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Response of Anabolic Capacities, Proline, Protein Patterns and Mineral Elements to Nickel and EDTA Stress in *Chorcorus olitorius*

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Abstract: *Chorcorus olitorius* grows in nutrient solution was treated with Ni (10 and 50µM) and EDTA (10, 50 and 100 µM) in different combinations. Shoot, root length, fresh and dry weight for shoot and root photosynthetic pigments and carbohydrates were decreased with increasing Ni supply. EDTA reduces the inhibition of Ni on chlorophyll and total carbohydrates formation. Proline content was increased significantly with increasing Ni concentration and decreased after EDTA treatment. Ni and EDTA treatment induced quantitative and qualitative changes in the protein PAGE profile of plant. New polypeptides of varying molecular weights were synthesized with the addition of Ni and others were stimulated by Ni and EDTA to nutrient solution. In general, the total number of protein bands were decreased by Ni treatment but increased after EDTA addition. It was noticed that, increasing Ni concentration in nutrient solution significantly increased uptake and accumulation of Ni in both shoot and root.The concentration of Ni in shoot and root was greater than in shoot. Accumulation of K, Ca and Fe were significantly decreased with increasing Ni concentration. The uptake of Ni is diminished in the presence of EDTA, suggesting that the Ni-EDTA complex is unable to penetrate the membrane. Increasing EDTA concentration combined with Ni, increased the concentration of Ca, K and Fe. In conclusion EDTA can ameliorate the toxic effects of Ni on *Chorcorus olitorius* plant.

Key words: *Chorcorus olitorius*, EDTA, Ni toxicity, protein profile

Introduction

Soil contamination with heavy metals has become a worldwide problem, leading to a loss in agricultural yield and hazardous health effects as they enter the food chain (Nellessen and Fletcher, 1993; Guo and Marschner, 1995). Man's energy and chemical consumption is the main cause of trace element pollution in the biosphere. The main sources of contamination in agricultural soils are fertilizer impurities and the use of refuge-derived compost and sewage sludge (Ni^{2+}). Disposal of household, municipal and industrial waste has given rise to even more widespread nickel soil concentrations (Alloway, 1995). Nickel has recently been defined as essential micronutrient, because of its involvement in enzymatic activity in legumes (Welch and Shuman, 1995). In most plants it is found at the level of 0.1-5 ppm (on a dry weight basis), with a wide threshold range of 40-246 ppm for toxicity symptoms, dependent upon the plant species (Kabata-Pendias and Pendias, 1992). The main common symptoms are chlorosis, and inhibited photosynthesis and respiration (Bazzaz *et al.*, 1974, Foy *et al.*, 1978). The mechanisms governing Ni^{2+} toxicity are not well understood. The dry matter of wheat was not affected by Ni treatment (Zeller and Feller, 1999). Nickel prevented any growth of redbeet plant (Kukier and Chaney, 2001). Cd caused reduction in dry weight and carbohydrate concentration in sugar beet plant (Greger and Bertell, 1992). EDTA reduces the inhibition of Cd^{2+} on sugar formation and accumulation (Greger and Lindberg, 1987). Exposure of Indian mustard to high concentrations of Pb and EDTA caused reductions in both the transpiration rate and the shoot water content (Andrew *et al.*, 1998). Excess EDTA to nutrient cations, reduce the root damage (Eskew *et al.*, 1984). After EDTA treatment, soluble heavy metals in soil water occurred mainly as metal-EDTA complexes and high concentrations of heavy metals in soil water after EDTA treatment could pose an environmental risk in the form of ground water contamination (Lambi *et al.*, 2001). Many plant species accumulate high concentrations of proline under stress conditions such as salt, drought, or heavy metals stress. Furthermore, proline serves as a compatible solute to protect macromolecular structures, as a radical scavenger, or as a rapid source of energy for recovery from stress (Hare and Cress, 1997; Nanjo *et al.*, 1999; Chakravarty and Srivastava, 1997). The concentration of free proline increased in sunflower and nontolerant ecotype of *Silene vulgaris* leaves in the presence of heavy metals (Kastori *et al.*, 1992). The function of proline under stress conditions is not fully understood. The mobility of Ni^{2+} is pH dependent (Adersson and Nilsson, 1974; Baker *et al.*, 1979) and other cations and chelating agents (EDTA) can reduce the mobility (Hardiman and Jacoby, 1984). Heavy metals decreased protein content and delay in the synthesis of certain polypeptides (Krupa, 1988 and Jana *et al.*, 1987). Increase in soil solutions concentrations of Ni resulted in increased Ni concentrations in plant tissues (Patrick *et al.*, 1989; Zeller and Feller, 1999; Brown *et al.*, 1989) and the accumulation of heavy metals was more intensive in root than in the shoot of sunflower (Kastori *et al.*, 1992). High heavy metals concentration reduced the iron (Fe) content of the tissues and total uptake of Fe in Sorghum (Kue and Mikkelsen, 1981). EDTA in hydroponics solutions caused significant inhibition of Cd accumulation by the roots and increased Fe concentrations in the shoots (Checkai *et al.*, 1987 and Chaney *et al.*, 1972). Ni^{+2} contamination in cucumber plants hinders the transport of K and Zn and leads to a higher accumulation of Mn in the roots (Varga *et al.*, 1999). The purpose of this study was to investigate the effect of different Ni^{2+} concentrations and EDTA on the growth, some metabolic products, electrophoratic patterns of proteins, nickel and some nutrient elements uptake by *Chorcorus olitorius* plant. Also, tried to eleviate the symptoms of Ni^{2+} toxicity by using different concentrations of EDTA.

