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ALLEVIATION OF NICKEL TOXICITY BY NITROGEN ADDED TO MAIZE (*Zea mays* L.) PLANTS

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ABSTRACT

It is known that phytotoxicity of nickel (Ni) varies within plant species and varieties according to its concentration in the root medium. Moreover, several nutrients can modify the plant response to Ni excess. Because nitrogen can be absorbed by plants as NO_3^- or NH_4^+ and N metabolism are closely related to Ni, a hydroponic experiment was conducted to study the effect of Ni toxicity on the growth of maize (*Zea mays* L.). Nutrients status of the different plant parts and leaf chlorophyll concentrations were also taken into consideration. A single cross hybride Giza 10 grown for 40 days with either forms of N supply. The obtained results indicated that a significant differences in the sensitivity of maize plants to Ni excess.Where,the plant tolerance to Ni toxicity was lowest when grown with NO_3^- supply alone.In this concern a high increase of Ni in the presence of NO_3^- as the only source of nitrogen caused a significant decreases of both maize dry weight and nutrient concentration.But ,when plants supplied with Ni in the presence of mixture of NO_3^- and NH_4^+ , the absorption of Ni by plant was less than those supplied with NO_3^- alone.This decrease reached to about three times.This clearly indicates that, there were great differences in Ni concentrations between treatments.The addition of nitrogen as NO_3^- alone , Ni supply led to severe growth inhibition. Just contrary, nitrogen supply as NO_3^- and NH_4^+ together not only reduced Ni toxicity, but Ni might enhance and stimulate the growth of maize plants.Therefore,confirm this Important role of the N form in the Ni behaviour in plants.

INTRODUCTION

Nickel is common element in some plant tissues. Its concentration in most plants ranges from 0.01 to 5 mg kg^{-1} dry weight (Mishra and Kar, 1974; Sherif and Amin, 2001 and El-Sayed, 1999a). Nickel is an essential element for some higher plants (Brown *et al.*, 1987; Eskew *et al.*, 1984; Welch, 1981 and El-Sayed and Salem, 2002). In other cases, a very low concentration of Ni has a beneficial effect on growth of species such as wheat, cotton, tomato, paprika and potatoes (Mishra and Kar, 1974; Al-Oud, 2002 and El-Sayed, 1999c). However, increased industrial and mining activities, mineral and organic fertilizers, pesticides and the disposal of urban and industrial wastes have led to the input of large amounts of Ni in the environment (Alloway, 1990 and Rizk and Abdel-Sabour, 2001).The easily absorption of Ni by plants from soils and nutrient solutions, may lead to high accumulation of Ni with a harmful effect on their growth. Thus,the concentrations of Ni in the range of 1-

2 mg kg^{-1} have been found to be toxic for a wide variety of plants, above all in nutrient solution experiments (Mishra and Kar, 1974 and Abou-El-Naga *et al.*, 1999). Alterations of fundamental physiological and biochemical processes have been attributed to an excess of Ni, as, e.g., leaf photosynthetic and transpiration activities (Jones and Hutchinson, 1988; Morgutti *et al.*, 1984 and Eissa and El-Kassas, 1999), leaf chlorophyll content (Pandolfini *et al.*, 1992; Piccini and Malavolta, 1992 and El-Dawwey, 1999) and impairing membrane permeability associated with enhanced extracellular peroxidase activity (Pandolfini *et al.*, 1992 and El-Desouky, 1999). Nickel toxicity can be lessened supplying extra levels of Ca, Mg, Fe, Cu and Zn (Heale and Ormrod, 1982; Heikal *et al.*, 1989; Lizukka, 1975; Robertson, 1985 and El-Gendi *et al.*, 1999), although it was also observed that Mg intensified Ni toxicity (Johnston and Proctor, 1981 and El-Kassas, 1999). So far the mechanisms responsible for decreasing or aggravating of Ni toxicity are not known (Proctor and Baker, 1994; El-Shebiny, 1998 and El-Sayed, 1998).

