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Mungbean: A Nickel Indicator, Accumulator or Excluder?

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Abstract Seeds of the two mungbean cultivars were exposed to 25, 30, 35 and 40 mg L^{-1} nickel along with control. Nickel stress decreased growth, photosynthetic pigments, yield attributes and cation (Na^+, K^+ and Ca^{2+}) accumulation in mungbean. This reduction was less at 25 mg L^{-1} as compared to 40 mg L^{-1} nickel. In addition, nickel was mainly stored in roots and restricted transfer to the aerial parts (shoot and least by leaves) was observed.

Keywords Mungbean · Nickel · Growth · Photosynthetic pigments · Na^+ · K^+ · Ni

Environmental pollution leads to undesirable changes in the physical, chemical and biological characteristics of air, water and soil that affects human beings, animals and plants (Misra and Mani 1991). Among these pollutants, metals are of particular concern because the crops grown in the contaminated soil accumulate metals in excessive quantities in various food parts, which in turn are transferred to human body causing numerous ailments (Kumar and Clark 1991). It is a hard fact that agricultural soils in developing countries including Pakistan are being depleted with metal contaminants derived from effluents of industrial establishment. This contamination becomes more drastic particularly in urban and agricultural soils which often receive organic manures heavily loaded with toxic metals including mercury, lead, copper, zinc and nickel (Ghafoor et al. 1995). In such cases, both treated and untreated municipal wastewater in the vicinity of large cities is used for vegetable production and when ingested by human they pose a health risk and can be lethal.

Although, some metals like Cd, Cr, Zn, Ni^{2+} are involved in a variety of processes in the cell, their excessive concentrations are highly toxic to many organisms including higher plants (Verkleij and Prast 1990). Metals may disrupt a number of plant phenomenon, for example they may cause essential nutrient deficiency and even change the concentration of basic nutrients such as nitrogen and phosphorus in plant tissues (Siedlecka 1995). Among metals, nickel toxicity has become of great concern due to its excessive use in different industries. Nickel is known to produce symptoms of stunting growth, leaf chlorosis and occasionally vein necrosis in plants (Seregin and Kozhevnikova 2006). Nickel significantly retards germination; inhibits growth and dry matter production (Nedhi et al. 1990) and affects nutrient uptake K, Ca and Mg in the different plant parts, particularly in shoot (Rubio et al. 1994). Similarly, it decreases number of flowers and fruits and subsequently yield (Balaguer et al. 1998) in different plant species. The current classification divides all plant species into three groups: (1) the accumulators that store metals mainly in the shoots under high and low metal concentration in soils; (2) the indicators, with plant metal concentrations reflecting the metal content in the environment; and (3) the excluders, with restricted transfer of heavy metals into the shoots whatever high are metal concentrations in the environment and the roots (Baker 1981; Antosiewicz 1992).

Keeping in view the increasing concern of nickel toxicity to crop plants in the developing countries like Pakistan, in the present study we accessed the response of mungbean to varying levels of nickel applied in rooting medium. In addition, we also accessed the placement of

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mungbean among metal indicators, accumulators or excluders.

Materials and Methods

Seeds of the two mungbean [*Vigna radiata* (L) Wilczek] cultivars M-1 and M-6 were collected form Ayub Agricultural Research Institute, Faisalabad. Ten seed were sown at 1 in. depth in pots containing 10 kg of sandy loom soil with a pH of 7.95. Seedlings were thinned to maintain four in each pot. The experiment was laid down in CRD manner with five replications. Thirty-days old well established plants were treated with 30 mL of solution containing 25, 30, 35 and 40 mg L^{-1} nickel sulphate [$Ni(SO_4)_2 \cdot 6H_2O$] and corresponded to the 0, 95,115, 133 and 152 μM final concentrations of nickel sulphate solution (Gajewska et al. 2006). Control plants were treated with distilled water only. The selected levels were used on the basis of nickel content reported in Pakistani soils (20–35 mg kg^{-1} soil at 0–90 cm depth) irrigated regularly by metal contaminated water (reported up to 0.05 mg L^{-1} Ni

