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Growth and Metal Ionic Composition of *Zea mays* as Affected by Nickel Supplementation in the Nutrient Solution

MUHAMMAD SABIR†, ABDUL GHAFOOR†, SAIFULLAH†, M. ZIA-UR-REHMAN†, HAMAAD RAZA AHMAD† AND TARIQ AZIZ[1]‡
Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan
‡Sub-Campus, University of Agriculture, Faisalabad at Depalpur, District Okara, Pakistan
[1]Corresponding author's e-mail: tasuaf@hotmail.com

ABSTRACT

Essentiality of nickel (Ni) as micronutrient for plants is not very old, yet handsome number of reports are available indicating its beneficial effects on growth of higher plants. We studied the effect of different levels of Ni on growth and micronutrient metal (copper, manganese, zinc & nickel) uptake by maize plants. Maize (cv. Neelam) was grown in pre-washed river-bed sand. Johnson nutrient solution was applied to supply nutrition with four levels of nickel at 0 (control), 17, 51 and 68 µM. Plants were grown for 50 days after sowing. Nickel addition significantly improved dry matter of maize at all levels compared to control, however maximum increase in shoot and root was observed at 68 µM. Addition of Ni in nutrient solution also increased Ni concentration in roots and shoots. Addition of Ni decreased Mn and Cu concentration in shoots, however it increased Zn concentration in shoots. Maximum decrease in manganese concentrations in shoots and roots (20% & 45%, respectively) was recorded with nickel at 68 µM and maximum decrease (17%) in copper concentration was observed with 68 µM in shoots, however copper concentration in roots increased with nickel at all levels.

Key Words: Nickel; Copper; Zinc; Manganese; Maize; Biomass; Metal phytoavailability

INTRODUCTION

Essentiality of nickel (Ni) for higher plants was first reported by Dixon *et al*. (1975) as an essential component of urease enzyme in plants followed by the findings of Polacco (1977), who reported that soybean cells had an absolute requirement for Ni, when grown with urea as a sole N source. Afterwards, several researchers reported essentiality of Ni for higher plants grown with urea as an N source (Shimada *et al*., 1980; Eskew *et al.,* 1984; Walker *et al.,* 1985). Nickel is involved in activation of urease enzyme, hence most of Ni essentiality studies were focused on legumes due to higher urease activity in seeds of legumes and transportation of absorbed N as ureids compounds within plant body, which requires urease (Holland *et al.,* 1987; Welch, 1981; Bollard, 1983; Walker *et al.,* 1985). Nickel is also required for hydrogenase enzyme, which is responsible for recycling of hydrogen produced during side reaction of nitrogenase in N_2-fixation process in legume plants (Albrecht *et al.,* 1979). Nickel deficiency decreases urease enzyme activity (Eskew *et al.,* 1984; Gad *et al.,* 2007) and some other enzymes responsible for nitrate reduction leading to decreased synthesis of protein and level of total N in plants (Brown *et al.,* 1990). Decrease in urease and other enzymes activities results in accumulation of nitrate, urea and amino acids (Eskew *et al.,* 1984; Shimada & Watanab, 2004), which results in chlorosis and necrosis at meristem in plants such as soybeans (Eskew *et al.,* 1984), parsley and cucumber (Shimada & Watanab, 2004). Nickel deficiency causes decrease in growth, early senescence and inhibition of grain development, grain viability and reduction in iron concentration in plants tissues. Improvement in yield of tomato biomass and fruit, fruit quality (Rahmatullah *et al.,* 2001; Karagiannidis *et al.,* 2002; Brake *et al.,* 2004), root and shoot growth, leaf area, leaf biomass, chlorophyll contents, carbohydrate and sugar in leaves of *Albizia lebbek* plants (Tripathi & Tripathi, 1999) were also reported due to Ni application. So it has been realized that inclusion of Ni in plant nutrition for sustaining the yield of commercial crops (Eskew *et al.,* 1984; Brown *et al.,* 1987) must be considered.

