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ORIGINAL ARTICLE

Hydroponic Phytoremediation of Nickel by Coriander (*Coriandrum sativum*)

Mozhgan Tagharobiyan, Vahid Poozesh*

Department of Botany, Faculty of Biology, Damghan University, Damghan, Iran

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KEYWORDS

Coriander;

Hydroponics;

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ABSTRACT: Environmental stresses are one of the most important factors of agricultural products reduction in the world. The influence of different concentrations of nickel nitrate (0, 100, 200 and 500 μM in Hoagland's solution) on dry matter, catalase enzyme, flavonoids, hydrogen peroxide, peroxidase Enzyme, MDA and accumulation of Ni were studied in coriander (*Coriandrum sativum*) plants. Treatment with Ni led to significant increase in flavonoids, hydrogen peroxide, MDA and other aldehyde. Exposure of coriander plant to Ni altered catalase enzymes, leading to significant decrease in their contents. In both shoots and roots of coriander plants, significant decrease in dry matter was observed. Ni accumulation increased significantly in shoots and roots. Ni increased in the roots more than the shoots. According to a more accumulation of Ni in the roots, the expansion of plants root can help to better adaptability with the toxicity of metals. It may be used as an indicator to illustrate the differences between plant species.

INTRODUCTION

Increasing environmental pollution related to the discharge of industrial wastewater containing toxic heavy metals in rivers and underground aquifers that are dangerous to human health [1]. The most common metals found in sewage can named as lead, zinc, copper, cadmium, chromium and nickel [2]. Coincides with the absorption of heavy metals by plant roots and transmitted to the shoot, the function of metabolism is disrupted and growth is reduced [3]. Similarly, to other micronutrients, nickel (Ni), when applied in excess, becomes toxic for plants causing various symptoms of injury such as growth inhibition, chlorosis, necrosis and wilting. Disturbance of mineral nutrition [4], photosynthesis [5], water relations, respiration [6], germination [7] as well as nitrogen metabolism [8] have been reported for plants subjected to Ni stress (Table 1).

* Corresponding author: poozesh@du. ac. ir (V. Poozesh).

Table 1. Review some of recent works on Ni

No.	Research works	References
1	Influence of nickel-contaminated soils on fenugreek (*Trigonella corniculata* L.) growth and mineral composition	[4]
2	Organ-distinctive changes in respiration rates of rice plants under nickel stress	[6]
3	Theeffectof different concentrations of nickel on germination and growth ofcoriander (*coriandrum sativum*) and milk thistle (silybum marianum) seedlings	[7]
4	Nickel-induced changes in nitrogen metabolism in wheat shoots	[8]
5	Effect of nickel on ROS content and oxidative enzyme activities in wheat leaves	[9]
6	The effects of different nickel concentrations on some morpho-physiological characteristics of parsley (*Petroselinum crispum*)	[18]
8	Phytotoxicity of nickel and its accumulation in tissues of three Vignaspeciesat their early growth stages	[34]
7	Nickel availability and its uptake by plant as influenced by nitrogen and zinc application	[37]
9	A note on effect of nickel application on rabi cereals	[38]

Mechanisms of negative influence of Ni on plants are not fully understood; however, numerous data indicate that this metal phytotoxicity cannot be attributed to any single factor, but seems to be a complex phenomenon involving several related and interacting processes. In recent years, attention of researchers has been focused on Ni-induced oxidative stress. Despite its relatively low redox potential, Ni induces accumulation of reactive oxygen species (ROS) and oxidatively modified macromolecules in plant tissues [9]. Increase in ROS level has been ascribed to their enhanced generation mediated by Ni, disturbances in metabolic processes associated with electron transport as well as decrease in antioxidative capacity of plant cells [9].

