in soil/soil solution. In excluder plant species, metals are accumulated mostly in roots, therefore roots are more sensitive for metal toxicity than shoots [62]. Germination of *Triticum aestivum* leads to growth of three roots. The longest root was measured for this species.

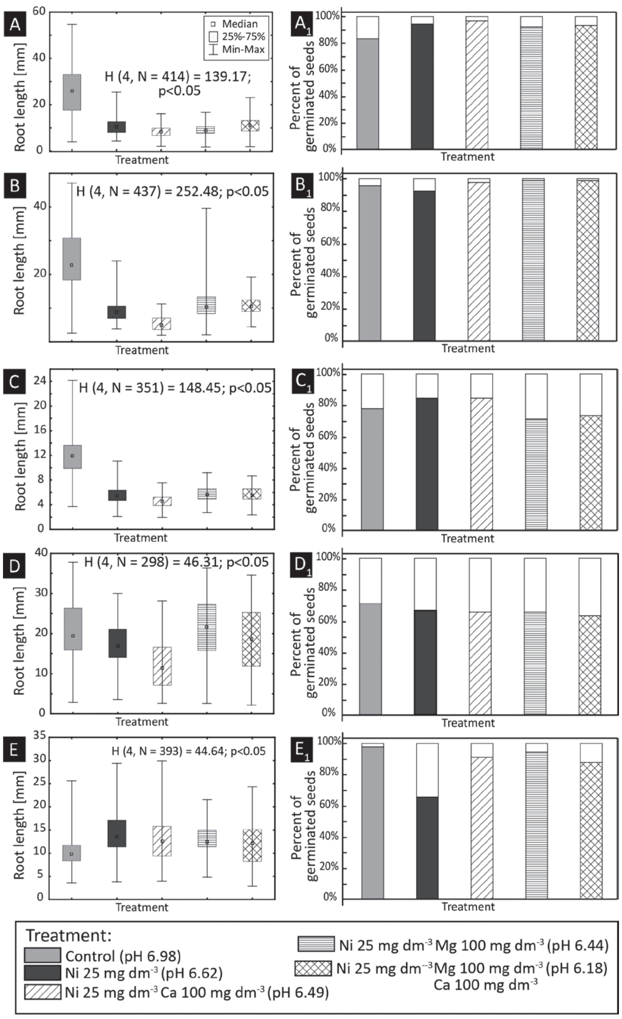

Fig. 1. Results of roots length (A-E) and percentage of germinated seeds (A1-E1) in low Ni^{2+} (25 mg dcm^{-3}) treatment (A- *Brassica napus*, B-*Raphanus sativus*, C-*Trifolium pratense*, D-*Triticum aestivum*, E-*Vicia sativa*).

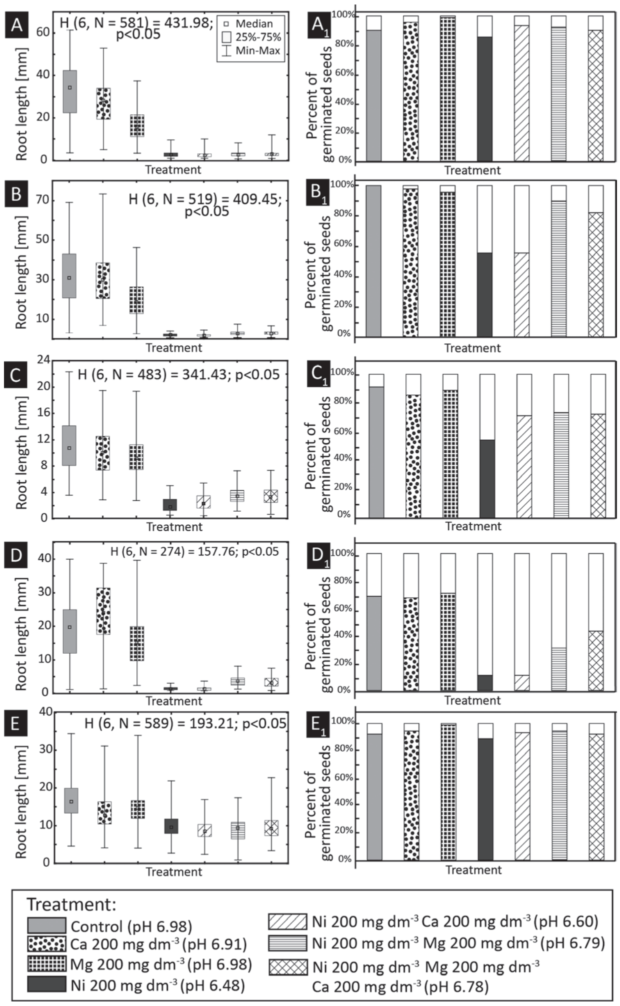

Fig. 2. Results of roots length (A-E) and percentage of germinated seeds (A1-E1) in high Ni^{2+} (200 mg dcm^{-3}) treatment (A-*Brassica napus*, B-*Raphanus sativus*, C-*Trifolium pratense*, D-*Triticum aestivum*, E-*Vicia sativa*).

