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Interaction of Ni and Cu in accumulation in leaves of the Ni-hyper accumulator, *Alyssum murale*

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ABSTRACT

Plants that can accumulate metals to exceptionally high concentrations in their shoots are so-called hyper-accumulators. To further quantify potential interactions between Ni and Cu, the *Alyssuem murale* grown in soils with factorial additions of $NiSO_4 \cdot 6\ H_2O$ (0, 50, 250, 500 and 750 mg kg^{-1} Ni = Ni-$T_{0,\ 50,\ 250\ \text{and}\ 750}$) and/or $CuSO_4 \cdot H_2O$ (0, 50, 250 and 500 mg kg^{-1} Cu = Cu-$T_{0,\ 50,\ 250\ \text{and}\ 500}$) salts were investigated. The experiments were carried out in pots in a greenhouse under controlled temperature, light conditions and ambient humidity. The test plants for biomass production were harvested three times; 30, 60 and 100 days after germination. Ni and Cu concentrations in the digests were determined by flame atomic absorption. The results showed that by each different levels of Ni, the maximum amount of absorbed Ni was achieved at 50 mg kg^{-1} Cu concentration. Also, with elevation of Cu concentration, Ni uptake decreased. These results indicated that the Ni-T_{750} and Cu-T_{50} at the third time period had the maximum average of 1585 µg kg^{-1} and was significantly different from the other treatments. The statistical analysis indicated that by the increased Ni levels from zero to 50 mg kg^{-1} in soil, the performance of the plant dry matter was significantly declined at the all Cu levels. In addition, by the Cu-T_{50} in the soil, the dry matter amount at the all Ni levels were higher than that of Cu-T_0, although the differences were not significant ($p < 0.05$).

Keywords: *Alyssuem murale*, Cu, Ni, Phytoextraction, Soil pollution.
Article type: Research Article.

INTRODUCTION

Nowadays, one of the most important environmental challenges is the gradual increase or accumulation of heavy metals due to their lack of decomposition by microorganisms. These metals have serious hazards on humans and other living organisms due to their potential for cytotoxic, carcinogenic and mutagenic effects. On the other hand, the entry and discharge of industrial and agricultural wastewater with sludge and waste disposal, as well as increased use and reuse of sewage sludge and compost fertilizer in agricultural areas, causes significant changes in the physical, chemical and biological quality of the soils of these areas. Consequently, it has increased the absorption and contamination of heavy metals in plants and agricultural products in the world. Remediation of polluted soils has been a matter of concern and for its remediation, many technologies like pneumatic fracturing (Wishart *et al.* 2009; Clark *et al.* 2012); soil flushing (Svab *et al.* 2009; Mena *et al.* 2015), solidification (Paria & Yuet 2006; Hunce *et al.* 2012), verification (Raicevic *et al.* 2005; Hashim *et al.* 2011), electrokinetic (Huang *et al.* 2012; Rozas & Castellote 2012), chemical reduction (Wuana & Okieimen 2011), soil washing (López-Vizcaíno *et al.* 2012; dos Santos *et al.* 2015) have been tried. But these traditionally used methods are limited in their application to selected areas because of some limitations. Currently, conventional remediation methods of heavy metals contaminated soils are expensive and environmentally destructive. Phytoremediation offers a low