However, little information is known about influence of Ni on the biochemical and morphological characteristics of the coriander plant. The aim of this study was to study the effect of Ni on dry matter, Ni - accumulation and biochemical parameters as catalase enzyme, flavonoids, hydrogen peroxide, lipid peroxides and MDA production

MATERIALS AND METHODS

Preparation of seedlings

Seeds of *Coriandrum sativum* were sterilized in 10% H_2O_2 (v/v) for 10 min followed by thorough washing in de-ionized water, and then germinated on transplantation tray for 25 d. The seedlings were removed from the peat soils and washed carefully under tap water to remove adhering particles. They were then transferred to PVC pots containing 1000 mL of Hoagland's solution containing: 0. 5mM KNO_3,0. 5 mM Ca $(NO_3)_2$, 0. 5 mM $MgSO_4$, 0. 1 mM KH_2PO_4,10 µM FeEDTA, 1 µM $ZnSO_4$, 0. 5 µM $CuSO_4$; 10 µM H_3BO_3, 0. 2 µM Na_2MoO_4. H_2O, 0. 5 µM $CuSO_4$. Coriander plants were planted into one pot with five holes, and with one plant in each hole. The nutrient solution was renewed twice a week and aerated continuously. Pots were randomly arranged every day during the growth period. The seedlings were grown in a greenhouse with 11/13 h light/dark cycles. The temperature was maintained at 25 ± 0. 5 ºC.

Treatments

After 2 d of pre-treatment in hydroponics for compatibility the plants, different concentrations of nickel nitrate (0, 100, 200 and 500 μM) was added to Hoagland's solution. Experiment was performed in randomized complete block design with 3 replications. After two weeks of treatment, the necessary measures were assessed. To determine the dry weight of the plant samples, initially they were placed for 72 h in the oven at 70 °C, and then the samples were weighed on scales 0. 0001.

Ni content

Nickel content in the coriander shoots and roots was determined by atomic absorption spectrometry using Varian Spectra 300 spectrometer (Varian Australia Pty. Ldt., Mulgrave, and Vic., Australia) equipped with deuterium lamp for background correction and an air/acetylene flame at 232 nm.

Hydrogen peroxide content

H_2O_2 concentration was measured according to the method of Alexieva et al. [10]. In this method, fresh plant samples (0. 2 g) were homogenized in an ice-cold mortar with 3 ml 1 % trichloroacetic acid. The homogenate was filtered and centrifuged (10 000 g, 15 min) and the supernatant (0. 5 ml) was added to 0. 5 ml of Potassium phosphate buffer (pH 7 with 10 Mm) and 1 ml of potassium iodide. Then, absorbance of hydrogen peroxide was read at a wavelength nm 390 using spectrophotometer.

Flavonoids

Flavonoids were measured by method of Krizek et al. [11]. In this method, leaf samples were homogenized in a mortar and pestle with 5 ml 1% acetic acid-ethanol solvent (1:99 v:v). The homogenate was centrifuged at 18,000 g for 30 min, and the supernatant was incubated in a water bath for 10 min at 80 °C and allowed to cool to room temperature. The amount of flavonoids was determined from the absorbance at 270.

Malondialdehyde (MDA) content

The MDA content was determined with the thiobarbituric acid (TBA) reaction. Briefly, a 0. 2 g tissue sample was homogenized in 3 ml 5% TCA. The homogenate was centrifuged at 4000 × g for 5 min.

To 1 ml aliquot of the supernatant, 4ml of 20% TC A containing 0. 5% TBA was added. The mixture was heated at 95 ^{o}C for 15 min and cooled immediately nice. The absorbance was measured at 532 nm. The value for the non - specific absorption at 600 nm was subtracted. The concentration of MDA was expressed using an extinction coefficient of 155 MCm^{-1} [12].

Other aldehyde (Propanal, Butanal, Hexanal, Heptanal and 2-Methyl-1-propyl acetate)

To calculate the concentration of these aldehydes, extinction coefficient (ε) of 45. 7 *1000 M $cm^{-1}$was used. The extinction coefficient is the average extinction coefficient of five aldehydes. The results were reported in terms of fresh weight. TBA-reactive substance MDA representing lipid peroxidation products were extracted as described previously by homogenization of 0. 2 g of tissue in 3 mL of solution containing 5% TCA [13]. The solution was incubated at 95 ºC for 1 h. The solution was then cooled and centrifuged for 10 min at 4000 rpm.

Peroxidase (POD) activity

Peroxidase (POD) activity was assayed according to the method of Plewa et al. [14] and based on the amount of tetraguaiacol absorbed after formation by oxidation of guaiacol catalyzed by this enzyme in 1 min at a wavelength of 420 nm.