Though Ni is essential for higher plants, yet its higher concentration may be toxic to plants (Farago & Cole, 1988) and could modify different physiological/biochemical processes (Morgutti *et al.,* 1984; Jones & Hutchinson, 1988; Pandolfini *et al.,* 1992; Seregin & Kozhevnikova, 2006). Nickel toxicity affects membrane structure decreasing permeability, which is associated with higher extracellular peroxidase activity (Pandolfini *et al.,* 1992). Nickel, when present at higher concentration in rooting medium could

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decrease Cu, Zn and Mn in some plant species. Nickel deficiency is not very much common in soils rather its toxicity is a matter of concern in soils of peri-urban areas. A major source of Ni contamination of agricultural soils is city effluents, which is being used for irrigation in peri-urban agriculture that contains considerable amount of Ni (Hussain *et al.,* 2006).

Maize is a multiple purpose crop being used as food, feed and fodder in Pakistan. As it is dominantly grown in peri-urban areas receiving raw city effluent, it could absorb Ni and contaminate the human food chain. We hypothesized that low levels of Ni may improve maize yields; however, it may decrease concentrations of other essential metal micronutrients (Yang *et al.,* 1996; Palacios *et al.*, 1998; Rahman *et al.,* 2005) thus causing malnutrition to the animals. The present study was therefore, conducted to evaluate the effect of different levels of Ni on growth of maize and secondly to study the influence of added Ni on Cu, Mn and Zn uptake by plants.

MATERIALS AND METHODS

The experiment was conducted in the green house having mean day temperature 30±5°C and night temperature 22 ± 3°C temperatures during the study period. During day time relative humidity decreased to 35%, while it increased to 85% during night. Light intensity varied during study period, but overall remained between 300 and 1400 µmol photon m^{-2} s^{-1}.

River-bed sand was washed with dilute HCl followed by tap water and distilled water before filling into PVC pots (2 kg per pot). Seeds of maize (*Zea mays* L. cv. Neelam) were treated with benlate to protect the seedlings from diseases and soaked in moist cloth. The germinating maize seeds were sown in sand filled pots without having leaching provision. Nutrition to maize seedlings was supplied through Johnson's nutrient solution (Johnson *et al.,* 1957) during the crop growth period of 50 days. The nutrient solution contained 8 mM nitrogen (N) both as (NH_4^+ & NO_3^-), 0.25 mM phosphorus (P as $H_2PO_4^-$), 2 mM calcium (Ca) as $Ca(NO_3)_2.4H_2O$, 1 mM magnesium (Mg) as $MgSO_4$, 0.5 mM sulfur (S) as as $MgSO_4$, 50 µM chloride (Cl) as KCl, 25 µM boron (B) as H_3BO_3, 2 µM manganese (Mn) as $MnSO_4.H_2O$, 2 µM zinc (Zn) as $ZnSO_4.7H_2O$, 0.5 µM each of copper (Cu) as $CuSO_4.5H_2O$ and molybdenum (Mo) as H_2MoO_4 and 50 µM Fe as Fe-EDTA.

There were four levels of Ni (0, 17, 51 & 68 µM) using $Ni(NO_3)_2.6H_2O$ solution. The pots were arranged according to completely randomized design with three replicates of each Ni level. The plants were harvested after 50 days of growth, washed with 1% HCl, followed by tap water and then rinsed in distilled water to remove aerially deposited material on plant shoots. After blotting with tissue paper, plants were separated into shoots and roots for fresh weight record. Dry weights of maize shoots and roots were recorded after drying at 65 ± 5°C to a constant weight in a forced air oven. Plant samples were then ground to powder in a mechanical grinder (MF 10 IKA, Werke, Germany). Shoot and root ground samples (0.5 g) were digested in 3:1 mixture of nitric acid and perchloric acid at 150°C (Miller, 1998). Concentration of Ni, Cu, Mn and Zn in shoot and root digests was determined using Atomic Absorption Spectrometer (Solaar S4 AA Spectro, Thermo Electron, Cambridge, UK). The data recorded were subjected to statistical analysis using window based program Statistix (Version, 8.1) and treatment effectiveness was evaluated using least significant difference (LSD) test.