Materials and Methods

Plant culture: Seeds of *Chorcorus olitorius* were obtained from the Field Crop Institute, Agricultural Research Center, Egypt. Seeds were germinated in pots containing acid washed sandy-loam soil for two weeks. After germination, the seedlings were transferred to pots containing nutrient solution based on half strength Hoagland solution at pH 6.8 for ten days of preculture. The nutrient solutions were constantly aerated and replaced every 2 days. The seedlings were kept in Precision low temperature incubator Model 818. 25/27 °C night/day temperature, 63-65 % relative humidity under 12 hrs photoperiod and the light intensity was 1100 Lux. This search was carried out in Botany Department, Faculty of Science, Suez Canal University during July and August 2001.

Exposure to Ni^{2+}: Seedlings were transferred to pots with fresh nutrient solution containing different concentrations of Ni (0, 10 and 50 µM). Ni was added as $NicL_2.6H_2O$. The nutrient solution were constantly aerated and replaced every 2 days.

Exposure to Ni^{2+} and EDTA: In a second experiment, to assess the effect of Ni and EDTA (ethylene diamine tetraacetic acid) on toxicity, seedlings were transferred to pots contained nutrient and different combinations of Ni (0, 10 and 50 µM) and EDTA (0,10,50 and 100 µM). EDTA was added as dipotassium salt. Plants were harvested separately after 2 weeks treatment.

Growth parameters: Four plants from each treatment were chosen randomly for the measurement of root and shoot length, fresh and dry weights for root and shoot. The plants were dried to constant weight at 70 °C to estimate the total carbohydrates and mineral elements content (K, Ca, Fe and Ni).

Determination of photosynthetic pigments: The concentration of pigments as µg/g was determined spectrophotometrically in fresh leaves according to Metzner *et al.* (1965).

Determination of total carbohydrates: Total carbohydrate contents were extracted according to Dubois *et al.* (1956) and estimated colorimetrically by the phenol-sulphoric acid method. The results were expressed as mg glucose/100mg dry weight.

Determination of proline: The proline content as µ mols/100 gm plant tissue was extracted with aqueous sulphosalicylic acid and estimated spectrophotometrically in fresh shoot system.