Catalase (CAD) activity

Catalase (CAD) activity was measured according to the method of Maehly and Chance [15] and based on the enzyme's capability for degrading H2O2 in 1 min at a wavelength of 240 nm.

STATISTICAL ANALYSIS

All experiments were done with 4 independent replications with a completely randomized design. Data means were used for Duncan's multiple range tests after that one way analysis of variance (ANOVA) with a significance level of 0. 05 were used for analyses of data with Graph Pad 6.

RESULTS AND DISCUSSION

Root and shoot biomass

The analysis of variance demonstrated that different concentrations of Ni had significant effect on dry matter of shoot and root. Ni stress significantly decreased total dry matter (Fig. 1). Root growth reduction could be due to high accumulation of metalsin plants and cell wall lignifications [16]. Besides, metal stress could prevent root and shoot growth with inactivate the sulfhydryl groups of cell membrane and disrupt in the cell division processes [17]. The significant of Ni stress on reducing the dry matter of plant is supported by the observations in rice [9]. On the other hand, reports indicate that root absorption of nutrient reduce under heavy metal stress and result in biomass and growth reduction [18].

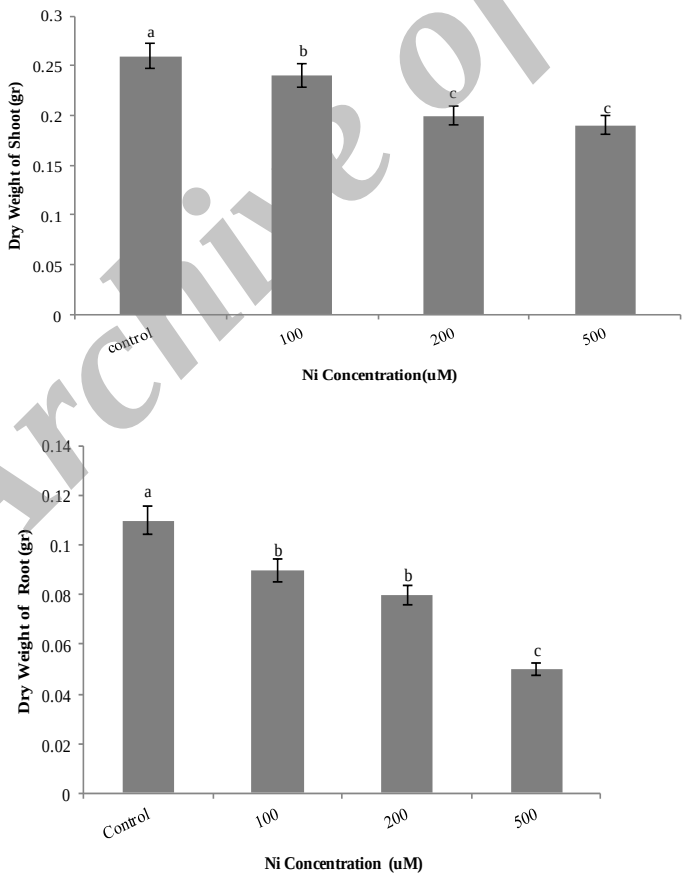

Figure 1. Effects of various Ni levels on dry weight of shoot and root

MDA and other aldehyde

Cytotoxic product of lipid peroxidation (MDA) and other aldehyde increased significantly in the plant of coriander treated with 100, 200 and 500 µM of Ni (Figure 2). MDA and other aldehyde formations in plants exposed to Ni metal stress are reliable indicators of free radical formation in the tissue, and are currently used as indicators of lipid peroxidation [19]. The maximum amount of MDA (which corresponds to a concentration of 500 mM nickel equal to 3. 05 micromoles per gram-wet weight) was increased 2-fold compared to control. Destruction of the cell membrane stability under stress of heavy metal accelerates peroxidation of lipid. Indeed, oxidative stress cause lipid peroxidation and lead to product malondialdehyde. Also, MDA can be considered as indicator of physiological stress and aging process [20].