RESULTS

Biomass production: There was a significant ($P < 0.05$) effect of Ni application on shoot and root fresh, as well as dry matter of maize (Table I). Nickel application increased shoot dry matter (SDM) and root dry matter (RDM) at all application levels and maximum shoot dry matter (1.07 g $plant^{-1}$) and root dry matter (1.04 g pot^{-1}) was recorded with application of Ni at 68 µM compared with that of control (0 Ni application level). Nickel application at 68 µM increased shoot dry matter and root dry matter of maize by 22 and 20%, respectively compared with that of control.

Ni concentration and uptake by maize shoots and roots: There was a significant ($P < 0.001$) effect of Ni application on its concentration and uptake by maize shoots and roots (Table II). The Ni concentration in maize shoots was increased with increasing Ni level. Maximum increase (2.6 fold) in Ni concentration in maize shoot compared with that of control was recorded at 68 µM Ni level. Nickel application significantly ($P<0.001$) increased Ni concentration in maize roots (Table II). Nickel application at 68 µM increased Ni in roots by 4.3 fold followed by 51 µM Ni application level that increased Ni concentration in root by 3.4-fold compared with that of control. Application of Ni significantly ($P<0.001$) affected Ni uptake by maize shoots and roots (Table I). Increasing Ni application levels increased Ni uptake by maize shoots and roots. Maximum Ni uptake in shoots and roots was recorded with application of Ni at 68 µM compared with that of control. Maximum increase in Ni uptake by maize shoots (4.5 fold) and roots (5.1 fold) was recorded with 68 µM compared with that of control.

Effect of Ni on ionic composition: Application of Ni significantly ($P < 0.05$) affected concentration and uptake of Mn in roots while non-significantly in shoots (Table III). Increasing application rate of Ni decreased Mn concentration and uptake in shoots and roots and maximum decrease was recorded with 68 µM Ni for shoots (45%) and roots (20%). Maize shoots uptake more Mn compared with roots. The Mn concentration in shoots decreased with the application of Ni at all levels compared with that of control. The Mn concentration in maize shoots was significantly, but negatively correlated ($r = -0.5123$, $P < 0.05$) with Ni concentration in maize shoots. The Mn concentration in maize roots was significantly ($P < 0.001$) affected by Ni

Table I: Shoot and root biomass (g pot^{-1}) of maize grown under different Ni levels

Treatments	SFM	SDM	RFM	RDM
Control	4.47 ± 0.34	0.87 ± 0.04	2.81 ± 0.09	0.87 ± 0.04
17 µM Ni	6.40 ± 0.67	0.96 ±0.02	3.72 ± 0.14	0.86 ± 0.02
51 µM Ni	5.62 ± 0.82	0.98 ± 0.04	3.17 ± 0.04	0.92 ± 0.07
68 µM Ni	5.66 ± 0.53	1.07 ± 0.03	3.29 ± 0.14	1.04 ± 0.06
α = 95%	*	*	*	*

SFM and SDM are shoot fresh and dry matter, while RFM and RDM are root fresh and dry matter

Table II: Nickel concetration (mg kg^{-1}) and uptake (µg pot^{-1}) in shoot and root of maize plants under different Ni levels

Treatments	Ni concentration		Ni uptake		Total Ni uptake
	Shoot	Root	Shoot	Root	
Control	0.71±0.02	2.00±0.05	0.43±0.27	1.74±0.07	2.17±0.33
17 µM Ni	1.41±0.05	3.33±0.30	1.35±0.04	2.84±0.24	4.20±0.20
51 µM Ni	1.31±0.09	7.41±0.49	1.29±0.13	6.82±0.78	8.11±0.78
68 µM Ni	1.84±0.23	8.52±0.90	1.95±0.23	8.79±0.55	10.74±0.77
α = 95%	*	*	*	*	*

Table III: Manganese concetration (mg kg^{-1}) and uptake (µg pot^{-1}) in shoot and root of maize plants under different Ni levels