Protein electrophoresis

Extraction of total proteins: Total protein extracts were prepared by extracting appropriate weight from the frozen plant material with 0.125 M tris/borate, pH 8.9. All the obtained extracts were kept at 4°C for 24 hr and then centrifuged at 10,000 rpm for 20 min. The supernatants were used for electrophoresis.

Gel electrophoresis: SDS polyacrylamide gel electrophoresis (PAGE) was carried out with gel slabs according to the method of Laemmli (1970). Protein subunit bands were stained with coomassie blue R-250 by standard techniques. The gel was scanned using Jel-Pro-Analyzer ver. 3.3 (Media Cybermetics, 93-97).

Determination of minerals in plant material: The wet ashing method described by Westerman (1990) was used for the estimation of Ni, Ca, K and Fe concentrations. Analysis was carried out using a Perkin-Elmer 3100 atomic absorption spectrophotometer. The data were recorded in ppm dry weight.

Statistical analysis: All parameters were statistically analyzed by a one way and two ways ANOVA followed by least significant difference (L.S.D.) at 5% level (p= 0.05). Also, Pearson Rank Correlation test was carried out at SPSS version 8. (R= correlation or regression value).

Results

Shoot and Root length, fresh and dry weight: When the seedlings of *Chorcorus olitorius* were treated with Ni^{2+}, shoot and root length, fresh and dry weight decreased with increasing Ni^{2+} concentration in comparison with the control (Table 1). With the addition of EDTA to nutrient solution containing 50 µM Ni^{2+}, all the above growth parameters increased significantly with increasing EDTA concentration. At 10 µM Ni^{2+}, root length correlated increased significant with increasing EDTA concentration (R= 0.736, P= 0.006) and other growth parameters increased but not significantly.

Photosynthetic pigments: The results in Table 2 show that, the total chlorophyll decreased significantly at 10 and 50 µM Ni^{2+} by 38.1 and 54.8 % respectively in comparison with the control. The chlorophyll content correlated significantly and increased with increasing EDTA concentration combined with 10 and 50 µM Ni^{2+} (R= 0.982, 0.192 & P= 0.000, 0.547 respectively).

Total carbohydrates: In *Chorcorus olitorius* plants grown in nutrient solution containing 10 µM Ni^{2+}, the carbohydrate content decreased but not significant and significantly decreased at 50 µM Ni^{2+} by 20.8% compared with the control. An increase in the supply of EDTA combined with 10 and 50 µM Ni^{2+}, the carbohydrate concentration significantly increased gradually (Table 2) (R= 0.934, 0.735& P= 0.000, 0.001 respectively).

Proline content: Table 2 shows that the proline content in *Chorcorus olitorius* significantly increased with increasing Ni concentration compared with the control. In the presence of EDTA in nutrient solution containing 10 and 50 µM Ni^{2+}, the proline content correlated and significantly decreased (R= -0.586, -0.647& P= 0.016, 0.006 respectively).

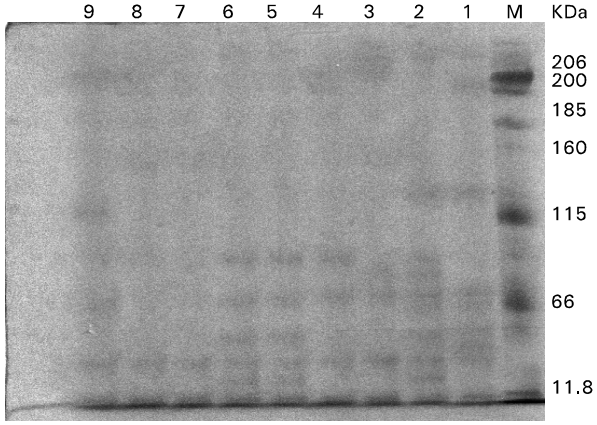

Fig. 1: Electrophotograph of Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of the total proteins of *Corchorus olitorius* plants, 1= untreated plants (control) (track1); 2= plants treated with 10 µM Ni^{2+} (track 2); 3,4 &5= plants treated with 10,50 and 100 µM EDTA combined with 10 µMNi^{2+} (tracks 3,4, & 5 respectively); 6= plants treated with 50 µM Ni^{2+} (track 6); 7,8 &9= plants treated with 10, 50 & 100 µM EDTA combined with 50 µM Ni^{2+} (tracks 7,8 and 9 respectively); M= Marker.

