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Uptake, Transport, Re-translocation and Chelation of Ni in One Tolerant and One Susceptible *Graminaceous* Species

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Abstract: From flora of Northwest Iran, two *Graminaceous* species including *Secale montanum* and *Dactylis glomerata* with different distribution and coverage on Ni-rich soils were selected for study of tolerance mechanisms to Ni concentrations up to 100 μM in hydroponic culture medium. Shoot and root growth of *D. glomerata* was inhibited up to 42 and 69% in response to 100 μM Ni, respectively, while growth reduction of *S. montanum* was as low as 20% in shoot and no growth inhibition of root was observed. Different growth response was not the result of different Ni accumulation, two studied species demonstrated a similar accumulation potential for Ni. A higher endogenous and Ni-induced malate accumulation did not cause a higher tolerance to Ni in *D. glomerata*. In contrast, high endogenous and Ni-induced accumulation of cysteine particularly in shoot was observed in Ni tolerant *S. montanum*. Distribution of Ni among various fractions of plant tissues studied by gel filtration chromatography suggested also an efficient chelation of excess Ni by low molecular weight fraction in *S. montanum* but not in *D. glomerata*. A higher re-translocation of Ni from mature into growing young leaves was one of the other causes of higher susceptibility of *D. glomerata* to excess Ni. Activity of peroxidase and catalase was much stronger inhibited in *D. glomerata* than *S. montanum* and activity of polyphenol oxidase was induced in response to Ni toxicity in roots of *S. montanum*. A possible role of this enzyme, involving in lignification, for higher Ni tolerance of *S. montanum* was discussed.

Key words: Graminaceous species, Ni, uptake, transport, re-translocation, toxicity

INTRODUCTION

The presence of phytotoxic amounts of heavy metals in the soil can lead to toxicity symptoms and the inhibition of growth of most plants. Uptake, accumulation, distribution and detoxification of heavy metals, have been studied in a wide range of plants species (Clemens, 2001, Hall, 2002). However, the mechanisms involved are still only partially understood. The response of plants is complex with considerable variation between species. Several sequestration and detoxification strategies are known to occur, different effects are also observed with different metals and metal concentrations (Hall, 2002).

Complexation with phytochelatin peptides has been identified as an important mechanism for detoxifying metals such as Cd, but they have no apparent role in the detoxification of Zn and Ni (Cobbett, 2000).

Complexation of Ni by organic acids was studied in some Ni accumulator plants and it was shown that nickel is bound primarily to malic and malonic acids in *Alyssum* and to citric acid in many other hyperaccumulators such as *Sebertia acuminata*, *Homalium* and *Hybanthus* (Brooks, 1998a). However, from stability constants for complexes between nickel and amino acids and carboxylic acids it is clear that nickel complexes with amino acids are

considerably more stable than those with carboxylic acids (Homer *et al.*, 1997). Works of many investigators (Krämer *et al.*, 1996) has displayed compelling evidence that nickel is bound to free histidine in *Alyssum* species. However, works on Ni-hyperaccumulator *T. goesingense* suggests that the histidine response might not be the general Ni-tolerance mechanism of plants (Persans *et al.*, 1999).

An association between metals and low molecular weight organic compounds suggests that metal detoxification occurs by vacuolar sequestration (Boominathan and Doran, 2003). However, other strategies for metal tolerance and accumulation, such as binding to the cell wall or localization in the apoplast, may also be involved (Hall, 2002). Therefore, study of metal-organic and -amino acid complexes using chromatography (Brooks, 1988a) could provide information on the distribution of metals within various fractions of plant tissues as an indirect indicator of detoxification mechanism.

Uptake, transport and re-translocation of heavy metals could provide also additional information about the mechanisms of tolerance. Although the effect of nutrients re-translocation on the efficiency of plant genotypes was extensively studied (Marschner, 1995), the significance of

re-translocation of elements to growing young leaves in the expressing of toxicity and/or differences among species was not studied.