Data Calculations and Statistical Analysis

At the end of the experiment, several parameters allowing us to compare the tolerance of plants for Ni^{2+}, Cr^{3+}, and Co^{2+} were calculated. The Root Tolerance Index (RTI) and Elongation Inhibition Rate (EIR) were calculated only for experiments with high concentrations of metals (200 mg dm^{-3}; with and without Ca^{2+}, Mg^{2+}).

Germination percentage (GP) was calculated as the ratio of a total number of seeds germinated in a solution containing metals (with and without Ca^{2+}, Mg; GS) to the total number of seeds used for the treatment (N = 90; TS; Equation 1).

$$GP = \frac{GS}{TS} \times 100\ [\%] \tag{1}$$

The RTI was determined according to the formula proposed by Rout et al. [63]. The RTI refers to the ratio of mean root elongation in a solution containing Ni^{2+}, Cr^{3+}, and Co^{2+} (with and without Ca^{2+}, Mg^{2+}; RLM) in relation to the mean root elongation in control solution (MiliQ; RLC; Equation 2).

$$RTI = \frac{RLM}{RLC} \times 100\ [\%] \tag{2}$$

The EIR was calculated as the difference between root elongation in the control solution (RLC) and a solution containing metals (RLM; [51]):

$$EIR = \frac{(RLC - RLM)}{RLC} \times 100\ [\%] \tag{3}$$

The differences in root lengths among treatments were analyzed using the Kruskal-Wallis test (non-parametric equivalent for one-way ANOVA). Before the selecting statistical test, the normality of results was verified by means of the Saphiro-Wilk test. Furthermore, results of RTI and EIR from high Ni^{2+}, Cr^{3+}, and Co^{2+} treatments (200 mg dm^{-3}) alone and in combination with Ca^{2+} and Mg^{2+} (200 mg dm^{-3}) were used for cluster analysis (Euclidean distance, Ward agglomerative

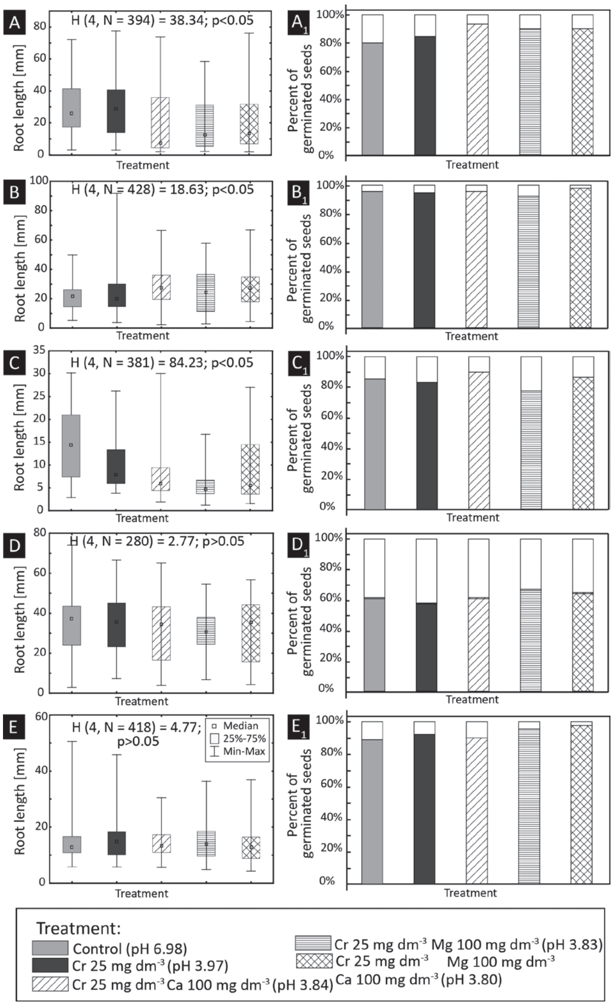

Fig. 3. Results of root length (A-E) and percentage of germinated seeds (A1-E1) in low Cr^{3+} (25 mg dcm^{-3}) treatment (A-*Brassica napus*, B-*Raphanus sativus*, C-*Trifolium pratense*, D-*Triticum aestivum*, E-*Vicia sativa*).