Nickel addition was found to stimulate the growth of soybean, rice and tobacco tissue cultures (Polacco, 1977 and El-Sayed, 1999b) and *Lemna paucicostata* (Gordon *et al.*, 1978 and Ghoneim *et al.*, 1997) in media where N was supplied as urea as the only source of N, but in other cases, Ni addition has a slight positive or no effect with other N sources (Eskew *et al.*, 1984; Gordon *et al.*,1978; Polacco, 1977 and El-Zoghbi and El-Kady, 1999). Several families of plants, such as Compositae, Leguminosae, Cucurbitaceae, Brassicaceae accumulate large amounts of soluble N in the form of ureides or guanidines in their tissues and these compounds are often considerably increased when NH_4^+ is present in the root medium. Ureides, therefore, are considered to be important for the N balance of these plant species and Ni might enhance growth because of its role in urease activity (Welch, 1981 and El-Sayed, 1999d). The aim of this study was therefore to examine the effect of Ni toxicity on maize plants under different forms of N supply.

MATERIALS AND METHODS

Grains of maize (*Zea mays* L.) a single cross hybride Giza 10 were germinated for 15 days in a greenhouse of Faculty of Agriculture , El – Shatby,Alexandria University, using perlite media moistened with deionized water. Afterwards, the seedlings were transferred to continuously aerated nutrient solution (Long Ashton) in 5-L plastic pots (each representing an experimental unit with two plants) and placed in the greenhouse.

Two nutrient solutions were prepared differing in NO_3^- : NH_4^+ ratios (100 : 0 and 60 : 40), but the same total N concentration (10 m *M*). The composition of the 100 : 0 solution was (m *M*) : 3.25 $Ca(NO_3)_2$; 3.5 K NO_3 ; 1.0 KH_2PO_4 ; 0.75 Mg SO_4 and 0.1 NaCL. The 60 : 40 solution had the following composition (m *M*) : 3.0 Ca $(NO_3)_2$; 4.0 NH_4HCO_3 ; 1.0 KH_2PO_4 ; 1.0 K_2SO_4 ; 0.75 $MgSO_4$; 0.25 $CaCl_2$; 1.5 KCL and 0.1 NaCL. The

micronutrients were supplied as follows (μg element ml^{-1}) : 2.0 FeEDDHA ; 0.54 H_3BO_3 ; 0.55 $Mn SO_4 . H_2O$; 0.064 $CuSO_4 . 5 H_2O$; 0.065 $Zn SO_4 . 7 H_2 O$ and 0.048 $(NH_4)_6 Mo_7O_{24} . H_2O$.While,nickel treatment,was kept at 2.0 μg Ni ml^{-1} as NiCl2. 6 H2O to each treatment of nitrogen . pH value of the nutrient solution was adjusted up to 6.0 and checked every two days when necessary. A complete change of the nutrient solutions took place once a week and deionized water was added regularly to replace water losses due to transpiration. The plants were grown with the Ni treatment for 15 days, afterwards Ni was removed from the nutrient solution when Ni toxicity was achieved.

There were three replicates for each treatment (NO_3^- : NH_4^+ ratio) and Ni supply in a randomized block design. At the end of the experiment (40 days after transplanting) .The whole plants of each treatment (100 : 0 ; 100 : 0 + Ni ; 60 : 40 ; 60 : 40 + Ni) were harvested, divided into roots, stem and leaves, dried at 70°C for 24 h to determine dry weights and plant nutrient concentrations. The concentrations of K, Ca, Mg, Fe, Mn, Cu, Zn and Ni were ânalyzed by atomic absorption spectrophotometry, N and B by means of a Technicon Autoanalyzer. For chlorophyll determination, the leaves were homogenized with 80% acetone and filtered twice in *vacuo*. The concentrations of chloropyll *a* and *b* were determined by reading the absorbance of the extract at 645 and 663 nm (Maclachlan and Zalik, 1963; Evenhuis and DeWaard, 1978; Cottenie, 1980; FAO, 1980 and Page *et al.*, 1982).

Mean separation was done by analysis of variance followed by a test for the least significant difference (LSD) with a probability of 0.05 (Steel and Torrie, 1982; SAS, 1985 and Bender *et al.*, 1989).