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cost and an environmentally friendly approach for decontaminating soils and waters of heavy metals. The term "hyperaccumulator" describes some plants that can accumulate metals to exceptionally high concentrations in their shoots and aerial organs without suffering phytotoxic effects (Rascio & Navari-Izzo 2011). The metals are concentrated at levels that are toxic to closely related species not adapted to growing on the metalliferous soils. Compared to non-hyperaccumulating species, hyperaccumulator roots extract the metal from the soil at a higher rate, transfer it more quickly to their shoots, and store large amounts in leaves and roots (Hossner *et al.* 1998 Rascio & Navari-Izzo 2011). Over 500 species of flowering plants have been identified as having the ability to naturally accumulate high levels of metals such as Cd, Cu, Co, Mn, Ni and Zn in their tissues (Sarma 2011). A. murale is a Ni-hyperaccumulating flowering plant from the family Brassicaceae that occurs widely in the eastern Mediterranean region and has been developed as a commercial crop for phytoremediation (Bani *et al.* 2018; Tappero *et al.* 2007; Chaney 2004; Li *et al.* 2003a). The ability of hyper-accumulator plants and the interaction of heavy metals and elemental associations in them has been evaluated in many studies. Many studies have been done in this regard. Broadhurst *et al.* (2009) studied the interaction of Ni and Manganese concentrations in the leaves of both species over Ni in A. murale corsicum and A. murale. They showed that the extraction of Ni rose by A. murale the plant has reduced by available manganese, and suggesting that more species of Ni by A. murale can be increased by the increasing manganese system. Sellami *et al.* (2012) studied the effects of the Ni concentration on the physiological properties of A. murale in soil mixed with sewage sludge agriculture. Their results showed that Ni accumulates in the stem more than the root and A. murale was able to save a high concentration of 12730 µg kg^{-1} of Ni in the leaves. In another study, Motesharezadeh and Savaghebi-Firoozabadi (2011) showed that by the concentration of Ni in the soil, its absorption by plants also increased significantly. Information regarding metals localization (e.g. stem and leave), the interaction of heavy metals and elemental associations in accumulator plants is crucial to understanding the mechanisms of hyper accumulation and tolerance. In this regard, to further quantify potential interactions between Ni and Cu, we have investigated A. murale grown in soils with factorial additions of Ni and Cu salts.

MATERIALS AND METHODS

Plant culture

The *A. murale* plant was used for the phytoremediation experiments to accumulate Cu and Ni. The soil was sieved (< 4 mm) and homogenized. The *Alyssum* seeds were planted in pots of 22 cm in diameter and 33 cm in height filled with 7.5 kg soil. The soil samples used in this study were collected from unpolluted sites located in Azad University of Tabriz. The soil used was sandy loam. The soil characteristics such as pH (U.S. Salinity Laboratory Staff 1954), EC (Rhoades 1982), organic matter (Nelson & Sommers 1996) and soil texture (Gee & Bauder 1986) were determined. The Physicochemical analyses of the study soil were shown in Table 1. The experiments were carried out in pots under the controlled conditions of a greenhouse at the research station of the Faculty of Agriculture and Natural Resources, the Islamic Azad University of Tabriz, with a longitude of 38° 02' 77.88" N and latitude of 46° 43' 97.07" E. Following the same method described for Cu and Zn spiking by Kim & McBride (2009) and Ni spiking by Tang & McBride (2018), the soil in each pot polluted with increasing series of $NiSO_4·6H_2O$ (0, 50, 250, 500 and 750 mg kg^{-1} Ni = Ni-$T_{0, 50, 250 \text{ and } 750}$) and/or $CuSO_4·H_2O$ additions (0, 50, 250 and 500 mg kg^{-1} Cu = Cu-$T_{0, 50, 250 \text{ and } 500}$), such that every combination was represented in nine replications (Table 2). Thus the experiment involved 20 treatments with 180 pots. The experiments were conducted in a greenhouse under controlled temperature and light conditions as well as ambient humidity for four months. The wet humidity of each pot was kept in field capacity with tap water. The test plants for biomass production were harvested in three times; a) after 30 days, b) after 60 days and c) 100 days after germination and in each time three pots were deleted.

Table 1. Physicochemical of the investigated soil before planting.

pH	EC (ds m^{-1})	Ɵ (cm^3 cm^{-3})	O.M %	Soil texture			
				Clay (%)	Silt (%)	Sand (%)	Texture class
7.8	>1	0.185	1.2	13	22	65	Sandy loam

Ni and Cu accumulations in plants

The plants grown under the above-mentioned conditions were rinsed with distilled water and divided into shoots and roots, dried at 70˚C for 24 h and then weighed and ashed in a 480˚C oven for 16 h. After cooling, the ash was digested in 10 mL 1N HCl, then, filtered through Whatman 40 filter paper and brought to volume in a 50 mL volumetric flask using 1N HCl (Gupta 2007). Ni and Cu concentrations in the digests were determined by flame atomic absorption (Shimadzu AA-670G; Gupta 2007). The experimental design used in this study was factorial with three replications. The analysis of variance (ANOVA) was performed in SAS software.

