http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (9): 1447-1452, 2000

Response of Bean Seedlings to Nickel Toxicity: Role of Calcium

A.E. El-Enany[1], M.A. Atia [2], M.H. Abd-Alla[1] and T. Rmadan
University of Assiut, Faculty of Science, Botany Department, Assiut, Egypt
University of South Valley, Qena Faculty of Science, Botany Department, Qena, Egypt

Abstract: Bean seedlings 7-days old were left to grow for 10 days in hydroponic cultures containing 0, 5, 15 ppm Nickel as $NiCl_2$ Calcium was added (1 mM or 5 mM) in combination with Nickel. Growth criteria, mineral contents, nickel distribution and metal-binding proteins were investigated. The fresh and dry weights of roots and shoots were sharply reduced especially at the higher dose (15 ppm Ni^{+2}). The content of some elements (K, Na , Ca, Mg and Mn) of roots and shoots were variably affected in presence or absence of calcium. Nickel was accumulated in roots more than in shoots. Fractionation of roots and shoot of bean plants revealed that the accumulation of Nickel was in the insoluble form (cell wall), while the remainder (the soluble form) was bound to proteins in the form of metalloproteins. Calcium reduced the toxic effect of nickel especially at the low dose (5 ppm). It was found that calcium reduced the uptake of nickel by roots and enhanced the synthesis of proteins, which form metal-binding proteins.

Key words: Bean, texicity, calcium

Introduction

Heavy metals increased over the years in many environments. Mining, smelting and chemical consumption is the main cause of trace elements in the biosphere. The main source of heavy metals in agriculture soils are fertilizers impurities (Cd^{+2}) and the use of refuge-derived compost and sewage sludge (Afloway, 1995). Heavy metals in agricultural soils led to losses in crop yields and hazardous health effect as they enter the food chain (Nellesson and Feletcher, 1993; Guo and Marschner, 1995; Salt *et al.*, 1995).

Nickel is defined as an essential micronutrients, because of its involvement in enzymatic activity (Welch, 1995). The Nickel requirement of plants is generally very low, 1.7 nmol g^{-1} or less in tissue dry biomass (Brown *et al.*, 1988; Dalton *et al.*, 1988). Symptoms of Ni toxicity include inhibition of root elongation and intervenal chlorosis and interfere in chlorophyll synthesis (Bazzaz *et al.*, 1974; Woolhouse, 1983; Foy *et al.*, 1987; Gabbrielli *et al.*, 1990; Brune and Dietz, 1995). High levels of Nickel are toxic to most plants. Plant species, and also genotypes within the same species, differ greatly in their ability to take up and transport these metals within the plants (Rebafka *et al.*, 1990 and Lubben and Sauerbeck, 1991). High retention of heavy metals in roots (Jarvis *et al.*, 1977) is practically desirable in many crops, where the roots are not utilized, thus reducing the heavy metals burden to animals and man. Heavy metals may interfere with other essential nutrients by competition for uptake system, and thereby disturb the mineral nutrition of the plants (Loneragan and Webb, 1987 and Ruano *et al.*, 1987).

Several defense strategies against metal stress are described in higher plants (Vangronsveld and Clijsters, 1994), e.g., compartmentation of metals in vacuoles (Rauser and Ackerely, 1987), complexation of metals by organic ligands, organic acids (Mutoh and Hayashi, 1991), phytochelatins (Grill *et al.*, 1985; El-Enany, 2000). The biological activity of heavy metal ions can be markedly affected by the presence of cations such as, magnesium and calcium (Gadd and Griffith, 1978; Ouzounidou *et al.*, 1998). Calcium is unique among the elements comprising living systems because of its role as a messenger for many different types of physiological processes and its unique chemical binding properties (McLaughlin and Wimmer, 1999). The toxicity of heavy metals can be ameliorated by calcium (El-Enany, 1995 and Issa *et al.*, 1995). The selective permeability and ion uptake through the membranes is regulated by calcium (Nakamura *et al.*, 1990; Abdel-Basset and Issa, 1994).

The objective of our study are to assess the sensitivity of plant growth to toxic Nickel level and to determine the ameliorative effect of additional Ca, using broad bean as a test crop plant. To test this possibility, we investigated the growth criteria, protein content, mineral composition and Nickel distribution within the plants treated with Ni or Ni-Ca combination.