Treatments	Mn concentration		Mn uptake		Total Mn uptake
	Shoot	Root	Shoot	Root	
Control	46.67±5.2	27.39±1.2	40.73±5.7	23.77±0.1	64.50±5.6
17 µM Ni	39.66±2.4	30.90±2.2	38.04±2.0	26.39±1.6	64.43±3.6
51 µM Ni	45.86±3.9	22.58±1.5	45.04±4.7	20.60±0.3	65.64±5.0
68 µM Ni	37.42±0.2	15.01±1.5	39.86±1.0	15.49±0.6	55.34±0.5
A = 95%	ns	*	ns	*	ns

addition.

Application of Ni significantly (P < 0.05) increased Zn concentration in maize roots while it non-significantly increased Zn concentration in maize shoots compared with that of control (Table IV). Nickel application increased significantly (P < 0.05) Zn uptake by maize shoots and roots and maximum increase in Zn uptake by maize shoots (48%) and roots (1.5 fold) was recorded with 68 µM Ni.

The Cu concentration in maize shoots remained statistically similar with all Ni application rates, being maximum (5.69 mg kg^{-1}) and minimum (4.46 mg kg^{-1}) with Ni addition at 51 and 68 µM, respectively (Table V). However, Cu concentration in roots was significantly affected with Ni application. The concentration of Cu remained higher in roots than in shoots with all the Ni application rates.

DISCUSSION

Shoot dry matter has significant positive correlation (r=0.81, P<0.001) with Ni concentration in shoot indicating positive effect of Ni application on maize growth (Narwal *et al.*, 1991; Yang *et al.*, 1996). Similarly, Ni application has significant correlation (r = 0.60, P < 0.05) with RDM. Maize appears to be tolerant to Ni stress as its dry matter production increased with application of Ni and toxicity symptoms of Ni like interveinal yellowing with general chlorosis were not observed on plants at any growth stage (Rahman *et al.*, 2005). It could be due to stimulating effect of Ni on N metabolism (Seregin & Kozhevnikova, 2006), which promoted plant growth (Parida *et al.*, 2003). Increase in SDM matter with application of Ni was also reported in tomato (Rahmatullah *et al.*, 2001; Seregin *et al.*, 2003; Gad *et al.*, 2007). Atta-Aly (1999) recorded an increase in leaf and root fresh and dry weights of parsley plants due to application of Ni. Nickel is required for N assimilation and protein synthesis in higher plants (Eskew *et al.*, 1984; Brown *et al.*, 1987 & 1990).

Table IV: Zinc concetration (mg kg^{-1}) and uptake (µg pot^{-1}) in shoot and root of maize plants under different Ni levels

Treatments	Zn concentration		Zn uptake		Total Zn uptake
	Shoot	Root	Shoot	Root	
Control	9.57±0.5	13.52±0.3	8.31±0.3	11.75±0.4	20.06±0.4
17 µM Ni	10.16±0.6	23.29±1.9	9.75±0.6	19.89±1.5	29.64±0.9
51 µM Ni	11.34±0.7	15.08±1.2	11.13±0.9	13.95±2.1	25.08±2.5
68 µM Ni	11.50±1.1	16.57±2.0	12.28±1.5	17.39±3.0	29.67±4.9
α = 95%	ns	*	ns	*	*

Table V: Copper concetration (mg kg^{-1}) and uptake (µg pot^{-1}) in shoot and root of maize plants under different Ni levels

Treatments	Cu concentration		Cu uptake		Total Cu uptake
	Shoot	Root	Shoot	Root	
Control	5.36±1.1	6.76±0.3	4.69±1.1	5.87±0.2	10.56±1.4
17 µM Ni	4.62±1.1	11.94±0.1	4.40±0.9	10.21±0.2	14.61±0.7
51 µM Ni	5.69±0.5	8.07±1.1	5.60±0.7	7.46±1.4	13.06±1.7
68 µM Ni	4.46±0.2	8.67±1.2	4.76±0.3	9.10±1.6	13.85±2.0
α = 95%	ns	*	ns	*	*

The Ni concentration in maize roots was considerably higher than in shoots, which indicated tendency of maize plants to retain more Ni in roots through its selective transport into shoots (Huillier *et al.*, 1996; Baccouch *et al.*, 1998). Similarly, Parida *et al.* (2003) recorded increased Ni concentration in shoots and roots of fenugreek with increasing Ni application levels. Present findings regarding uptake of Ni by roots and shoots suggest the possibility of retention of absorbed metals in roots and its restricted translocation to shoots.