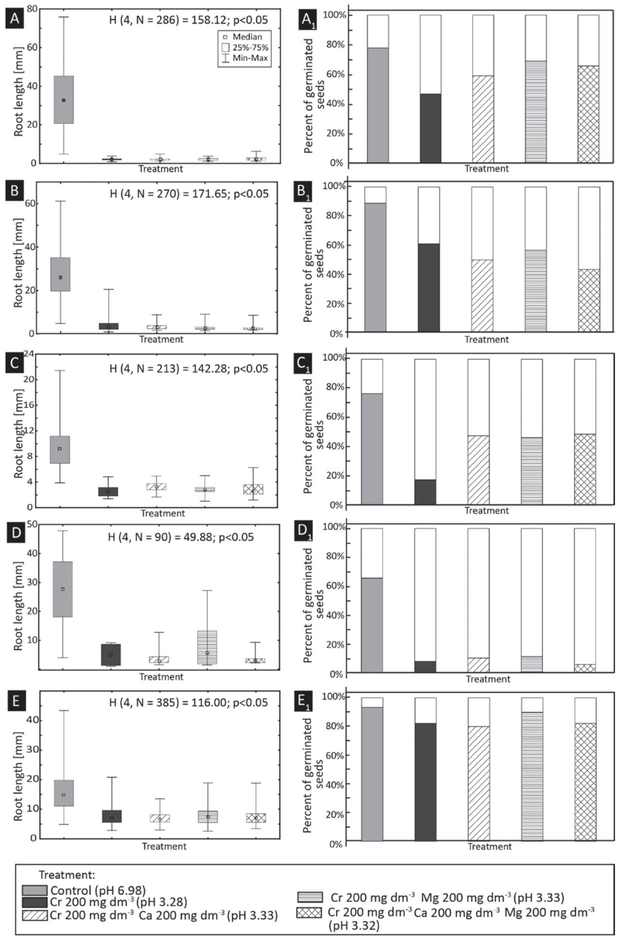

Fig. 4. Results of root length (A-E) and percentage of germinated seeds (A1-E1) in high Cr^{3+} (200 mg dcm^{-3}) treatment (A-*Brassica napus*, B-*Raphanus sativus*, C-*Trifolium pratense*, D-*Triticum aestivum*, E-*Vicia sativa*).

method) to confirm the tolerance among studied species. In order to separate plant groups (cluster numbers), the Sneath's index was calculated [64]. The less restrictive significance criterion refers to 2/3 Dmax and the strict significance criterion to 1/3 Dmax. Significance level (α) in this study was 0.05. All statistical analyses were done in Statistica 12.5 (StatSoft).

Results and Discussion

Germination and Root Growth

Results of germination and root elongation test are presented in Figs 1-6. Our study shows that low concentrations of Ni^{2+}, Cr^{3+}, and/or Co^{2+} (25 mg dm^{-3}) cause an unexpected increase in seed germination of several plant species (Figs 1, 3, 5). For example, low concentrations of Ni^{2+} result in enhanced germination of *Brassica napus* of from 83% in control up to 94% in metal salt solution. A contrasting trend is observed in the example for *Raphanus sativus* and *Vicia sativa* during low Ni^{2+} concentration treatments. Low concentrations of metals play different roles relative to root length. In some cases, no significant effect of low concentrations of Ni^{2+} (25 mg dm^{-3}) on root length is visible (i.e., for *Triticium aestivum* in Ni^{2+} treatment M = 17.11 mm SD = 5.75 mm Me = 16.88 mm and in control M = 20.44 mm SD = 7.42 mm Me = 19.40 mm; $H(4,N = 298) = 46.31$, $p<0.05$; $z = 2.43$, $p>0.05$). On the contrary, Ni^{2+} (25 mg dm^{-3}) significantly inhibits the growth of *Trifolium pratense* roots (Fig. 1C; ($H(4,N = 351) = 148.45$, $p<0.05$; $z= 8.39$, $p<0.05$). On the other hand, enhanced root elongation is observed in *Vicia sativa* in low Ni^{2+} treatment compared to control (Fig. 1E; $H(4, N = 393) = 44.64$, $p<0.05$; $z = 5.96$, $p<0.05$). Low concentrations of Cr^{3+} and Co^{2+} do not reduce root growth of all species compared to control except for Co^{2+} in *Trifolium pratense* ($H(4, N = 377) = 89.51$, $p<0.05$; $z = 5.96$, $p<0.05$). Furthermore, our results for some plants show that low metal concentrations do not affect germination and root elongation in the same way. In *Brassica napus*, nickel is responsible for inhibition of root growth,