Materials and Methods

Broad bean plants (*Vicia faba*) were grown in 22/16 1C and 12/12 h (light/ dark) regimes with light. Five-day-old seedlings were placed on a plastic pot (1 L). The plants were grown in aerated Hoagland's solution 1/2 strength and exchanged every 2-days. Five-days after transfer of seedlings to the Hoagland's solution, Nickel as ($NiCl_2$) at final concentrations (0, 5 and 15 mg/l) was added to the nutrient solution. Calcium was added as $CaCl_2$ to the nutrient solution, in combination with or without nickel. Each treatment was carried out at 3-replicates. The pH value of the nutrient solution was adjusted at 5.7 ± 0.2. All plant parts were rinsed briefly with deionzed water, blotted dry and weighted. Two samples of roots (2-3 g fresh weight) from each treatment were collected, and stored frozen for gel-filtration. Remainder of shoots and of roots was oven-fried at 70°C for 48 h and dry weights were determined.

Determination of protein: Protein was determined spectrophotometically according to Bradford (1976).

Chemical analysis of plant samples: About 0.2 g powdered tissue received 2 ml concentrated HNO_3 in an acid washed tube, and the samples were digested in a heating block at 180 1C untill the remaining solution appeared clear (Kraemer *et al.*, 1997). Adding I ml of 30 % (w/v) H_2O_2 and heating to 120 °C until effervescence ceased from the decolorized digests. Made up to 25 ml with distilled water. The insoluble centrifugation fractions was similarly digested in 5 ml concentrated HNO_3. Ni, Mn,Ca, Na, K and Mg were measured by Atomic Absorption Spectrophotometry (Model Varian AA 55).

Gel-Filteration: One gram of frozen roots were homogenized in 2 ml 10 mM Tris-HCl (pH 8.0 + 0.1 M 1 NaCl) buffer solution in a mortar. The homogenate was centrifuged at 6000 rpm for 15 min. at 4 °C and the liquid fraction was centrifuged at

12,000 rpm for 30 min. at 4°C. The residue of both centrifugation were combined (Insoluble fraction). The supernatant liquid of about 2 ml was confused at 2000 rpm for 15 min. at 4 °C. The concentrate supernatant (soluble fraction) was put on a (50 ± 2.5 cm) column of sephadex G-100 (fine) and eluted with 10 mM Tris-HCl (pH 8.0 + 0.1 M NaCl) buffer at a flow rate of 0.5ml /min. Eluant buffer was collected in linear fraction collector type (HAAK Bechle LC, 2000) at 4°C, each fraction 5 ml volume. The concentration of Ni and Ca in each fraction was measured by AAS. The absorbency of each fraction was recorded spectrophotometry at 280 nm.

Results and Discussions

Plant Growth: The fresh and dry matter of the roots and shoots of broad bean plants were adversely affected in this study by nickel added to the nutrient solution Fig. (1 and 2). The dry weight of roots was severely reduced by about 68% and 65% of the control, respectively (Fig. 1 b). Nickel inhibits fresh weigh of shoots by about 54 and 70% of control plants at 5 ppm and 15 ppm, respectively (Fig 2a). The addition of Ca^{+2}, especially at 5 mM variably minimized the inhibitory effect of Ni^{+2} especially at the high dose (15 ppm).

Nickel inhibits protein contents by about 50% at low and high doses. While Ca per se, stimulate protein synthesis by about 12 and 58% at 1 mM and 5 mM, respectively. Ca-Ni combination raised the protein content in roots and shoots at different levels (Fig. 3 a,b).

Critical values for metal-toxicity were calculated from the relationship between growth-plant-dry matter yield and element concentration (Macoqut *et al.*,1996). Our results demonstrated a marked decrease in the fresh and dry weight of roots and shoots of broad bean seedlings (Fig. 1 and 2). Many investigators recorded the observed decrease in fresh weight as a result of Ni toxicity (Macoqut *et al.*,1996; Paivoeke, 1983; Barceleo *et al.*, 1986). The observed decrease in fresh weight is a result of change in the plant water status. Nickel accumulation decreased water uptake or enhanced water loss, both of which may cause membrane damage. Plant cell membranes are generally considered primary sites of metal injury (Barceleo and Poschenrieder, 1990). Decreased water contents in plants grown on solution containing toxic levels of micronutrients have also been reported (Paivoeke, 1983; Barceleo and Poschenrieder, 1990), which may be result of decreased water uptake or enhanced water loss.