RESULTS AND DISCUSSION

The effect of different concentrations of Cu and Ni interval on Cu absorption by *A. murale*

Based on the results of the average comparison for Cu absorption by the *A. murale*, the Cu-T_{50} by the Ni-T_0 in the third time-period has allocated the most average (2115.78 µg kg^{-1}) and was significantly different from the rest of the treatments. Fig. 1 shows the concentration of Cu absorption by the *Alyssum* in all treatments at different Cu concentration. It was observed that Ni-T_0 at the third time-period (100 days after planting) and Cu-T_{50} exhibited the highest Cu absorption in *A. murale* in the presence of the other Ni levels. These results indicate that at each of the different Ni levels in soil, by elevated Cu levels, the absorption of Cu by plant increases, so that the maximum Cu absorption was found in the third time-period. In addition, the results showed that the Ni in the soil was impressive on the rate of Cu absorption. By the upraised Ni in soil from zero to 50 mg kg^{-1}, the Cu absorption was significantly declined; whereas the other Ni concentrations in the soil did not display a significant decline in the absorbed Cu. So, *A. murale* is a proper plant for the extraction of Cu under different Ni concentrations in the soil. Several studies have also concluded that the *Alyssum* is more suitable plant than others for accumulating the Ni (Asemaneh *et al.* 2006; Ghaderian *et al.* 2007; Broadhurst & Chaney 2016).