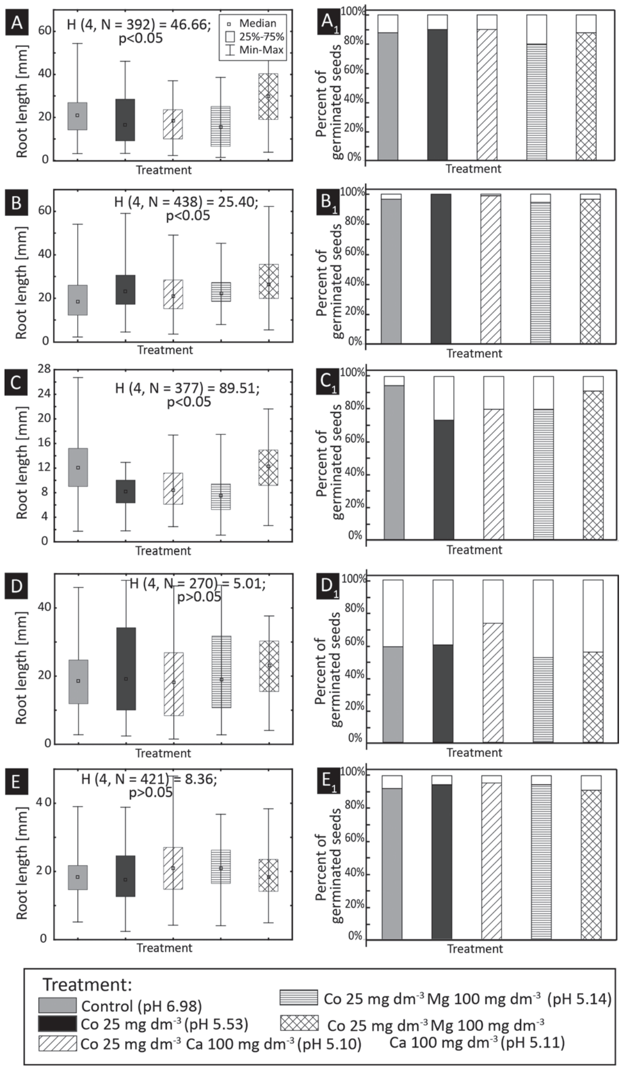

Fig. 5. Results of roots length (A-E) and percentage of germinated seeds (A1-E1) in low Co^{2+} (25 mg dcm^{-3}) treatment (A-*Brassica napus*, B-*Raphanus sativus*, C-*Trifolium pratense*, D-*Triticum aestivum*, E-*Vicia sativa*).

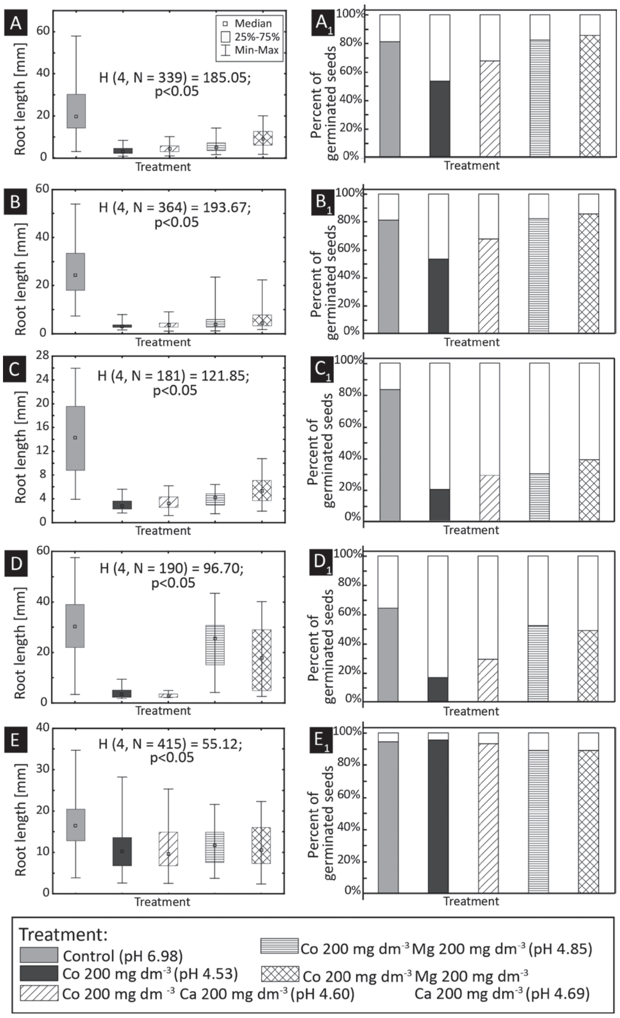

Fig. 6. Results of roots length (A-E) and percentage of germinated seeds (A1-E1) in high Co^{2+} (200 mg dcm^{-3}) treatment (A-*Brassica napus*, B-*Raphanus sativus*, C-*Trifolium pratense*, D-*Triticum aestivum*, E-*Vicia sativa*).