Heavy metals are known to produce active oxygen species (Halliwell and Gutteridge, 1984). Activity of enzymes involving in the metabolism of free oxygen radicals e.g., peroxidase and catalase, was reported to be increased or decreased in response to metal toxicity depending on metal concentration, treatment duration or plant species (Chen *et al.*, 2000; Sandalio *et al.*, 2001; Schützendübel *et al.*, 2001; Boominathan and Doran, 2002). Activity of polyphenoloxidase, involving in the biosynthesis of lignin, may be also influenced by metal toxicity as a consequence of common response of plants to stress. The extent of changes in the activity of these enzymes could affect the response of a given species to heavy metal toxicity.

Regarding contradictory results of chelation form and distribution of Ni between various cell fractions as well as effect of antioxidant capacity in determinations of plants response to heavy metal toxicity, it has been suggested that cellular mechanisms of heavy metal detoxification are highly species specific (Schat and Kalffl, 2000). In our previous study approximately 73 species were collected and identified from heavy-metal rich soils of Northwest of Iran (Hajiboland *et al.*, 2002). Mechanisms of Ni tolerance of species growing on these soils are completely unknown. Moreover, some contrasting species concerning frequency and coverage on Ni-rich soils were introduced in this work (Hajiboland *et al.*, 2002). From these contrasting species, *Secale montanum* and *Dactylis glomerata* both from Poaceae were of special interest and selected for further studies.

This research was aimed to study the mechanisms of Ni tolerance and/or susceptibility of two contrasting graminaceous species with special emphasize on chelation, fractionation in plants tissues as well as re-distribution of Ni among various organs in order to find the reason of different frequency and coverage of these two species on Ni-rich areas of Northwest of Iran.

MATERIALS AND METHODS

Plant materials: Two species were used in this study including *Secale montanum* and *Dactylis glomerata*. Both of plant species have been previously determined as members of flora of Ni rich soils in NW Iran. Seeds were collected from Ni rich areas near Khoy (West Azarbaijan Province, Iran).

Plants culture: The experiments were conducted in a growth chamber under controlled environmental conditions. Seven-days-old seedlings were transferred to the nutrient solution (Cakmak *et al.*, 1996) and pre-cultured for 3 days. Nickel treatments were started for 10-days-old plants, consisted of five levels of $NiSO_4$ at 0 (control), 25, 50, 75 and 100 μM.

Harvest: After 12 days treatment, plants were harvested. For removing of the apoplasmic Ni from roots, plants were placed for 1 h in 5 mM $CaCl_2$+25% nutrient solution without Ni. Thereafter, roots were washed with distilled water, divided into shoots and roots, weighed and blotted dry on filter paper and dried at 70°C for 2 days to determine plant dry weight. Nickel concentration was determined by atomic absorption spectrophotometry (Shimadzu, AA 6500).

TLC: Fresh tissue samples were extracted in 20 mM Tris-HCl (pH = 8.0). The crude extract was centrifuged and applied to the plates according to the method described by Oven *et al.* (2002). Tissue extract was applied to a cellulose plate (formic acid/ethanol/water 48.8:48.8:2.4, v/v/v as mobile phase) for organic acids and to a silica gel plate (isobuthanol/acetic acid/water 50:25:25, v/v/v as mobile phase) for amino acids. After drying for 1 day at room temperature, dichlorophenol-indophenol and ninhydrin reagents were used for detection of organic and amino acids, respectively. The standard solutions of citric acid, malic acid, histidine, cysteine, glutamine and asparagines were applied simultaneously to the plates.

After detection of spots using an UV-detector, the margins of spots were lined and presence or absence as well as relative amount of each compound were reported using-or+characters. Each TLC experiment was conducted in triplicate.

Cysteine assay: Extraction of cysteine was carried out according to the method was described for TLC method. A quantitative determination of cysteine in plant tissues was conducted using a photometric method (Gaitonde, 1967).

Size exclusion chromatography: The fractionation of tissue extract of plants treated for 1 week with 50 μM Ni, was carried out according to the method described by Guo (1995). Fresh shoot or root tissues were homogenized in 10 mM Tris-HCl (pH = 8.0, +100 mM NaCl) in a chilled mortar then centrifuged at 2000 xg for 10 min at 4°C. The liquid fraction was re-centrifuged at 30000 ×g for 45 min at 4°C. The supernatant (soluble fraction) was applied on a column of Sephadex G-50 fine (50×2.5 cm) pre-equilibrated with running buffer and eluted at a flow rate of 60 mL h^{-1}. The running and pre-equilibration buffer was the same and contained 10 mM Tris-HCl (pH = 8.0)

supplemented by 100 mM NaCl. Elutant solution was collected in 60×1 mL samples. The concentration of Ni in each fraction was determined by AAS.