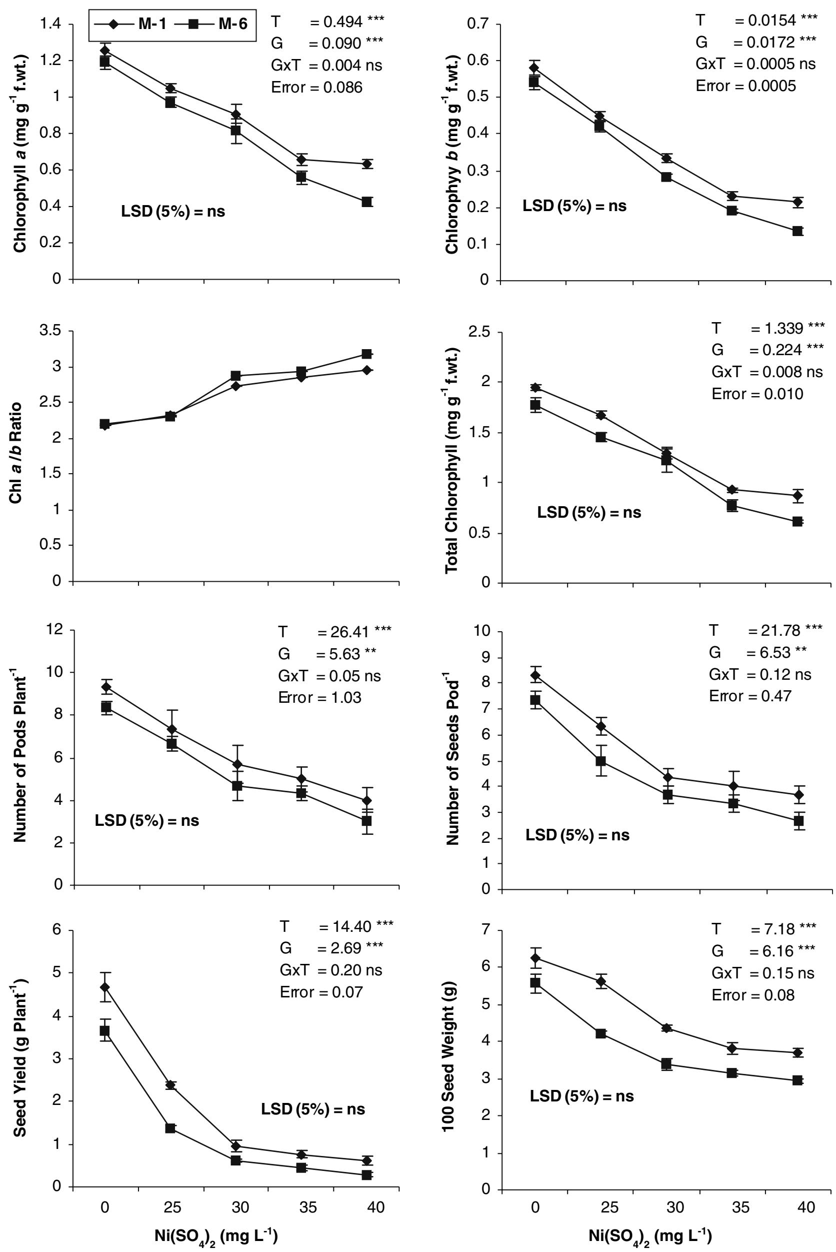
Table 1 Relative increase/decrease in growth parameters of mung bean [*Vigna radiata* (L.) Wilczek] when 30-day old plants were subjected to varying levels of nickel

Parameter	Harvest	Varieties	Nickel (mg L^{-1})				
			Control	25	30	35	40
Shoot fresh weight (g day^{-1})	1	M-1	3.5	3.1	2.8	2.5	2.1
		M-6	2.8	2.6	2.4	2.1	2.0
	2	M-1	5.4	5.1	4.8	4.5	4.1
		M-6	4.8	4.6	4.4	4.2	3.9
	3	M-1	6.3	6.0	5.8	5.6	5.4
		M-6	5.2	5.0	4.8	4.7	4.5
Root fresh weight (g day^{-1})	1	M-1	2.9	2.6	2.5	2.2	2.0
		M-6	2.4	2.3	2.1	2.0	1.8
	2	M-1	3.4	3.2	3.0	2.9	2.7
		M-6	2.7	2.5	2.3	2.1	2.0
	3	M-1	3.7	3.6	3.4	3.1	2.9
		M-6	3.0	2.9	2.8	2.7	2.5
Shoot dry weight (g day^{-1})	1	M-1	2.9	2.6	2.4	2.2	1.9
		M-6	2.3	2.2	2.0	1.8	1.7
	2	M-1	4.9	4.7	4.5	4.3	4.0
		M-6	3.9	3.8	3.6	3.5	3.3
	3	M-1	5.1	4.9	4.7	4.5	4.3
		M-6	4.3	4.2	4.0	3.9	3.7
Root dry weight (g day^{-1})	1	M-1	1.11	1.08	1.05	1.03	0.90
		M-6	1.09	1.07	1.05	1.02	0.90
	2	M-1	1.12	1.10	1.08	1.02	1.00
		M-6	1.09	1.08	1.07	1.06	1.05
	3	M-1	1.18	1.16	1.14	1.13	1.11
		M-6	1.13	1.12	1.11	1.10	1.09
Shoot length (cm day^{-1})	1	M-1	3.8	3.4	3.1	2.9	2.6
		M-6	3.2	3.0	2.8	2.5	2.3
	2	M-1	5.7	5.4	5.1	4.7	4.5
		M-6	3.9	3.7	3.6	3.4	3.2
	3	M-1	6.7	6.4	6.1	5.9	5.6
		M-6	5.3	5.1	4.9	4.7	4.5
Root length (cm day^{-1})	1	M-1	1.11	1.08	1.05	1.03	0.9
		M-6	1.09	1.07	1.05	1.02	0.9
	2	M-1	1.12	1.10	1.08	1.02	1.00
		M-6	1.09	1.08	1.07	1.06	1.05
	3	M-1	1.15	1.13	1.11	1.09	1.07
		M-6	1.11	1.10	1.09	1.08	1.07