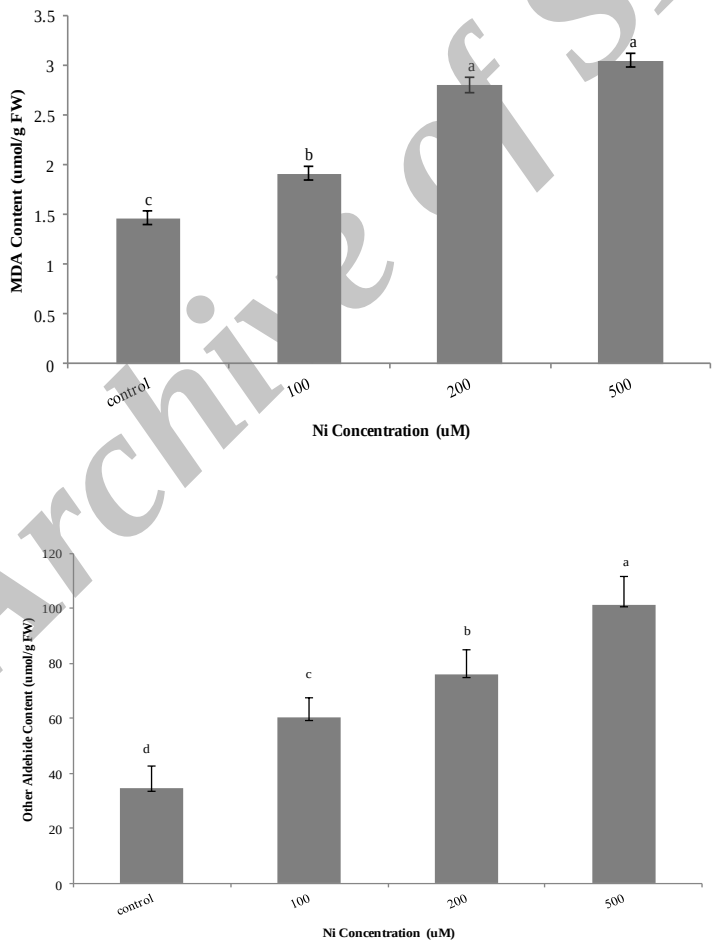

Figure 2. MDA and Other Aldehyde contents (umol/g FW) of coriander in various concentration of Ni (µM)

Guaiacolperoxidase activities

Analysis of variance showed that amount of Guaiacol peroxidase enzyme was significantly increased in the coriander plant (Figure 3). Numerous evidences indicate that the Ni toxicity can be increased enzymes comme Guaiacol peroxidase [21]. The main activity of peroxidase is removal of H_2O_2 and defense against oxidative stress in plant cells [22]. The enzyme is involved in several processes such as cell growth, auxin catabolism, lignifications as well as abiotic and biotic stress responses [23]. The peroxidase activity levels as a potential indicator can be used to evaluate the stress intensity.

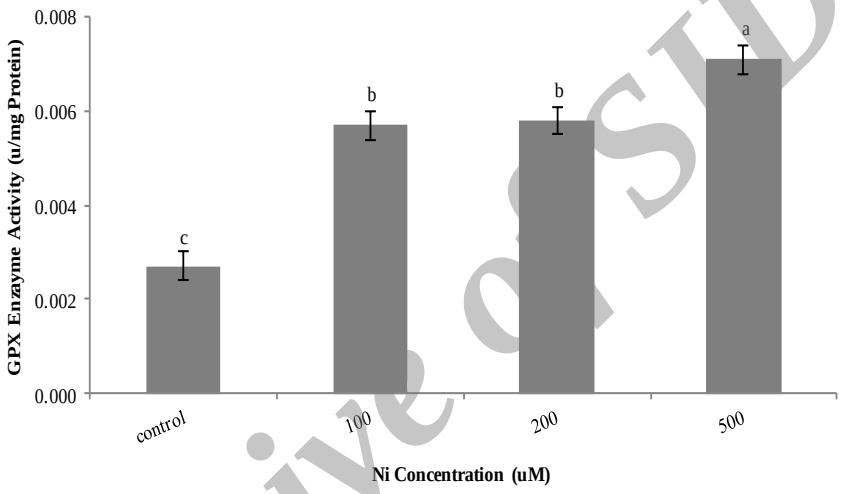

Figure 3. Guaiacol peroxidase Activities (u/mg Protein) of coriander in various concentration of Ni (µM)

Flavonoids

In this study, flavonoids contents of plants under Ni treatments showed a significantly increase compared to control in the wavelengths of 270 nm (Figure 4). It has been reported about ecophysiological role of these compounds in protecting plants against environmental stresses [24]. Flavonoids available in vacuoles can neutralize reactive oxygen species, particularly hydrogen peroxide [25]. These activities are essential in the plant, because they provide optimal conditions for plant growth. In this experiment, it was observed the reddish color in the treatments with high concentrations of nickel, which results in the accumulation of flavonoids.