Re-translocation experiment: The experiment was carried out using method described previously for Cu (Hajiboland and Boiadi, 2005). The first harvest was performed at the end of root washing and four subsequent harvests were carried out each time after that a new leaf had emerged and partially expanded. Four replicates, consisting of defined leaves of four plants, were harvested for each treatment and harvest time. Samples were washed with double distilled water, oven-dried samples were ashed and Ni concentration was determined by AAS.

Assay of enzymes activity: Catalase (EC 1.11.1.6) activity was assayed by monitoring the decrease in absorbance of H_2O_2 at 240 nm (Lück, 1962). Unit activity was taken as the amount of enzyme, which decomposes 1 M of H_2O_2 in one min. Peroxidase (EC 1.11.1.7) activity was determined using the guaiacol test (Chance and Maehly, 1955), the absorption of tetraguaiacol formed in the reaction was measured at 470 mm. The enzyme unit was calculated as enzyme protein required for the formation of 1 μM tetraguaiacol for 1 min. Polyphenoloxidase (EC 1.10.31) activity was assayed by monitoring the absorbance at

430 nm (Halder *et al.*, 1998). The enzyme unit was calculated as enzyme protein required for color change for 1 min.

Determination of protein in tissue extracts for calculation of enzymes activities was carried out according to Bradford (1976).

RESULTS

Plants growth and accumulation of Ni in shoots and roots: Shoot and root dry weight of both species decreased in response to toxic levels of Nickel. However, growth reduction of *D. glomerata* was much higher than that of *S. montanum*. Shoot and root growth of *D. glomerata* was inhibited up to 42 and 69% in response to 100 μM Ni, respectively. The growth inhibition in *S. montanum* was only 20% in shoot. In roots no significant growth inhibition was observed at Ni treatment as high as 100 μM (Table 1).

Concentration of Ni in roots of plants grown at high Ni levels in medium was much higher than shoots. On the other hand, the extent of Ni accumulation was similar in two studied species (Table 1).

Accumulation of organic acids, amino acids and amides: In shoot extract of *S. montanum* organic acids were not

Table 1: Growth and concentration of Ni in shoot and roots of *Dactylis glomerata* and *Secale montanum* grown at toxic levels of this metal in the nutrient solution

Treatments	Dry weight (mg plant⁻¹)		Ni concentration (μg g⁻¹DW)	
	Shoot	Root	Shoot	Root
Dactylis glomerata				
0 μM	15.2±1.8[a]	4.5±1.7[a]	43±18[b]	259±72[b]
25 μM	10.9±2.6[b]	3.4±1.4[ab]	269±60[ab]	1760±544[a]
50 μM	8.1±3.1[b]	2.4±0.8[ab]	309±154[a]	1785±114[a]
75 μM	11.5±3.8[ab]	3.2±1.1[ab]	417±184[a]	1802±766[a]
100 μM	8.8±2.0[b]	1.4±0.4[b]	513±197[a]	2130±393[a]
Secale montanum				
0 μM	35.0±4.4[a]	9.9±1.0[ab]	27±5[c]	79±29[d]
25 μM	34.9±4.4[a]	11.8±1.3[a]	48±12[c]	883±297[c]
50 μM	38.0±2.5[a]	9.9±0.9[ab]	58±8[c]	943±69[c]
75 μM	34.5±3.7[ab]	9.0±2.2[b]	338±83[b]	1348±88[b]
100 μM	28.1±2.9[b]	8.1±0.9[b]	565±258[a]	2057±96[a]

Values in each column within each plant species followed by the same letter(s) are not significantly different (p<0.05)

Table 2: Detection of the presence of organic acids by TLC in tissue extract of *Dactylis glomerata* and *Secale montanum* grown at toxic levels of Ni in the nutrient solution

Treatments	Shoot		Root	
	Malic acid	Citric acid	Malic acid	Citric acid
Dactylis glomerata				
0 μM	++	++	++	++
50 μM	+++	++	+++	++
100 μM	++	++	++	++
Secale montanum				
0 μM	−	−	++	++
50 μM	−	−	++	++
100 μM	−	−	++	++

The number of +signs is correlated with the concentration

detectable with TLC method. But in shoot extract of *D. glomerata* not only malate and citrate were detected, but also an increase in malic acid content in plants treated by 50 μM Ni was observed. In root extract of both species detectable amounts of malate and citrate was found, but similar with shoot, the increase in malic acid content was observed only in *D. glomerata* and no detectable change in citric acid amounts was observed in both species (Table 2).