Protein electrophoresis: The gel electrophoretic technique revealed both quantitative (band intensity and relative mobility) and qualitative (disappearance of some bands or appearance of new bands) changes in SDS-PSGE protein patterns of *Chorcorus olitorius* plant in response to Ni^{2+} and nickel combined with different concentrations of EDTA (Table 3 and Fig. 1). In general, the number of protein bands decreased at 10 and 50 µM (4 and 5 bands respectively). These bands increased when the plants treated with EDTA Ni^{2+} in combination. It was noticed that, the band number increased at 50 µM Ni^{2+} combined with 10, 50 and 100 µM EDTA (8,11 and 10 bands respectively). But when the plants were treated with 10 µM Ni^{2+} combined with 50 and 100 µM EDTA, the total bands of protein were 7 , 10 and 50 µM Ni^{2+} completely inhibited the synthesis of a 129.4 KDa polypeptide, but EDTA (50 and 100 µM) combined with 50 µM Ni^{2+} were able to synthesizes this polypeptide again (149.40 KDa). With addition of 50 and 100 µM EDTA to nutrient solution containing 10 µM Ni^{2+}, the new polypeptides were synthesized at 90.46, 72.4 and 16.46 KDa and the concentration of these polypeptides were increased with increasing EDTA concentration. The highest Ni^{2+} concentration (50µM) added to nutrient solution containing EDTA induced the synthesis of new polypeptides at 236.65, 227.68, 216.57, 202.75, 129.40, 83.36 and 66.0 Kda compared with lower Ni concentration (10µM).

Minerals in plant tissues: Table 4 shows that, increasing Ni^{2+} concentration in nutrient solution strongly increased the concentration of Ni^{2+} in both cshoot and root of *Chorcorus olitorius*. The concentration of Ni was greater in roots than shoots and was statistically significant. Ca, K and Fe concentrations were decreased in shoot and root of *Chorcorus olitorius* with increasing Ni concentration in nutrient solution. It

Table 1: Effect of different concentrations of Ni^{2+} (µM) and EDTA (µM) in nutrient solution on vegetative growth parameters of *Chorcorus olitorius* plant. Values are means of three replica

Treatments (µM)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
0.00 (Control)	26.0	12.7	6.91	0.91	5.70	0.36
10 Ni^{2+}	21.3	10.0	5.81	0.72	3.12	0.25
10Ni+ 10EDTA	16.7	11.0	5.50	0.76	3.97	0.31
10Ni+ 50EDTA	17.7	13.7	5.93	0.74	3.83	0.31
10Ni+ 100EDTA	16.7	13.0	5.90	0.75	3.84	0.30
F-ratio	8.039	15.167	7.644	0.076	2.785	51.038
p-value	0.008	0.001	0.037	0.971	0.110	0.000
50 Ni^{2+}	18.3	7.0	3.67	0.55	1.96	0.16
50Ni+ 10EDTA	20.3	9.7	3.62	0.55	2.48	0.24
50Ni+ 50EDTA	20.0	12.0	5.85	0.78	2.64	0.29
50Ni+ 100EDTA	26.0	14.7	5.3	0.74	3.10	0.27
F-ratio	50.167	19.267	62.626	6.881	51.038	30.154
p-value	0.000	0.001	0.000	0.013	0.000	0.000

Table 2: Effect of different concentrations of Ni^{2+} (µM) and EDTA (µM) in nutrient solution on total chlorophyll (mg/g fresh wt.), total carbohydrates (mg glucose/100mg dry wt.) and proline content (µM/100 g fresh wt.) of *Chorcorus olitorius* plant