whereas an increase of germination is observed at the same time. Based on this result, it may be assumed that production of abscisic acid (the multifunctional hormone responsible for regulation of biochemical processes ongoing during plant development) was not affected by the presence of low nickel concentrations (since germination was obtained on an optimal level). On the further stages of plant development inhibition of root elongation likely occurred due to the abscisicacid-mediated response to encountered stress conditions [65]. Furthermore, our data for some plant species is not consistent with the results published by Léon et al. [54], who demonstrated that Ni^{2+} concentration even at 5 mg dm^{-3} causes a decrease in *Grevillea exul* var. *rubiginosa* seed germination of approximately 20% relative to water. The increase of Ni^{2+} concentration in solution also caused a considerable reduction in germination of *Raphnus sativus* [66]. On the other hand, Visioli et al. [51] reported no effect of Ni^{2+} on seed germination at 3-6 mg dm^{-3}. Furthermore, increases of percentage germination were noted for *Ambrosia artemisiifolia* of from 83% in control up to 85% at 50 mg dm^{-3} Ni^{2+} [67]. Enhanced germination in low Ni^{2+} treatment in our study is probably due to the stimulation of germination by slight oxidative stress [68]. Bailly et al. [69] consider that reactive oxygen species behave as cellular signaling pathways or as a toxic product during stress conditions. In seeds, reactive oxygen species can be beneficial for germination if production is regulated by removal. Additionally, the increase of reactive oxygen species should lead to an increase in lipid peroxidation [70]. Nevertheless, the authors observed a decrease of lipid peroxidation in *Vicia sativa* in low Ni^{2+} content solution relative to pure water, which was explained by the presence of antioxidants in seedlings against reactive oxygen species [70]. In a real plant system, excretion of metal-chelating agents under stress conditions often occurs and limits metal transfer to root and plant tissue [71]. In field conditions, the role of mycorrhiza and microorganisms should not be neglected. Some studies have demonstrated that the Ni-tolerant ectomycorrhiza (*Pisolithus albus*) isolated from Ni-mine in New Caledonia strongly enhances the growth of *Eucalyptus globulus* under Ni stress [72]. Moreover, the requirement of low amounts of Ni for urease (an enzyme that hydrolyzes urea) offers another possible explanation for our observations [73, 74]. An experiment with *Lactuca sativa* demonstrated that the addition of Ni^{2+} to the nutrient solution significantly increases leaf and root growth in plants amended with urea [75].

The decrease of germination is markedly observed for all species in high metal content solutions compared to control. For example, the percentage of germinated seeds changes from 100% in the control up to 56% in high Ni^{2+} conditions (200 mg dm^{-3}) for *Raphanus sativus*. High concentrations of all studied metals significantly reduce root growth of all species compared to control (i.e., for Co in *Trifolium pratense* M = 2.96 mm, SD = 1.04 mm, Me is 2.76 mm and for control M = 14.27 mm, SD = 6.04 mm, Me = 14.25 mm; H(4, N = 181) = 121.85, $p<0.05$; z = 7.50, $p<0.05$). These results confirm the toxic effect of high concentrations of metals and are in agreement with other studies [66, 76-84]. For example, Yadav et al. [66] explained poor germination and root elongation in high Ni^{2+} conditions by the hydrolyzing role of Ni in amylase. In germination test with *Helianthus annuus* under Ni, stress demonstrated that growth inhibition may be due to Ni-affected biochemical metabolism (mainly related to enzyme production) in a way that the availability of organic compounds necessary for energy production and protein synthesis are both reduced [85]. In addition, the observed inhibition effect has an important implication for a real plant system, because it is possible that Ni indirectly affects photochemical processes such that ATP and NADPH, which are both accumulated in large concentrations and which in turn cause a disturbance in electron transport [86].

The second part of the study was carried out in order to determine the role of Ca^{2+} and Mg^{2+} in metal toxicity related to changes in seed germination and root growth. In general, the effect of Ca^{2+} (200 mg dm^{-3}) on germination is not visible relative to control because Ca^{2+} is an essential element that is required in cell wall and membranes [87]. Furthermore, it is counter-cation for anions in vacuoles and it is intercellular messenger controlling responses to environmental challenges. For Mg^{2+}, the effect is not observed for *Trifolium pratense*, *Triticum aestivum*, and *Vicia sativa* (Fig. 2) due to the biological role of this element. Magnesium is incorporated mostly in cellular components [88]. This element participates in protein synthesis, coordinates with nucleotides, and affects phloem loading of carbohydrates. In contrast, roots of *Brassica napus* (M = 16.56 mm, SD = 7.82 mm, Me = 15.62 mm) and *Raphanus sativus* (M = 19.61 mm, SD = 9.14 mm, Me = 18.79 mm) in high Mg^{2+} solution are significantly shorter than roots in control (M = 32.05 mm, SD = 14.23 mm Me = 34.31 mm for *Brassica napus* and M = 32.76 mm, SD = 14.78 mm, Me = 30.83 mm for *Raphanus sativus*). These results correspond to a well-known Mg toxicity for plants (reviewed in [24]). The toxic effect of Mg was previously confirmed in a non-serpentine population of *Agrostis canina* [89]. Even though the serpentine population of this species is Mg-tolerant compared to the non-serpentine population, high Mg content in ultramafic soils is considered as the main factor controlling serpentine syndrome [23]. For example, plant tolerance to Mg-rich ultramafic soils can be related to phosphatase activity [90]. In *Alyssum bertolonii* (endemite in ultramafic soils), high requirements of Mg were observed in increase phosphatase activity compared to *Alyssum saxatile*, where Mg was toxic.