The results in (Fig. 1 and 2) revealed that Ca -Ni combination lessened the toxic effect of nickel. Several observations revealed that Ca-deficiency can intensify some heavy metal tonicities and that high levels of Ca tend to ameliorate the tonicities (Wallace *et al.*, 1977 and 1980). The metal-induced changes in membrane properties not only affect K^+ and H^+ extrusion, the function of membrane carrier and ion channels. Plant cell membranes are generally considered primary sites of metal injury (Barcelo and Poschenrieder, 1990). Ca may act through membrane stabilization of stressed plant cells (Leopold and Willing , 1984; Issa *et al.*, 1995) or through the decrease uptake of Ni^{+2} from the nutrient solution (Wallace *et al.*, 1971; Barsukuva and Gamzikova, 1999).

The application of Ni^{+2} treatments to the nutrient solution induced changes in the elemental distribution in roots and shoots of broad bean plants (Table 1 and 2). Increasing Ni in the nutrient solution significantly decreased the Ca content of roots and shoots. While addition of Ca to the solution medium minimize the inhibitory effect of Ca accumulation, especially at 1 mM. Mn contents consider a sensitive indicator of heavy metal stress. Ni completely inhibits the accumulation of Mn in roots and shoots at both treatments (5 ppm and 15 ppm). As Ca^{+2} added to the solution in combination with Ni^{+2}, the Mn contents were recovered, especially in the shoots. Nickel stress at high level (15 ppm) decreased Mg^{+2} in roots or shoots. While Mg was unchanged at 5 ppm Ni^{+2}. In contrast, Ca-Ni combination inhibits accumulation of Mg in shoots and roots. Potassium and sodium content showed their highest depression in Ni-treated plants. While K^+ and Na^+ content tend to be unaffected under Ca^{+2} treatments, although Ca-Ni combination treatment reduce the inhibitory effect.

Heavy metal-induced disturbance of mineral distribution within plants (Brune and Dietz 1993; Arduini *et al.*, 1998). Our results show a decreased in Mn content of roots as Ni elevated in the culture medium (Table 1). Arduini *et al.* (1998) suggested that nickel may interference Mn uptake. Nickel under severe stress decreases Mg content of shoots and roots. In the contrary Brune and Dietz (1993), found that leaf Mg content increased by more than 25% at elevated Ni concentration in the rooting medium.

The Mg contents of shoots was unchanged under Ni stress, while the Mg content of roots was reduced. The difference in response of Mg and Ca (Table 1) suggest that the cause for the reduction in root contents was not competition for binding sites of the cell wall but specific inhibition of uptake processes into the root cells. Nickel was manifested in the behavior of calcium, magnesium and potassium (Barsukova and Gamzikova, 1999). Arduini *et al.* (1998) found that the Mn and zn content of roots decreased in some tree seedlings as heavy metals increasing, whereas the Ca and Mg uptake and distribution depend on species and plant type. Displacement of divalent cations from anionic cell wall binding sites should result in a decrease in Ca content, since Ca is known to be a major ligand of the cell wall (Grigono and Sentenac, 1991).

The distribution of Ni^{+2} in roots and shoots of broad bean plants are presented in Fig. 4a and b. Large differences between roots and shoots were founds in the nickel concentration. Nickel was accumulated in roots more than shoots at different treatments. The Ni^{+2} concentration in roots and shoots markedly increased as Ni^{+2} elevated in the nutrient medium. Calcium addition to the medium (Fig.4 a,b) low Ni^{+2} accumulation in the roots and shoots of broad bean plants especially at 5 mM. The insoluble fraction of roots (cell walls) contained about 63 and 61% of Ni^{+2} at low and high doses, respectively. These percentages were decreased by about 40 and 32% in Ca^{+2}-Ni combination present in the nutrient medium Fig. (4a). While, the insoluble fraction of roots (cell free extract) contained about 33 and 39% of total Ni at 5 ppm and 15 ppm, respectively. These percentages are raised as Ca^{+2} applied to the solution (Fig. 4a). A higher proportion of Ni^{+2} in the soluble fraction was observed in roots than shoots. In higher plants, roots are the first organ to contact with the toxic metal concentrations, and roots usually accumulate significantly higher metal amount than upper parts (Malan and Farrant, 1998). Under long-term exposure, heavy metals generally reduce root growth more intensively than shoot growth, decreasing the root/ shoot ratio, but the amount of metal uptake and intensity of root growth inhibition may vary with the plant species and the growth conditions (Foy *et al.* 1987; Barcelo and Poschenrieder, 1990). These results are in agreement with our data presented in Fig. 4a, b, which revealed that the bean roots accumulated Ni more than shoots and most of Ni was found in the insoluble form (cell wall)