RESULTS AND DISCUSSION

Ni uptake and its accumulation in plant organs :

The concentrations of Ni in the different parts of the plant and its uptake by the maize plants grown under various forms of N supply are given in Table (1). It is seen that the absorbed amount of Ni by the plants grown in a mixture of NO_3^- and NH_4^+ was less than those supplied with NO_3^- alone (100 : 0 + Ni) by about three times. This results are completely differed in case of Ni accumulation. Wheres , Ni concentration in the leaves, stem and root of the plants supplied with a mixture of NO_3^- and NH_4^+ (60 : 40 + Ni) was lower than those in the corresponding 100 : 0 + Ni treatment. This percentage decrease for leaves,stem and root reached to 60,75 and 13% respectively.

According to literature, Ni uptake and its distribution within different tissues of plant determines the plant tolerance to Ni toxicity (Yang *et al.*, 1996 and Abou-El-Naga *et al.*, 1999). In this case, irrespective of the NO_3^- : NH_4^+ assayed, the highest concentrations of Ni were found in the roots. However,

proportionally to the Ni absorbed, the roots of the plants with the (60 : 40 + Ni) treatments retained less Ni than those with the (100 : 0 + Ni) treatment. Despite severely reduced total Ni uptake, root Ni concentrations of $NH_4 NO_3$ plants were almost equal to those of NO_3^- fed plants. Therefore, the Ni translocation to the shoot was more limited when NH_4^+ was present in the nutrient medium than when the maize plants were grown with pure NO_3^- as N source. The retention of Ni in the root is particularly desirable in crops where the root is not used for human consumption. The Ni concentrations in maize tissues decreased in the following order: root > leaves > stem, independently of the NO_3^- : NH_4^+ ratio assayed. A similar ranking order was obtained with other crops (Heale and Ormrod, 1982; Yang *et al.*, 1996; Ghoneim *et al.*, 1997 and Al-Oud, 2002).

Table (1): Nickel uptake (mg plant $^{-1}$) and Ni concentration (mg kg^{-1} DW) in the different organs of maize plants supplied with different NO_3^- : NH_4^+ ratios with or without extra Ni in solution during the first 15 days; nd = not detectable.

Organs	Treatments				
	100 : 0	100 : 0 + Ni	60 : 40	60 : 40 + Ni	$LSD_{P=0.05}$
Ni uptake	nd	0.61	nd	0.22	0.11
Leaves	nd	98	nd	40	14
Stem	nd	57	nd	15	7
Root	nd	201	nd	176	25

Plant Growth :

With regard to the effect of nitrogen form on Ni toxicity to plant ,the results indicated that Ni toxicity symptoms appeared after 15 days of Ni supply, but the symptoms were more effective when the plants supplied with NO_3^- alone (100 : 0 + Ni) where,in case of this treatment the growth of root and shoot was stunted, leaves were abnormally small and the younger leaves suffered from intervinal chlorosis and necrosis, while with time the older leaves turned to dark green.But in case of the (60 : 40 + Ni) treatment, chlorosis and necrosis of leaves were weak, even of fully expanded new leaves.

As regard to Table (2), the dry weight of the different plant organs supplied with NO_3^- alone was negatively affected by the presence of Ni in the growth medium,where a reduction of the dry weight was markedly observed . This reductions were between 52 and 62%, in comparison to those of the (100 : 0) treatment. By contrast, when the plants supplied with a combination of NO_3^- + NH_4^+, exposed plants to Ni produced higher dry weights of leaves (40%), stem (70%), root (192%) and total (73%) compared to corresponding control treatment (60 : 40). This must be attributed to the

presence of Ni, since when the maize was grown at the same NO_3^- : NH_4^+ ratio, but without Ni, its growth did not change significantly. Brown *et al.* (1987) reported that Ni was necessary for optimal development of barley, oat and wheat supplied with NH_4^+ as N source. Several authors (Yang *et al.*, 1996; Eissa and El-Kassas, 1999 and El-Sayed and Salem, 2002) reported that, when plants take up and transport small amounts of Ni from roots to shoots, they grow better than those that taken up and transport high amounts of Ni. Based on this concept, it is clear that , the (60 : 40 + Ni) gave yield more than other treatments.This was true for the different plant organs. As mentioned before the accumulation and absorption of Ni with this treatment with less than other treatments Table (1).

Table (2): Dry weights of maize plants (g plant $^{-1}$) supplied with different NO_3^- : NH_4^+ ratios with or without extra Ni in solution during the first 15 days.