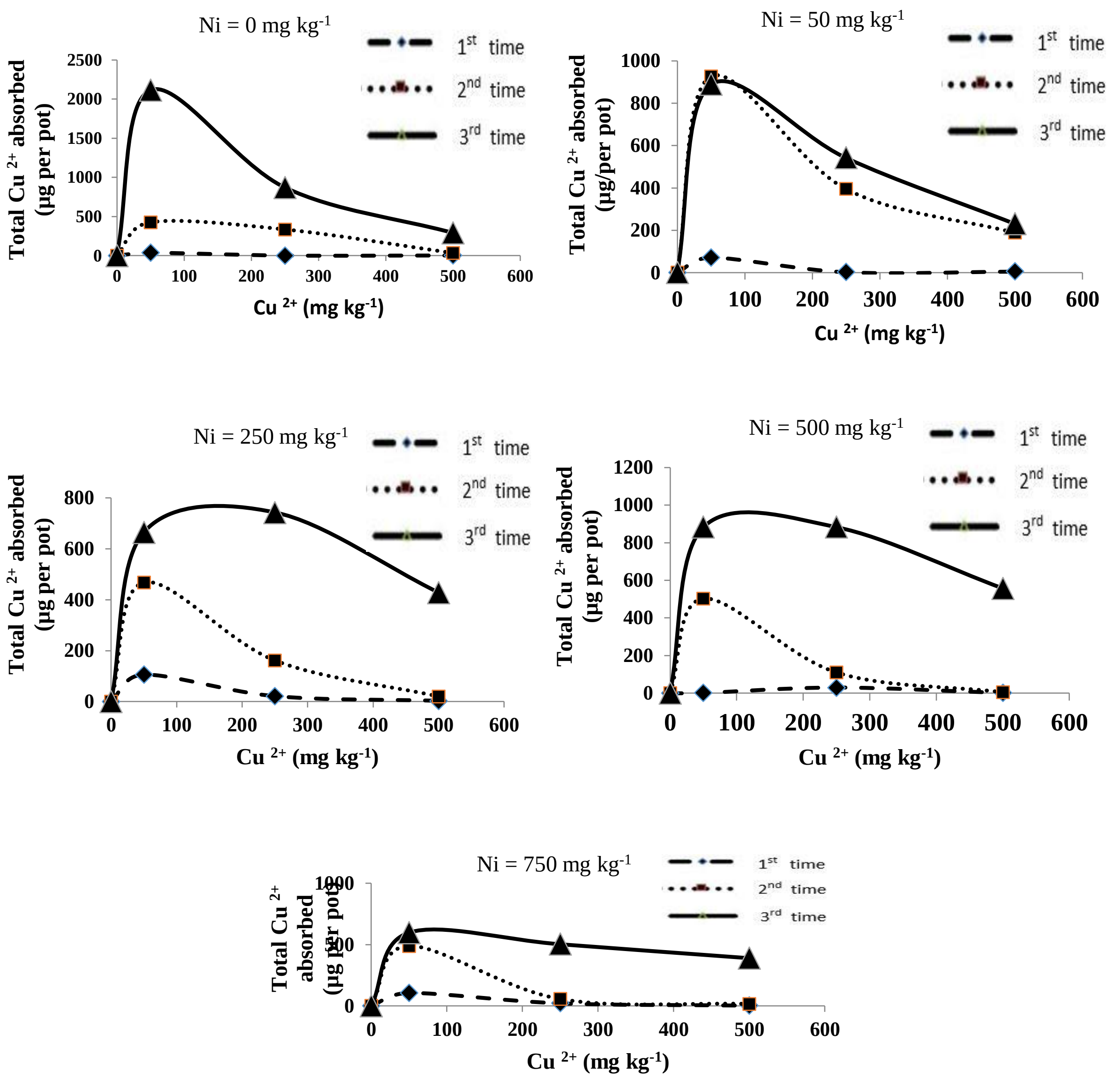

Fig. 1. Comparison of the averaged mutual effects of different Cu levels and time in the different Ni concentrations on the total absorbed Cu by *A. murale*.

The effect of the different Cu levels and time-period at the different concentrations of Ni on the capability of Ni absorption by *A. murale*

Fig. 2 illustrates the effect of interacted effect of different Ni and Cu levels in three different time interval on the total amounts of absorbed Ni by the *A. murale*. The results showed that by each different levels of Ni, the maximum amount of absorbed Ni was achieved at 50 mg kg^{-1} Cu concentration (Cu-T_{50}). Also, by elevation of Cu concentration, Ni uptake decreased. These results also indicated that the Ni-T_{750} and Cu-T_{50} at the third time period had the maximum average of 1585 µg kg^{-1} which was significantly different from the other treatments. Given the amount of absorbed Ni at different levels, Ni and Cu exhibited significant differences in the periods. In addition, the maximum absorbed Ni was achieved in the third time-period (100 days after planting). In the first interval of time (30 days after planting), due to the lower plant growth, Ni and Cu are little in the soil, while increase over time. The effect of time or the plant growth stage on the metal extraction efficiency from contaminated soils should be considered. Cassina *et al.* (2011) used exogenous plant growth regulators (cytokinin) to increase the efficiency of plant metal extraction from contaminated soils and concluded that *A. murale* was a plant species sensitive to cytokinin treatment and that cytokinin treatment was potentially useful in increasing the phytoextraction capability by increasing biomass.