The role of Ca^{2+} and Mg^{2+} in low-content metal solutions is diversified. For example, no effect of Ca^{2+} and Mg^{2+} on the germination of *Triticum aestivum* with Ni^{2+} is visible. On the other hand, in *Vicia sativa*, an increase of germination is observed in Ni^{2+}-Ca^{2+} treatment (91%) relative to Ni^{2+} treatment (66%). On the contrary, magnesium inhibits the germination of *Trifolium pratense*. Rather, Ca^{2+} and Mg^{2+} do not affect root length in low Ni^{2+} treatment except for *Triticum aestivum*. Roots of this species in Ni^{2+}-Mg^{2+} treatment are longer (M = 20.49 mm, SD = 9.10 mm, Me = 21.62 mm) compared to Ni^{2+} treatment (M = 17.11 mm, SD = 5.75 mm, Me = 16.88 mm). However, the difference is not important statistically (H(4, N = 298) = 46.31, $p<0.05$; z = 2.26, $p>0.05$). In low concentration Cr^{3+} and Co^{2+}-treatments, the alleviating role of Ca^{2+} and Mg^{2+} in germination is visible only in some plant species (i.e., *Trifolium pratense* for Co^{2+}). In most cases with high concentrations of metals (200 mg dm^{-3}), Ca^{2+} and/or Mg^{2+} play an alleviating role with regard to germination (Figs 2, 4, 6). For high Ni^{2+} treatment, both macronutrients enhance root growth in all plant species except for *Vicia sativa*. For example, roots of *Trifolium pratense* are longer in Ni^{2+}-Ca^{2+} (M = 2.59 mm, SD = 1.17 mm, Me = 2.31 mm), Ni^{2+}-Mg^{2+} (M = 3.61 mm, SD = 1.33 mm, Me = 3.44 mm), and Ni^{2+}-Ca^{2+}-Mg^{2+} (M = 3.47 mm, SD = 1.35 mm, Me = 3.377 mm) treatments compared to Ni^{2+} (M = 2.20 mm, SD = 1.23 mm, Me = 1.78 mm). However, it is not important statistically (Fig. 2). For high Cr^{3+} treatment, an increase of root length with Ca^{2+} and Mg^{2+} is observed only for *Brassica napus* and *Trifolium pratense*. The ameliorative influence of Ca^{2+} and Mg^{2+} for Co^{2+} toxicity is visible in all studied plant species. For example, roots of *Triticum aestivum* are longer in Co^{2+}-Mg^{2+} (M = 23.24 mm, SD = 10.61 mm, Me = 25.49 mm) and Co^{2+}-Ca^{2+}-Mg^{2+} (M = 18.03 mm, SD = 12.15 mm, Me = 18.05 mm) treatments compared to Co^{2+} (M = 3.85 mm, SD = 1.99 mm, Me = 3.45 mm). The differences between Co^{2+} high metal treatment with and without Ca^{2+} and Mg^{2+} are important statistically for all studied plants except for *Vicia sativa*. In general, our results demonstrate that Ca^{2+} and Mg^{2+} are able to reduce stress caused by high concentrations of metals (especially Ni^{2+} and Co^{2+}) in plants during germination. Gabbrielli and Pandolfini [90] showed that Ca reduces the toxicity of Ni in relation to root elongation in *Alyssum bertolonii*. The addition of Ca also reduced Ni uptake by this species. For *Alyssum* species, Chaney et al. [91] reported that Ca addition to ultramafic soils reduces Ni toxicity and improves annual phytoextraction. In other studies, Léon et al. [54] revealed that Ca-crystals in the seed coats of *Grevillea exul* var. *rubiginosa* that can mitigate Ni toxicity. The heterogenic nucleation of Ca-crystals allows Ni incorporation, hence Ni does not enter the seed interior. Therefore, less inhibition of seed germination and root elongation was observed. In *Arabidopsis thaliana,* calcium restored the growth inhibition under Pb and Zn stress [92]. The affinity between Ni^{2+} and Ca^{2+} was confirmed also in a germination experiment with *Raphanus sativus* [78]. The authors observed the release of Ca^{2+} into test solution under Ni^{2+} stress. Furthermore, in *Triticum aestivum*, calcium improved growth and physiological parameters of plants germinated from seeds soaked with Ni^{2+} and Ca^{2+} together [93]. In this species, the EC_{50} simultaneously increased when Mg^{2+}activity increased [94]. Other species belonging to Poaceae (*Hordeum vulgare*) demonstrated that Ni toxicity decreased with increasing Ca and Mg [95]. Furthermore, Shen et al. [96] showed that Mg^{2+} added to solution improves the growth of Pb-stressed seedlings. Lead toxicity mitigation was reflected in increased roots growth and chlorophyll content. Interactions between Ni^{2+} and Mg^{2+} probably result from the high chemical affinity of both elements [53].