Treatments (µM)	Total chlorophyll	Total carbohydrates	Proline
0.00 (Control)	4.53	0.48	0.38
10 Ni	2.80	0.41	1.35
10 Ni+ 10 EDTA	2.93	0.38	0.41
10 Ni+ 50 EDTA	3.50	0.56	0.52
10 Ni+ 100 EDTA	4.97	0.65	0.41
F-ratio	1708.5	3.084	6.215
P-value	0.000	0.090	0.017
50 Ni	2.05	0.38	1.42
50 Ni+ 10 EDTA	4.77	0.47	0.48
50 Ni+ 50 EDTA	4.80	0.47	0.45
50 Ni+ 100 EDTA	3.53	0.53	0.38
F-ratio	5787.5	0.995	9.073
P-value	0.000	0.443	0.006

Table 3: Comparitive analysis of relative concentrations, molecular weight and mobility rate (Rm) of proteins in *Chorcorus olitorius* plants treated with different combinations of Ni^{2+} and EDTA.These bands were separated using SDS-PAGA technique.

Band No.	Treatments and band %									Rm	M. Wt. (KDa)
	1	2	3	4	5	6	7	8	9		
1	-	-	-	-	-	-	2.18	-	-	0.05	236.65
2	-	-	-	-	-	-	2.44	4.37	3.52	0.081	227.68
3	8.7	-	-	-	-	-	3.45	-	-	0.12	216.57
4	-	-	-	-	-	-	-	-	-	0.16	206.00
5	-	-	-	-	-	-	-	-	19.3	0.19	202.75
6	-	-	-	-	-	-	-	-	-	0.27	185.00
7	-	4.01	-	-	-	-	-	-	-	0.33	160.00
8	4.24	-	-	-	-	-	-	2.43	3.27	0.44	129.40
9	-	-	-	-	-	-	-	-	-	0.51	115.00
10	-	-	-	1.68	1.81	2.99	-	1.94	-	0.61	90.64
11	-	-	-	-	-	-	2.18	2.39	-	0.64	83.36
12	5.68	-	-	5.16	5.11	1.78	3.47	4.37	1.93	0.7	72.4
13	-	-	-	-	-	-	-	3.03	1.12	0.74	66.00
14	-	-	-	-	-	-	-	-	0.532	0.79	45.36
15	-	-	-	1.64	2.23	-	-	2.12	0.893	0.84	32.06
16	2.65	1.78	2.15	2.31	2.31	3.22	1.69	2.68	1.38	0.88	23.29
17	-	-	-	0.721	1.13	-	-	1.14	-	0.92	16.46
18	4.38	3.65	8.54	4.8	4.47	5.09	4.97	4.58	7.07	0.97	11.16
19	41.3	35.9	40.2	27.4	30.7	43.5	40.8	28.6	28.8	0.99	10.12
Total No. of protein bands	6	4	3	7	7	5	8	11	10		

Treatments: 1= untreated plants (control); 2= 10µM Ni^{2+}; 3,4 &5= 10, 50 &100 µM EDTA+ 10µM Ni^{2+} respectively; 6= 50µM Ni^{2+}; 7,8&9= 10, 50 &100 µM EDTA+ 50µM Ni^{2+} respectively;

was noticed that, the concentrations of Ca and K were greater in shoots than roots. Increasing the concentration of EDTA combined to Ni increased the concentration of Ca, K and Fe in both shoots and root and decreased Ni concentrations in *Chorcorus olitorius* tissues.

Discussion

The present data (Table 1) showed that, shoot and root length, fresh and dry weight decreased at 10 and 50µM Ni^{2+} due to the inhibitory effect of heavy metals on cell division and cell elongation and enzyme activity (Pahlsson, 1989). Nickel prevented any growth of redbeet plant (Kukier and Chaney, 2001). Also, this decline in growth due to an accumulation of heavy metals in plant tissues (Bonnet *et al.*, 2000). With the addition of EDTA to nutrient solution containing Ni^{2+}, the growth parameters increased with increasing EDTA concentration. Chelators may protect plants from damage caused by heavy metals. EDTA reduced Ni^{2+} toxicity (Singh and Pandey, 1981).