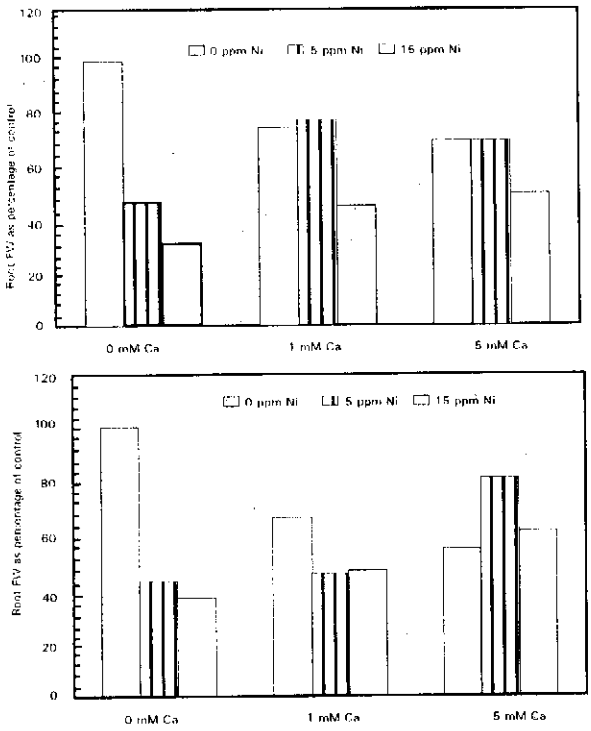

Fig. 1: Fresh weight (a) and dry weight (b) of board bean roots grwon for 150-days in nutrient solution at different treatment

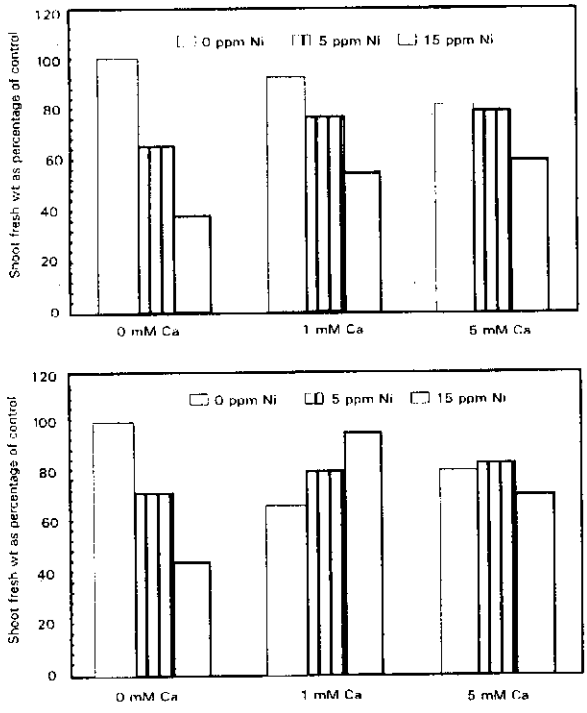

Fig. 2: Fresh weight (a) and dry weight (b) of board bean shoots grwon for 150-days in nutrient solution at different treatment

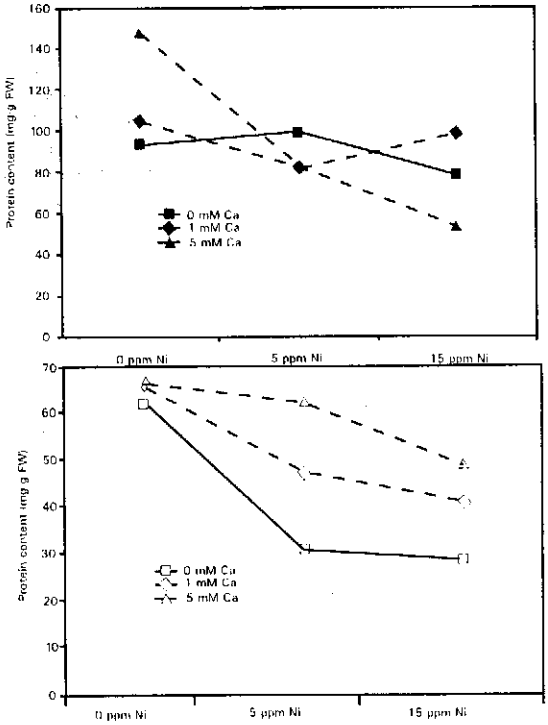

Fig. 3: Protein content (mg/g fresh weight) of roots (a) and shoots (b) of broad bean plants grown for 15-days innutrient solution containing different treatments