Hydrogen Peroxide (H_2O_2)

Analysis of variance showed that amount of hydrogen peroxide was significantly increased in the coriander plant. The maximum amount of H_2O_2 was found at 500 µmol of nickel with 0. 03 nmol H_2O_2 per gram fresh weight of plant and the lowest was observed in the control treatment with 0. 01 nmol H_2O_2 per gram fresh weight of plant (Figure 4). Hydrogen peroxide (H_2O_2), a major kindof ROS plays an important role in signal transduction for abiotic stress tolerance, although $H_2O_2$is toxic at high concentrations [26].

Gao [27], He [28] and Morita [29] reported that the application of H_2O_2 at low concentrations could improve plant tolerance to abiotic stresses such as drought, heat, and heavy metal stresses.

Phenolic compounds

The phenolic compounds increased significantly with increasing levels of Ni. The maximum amount of phenolic was found at 500 µM of nickel with 0. 054 µg phenoliccompounds per gram fresh weight of plant that showed a 5-fold increase compared to control (Figure 4). All plants produce an amazing diversity of secondary metabolites. One of the most important groups of these metabolites is phenolic compounds. These compounds are the main option for coping with free radicals the stress resulting of heavy metals [9]. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. In this study, increase in phenolic compounds with increasing heavy metal tension is in general agreement with that reported previously [30].