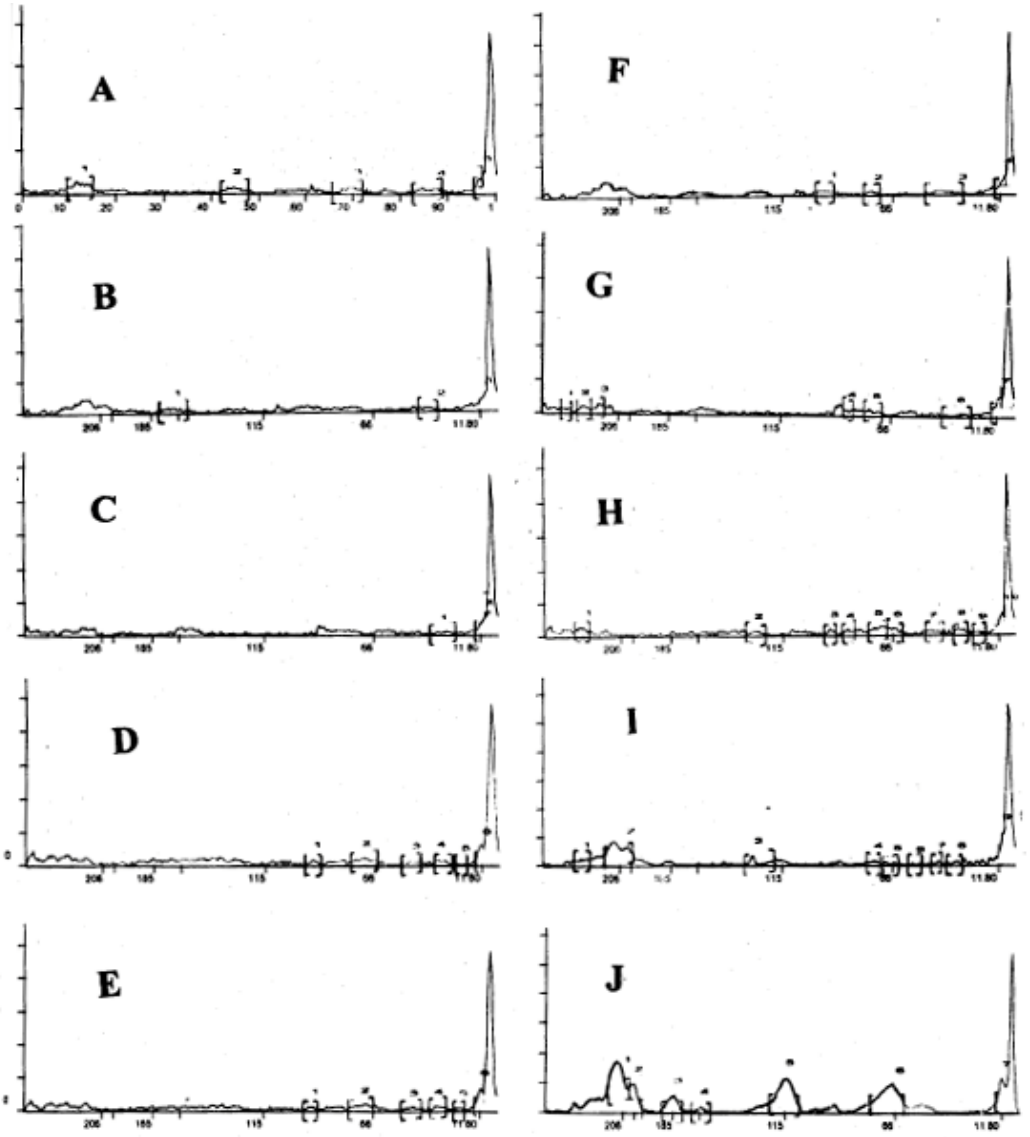

Fig. 2: Scans of the tracks in Fig.1 A = scan of the track M (mol. Wt. Markers); B= scan of the track 1 (control); C= scan of the track 2 (10μM Ni^{2+}); D, E, & F = scan of the tracks 3, 4, & 5 (10, 50 & 100 μM EDTA combined with 10 μM Ni^{2+}); G= scan of the track 6 (50μM Ni^{2+}); H, I, & J= scan of the tracks 7, 8 & 9 (10, 50 & 100 μM EDTA combined with 50 μM Ni^{2+}).

Table 4: Effect of different concentrations of Ni^{2+} (μM) and EDTA (μM) in nutrient solution on Ca, K and Fe content in plant tissues of *Chorcorus olitorius* plant. Values expressed as ppm/dry weight

Treatment (μM)	Ca		K		Fe		Ni	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0.0 (control)	86.1	24.3	1333.5	658.7	68.4	58.2	0.1	0.6
10 Ni	82.8	21.9	741.9	520.3	16.7	15.1	3.3	28.5
10Ni+ 10EDTA	88.7	22.0	955.7	694.0	25.6	22.6	1.5	8.0
10Ni+ 50EDTA	82.9	24.5	1138.3	720.9	25.1	26.0	1.3	1.6
10Ni+ 100EDTA	88.3	36.8	1304.6	932.9	22.5	24.6	1.1	1.9
F-ratio	7.25	21.65	17.64	317.2	3.74	15.62	133.4	213.4
P-value	0.011	0.000	0.000	0.000	0.1000	0.000	0.000	0.00
50 Ni	75.5	21.9	937.9	506.4	15.7	12.6	17.4	71.1
50Ni+ 10EDTA	63.4	27.4	963.4	697.7	24.1	39.3	8.6	64.8
50Ni+ 50EDTA	63.1	23.5	1220.8	945.1	30.1	41.4	2.6	19.6
50Ni+ 100EDTA	98.0	42.3	1458.3	937.9	51.4	70.5	1.6	2.9
F-ratio	104.0	48.76	34.57	952.2	172.39	170.4	411.1	783.2
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00

Excess EDTA to nutrient solution, reduce the root damage (Eskew *et al.*, 1984).

The results of photosynthetic pigments in Table 2 showed that, chlorophyll content decreased with increasing Ni concentration in nutrient solution. The main common symptoms of heavy metals are chlorosis and inhibited photosynthesis and respiration (Bazzaz *et al.*, 1974 and Foy *et al.