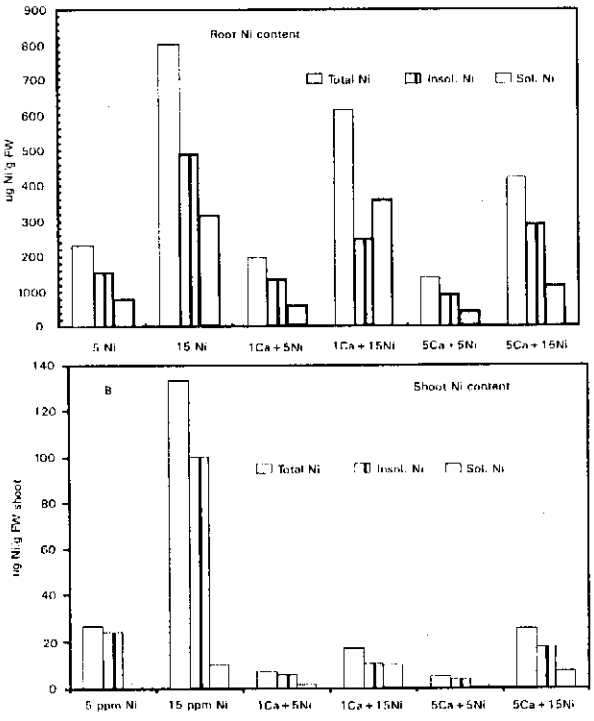

Fig. 4: Total, Insoluble and soluble nickel contents of roots (a) and shoots (b) of broad bean plants grown for 15-days in nutrient solution at different treatments

Table 1: Mineral concentrations of *Vicia faba* seedling roots, after 15-days exposure to different treatments. Values are means of three values

Treatment (ppm)	Metal concentrations (mg/g dry wt)				
	K	Na	Ca	Mg	Mn
Control	63.00	15.50	8.26	1.05	0.32
Ni (5 ppm)	34.25*	7.15*	7.25*	1.26	0.012*
Ni (15 ppm)	20.70*	3.15*	5.14	0.72*	0.015*
Ca (1 mM)	60.90	11.40*	7.33*	0.93	0.25*
Ca (5 mM)	51.05*	10.25*	5.50	1.16	0.34
Ca (1 mM) + Ni (5 ppm)	51.5*	7.04*	6.41	2.06	0.12*
Ca (1 mM) + Ni (15 ppm)	51.05*	3.56*	6.65	1.80	0.11*
Ca (5 mM) + Ni (5 ppm)	27.3*	10.25*	6.67	1.01	0.08*
Ca (5mM) + Ni (15 ppm)	16.1	11.4	6.34	0.32*	0.075
L.S.D. 5%	4.3	2.91	1.84	0.288	0.03

* Significantly differences ($P < 0.05$).

Table 2: Mineral concentrations of *Vicia faba* seedling shoots, after 15-days exposure to different treatments. Values are means of three values

Treatment (ppm)	Metal concentrations (mg/g dry wt)				
	K	Na	Ca	Mg	Mn
Control	76.8	4.53	12.42	5.54	1.05
Ni (5 ppm)	36.4*	1.54	8.97*	5.02	0.02
Ni (15 ppm)	40.88*	4.83	8.35*	3.77*	0.02
Ca (1 mM)	66.03	12.61*	11.67	4.75	1.30
Ca (5 mM)	81.2	7.60	12.83	5.26	3.81*
Ca (1 mM) + Ni (5 ppm)	78.8	14.24*	8.21*	4.55	3.73*
Ca (1 mM) + Ni (15 ppm)	61.37	12.29	6.02*	2.62	3.82
Ca (5 mM) + Ni (5 ppm)	72.76	5.23	9.96*	1.10*	2.77
Ca (5mM) + Ni (15 ppm)	66.32*	5.01*	7.32*	1.66*	2.37
L.S.D. 5%	12.1	3.2	2.08	1.5	1.01

* Significantly differences ($P < 0.05$)