Histidine was not dateable in both shoot and root extracts and in both studied species neither in control nor in Ni treated plants. Cysteine was found in prominent amounts and in both plant organs. An increase in cysteine amount in response to Ni treatment was observed in shoot of *S. montanum*. Glutamine but not asparagine was detected in both shoot and root extract of *D. glomerata,* but not of *S. montanum.* However, no change in response to Ni treatment was observed in glutamine content of shoot and root (Table 3).

Data of cysteine concentration which were determined spectrophotometrically, were in accordance with TLC experiment, so that a significant increase in cysteine concentration of shoot in response to 50 μM Ni was observed in *S. montanum.* In *D. glomerata* cysteine concentration of shoot rather decreased in response to 50 μM Ni. In roots of *D. glomerata* a significant reduction and in *S. montanum* an increase in cysteine concentration was observed in response to Ni (Table 4). Changes in cysteine concentration of root in response to Ni treatment could not be detected by TLC method.

Distribution of Ni between various fractions of shoot and root: To characterize the binding state of Ni in shoot and roots, the soluble fraction was eluted through a Sephadex column. In the soluble fraction, phytochelatins can be separated from proteins and Low-Molecular-Weight (LMW) solutes by gel filtration (Mutoh and Hayashi, 1991). Accordingly, the soluble fraction of shoot and root extracts was separated into 60 fractions. The concentrations of Ni in these fractions are presented in Fig. 1. The first peak between fraction 18 and 22 represents High Molecular Weight Compounds (HMWs), the second peak between fraction 28 and 41 most likely contains PCS (Becher and Höfner, 1994) and the third peak between fraction 45 and 59 corresponds to LMW, such as organic acids, amino acids and probably also free metal ions (Mutoh and Hayashi, 1991). In the chromatogram (Fig. 1), it could be detected two peaks in shoot and one peak in root extract. The peaks of root extract were similar in two studied species and an exact coincidence was not observed between these two chromatograms with given data by other workers for elution time of three mentioned group of compounds. These peaks could represent the MMWs (phytochelatins) or may represent the LMWs, organic and amino acids as well as free Ni.

The chromatogram of the shoot extract was different between two species. On the other hand, according to elution time, it is possible to determine with a high confidence the nature of compounds to them Ni was chelated. In *D. glomerata*, the first peak was coincided on the HMWs, possibly proteins and other soluble macromolecules and the second peak represented the MMWs, possibly phytochelatins and other small peptides. In *S. montanum*, the first peak was coincided on the MMWs, possibly phytochelatins and the second peak represented the LMWs, organic and amino acids

Table 3: Detection of the presence of amino acids by TLC in tissue extract of *Dactylis glomerata* and *Secale montanum* grown at toxic levels of Ni in the nutrient solution.

Treatments	Shoot				Root			
	Cysteine	Histidine	Glutamine	Asparagine	Cysteine	Histidine	Glutamine	Asparagine
Dactylis glomerata								
0 μM	+	–	+	–	+	–	+	–
50 μM	+	–	+	–	+	–	+	–
100 μM	+	–	+	–	+	–	+	–
Secale montanum								
0 μM	+	–	–	–	+	–	–	–
50 μM	++	–	–	–	+	–	–	–
100 μM	+	–	–	–	+	–	–	–

The number of +signs is correlated with the concentration

Table 4: The concentration of cysteine (μg g⁻¹FW) detected spectrophotometrically in tissue extract of *Dactylis glomerata* and *Secale montanum* grown at toxic levels of Ni in the nutrient solution

Ni treatment	*Dactylis glomerata*		*Secale montanum*	
	Shoot	Root	Shoot	Root
0 μM	803±58[a]	511±21[a]	1305±425[b]	614±35[b]
50 μM	496±187[b]	425±15[b]	3538±786[a]	825±41[a]
100 μM	722±185[ab]	453±23[b]	1271±542[b]	768±18[a]