*, 1978). In *Brassica nepus*, heavy metals significantly decreased root dry weights and totals chlorophyll contents (Larsson *et al.*, 1998). In wheat, the inhibited growth by increased heavy metal concentrations was attributed to indirect effects of heavy metals on contents of essential nutrients or structural damages of chloroplasts (Ouzounidou *et al.*, 1997). Chlorosis occurs due to Fe and Mg deficiency symptoms (Greger and Lindberg, 1987). Mg is necessary for chlorophyll formation and Fe is needed in ferredoxins in the photosynthetic redox system (Khan and Khan 1983). Also, the chlorophyll synthesizing system and chlorophyllase activity were affected by heavy metals (Jana *et al.*, 1987). EDTA increased the chlorophyll content when added to nutrient solution containing Ni^{2+} due to the formation of heavy metal-EDTA complex and this complex is unable to penetrate the plant membrane (Greger and Lindberg, 1987). Also, EDTA reduces the mobility of heavy metal and then decreases its toxicity (Hardiman and Jacoby, 1984). The present investigation shows that, carbohydrate formation is inhibited by Ni (Table 2).This could be an effect of a reduced photosynthesis as the contents of all the investigated sugars decrease (Vallee and Ulmer, 1972). Cd caused reduction in dry weight and carbohydrate concentration in sugar beet plant (Greger and Bertell, 1992). This could be an effect of a reduced photosynthesis as the contents of all the investigated sugars decrease (Kremer and Markham, 1982). EDTA reduces heavy metal toxicity (Singh and Pandey, 1981) which agrees with this experiment that the Ni inhibition on the carbohydrate content in *Chorcorus olitorius* was suppressed by EDTA (Table 2) due to the formation of heavy metal-EDTA complex and this complex is unable to penetrate plant membrane (Greger and Lindberg, 1987).