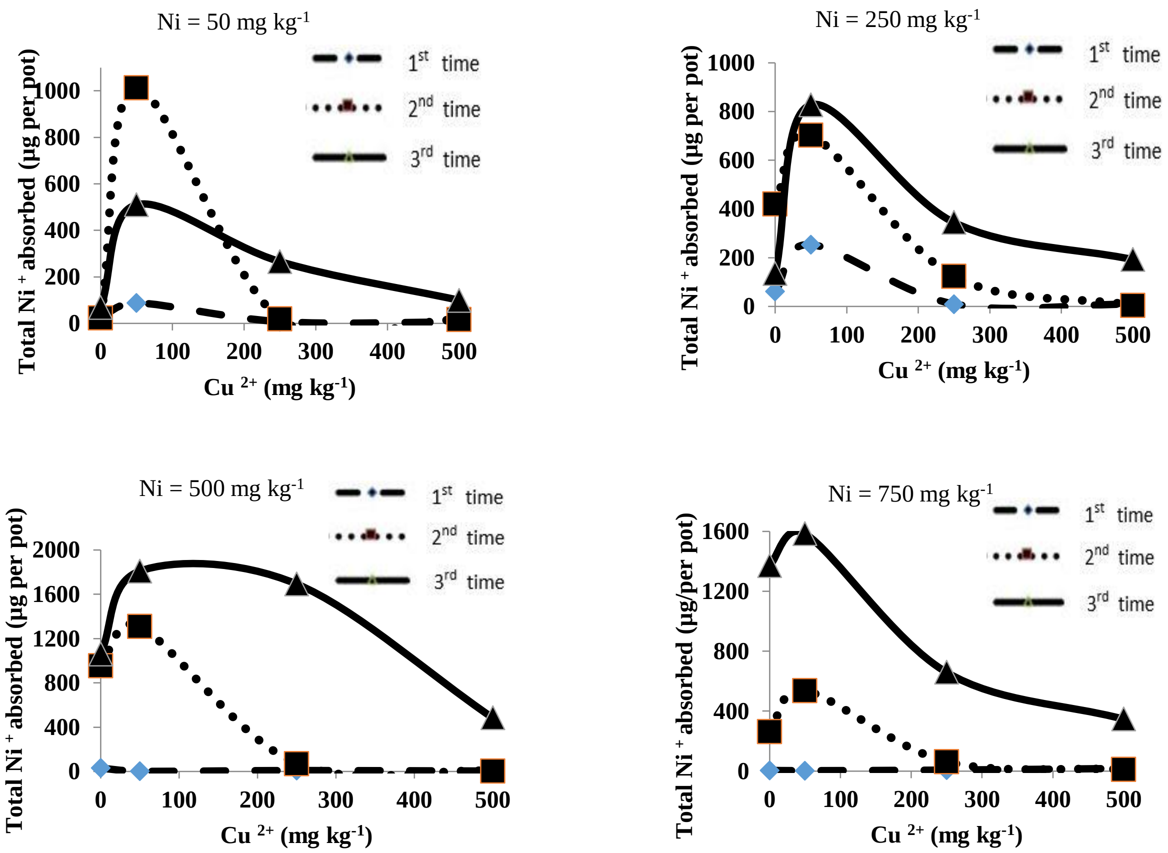

Fig. 2. Comparison of the averaged mutual effects of different Cu levels and time in different Ni concentrations on the total absorbed Ni by *A. murale*.

The effect of Cu levels and time-period in the different Ni concentrations on the Ni absorption capability by *A. murale* plant stem

Fig. 3 depicts the effect of interaction between different Ni and Cu levels in three different time interval on the total Ni absorption by *A. murale* plant stem. By the increased Cu concentration, the amount of absorbed Ni was significantly declined. In each different Ni levels, the maximum and minimum Ni absorption were achieved at Cu-T50 (50 mg kg^{-1} Cu concentration) and Cu-T500 (500 mg kg^{-1} Cu concentration), respectively by the plant stem. In addition, results of the comparison of the averages showed that the maximum absorbed Ni (300 µg kg^{-1}) at the *A. murale* stem was achieved by the Cu-T_{50} and in the third time-period with no significant difference between Ni levels ($p < 0.05$). Given the absorbed Ni at the different levels, Ni and Cu exhibited a significant difference in the time periods and the maximum absorbed Ni was achieved at the third time-period (100 days after planting). Generally, by the increased level of Cu in any Ni level, the amount of absorbed Ni was declined. Tappero *et al.* (2007) concluded that *A. murale* relies on a different metal storage mechanism for Cu (exocellular sequestration) than for Ni (vacuolar sequestration).