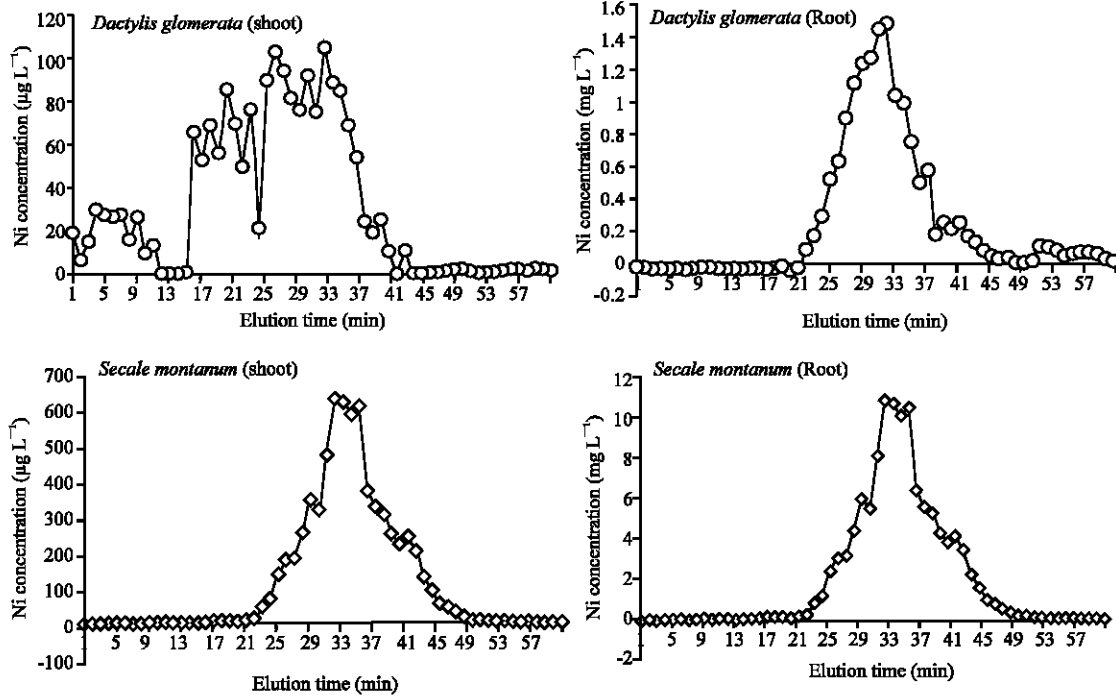Values in each column followed by the same letter(s) are not significantly different (p<0.05)

Fig. 1: Concentration of Ni in the eluted fractions of shoot (µg L^{-1}) and root (mg L^{-1}) extracts from gel-chromatography on a Sephadex G$_{50}$ column in *D. glomerata* and *S. montanum* grown at 50 µM Ni in medium

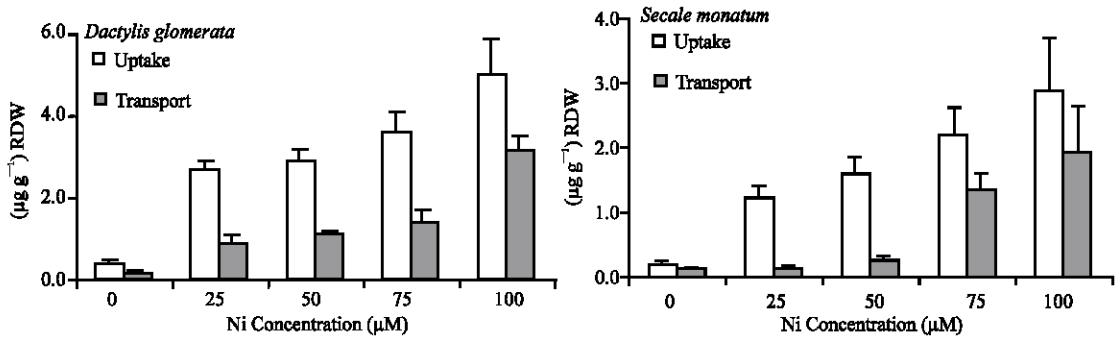Fig. 2: Uptake (µg g^{-1} RDW) and transport (µg g^{-1} RDW) of Ni in *D. glomerata* and *S. montanum* at toxic concentrations of Ni in medium

as well as free Ni. In LMWs fraction, Ni could not be detected in *D. glomerata* and in HMWs fraction in *S. montanum* (Fig. 1).