Results of RTI and EIR indicate that plants differ in metal tolerance (Fig. 7, A-I).The most tolerant species for Ni^{2+} is *Vicia sativa*, followed by *Trifolium pratense*, *Brassica napus*, *Triticum aestivum,* and *Raphanus sativus* being the least tolerant. For Cr^{3+}, the most tolerant is *Vicia sativa* followed by *Trifolium pratense*, *Triticum aestivum*, *Raphanus sativus*, and *Brassica napus*. Furthermore, the results show that Co^{2+} toxicity increases in the following order: *Vicia sativa*<*Trifolium pratense*<*Brassica napus*<*Raphanus sativus*<*Triticum aestivum*. Results of EIR show that

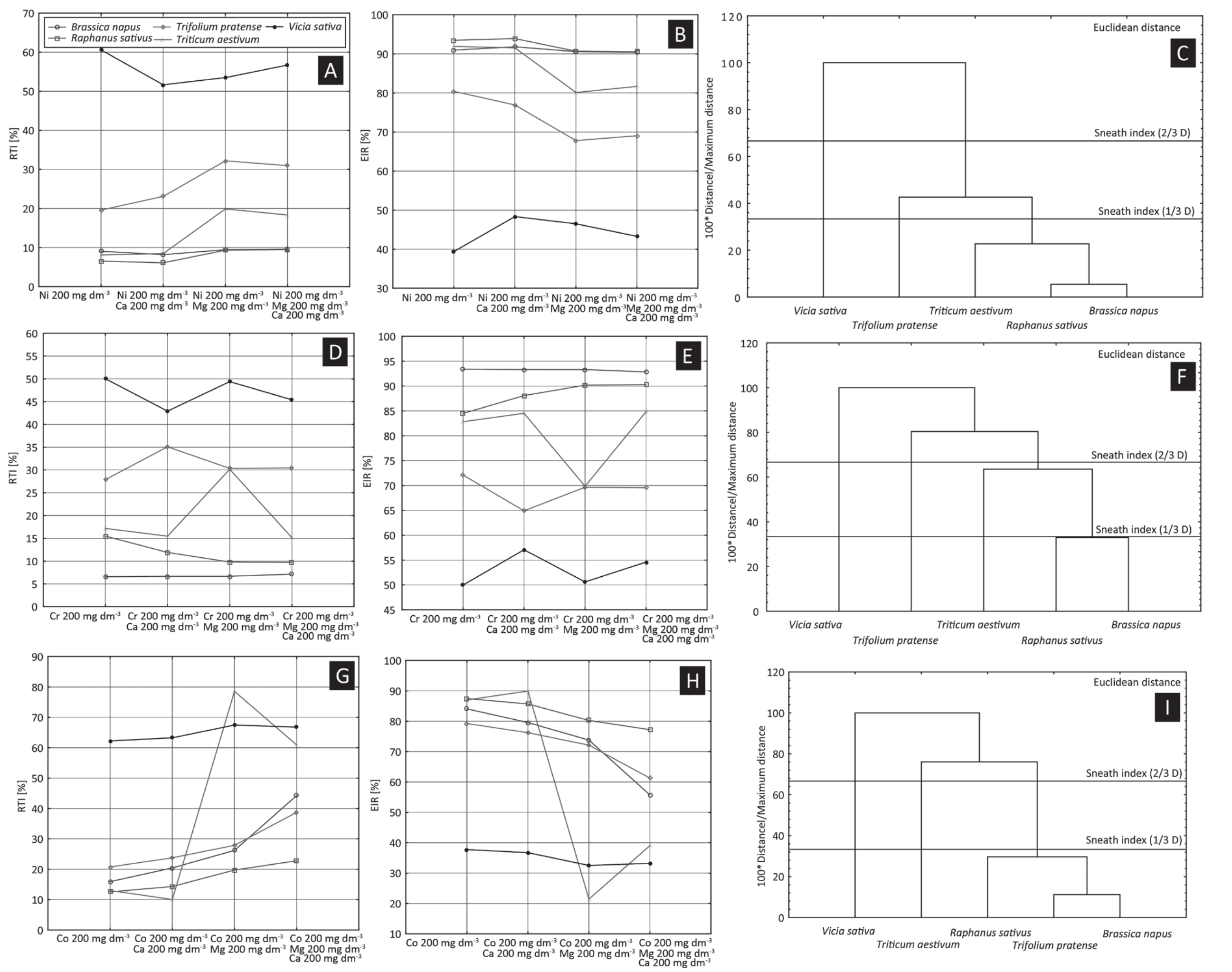

Fig. 7. Root tolerance indexes (A, D, G), elongation inhibition rates (B, E, H), and cluster analysis (C, F, I) in high Ni^{2+}, Cr^{3+}, and Co^{2+} treatments.

metals are able to inhibit root growth of *Vicia sativa* at the lowest extent and *Raphanus sativus*, *Brassica napus* and *Triticum aestivum* at the highest extent. Metal tolerance among plants is confirmed in cluster analysis (Fig. 7). More restrictive criterion divides species into three groups for Ni^{2+} and Co^{2+}. For Cr^{3+} the situation is more variable. In general, *Vicia sativa* is the most tolerable species, hence it is a good candidate for long-time phytotoxic experiments and soil rehabilitation. Seeds of *Brassica napus* and *Raphanus sativus* are very sensitive. Therefore, there are good candidates for short-term experiments leading to testing metals, polluted soils, and wastes. Nevertheless, tolerance of plants to metals changes among species, populations [97] and even amongst plant cultivars as observed for *Brassica napus* under Cr stress [98]. In other studies, differences in Ni tolerance of *Brassica napus* cultivars were explained by developing complexes with histidine, serine, and cysteine [99]. The origin of seeds should be also taken into account during consideration of metal tolerance. Previous studies with seeds of plants growing in ultramafic soils demonstrated metal adaptation in *Arabidopsis lyrata* ssp. *lyrata* and *Echinochloa colona* [63, 100]. In Poland, the serpentine population of *Silene vulgaris* has a higher tolerance to Ni^{2+} (i.e., in relation to seed germination) compared to non-serpentine populations [101]. Nevertheless, all populations of *Silene dioica* have the genetic and ecological tolerance to grow in ultramafic soils regardless of seed origin [102].