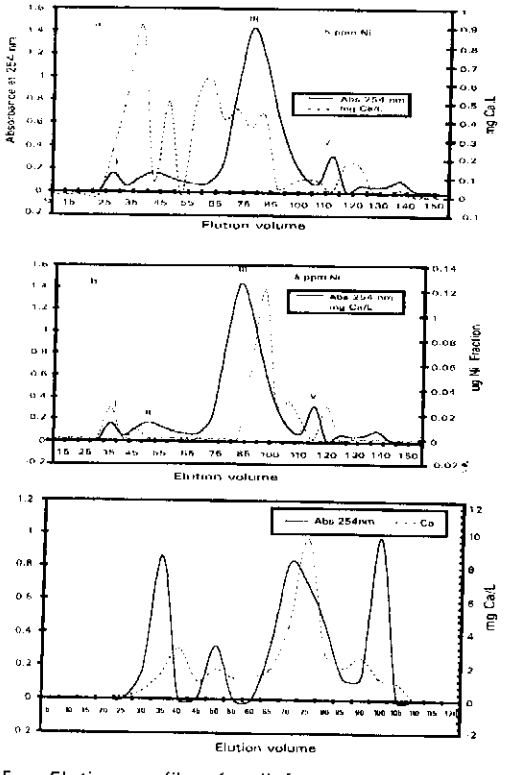

Fig. 5: Elution profile of cell free extract of bean roots grown for 15-days in nutrient solution containing 5 ppm Ni. Faction volume 5 ml. Flow rate 0.5 mL

debris. In this respect, Khan *et al.* (1992) reported that Cd mainly associated with cell wall, whereas Velazquez *et al.* (1992) found the accumulation of Cd in vacuoles of bean roots. Whereas, other authors (Khan *et al.*, 1984; Lozano-Rodrigner *et al.*, 1997; Gadalla and El-Enany, 1999) observed that the accumulation of Cd and Zn are in the cell walls. Metal retention in cell walls might due to cross-linking of metal to carboxyl groups of the cellulose (Landberg and Greger, 1984; Barceleo and Poschenrider , 1990). This was in contrast with Cataldo *et al.* (1981) and Weigel and Jager (1980), who reported that over 5% of the total Cd concentration in legumes plants was in the soluble form. The higher proportion of Ni in the soluble fractions of shoots especially at Ni-Ca combination (Fig. 4a, b). This in agreement with results obtained by Guo and Marchner (1995) and Malan and Farrant (1998) who observed the high mobility of Ni within the plant

Gel-filtration of the soluble fraction from roots: To characterize the binding state of Ni^{+2} and Ca^{+2} in the soluble fraction of roots, the soluble fraction was eluted through a sephadex G-100 column (Fig. 5 a, b). In the soluble fraction, low and high molecular weight proteins can be separated by gel-filtration. The soluble fraction of root extracts was separated into 30 fractions. The concentrations of Ca and Ni in these fractions are presented in fractions diagram. The elution diagrams revealed 5-protein peaks. The first peak (I) of Mr. 170 kD was induced only in nickel treated plants and retained about 11.25% of soluble Ca and 12.2% of soluble Ni^{+2} fraction. Peaks II, III and V were appeared in roots at different treatments. The main peak of Mr. (75-80 kD) retained about 57% soluble Ca^{+2} and 80% of soluble Ni^{+2} in roots of Ni or Ca^{+2} -Ni treated plants. This peak retained small amount of Ca^{+2} and Ni^{+2}, in roots of Ca-Ni combination. Treated plants with Ni^{+2} (5 ppm) induced proteins of low molecular weights

(30 kD and 12 kD), these proteins contained about 22.4 and 7.5 % of Ni^{+2} cell free extract, respectively (Fig.5 c). In contrast these proteins were disappeared in control (Fig.5 a) or in untreated plants received Ca^{+2} only (Fig. 5b).

The results of gel filtration in Fig. (5, a, b, c) showed that Ca and Ni are bind to proteins of low and high molecular weights. These are in agreement with many investigators (Gadalla and El-Enany, 1999). Blackbourn *et al.* (1991) found that Ca stimulate the synthesis of Ca-binding proteins of Mr. (53-33 kD) in maize plants. Makymiec and Baszynski (1999) stated that calcium play an important role in signal transudation during stress condition.

References

Abdel-Basset R. and A. A. Issa, 1994. Membrane stabilization and survival of dehydrated *Chlorella fosca* cells induced by calcium. Biologia Plantarum, 36:389-395.

Alloway B.J., 1995. The origin of heavy metals in soils. In Alloway B. J. (ed.): Heavy metals in soils. Blackie Academic and Professional, London, pp: 39-57.

Arduini I., D. L. Godbold, A. Onnis and A. Stefani, 1998. Heavy metals influence mineral nutrition of tree seedlings. Chemosphere, 36: 739-744.

Barceleo J. and C. Poschenrieder, 1990. Plant water relations as affected by heavy metal stress: A review. J. Plant Nutr., 13: 1-37.