Organs	Treatments				
	100 : 0	100 : 0 + Ni	60 : 40	60 : 40 + Ni	$LSD_{P=0.05}$
Leaves	1.99	0.72	2.06	2.87	0.52
Stem	1.47	0.68	1.22	2.07	0.41
Root	1.03	0.50	0.63	1.82	0.29
Total	4.47	1.88	3.89	6.74	1.17

Nutrients status of the different plant parts :

The effect of Ni on the macro and micronutrient concentrations in the maize plants supplied with NO_3^- alone or in combination with NH_4^+ is given in Tables (3 and 4), respectively. With (100 : 0 + Ni) treatment , a remarked decrease of the concentrations of N, P, Ca, Mg, Fe, Mn and Cu in the leaves was observed, this decrease relative to the control treatment . (100:0) for these nutrients reached to 20, 23,20,25,52,41and 52% respectively. While for root the decrease percent for P,K,Ca,Mn and Cu was 35,25,18,32 and 44% respectively, whereas the reverse was true with Fe (24%) and Zn (34%). The stem of these plants contained lower concentrations of almost all nutrients, except K and Zn, which increased, and Mg and B, which did not vary.

Adding Ni to the NH_4^+ and NO_3^- mixture, the plants did not cause variation in the concentrations of the nutrients in the leaf, except N, which decreased. But in the root an increase of Ca, Mg and Fe by 37,64 and 34% respectively, was attained, while the decrease in the sconcentration of N was 32% when compared with the control treatment (60 : 40). With (60 : 40 + Ni) treatment the stem plants contained lower concentration of N, P, Cu and Zn, whereas the others did not change.

Table (3): Macronutrient concentration (g kg^{-1} DW) in the maize plants supplied with different NO_3^- : NH_4^+ ratios with or without extra Ni in solution during the first 15 days.

Macronutrient	Treatments					
concentration(g kg^{-1} DW)	Organ	100 : 0	100 : 0+Ni	60 : 40	60 : 40+Ni	$LSD_{P=0.05}$
N	Leaves	63.7	50.8	69.4	29.2	3.9
	Stem	32.8	26.1	36.1	27.4	2.7
	Root	35.6	34.2	55.2	37.8	5.7
P	Leaves	8.0	6.2	9.7	10.2	1.4
	Stem	5.3	4.7	6.9	5.4	0.6
	Root	16.8	10.9	17.0	15.9	2.1
K	Leaves	46.3	49.7	47.5	46.5	5.4
	Stem	52.7	68.0	65.1	62.3	6.4
	Root	65.1	48.9	49.1	42.5	7.0
Ca	Leaves	19.8	15.9	16.0	15.3	1.5
	Stem	19.5	16.6	20.6	20.5	1.9
	Root	9.4	7.7	6.0	8.2	1.4
Mg	Leaves	3.7	2.8	3.0	3.6	0.8
	Stem	3.7	4.2	6.5	5.9	1.0
	Root	1.6	1.8	1.5	2.4	0.4

Table (4): Micronutrient concentration (mg kg^{-1} DW) in the maize plants supplied with different NO_3^- : NH_4^+ ratios with or without extra Ni in solution during the first 15 days.

Micronutrient	Treatments					
Concentration (mg kg^{-1} DW)	Organ	100 : 0	100 : 0+Ni	60 : 40	60 : 40+Ni	$LSD_{P=0.05}$
Fe	Leaves	135	65	123	108	21
	Stem	61	33	54	48	12
	Root	1323	1638	699	934	196
Mn	Leaves	306	181	119	115	41
	Stem	124	105	79	81	18
	Root	339	230	144	199	67
B	Leaves	48	48	62	57	14
	Stem	8	11	13	11	5
	Root	10	10	8	9	4
Cu	Leaves	22	11	22	20	10
	Stem	13	7	13	9	4
	Root	258	144	252	292	44
Zn	Leaves	29	25	21	15	9
	Stem	28	36	27	11	8
	Root	62	83	41	30	14