Implications for Plants Growing in Ultramafic Soils Developed under Temperate Climate

Our results show that Ca^{2+} and Mg^{2+} generally enhance seed germination and/or growth of roots in high metal (mainly Ni^{2+} and Co^{2+}) conditions, even if it is confirmed statistically only for Co. Therefore, our observations can be linked to ultramafic soils developed under temperate climate. Serpentinization is a low-temperature metamorphic process causing alteration of peridotite to serpentinite [103, 104]. As the consequence of serpentinization involving the hydration of mafic minerals from peridotites (olivine, pyroxenes), serpentine minerals (antigorite, chrysotile, lizardite) crystallize. Therefore, peridotites contain low amounts of water compared to serpentinites (average 13% of H_2O; [105]). The reaction of water with peridotites leads to a release of Ca. When fluids from serpentinization mix with cold seawater (containing CO_2), the carbonates (i.e., calcite) crystallize as deposits that are a sink for Ca [103, 106, 107]. Additionally, calcium can be trapped in rodingites (rocks rich in Ca-silicates that result from gabbro dike hydration), which can co-exist with other rocks in ultramafic complexes. Therefore, very low content of Ca in proper serpentinites is expected, which suggests that Ca-minerals identified in peridotite-derived soils can be a source of Ca that alleviates toxicity of metals resulting in enhanced seed germination and/or root growth.

The influence of ultramafic parent material on seed germination and root elongation depends on the susceptibility of minerals to weathering. The most important Mg-phase in peridotites is olivine, and serpentine in serpentinites. Olivine is more susceptible to weathering than serpentine [108], hence Mg can be more available in peridotite-derived soils than serpentinite-derived soils. Therefore, enhanced germination and root growth can be expected in soils developed on peridotites.

Studies of the influence of the type of ultramafic parent rock on soils and plants should be linked not only to the total chemical composition of soils and mineralogy studied by X-ray diffraction, but also to microscopic observations, electron microprobe analysis, and knowledge about the ability of minerals to weather/dissolve together. Some proper serpentinites have pseudomorphic textures after olivine (mesh texture) and after pyroxenes (bastite) visible under a polarizing microscope. Deschamps et al. [105] observed that serpentines representing pseudomorphosis after olivine are enriched in Mg and Ni. Less resistance to weathering of pseudomorphic serpentines can be expected compared to non-pseudomorphic serpentines. A study of ultramafic soils in southwestern Poland confirmed that Ni is more mobile in soils developed on pseudomorphic serpentinite compared to soils developed on proper serpentinite [39]. Based on these observations, enhanced release of Mg and Ni in soils developed on pseudomorphic serpentinites that affect seed germination can be predicted.

Mineral and chemical composition of ultramafic rocks may be affected by processes taking place after serpentinization. For example, Kukuła et al. [109] stated that hornblende ($Ca_2[Mg_4(Al, Fe)]Si_7AlO_{22}(OH)_2$) in peridotite from the Popiel Hill (southwestern Poland) originates from contact metamorphism with the Karkonosze Granite Intrusion. Therefore, post-serpentinization processes change the Ca and Mg budget in ultramafic soils. Additionally, in some ultramafic sites, allogenic minerals like quartz and feldspars may affect the chemical composition of ultramafic soils and, consequently, serpentine syndrome [110-112].

Conclusions

Metal toxicity in plants can be successfully determined using germination experiments. Our results demonstrate that low concentrations of Ni^{2+} increase seed germination and root elongation for some plant species. Based on the metal salt solution experiment, we consider that in soils containing low concentrations of Ni, Cr, and Co, the effect of macronutrients such as Ca and Mg is insignificant. However, in the case of ultramafic soil – an environment naturally enriched in some metallic elements – the mitigation effect of Ca and Mg on metal toxicity in high Ni and Co conditions is observed. This shows that the results of our laboratory experiments can be linked with ultramafic soils developed in a temperate climate. Enhancement of germination and root growth is expected in peridotite-derived soils compared to serpentinite-derived soils because of the presence of Ca minerals in peridotites. In order to determine the toxicity of metals whose origin is geogenic, other factors like post-serpentinization processes, the rate of weathering of minerals, seed origin, and the presence of allogenic minerals in soils should be taken into account. Among studied species, *Brassica napus* and *Raphanus sativus* are good candidates for short-term experiments testing chemicals due to the fast response to limiting factors.

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Conflict of Interest

The authors declare no conflict of interest.

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