Accumulation of more Ni in roots compared with that of shoot seems to be an important plant characteristic with respect to Ni toxicity tolerance mechanism (Yang *et al.*, 1996; Parida *et al.*, 2003). Increasing level of Ni application further decreased distribution of Ni towards shoots indicating translocation of more Ni towards shoots at lower Ni application levels compared with that at higher Ni application level (Seregin & Kozhevnikova, 2006). However, higher root Ni concentration is of practical significance and seems to be genetically controlled feature of maize plants. It favored inclusion of maize crop in cropping pattern of peri-urban agriculture to decrease Ni entry into human food chain.

The Ni addition at 17 μM increased Mn concentration in maize roots, but decreased it at higher levels (51 & 68 μM). The Mn concentration in maize roots had significant negative correlation ($r = -0.65$, $P < 0.05$) with Ni concentration in maize shoots indicating antagonistic effect of Ni on Mn concentration in roots. Similarly, decrease in Mn concentration in shoots of rye grass (Khalid & Tinsley, 1980) and barley (Rahman *et al.,* 2005) in response to application of Ni has been reported. Decrease in Mn concentration at higher Ni level could be due to its competition with Ni to be absorbed by the plant roots (Rahman *et al.,* 2005). Our results are partially in accordance with the findings of Brune and Dietz (1995) and Rahman *et al.* (2005), who reported that excess Ni in rooting medium decreased root Mn concentration. The role of Mn in activating enzyme-catalyzed reactions is well recognized. Deficiency of Mn, therefore, greatly affects respiration, amino acid synthesis, lignin biosynthesis and the level of hormones in plants (Rahman *et al.,* 2005).

Maize roots accumulated more Zn compared with that of shoots at all Ni levels, but it was non-significantly, but positively correlated with Ni concentration in maize shoots and roots. The increased Zn in Parsley plants with Ni was attributed to synergistic effect of Ni application on Zn absorption. (Atta-Aly, 1999), however our results contracted with findings of Khalid and Tinsely (1980), who reported decrease in Ni concentration with increasing Zn levels in rye grass, maize and pearl millet, respectively.

There was not any definite relationship between Ni levels and Cu concentration in maize shoots and Cu concentration in maize shoots and roots was non-significantly but negatively correlated with Ni concentration. These results are in agreement with findings of Patel *et al.* (1976). Copper concentration in roots was significantly higher than in shoots (Table IV). Parida *et al.* (2003) found 3-fold increase in Cu concentration in roots compared with that of shoots in fenugreek with application of Ni. Similarly, Rahman *et al.* (2005) reported increased Cu concentration in roots with Ni application (upto 100 μM), while opposite trend was recorded in shoots of barley. Contrarily, Narwal *et al.* (1994) reported that Ni application decreased Cu concentration in plants due to competition between Ni and Cu on exchange sites of roots. It seems that generally Ni application decreased influx of Cu into roots from growth medium and later on translocation of Zn, Cu and Mn from roots to shoots of maize (Yang *et al.,* 1996).

CONCLUSION

Application of Ni at ambient levels improved growth of maize. Furthermore, More Ni was retained in roots than its onward translocation to shoots, suggested that maize is a promising crop for peri-urban areas receiving raw city effluent generally containing considerably high levels of metals particularly Ni. However, further research is warranted to study the role of Ni on uptake and absorption of other micronutrients in fodder maize to prevent the nutritional imbalances in livestock.

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