Uptake, transport and Re-translocation of Ni from mature to young leaves: As expected, uptake and transport of Ni increased with increasing concentration of metal in the medium. However, transport into the shoot increased more distinctly at higher Ni treatments, particularly in *S. montanum*. The Ni transport into shoot amounted only 14% of total Ni taken up at Ni concentration of 50 µM,

while up to 67% of total Ni in plants treated with 100 µM Ni, was transported into aerial part (Fig. 2).

Ni content of each distinct leaves and roots changed significantly during the experimental period as simultaneous increase of leaf and root weight occurs. Ni content of roots decreased continuously toward the end of experimental period. This reduction was higher in *D. glomerata* than *S. montanum*. In various fractions of shoots, however, more distinct difference between two studied species was observed. Nickel content of mature leaves decreased in *D. glomerata* but increased

Table 5: Changes in the content (μg plant part^{-1}) and weight (mg plant part^{-1}) of various shoot fractions of *Dactylis glomerata* and *Secale montanum* loaded by Ni concentration of 100 μM in the nutrient solution for 2 days and followed during various harvest intervals

Treatments	1st+2nd leaves		3rd+4th leaves		Roots	
	Ni content	Weight	Ni content	Weight	Ni content	Weight
Dactylis glomerata						
Harvest 1	1.15±0.36	17.5±1.8	---	---	7.20±1.4	8.5±1.9
Harvest 2	2.71±0.11	41.4±5.1	4.5±0.45	69.4±8.9	6.63±1.8	28.7±8.0
Harvest 3	0.72±0.35	154.4±13.7	4.02±0.28	127.3±12.3	1.98±1.0	105.1±1.8
Harvest 4	0.63±0.28	91.4±11.1	1.97±0.32	70.2±9.1	1.20±0.98	194.7±15.8
Harvest 5	0.61±0.22	87.3±9.3	1.50±0.48	69.0±5.2	1.18±1.0	2358±17.33
% Change	-47		-66		-84	
Secale montanum						
Harvest 1	1.32±0.48	20.4±4.5	---	---	10.53±1.4	15.6±2.4
Harvest 2	1.65±0.55	37.3±8.7	1.35±0.28	32.8±3.9	8.21±1.2	26.4±5.7
Harvest 3	1.79±0.49	48.3±7.9	2.80±0.32	27.7±5.7	7.43±0.98	22.3±4.8
Harvest 4	1.92±0.44	53.2±8.3	3.28±0.41	230.8±17.9	5.98±0.83	39.2±3.9
Harvest 5	2.08±0.33	42.8±2.7	3.91±0.38	198±12.9	5.14±0.84	43.7±5.8
% Change	+58		+189		-51	

Table 6: Activity of peroxidase, catalase and polyphenoloxidase (unit mg^{-1} Pro) in tissue extract of *Dactylis glomerata* and *Secale montanum* grown at toxic levels of Ni in the nutrient solution

Treatments	*Dactylis glomerata*		*Secale montanum*	
	Shoot	Root	Shoot	Root
Peroxidase				
0 μM	116.3±3.82[a]	3.85±0.08[a]	50.15±0.95[a]	1.64±0.01[a]
50 μM	93.1±1.45[b]	3.52±0.03[b]	42.04±0.25[b]	1.53±0.01[b]
Catalase				
0 μM	1368.1±33.42[a]	1598.9±33.71[a]	609.9±6.0[a]	718.4±5.82[a]
50 μM	986.1±26.21[b]	1472.5±19.37[b]	502.4±6.95[b]	670.0±6.55[b]
Polyphenol oxidase				
0 μM	22.89±0.41[a]	0.66±0.03[a]	8.14±0.17[a]	0.29±0.01[b]
50 μM	16.05±0.19[b]	0.58±0.1[a]	5.95±0.13[b]	0.44±0.01[a]

Values in each column within each enzyme activity followed by the same letter(s) are not significantly different (p<0.05)

in *S. montanum*. It means that in *D. glomerata* after appearance of new growing young leaves, each mature leaf lost significant amounts of Ni loaded previously in these leaves and obviously re-translocate it to young leaf. In *S. montanum* such a reduction of Ni content of mature leaves did not occur and rather an increase in Ni content of old leaves was observed (Table 5).