It is known that Ni excess can inhibit the uptake of essential elements, inducing nutrient deficiences. In particular, Ni competes with other divalent cations such as Ca, Mg, Fe, Mn, Cu and Zn (Clarkson and Lüttge, 1989 and Marschner, 1995). According to the reference list of critical maize concentrations (Reuter and Robinson, 1986 and El-Sayed 1999a,b,c&d), most values are in the adequate range, except Fe which is rather low in the leaves of (100 : 0 + Ni) plants. Leaves showing Ni-induced chlorosis are found to contain suboptimal levels of Fe, suggesting an effect of Ni in the translocation of Fe from roots to shoots (Aller *et al.*, 1990; Foy *et al.*, 1978 and El-Sayed, 1998). The intensity of toxicity symptoms has been related closely to the Ni : Fe ratio in the plant tissue rather than to the concentration of either element. If the Ni : Fe ratio is higher than 1, there appears strong Ni damage (Khalid and Tinsley, 1980; El-Desouky, 1999 and El-Shebiny, 1998). In some plants, an excess of Ni induced chlorotic symptoms similar to chlorosis caused by Fe deficiency, but in other crops Ni toxicity did not cause chlorosis by decreasing the level of Fe in the leaves (Mishra and Kar, 1974; El- Kassas, 1999 and El-Gendi *et al.*, 1999). In this case, the addition of Ni decreased the translocation of Fe in the plants treated by (100 : 0 + Ni) and as a result, the Fe concentration was reduced (50%) in the aerial parts of these plants.

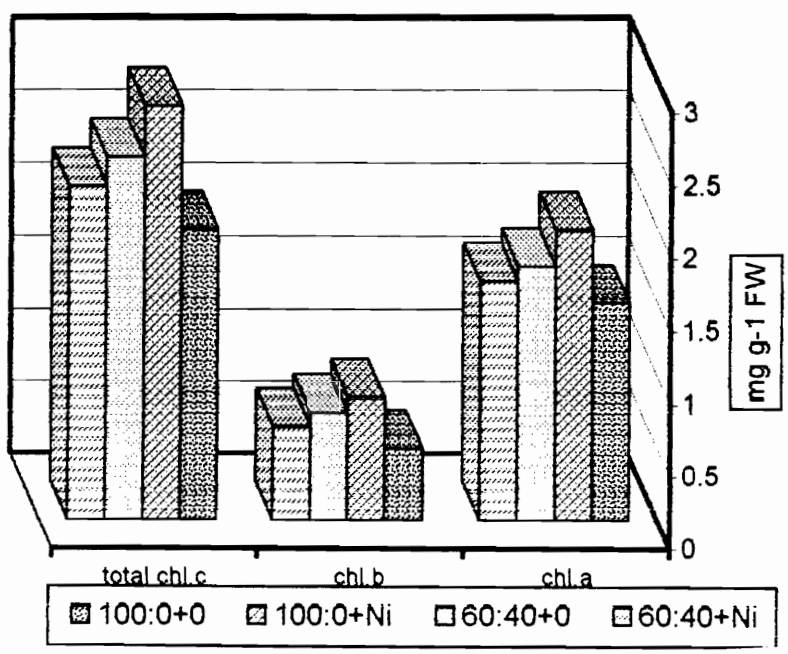

Figure (1) : Leaf chlorophyll concentrations in maize plants supplied with different NO_3^- : NH_4^+ ratios with or without extra Ni in solution during the first 15 days. Vertical lines indicate LSD P = 0.05.

Leaf chlorophyll concentrations :

The concentrations of chlorophyll in the leaves of plants supplied with different forms of N with or without extra Ni in solution during the first 15 days are presented in Figure (1). The leaves with the (100 : 0 + Ni) treatment had higher concentrations of chlorophyll *a* (52%), *b* (17%) and total (41%) than those with the 100 : 0 treatment. By contrast, the (60 : 40 + Ni) treatment did not lead to variations in the leaf chlorophyll concentration if compared with that of 60 : 40 treatment. According to Pandolfini *et al.* (1992); Piccini and Malavolta (1992) and El-Dawwey (1999), the application of excessive levels of Ni caused a marked depression in the chlorophyll concentration, whereas, with intermediate levels of Ni, it did not vary. These authors suggested that excessive Ni addition probably depresses the chlorophyll concentration of the leaves by inhibiting the incorporation of Mg in the protoporphyrin molecule, which is the precursor of chlorophyll. The first visual symptoms detected in plants supplied with high Ni levels was chlorosis both in the younger and older leaves, followed by dark pigmentation, probably due to an accumulation of phenolic compounds (Rauser, 1978 and El-Zoghbi and El-Kady, 1999).