Barsukova V.S. and O. I. Gazikova, 1999. Effect of excessive nickel on the elemental composition of wheat cultivars. With contrast resistance to nickel. Agrokhimiya, 0: 80-85.

Bazzaz F.A., R. W. Carlson and R. L. Rolfe, 1974. The effect of heavy metals on plants. Environ. Pollut., 7: 241-246.

Blackbourn H.D., J. H. Walker and N. H. Batley, 1991. Calcium-dependent phospholipid binding proteins in plants. Their characterization and potential for regulating cell growth. Planta, 184: 67-73.

Bradford M.M., 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem., 72:248-254.

Brown P.H., R. M. Welch, E. E. Cary and R. J. Checkai, 1988 . Nickel: a micronutrient effectual for higher plants. Plant Physiol., 85: 801- 803.

Brune A. and K. J. Dietz, 1993. A comparative analysis of element composition of roots and leaves of barley seedlings grown in the presence of toxic cadmium, molybdenum, nickel and zinc concentrations. J. Plant Nutr., 14: 853-868.

Cataldo D.A., R. G. Thomas and R. D. Wildung, 1981. Cadmium distribution and chemical fate in soybean plants. Plant Physiol., 68: 835-839.

Dalton A.D., S.A. Russell and H. S. Evan, 1988. Nickel as a micronutrient element for plants. Biofactors, 1: 11-16.

El-Enany A.E., 1995. Alleviation of cadmium toxicity on maize seedlings by calcium, 37:93-99.

Foy C. D., R. L. Cheny and M. C. White, 1987. The physiology of metal toxicity in plants. Annu.Rev. Plant. Physiol., 29: 511-566.

Gabbrielli R., T. Pandolfini, G. O. Vergnano and M. R. Palandri,1990. Comparison of two serpentine species with different nickel tolerance strategies. Plant Soil, 122: 271-277.

Gadallah M.A.A. and A. E. El-Enany, 1999. Role of kinetin in alleviation of copper and zinc toxicity in *Lupinus termis* plants. Plant Growth Regul., 29: 151-160.

Gadd G.M. and A. J. Griffith, 1987. Microorganisms and heavy metal toxicity. Microbial Ecology, 21:134-146.

Grigon C. and Sentenac, 1991. pH and ionic conditions in the apoplast. Annu. Rev. Plant Physiol. Plant Mol. Biol., 42:103-128.

Grill E., E. L. Winnacker and M. H. Zenk, 1985. Phytochelatins, the principal heavy metal complexing peptides of higher plant, Sci., 230: 674-676.

Guo, Y. and H. Marschner, 1995. Uptake, distribution, and binding of cadmium and nickel in different plant species. J. Plant Nutr., 18: 2691-2706

Issa A.A., R. Abdel-Basset and M. S. Adam, 1995. A bolition of heavy metal toxicity on *Kirchneriella lunaris* (Chlorophyta) by calcium. Annals of Botany, 75:189-192.

Jarvis S. C., L. H. P. Jones and M. J. Hopper, 1976. Cadmium uptake from solution by plants and its transport from root to shoot. Plant and Soil, 44: 179-191.

Khan D.H., J. G. Duckell,B. Frannkland and J. B. Kirkham, 1992. An x-ray microanalytical study of distribution of Cd in roots of *Zea mays* L. J. Plant Physiol., 115: 19-28.

Kraemer U., D. R. Smith, W. V. Wenzel, I. Raskin and D. E. Salt, 1997. The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesigense* Halacsy. Plant Physiol., 115: 1641-1650.

Landberg T. and M. Greger,1984. Influence of selenium on uptake and toxicity of copper and cadmium in pea (*Pisum sativum*) and wheat (*Trifolium aestivum*). Physiol. Plant, 90: 637-644.

Leopold A.C. and R. P. Willing, 1984. Evidence for toxicity of salt on membranes. In: Stoples R.C., Toennissen G.H. (eds). Salinity tolerance in plants. Strategies for crop improvement. New Yourk: J. Wiley and Sons, 67-91.

Loneragan J.F. and M. J. Webb, 1993. Interaction between zinc and other nutrients affecting the growth of plants, pp: 119-134. IN: A.D. Robson (ed.) Zinc in Soils and Plants. Kluwer Academic Publishers, Boston. MA.

Lozano-Rodriguez E., L. E. Hernandez, P. Bonay and R. O. Capena-Ruiz, 1997. Distribution of cadmium in shoot and root tissues of maize and pea plants.: Physiological disturbances. J. Exp. Bot., 48: 123-128.