Changes in the activity of enzymes in response to Ni treatment: Activity of three studied enzymes decreased in response to Ni treatment in both shoot and root. The extent of reduction was higher in shoots than roots in both species. In shoots % reduction of enzyme activities were higher in *D. glomerata* than *S. montanum*. The % reduction of peroxidase, catalase and polyphenoloxidase activity were 20%, 28 and 30% in *D. glomerata* but 16%, 18 and 27% in *S. montanum*. In roots the inhibition of enzyme activity in two studied species was similar. The activity of polyphenoloxidase was induced in roots of *S. montanum* in response to treatment with 50 μM Ni (Table 6).

DISCUSSION

A clear different growth response to excess Ni was observed between *D. glomerata* and *S. montanum*. This is in accordance with our observation on Ni rich soils. *S. montanum* is one of the important elements of Ni flora in NW Iran (Hajiboland, 2002). In contrast, the distribution of *D. glomerata* is mainly restricted to marginal areas of Ni soils with low Ni availability. A high tolerance to Zn toxicity was observed in *S. montanum* than *D. glomerata* in our previous work (Hajiboland *et al.*, 2006).

The main cause of difference between two studied species was not different uptake and accumulation of Ni. Both species took up Ni and accumulated it in shoot with similar extent. Hyperaccumulators of Ni have a concentration of Ni in aerial parts as high as 1000 μg g^{-1} DW (Brooks, 1998b). In this research, the accumulation of Ni in leaves was 500 μg g^{-1} DW, therefore, these species do not quite qualify for hyperaccumulator status. The amounts of Ni concentration of plant samples collected from Ni soils, was in the range of 50-100 μg g DW.

Therefore, these two species could be classified only as accumulator species but with different tolerance to Ni toxicity.

Carboxylic acids and amino acids such as citric, malic and histidine are potential ligands for heavy metals and so could play a role in tolerance and detoxification (Rauser, 1999; Clemens, 2001), however, strong evidence for a function in tolerance, such as a clear correlation between amounts of acid produced and exposure to a metal, has not been produced to support a widespread role (Hall, 2002).

A clear difference between two species was observed in malate accumulation and induction in response to Ni in this work. However, a high endogenous and Ni-induced malate concentration (at 50 µM Ni) in *D. glomerata* was not associated with higher tolerance, suggesting that malate had no effect on tolerance of this species. The effect of malate as a Ni chelating and detoxifying molecule was reported for *Psychotria douarrei* (Kersten *et al.,* 1980), but Ni chelation by malate was not observed in *Sebertia acuminate*, *Homalium* and *Hybanthus* (Brooks, 1988a). Malate is one of the important metabolites in TCA cycle and fluctuation of its concentration could be the result of a wide range of factors affecting the activity of TCA cycle enzymes.

In contrast to reports on Ni chelation by histidine in *Alyssum lesbiacum* (Krämer *et al.,* 1996), in this study detectable amount of this amino acid was not found by TLC method, therefore, further study on histidine concentration was not carried out. It was also reported that, histidine response is not involved in Ni tolerance of all Ni hyperaccumulators (Persans *et al.,* 1999). In contrast to histidine, a clear cysteine response was observed in Ni treated plants. Both TLC and spectrophotometry methods showed that, cysteine accumulated in response to Ni and induction of cysteine was associated with higher Ni tolerance in *S. montanum.* Apart from inductive effect of Ni, the absolute amounts of cysteine were significantly higher in *S. montanum* which could be an indication of a higher potential for tolerance to heavy metals in this species. For cobalt, one of the heavy metals that do not activate PC synthesis, the cysteine and citrate concentrations are elevated in the hyperaccumulator *Crotalaria cobalticola* (Oven *et al.,* 2002). Our previous work showed also the effect of high endogenous and Cu-induced cysteine content of shoot in Cu tolerance of *Astragalus echinops*, a Cu tolerant species growing around Cu mines of Northwest of Iran (Hajiboland *et al.,* 2006).