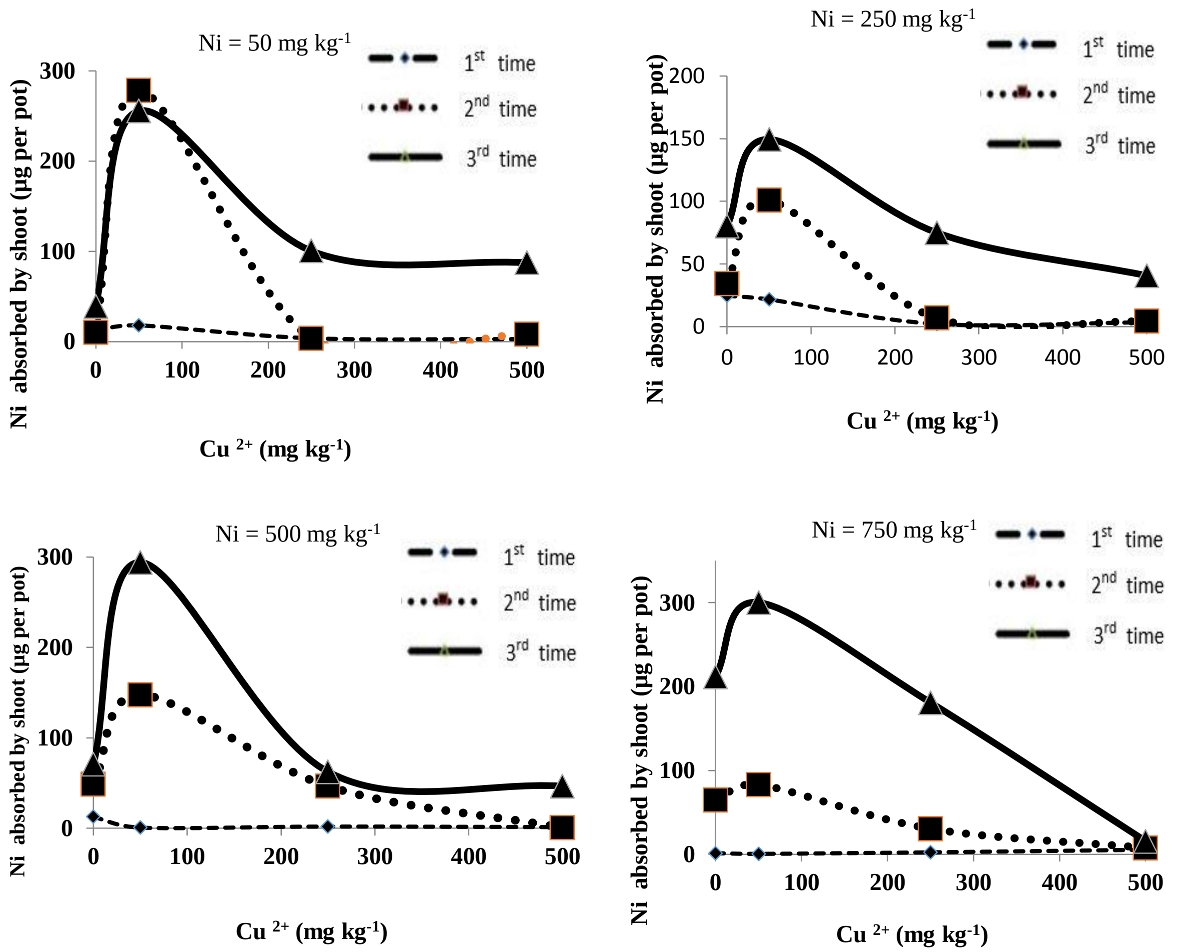

Fig. 3. Comparison of the averaged mutual effects of different Cu levels and time in different Ni concentrations on the total absorbed Ni by the stem of *A. murale*.

The analysis of variance (ANOVA)-Factorial design with three replications-including all different levels of Ni and Cu and all possible interaction terms between them are shown in Table 2, exhibiting the performance values of its dry matter in total plant and in its stem in the different Ni and Cu treatments. By the elevated Ni concentrations from zero to 50 mg kg^{-1} in soil, the performance of the plant dry matter was significantly declined in the all Cu levels ($p<0.05$). The declined performance of the plant dry matter in Cu-T_{50}, Cu-T_{250}, Cu-T_{500} and Cu-T_{750} by different Ni levels were not significant. In addition, Cu-T_{50} in soil, the amounts of dry matter at all levels of Ni were higher than in Cu-T_0, although the difference was not significant.