Finally, in this study significant differences were found with regard to the susceptibility of maize plants to Ni toxicity depending on the N source supplied. Tolerance was lower when NO_3^- was the only N source (100 : 0 + Ni). These plants showed a strong decreased of root dry weight, which may be attributed to factors, such as membrane damage, stiffening of the expansion zone tissue and destruction of the integrity of the root meristems (Barcelo and Poschenrieder, 1990; Robertson, 1985 and Sherif and Amin, 2001). These early events led to a large range of secondary effects, such as alterations of mineral nutrition, photosynthsis, water balance, photosynthate translocation, which further enhanced the metal-induced plant growth reduction (Barcelo and Poschenrieder, 1990 and Rizk and Abdel-Sabour, 2001). In this case, compared with other crops (100 : 0 + Ni) plants showed similar Ni damage, as e.g., strong reduction of dry weights, high capacity of Ni uptake and translocation, lower concentrations of nutrients in the plant parts.

CONCLUSIONS

By contrast, the simultaneous presence of Ni and NO_3^- plus NH_4^+ improved growth as these plants surpassed the growth of both control treatments (100 : 0 and 60 : 40). Although the roots of maize plants accumulated high levels of Ni, its concentration increased proportionally to the Ni absorbed when NH_4^+ was present in the nutrient medium, but the presence of NH_4^+ decreased the Ni concentration in the upper parts of the plants. The alleviation of Ni toxicity by the presence of NH_4^+ in maize plants may be related to their special and partially unknown metabolism found in the

crops when they are grown with high at NH_4^+ levels. This viewpoint is mainly of interest to the maize plants as it transports the greater part of soluble N in the form ureides, which are greatly enhanced by NH_4^+ nutrition. Further research is required in order to study the protection of Ni excess afforded by NH_4^+ supply.

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تخفيف سمية النيكل بإضافة النيتروجين لنباتات الذرة الشامية

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تختلف سمية النيكل للنبات ويتوقف ذلك علي صنف ونوع النبات وعلي تركيزه في منطقة نمو الجذور حيث وجد أن بعض المحاليل المغذية يمكن أن تخفف من سمية النيكل بالنبات من خلال صورة النيتروجين المضافة(أمونيوم أو نترات) وكلاهما تمثل الصورة القابلة للإفادة للنبات ونظراً لتأثير النيتروجين علي التمثيل الغذائي لعنصر النيكل أجريت هذه الدراسة لمعرفة تأثير سمية النيكل علي النمو وتركيزة في الأجزاء المختلفة من نبات الذرة الشامية هجين فردي صنف جيزة ١٠ وكذلك تركيز الكلوروفيل في وجود صور ونسب مختلفة من النيتروجين المضاف في المحلول المغذى .

وأظهرت الدراسة أن النباتات النامية لمدة ٤٠ يوماً تم إمدادها بالنيتروجين علي إحدى صورتين) الأمونيوم أو النترات) أو بنسب مختلفة بينهما . إن النتائج قد أظهرت فروقاً معنوية في حساسية نبات الذرة الشامية لزيادة النيكل حيث كان انخفاض مقاومة الذرة الشامية للنيكل عند نموه في النترات فقط ملحوظاً وكان ذلك واضحاً في انخفاض الوزن الجاف في وجود تركيز مرتفع من النيكل في وجود النترات كمصدر وحيد للنيتروجين.

ولكن عند إضافة خليط من كل من النترات و الأمونيوم في وجود النيكل حدثت اختلافات كبيرة بين المعاملات.

حدث تثبيط بسيط للنمو في وجود محلول مغذي من NO_3^- 100 % N-

مضافاً إليه نيكل . وبناء علي ما تقدم فقد أوضحت النتائج أن صورة النيتروجين المضافة لها دور فعال في تخفيف الأثر الضار و السام لسلوك النيكل في نباتات الذرة الشامية.