In shoots a clear difference in distribution of Ni among various parts of soluble fraction between two studied species was observed. A high Ni concentration was found in LMW fraction of shoot in *S. montanum,* most likely bound with cysteine as chelating molecule in this fraction. Ni concentration in LMW fraction of shoot extract of *D. glomerata* was negligible, therefore, a high sensitivity of *D. glomerata* to Ni toxicity, could be attributed to the lack of Ni in this fraction.

Considerable amounts of Ni in *D. glomerata* were found in HMWs, implicating the association of Ni with proteins and structural macromolecules instead of chelation with low molecular weight compounds such as cysteine.

The presence of Ni in the MMWs, was similar between two species. This fraction may contain phytochelatins (Becher and Höfner, 1994). Although phytochelatin-Ni complex by phytochemical methods has not been detected (Cobbett, 2000), chelation of Ni to phytochelatins in these species could not be ruled out. However, similar portion of Ni in MMWs between two species suggested that chelation of Ni with compounds in this fraction has obviously no distinct role in detoxification likely because of a poor compartmentation.

This is the second report on the re-translocation of a heavy metal at toxic levels in a long term experiment using root-fed plants. Results suggested that the distribution of Ni among leaves of various age i.e. different effect on growth response, could be important than total Ni content. A high Cu re-translocation resulting in a higher susceptibility to excess Cu was reported for maize and sunflower (Hajiboland and Boiadi, 2005). The results for the Ni re-translocation showed also that, substantial quantities of Ni, loaded in roots, were transported into the shoots shortly after loading. The extent of root-shoot translocation (reflected in %reduction of Ni content of root) was higher in *D. glomerata* compared to *S. montanum.* On the other hand, in *D. glomerata* after 2-3 harvest intervals (18-27 days growth), the mature leaves became source of Ni and began to re-translocate the loaded Ni to younger leaves which was well reflected in the reducing amounts of Ni in mature leaves during the 3rd and 5th harvests in this species. In contrast, in *S. montanum* Ni content of mature leaves was not reduced during the experimental period. Therefore, the mature leaves of *S. montanum* did not act as a source of Ni for young leaves and simultaneous, acted as a sink for Ni loaded previously in roots.

Reduction in the activity of antioxidant enzymes e.g., peroxidase and catalase, was reported under toxicity of heavy metals including Ni (Boomanathan and Doran, 2002). Higher reduction of the activity of antioxidant enzymes in *D. glomerata* than *S. montanum* under Ni toxicity leading to oxidative stress, could be one of the other factors determining responses of two tested species to Ni toxicity. However, the activity of polyphenol oxidase in roots was not either influenced by Ni toxicity

(*D. glomerata*) or rather increased (*S. montanum*). Polyphenol oxidase is involved in lignification of cell walls. Higher activity of polyphenol oxidase in roots of *S. montanum* in the presence of excess Ni could be one of the reasons for higher tolerance of this species particularly regarding to root growth. The heavy metal induced membrane damages and leakage of solutes from roots is well documented (Murphy *et al.*, 1999). Therefore, induction of lignification could prevent leakage of solutes from roots. It was reported that, Cd (Schützendübel *et al.*, 2001) and Cu (Chen *et al.*, 2000; Díaz *et al.*, 2001; Jouili and Ferjani, 2003) toxicity induce xylogenesis. In this study, a higher polyphenol oxidase activity likely leads to an induction of lignification and thereby providing a mechanism for adaptation against higher Ni concentrations for *S. montanum*. A possible role for lignification in establishing an apoplastic transport barrier in roots was hypothesized under Cu and Zn toxicity (Cuypers *et al.*, 2002).

CONCLUSION

Different sensitivity to Ni toxicity between *D. glomerata* and *S. montanum* grown in hydroponic medium was in accordance with our ecological observation in terms of frequency and coverage of these species on Ni-rich areas in Northwest of Iran. Study of physiological mechanisms involving in their contrasting response to excess Ni revealed that, although both species have similar uptake and accumulation of Ni, different re-translocation potential for Ni in association with a different sensitivity at tissue level due to different extent of Ni chelation are from the important reasons of contrasting responses of these species to excess Ni.

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