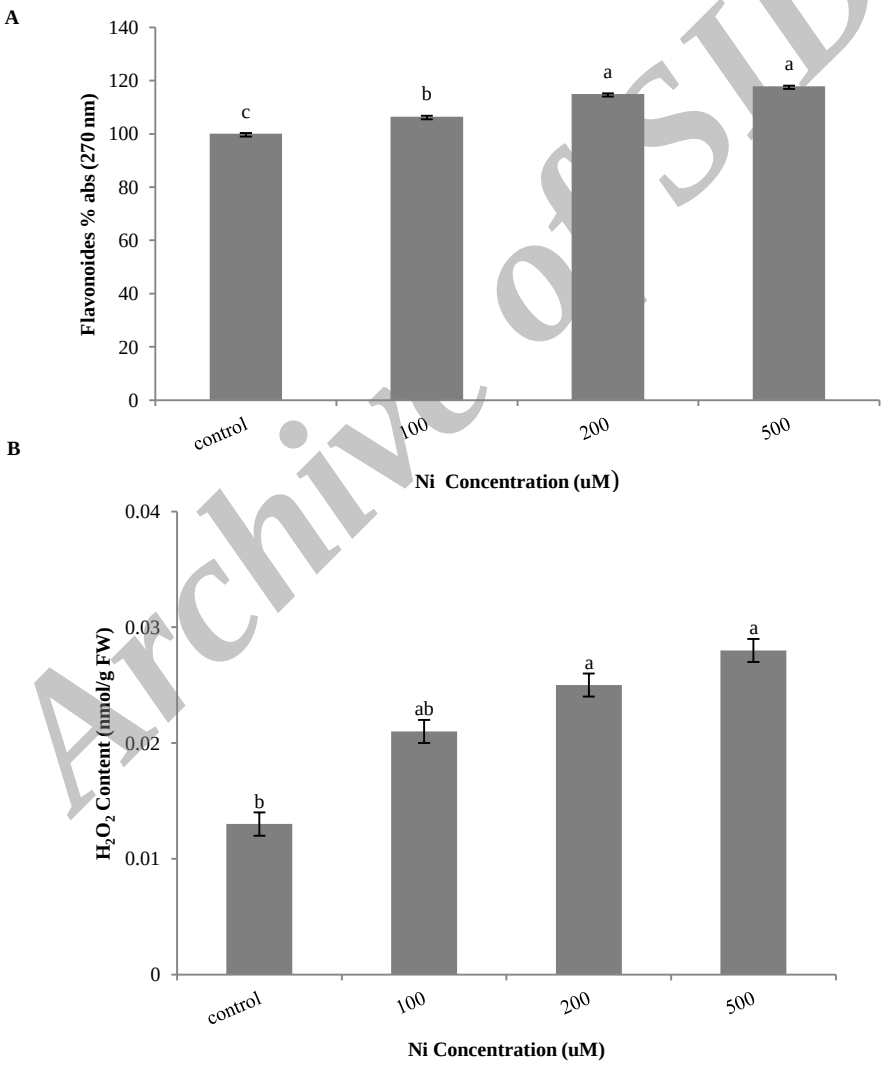

Figure 4. Effect of different levels of nickel on various biochemical attributes flavonoides % abs (A), Hydrogen peroxide (B) in coriander species after 2 weeks growth. Values presented are means across three replicates. Vertical lines indicate + S. E

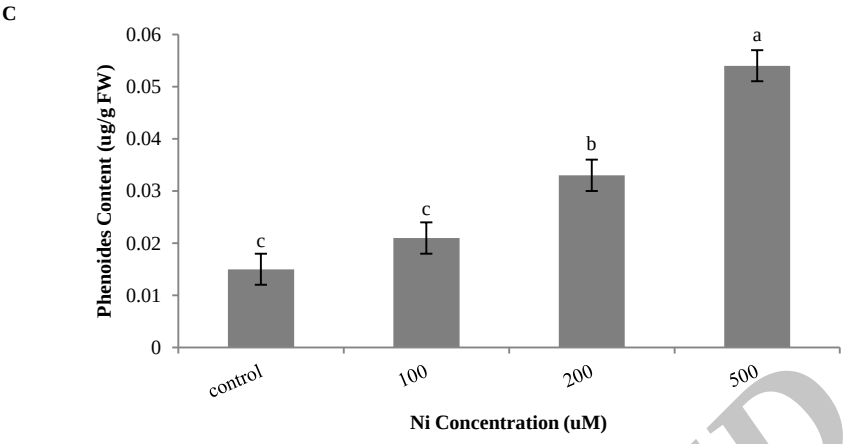

Figure 4 Continued. Phenolic compounds (C)

Catalase activity

The catalase activity decreased significantly with increasing levels of Ni (Figure 5). Catalase activity was severely affected by the highest (200 and 500 µM) Ni level. At low concentration of Ni (100 µM), catalase activity was greater. With doubling of the Ni concentration from 100 to 200 µM, the percentage of catalase activity declined by 42%.

Catalase activity too low declined (less than 0. 9%) when nickel was added at the concentration of 500 µM.

Thus, catalase activity was severely (P<0. 005) affected by the presence of Ni. Hence, heavy metals especially in high concentrations can inhibit of the catalase activity [31]. Heavy metals reduce catalase activity in *Brassica junica* [32]. In the present study, inhibition of catalase activity was also observed and consequently catalase activity failed to increase owing to the toxicity of Ni [33].

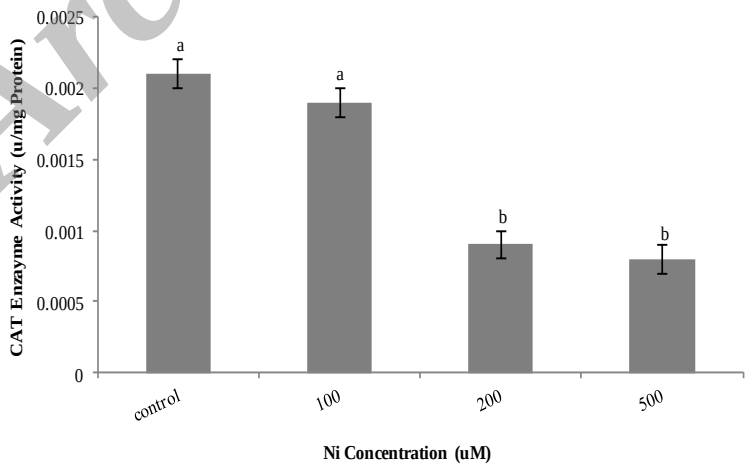

Figure 5. Catalase enzyme activity (µ / mg protein) of coriander in various concentration of Ni (µM)