Lubben S. and D. Sauerbeck, 1991. Transferfaktoren und Transferkoef-fizienten fur den Schwermetallubergang Boden-Pflanzen. In D. sauerbeck and S. Luebben (eds) Auswirkinng von Siedlun-gsabfallen auf Boden, Boden-organismen und Pflanzen. Berichte aus der Okologischen Forschung. Volume 6. Forschungzentrum, Julich, Germany.

Makymiec W. and T. Baszynski, 1999. The role of calcium ions in modulating changes induced in bean plants by an excess of Cu^{+2} ions. Chlorophyll fluorescence measurements. Physiol. Plant, 105: 502- 568.

Malan H.L. and J. M. Farrant,1998. Effect of the metal pollutants cadmium and nickel on soybean seed development.. Seed Sci. Res., 8: 445-453.

McLaughlin, S. B. and R. Wimmer, 1999. Calcium physiology and terrestrial ecosystem processes. New Phytol., 142., 373-417.

Mocquot B., J. Vangronveld, H. Clijsters and M. Mench, 1996. Copper toxicity in young maize (*Zea mays* L.) plants: Effects on growth, mineral and chlorophyll contents and enzyme activities. Plant Soil, 182: 287-300.

Mutoh N. and Y. Hayashi, 1991. Sulfur -containg cadystin-cadmium complexes. Methods Enzymol., 205: 341-347.

Nakamura Y, K. Tanaka, E. Ohta and M. Eakata, 1990. Protective effect of external Ca+2 on elongation and bean roots under high NaCl stress. Plant Cell Physiol., 31: 815-821.

Nellessen, J.E. and J. S. Fletcher, 1993. Assessment of published literature on the uptake, accumulation, and translocation of heavy metals by vascular plants. Chemosphere, 9: 1669 -1680.

Ouzounidou G., I. Ilias, Tranopoulou and S. Karataglis, 1998. Amelioration of copper toxicity by iron on spinach physiology. J.Plant Nutr., 21: 2089-2101.

Paivoeke A., 1983. The short-term effects of zinc on the growth anatomy and acid phosphatase of pea seedlings. Ann.Bot. Fenn., 20:197-203.

Rauser W.E. and C. A. Ackerley, 1987. Localization of cadmium in granules within differentiating and mature root cells. Can. J. Bot., 65:643-646.

Rebafka F. P., R. Schulz and H. Marschner, 1990. Erhebungsuntersuchngen zur Pflanzenverfugbarkeit von Nickel auf Boden mit hohen geogenen Nickelgehalten. Angew. Botanik, 64: 317-328.

Ruano A.J., J. Barceleo and C. Poschenrieder, 1987. Zinc-induced variations of mineral element composition in hydroponically grown bush bean plants. J. Plant Nutr., 10: 373-384.

Salt D.E., M. Blaylock kumar, V. Dusenkov, B. D. Ensly and C. I. Raski, 1995. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Bio. Technol., 13: 489-474.

Vangronsveld J. and H. Clijsters, 1994. In Farago, M.E. (ed.): Plant and Chemical elements Biochemistry, Uptake, Tolerance and Toxicity, pp: 150-177.

Velazquez M.D., C. H. Poschenrieder and J. Barcelo,1992. Ultrastructural effects and localization of low cadmium concentrations in bean roots. New Phytol., 120: 215-226.

Wallace A., 1971: Calcium and chelating agent regulation of mineral elenment composition of bush beans grown in solution culture. In

Wallace A., J. W. Cha and G. V. Alexander, and Rommn, 1977. Calcium deficiency and $CaCO_3$ on micronutrients status of plant grown in solution culture. Commun.Soil Sci. Plant Anal., 8: 781-785.

Wallace A., E. M. Romny, R. T. Mueller and Alexander G.V., 1980. Calcium interaction in soybean plants. J. Plant Nutr., 2: 79-86.

Weigel H.J. and H. J. Jager,1980. Subcellular distribution and chemical form of cadmium in bean plants. Plant Physiol., 65: 480-482.

Welch R.M., 1995. Micronutrient nutrition of plants. Crit Rev Plant Sci., 14: 49-82.

Woolhouse H.W., 1983. Toxicity and tolerance in responses of plants to metals. In O.L. Lange, Nobel, P.S. Nobel, C.B. Osmond, Ziegler H. (eds), Encyclopedia of plant Physiol, New Series, Vol. 12C. Springer-Verlag, Berlin, pp: 245-300.