Table 2. The analysis of variance (ANOVA) results.

Ni in the soil (mg kg^{-1})	Performance of the *A. murale* dry matter			
	Different levels of Cu in the soil (mg kg^{-1})			
	0	50	250	500
0	16075.55 ^{a,a}	16875 ^{a,a}	4695^{a,b}	772 ^{a,b}
50	10610 ^{b,a}	11052 ^{b,a}	801 ^{b,b}	948 ^{b,b}
250	10202 ^{b,a}	7078 ^{b,a}	593 ^{b,b}	219.89 ^{b,b}
500	6820 ^{c,a}	1208.26 ^{c,a}	932.17 ^{c,b}	352.64 ^{c,b}
750	545.88^{c,a}	708.17 ^{c,a}	242.31 ^{c,b}	124.42 ^{c,b}

Note: Superscript letters of the first and second, respectively, indicating a significant difference ($P \leq 0.05$) in each column and row. Averages of the same letters are not significantly different according to Duncan's multiple range tests.

Decreased dry weight of *Alyssum*, by the upraised Ni and Cu levels in different treatments, may be due to the elevated Ni concentration in its stem. Ni toxicity in plants causes impaired absorption of nutrients, cellular respiration and photosynthesis, which is a major cause of the dry matter weight loss. Therefore, by the elevated Ni and Cu concentrations, the plant dry weight decreased. One of the reasons for the *Alyssum* dry weight loss in the Ni-contaminated soils is a declined absorption of nutrients by the plant. The most decreased plant dry weight was achieved in Ni-T_{750} and Cu-T_{500} respectively. The results of this study are consistent with the results of Parida *et al.* (2003), Fuentes *et al.* (2007) and Papazoglou *et al.* (2005) who emphasized that heavy metals exhibit a negative effect on plant biomass by upraising the Ni concentration.

CONCLUSION

In recent years, the ability of plants to accumulate pollutants has received significant attention and hyper-accumulator species are primary candidates for use in phytoremediation because they play a major role in the removal of heavy metals and toxic trace elements from contaminated soils. In this study, the Ni-hyper accumulator, *A. murale* was used for the phytoremediation experiments to accumulate Cu and Ni. In addition, to quantify potential interactions between Ni and Cu, the *A. murale* grown in soils with factorial additions of Ni and Cu salts was investigated. The experiments were carried out in pots under the controlled conditions of a greenhouse at the research centre of the Islamic Azad University, Tabriz, Iran. In the case of statistical analysis, the experimental design used in this study was factorial with three replications. The results showed that by increasing the Ni and Cu concentrations, the dry weight of plant decreased. The decreased *Alyssum* dry weight, by the upraised Ni and Cu levels in different treatments, may be due to the elevated Ni concentration in the plant stem. Ni toxicity in plants causes impaired absorption of nutrients, cellular respiration and photosynthesis, which is a major cause of the dry matter weight loss. In general, *A. murale* is an appropriate plant for the extraction of Ni and Cu. However, it was not able to fix a high range of Ni and Cu, because of conducting this experiment in a greenhouse situation, hence, the plant could not achieve its maximum function. In the field situation, the plant reaches the high biomass. Therefore, this plant can be used for the bioremediation of soils contaminated with Ni and Cu.

Authors' contribution

In this study, the authors contributed as follows: Mohammad Reza Dalalian performed experimental part and wrote the manuscript draft, and Sh. Shahmohammadi-Kalalagh did writing-review and editing. All authors have read and agreed to the published version of the manuscript.

Declarations

It is confirmed that the present work has not been published, not under consideration for publication elsewhere, approved by all authors and, if accepted, it will not be published elsewhere in the same form in English or in any other languages.

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