Nickel accumulation in root and shoot

Accumulation of Ni in plant tissues was concentration dependent. A steady increase in metal content in both tissues (root and shoot) was observed with an increase significantly in Ni level in the growth medium (Figure 6). The metal content of roots was considerably higher in coriander as compared to shoots. At the highest Ni level (500 µM), 52 % of the metal uptake was carried out by the roots. Metal transport was lower in the shoots as only 47 % of the Ni was transported from soil to the aerial tissue. Thus, metal uptake from soil to roots was higher as compared with its transfer to the shoots [4, 34].

Hence, the capacity of species to accumulate Ni in their tissues vary markedly, metal seems to be restricted in the roots and a limited transfer to the aerial tissues became evident [34]. Several other studies also demonstrated differential transport of metals from soil to plant tissues [35, 36] and hyper-accumulators species can accrue far exceeding levels of metals than present in the soil [34]. Research results over the millet and spinach [37], lentils and peas [38] demonstrated that increasing the concentration of nickel in plant tissues is related to the increment of Ni concentration in the soil. The high metal concentrations in the roots than the shoots have also been reported in other species. The sensitivity of the shoot can be attributed to higher fraction of cystolic free Ni in the shoots than in the roots thus rendering the aerial tissue more sensitive to metal toxicity despite the fact that the roots accumulated more Ni [34].

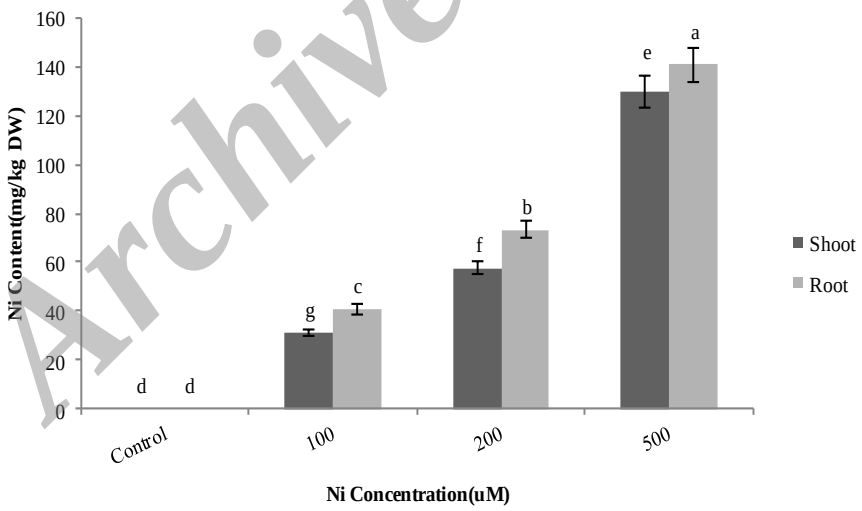

Figure 6. Ni content (mg / kg DW) of coriander in various concentration of nickel nitrate (µM).

CONCLUSIONS

Parameters of biochemical and vegetative in coriander specie were influenced by increasing Ni concentrations in the growth medium. However, catalase activity, shoot and root dry weight appeared to be more susceptible in the studied coriander specie in response to Ni contamination. These results suggest that the adverse effects of Ni stress can induce antioxidant defense activity in plants to remove the possible toxic effects of free radicals, making the plants more resistant to heavy metal stress. In addition, according to a more accumulation of Ni in the roots, the expansion of plants root can help to better adaptability with the toxicity of metals. It may be used as an indicator to illustrate the differences between plant species. In this study, phyto-extraction is the best-developed subjects of toxic metal phytoremediation nearing commercialization. The researchers become interested in this area. In addition, the ability of plants to accumulate toxic metals in their shoots and roots may be enhanced with specific chemicals (mainly metal-chelating agents) that facilitate the acquisition and transport of metals. Parallel evolutions in environmental and agricultural engineering should have a major impact on the efficiency of plant cultivation and excretion of metal-enriched biomass. More studies under controlled environmental conditions are required for evaluating the utility of these species in phytoremediation technologies.

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