In *Chorcorus olitorius* treated with Ni^{2+}, the proline content was increased. The concentration of free proline increased in sunflower leaves and *Silene vulgaris* in the presence of heavy metals (Kastori *et al.*, 1992). The possibility of proline involvement in tolerance mechanisms to heavy metals (Raman and Shanti, 1999). Sharp rise in free proline under stress due to protein proteolysis and the activities of protein biosynthetic enzymes (Chang and Lee, 1999). EDTA reduces the proline concentration due to the formation of heavy metal-EDTA complex.

The present study shows that, the number of protein bands decreased when the plant was treated with Ni^{2+}. Heavy metals caused a delay in synthesis of certain polypeptides (Krupa, 1988). The decrease in protein content revealed that soluble proteins may be leaked or diffused out of the plant material, or possibly the catabolic enzymes were induced and destroyed the protein (Jana *et al.*, 1987). These bands increased by addition of EDTA to nutrient solution containing Ni. The plants synthesize new polypeptides to alleviate Ni-stress effect or detoxification of Ni. The synthesis of certain polypeptide of specific molecular weights has been known as metal-binding polypeptides (Jackson *et al.*, 1985; Rauser, 1984; Reese and Wgner, 1987; El-Enany and Abd-Allah, 1995).

The content of Ni increased in both shoot and root of *Chorcorus olitorius* as the Ni supply increased (Table 4) and its concentration in root was more than in shoot. Increase in soil solutions concentrations of Ni resulted in increased Ni concentrations in plant tissues (Patrick *et al.*, 1989; Zeller and Feller, 1999; Brown *et al.*, 1989) and the accumulation of heavy metals was more intensive in root than in shoot of sunflower (Kastori *et al.*, 1992). Concentration of Ca, K and Fe was decreased in shoot and root of *Chorcorus olitorius* with increasing Ni concentration in nutrient solution). Heavy metals inhibited the absorption and accumulation of K, Ca and Fe in plant tissues (Trvedi and Erdei, 1991; Woolhouse, 1983; Boardman and McGuire, 1990 and Simon *et al.*, 1994). Zhang *et al.* (1991) and Nicolaus *et al.* (1999) showed that mobilization of Fe by phytosiderophores slightly inhibited by Zn and strongly inhibited by Cu. Nicotinamine (NA) occurs in all plants and chelates metal cations include Fe^{+2} and have an important role in scavenging Fe and protecting cell from oxidative damage. This reduction of Fe in shoot resulting from coating occurred on root surface and intensified with increasing heavy metal concentrations in the substrate or could be attributed to the formation of high valent heavy metal oxides on the root surfaces which may certain Fe and reduce its absorption by sorghum. The presence of EDTA in nutrient solution diminished the Ni uptake probably by chelating Ni. Hardiman and Jacoby (1984) obtained similar results on bush beans. The uptake of heavy metals is reduced in the presence of EDTA, suggesting that heavy metal-EDTA complex formation is unable to penetrate the membrane (Greger and Lindberg, 1987). In nutrient solution containing FeEDTA, other metals may form their respective EDTA chelates by displacing some Fe from FeEDTA. These reactions may lower the concentration of the free cations of many micronutrient metals while increasing the concentration of Fe (Eskew *et al.*, 1984).

From the results of this experiment, it could be concluded that, *Chorcorus olitorius* have the ability to uptake and accumulate Ni from the nutrient solution in its tissues to toxic level and DETA ameliorate this toxic effect of Ni.

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