in some cases) in the vicinities of large cities like Faisalabad (Ensink et al. 2002; UNIDO 2002; van der Hoek et al. 2002; Ensink et al. 2007). In this study, slightly higher levels of $Ni(SO_4)_2$ were used in view of the fact that alkaline pH of the root medium decreases the bio-availability of metals (Wahid, 2007).

Data for shoot and root fresh weights, and shoot and root lengths were recorded for three 7-day harvest intervals starting from 10 days after nickel application. Plants were uprooted carefully and washed with tap water with a subsequent washing with distilled water and fresh weights were taken. Plants were oven-dried at 65°C to constant dry weight and weights determined. Chlorophyll *a*, *b* and total chlorophyll were determined in second harvest. The fresh material grinded and extracted in 80% acetone, absorbance was read (at 645, 480 and 663 nm for Chl. *a*, Chl. *b* and total chlorophyll, respectively) by using a spectrophotometer (Hitachi-220, Japan) and the amount of Chl. *a*, *b* and total chlorophyll were calculated (Arnon 1949). Plants grown up to maturity were harvested (4th and final harvest) for yield components and number of pods per plant, number of seeds in each pod, yield per plant and 100-seed weight were was measured. Na^+, K^+, Ca^{2+} and Ni^{2+} contents in leaves, shoots and roots were detrimined in acid digest HNO_3 using an atomic absorption spectrometer (AAnalyst 300, Perkin-Elmer,

Fig. 1 Yield attributes and chlorophyll contents of mung bean [*Vigna radiata* (L.) Wilczek] when 30-day old plants were subjected to varying levels of nickel. *, **, *** significant at 0.05, 0.01 and 0.001 levels, respectively, *ns* non-significant (*df*, T = 4, G = 1, TxG = 4, Error = 40)



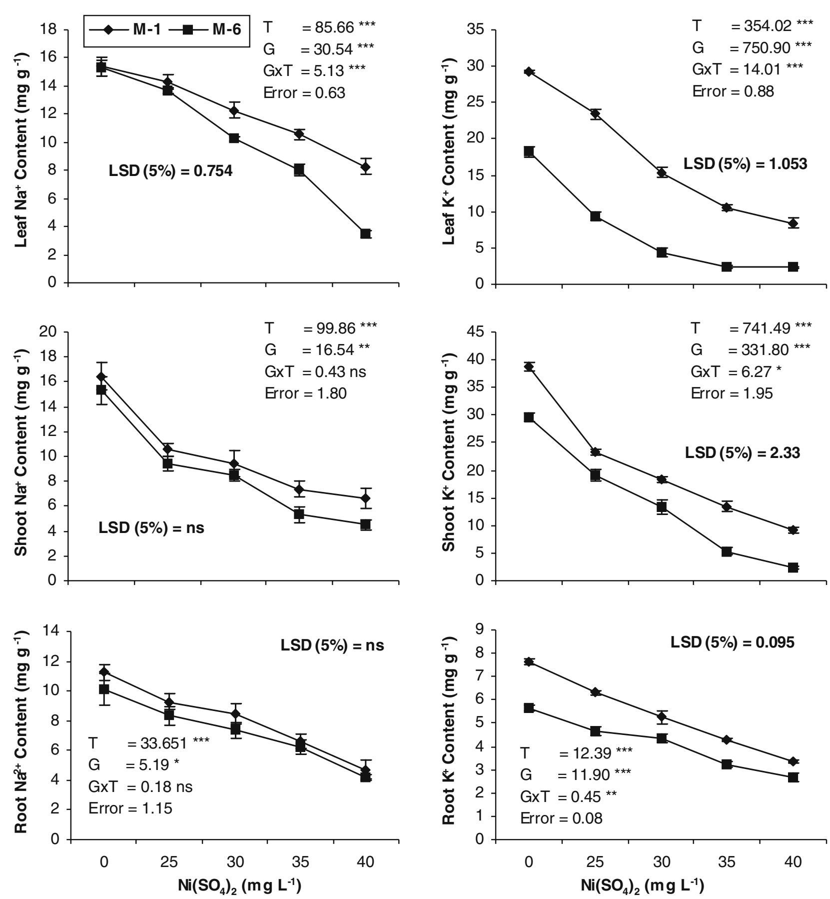
Germany). The data were subjected to statistical analysis using a COSTAT computer package (CoHort Software, 2003, Monterey, California).

Results and Discussion

Exogenously applied Ni has a negative effect on root and shoot lengths and fresh and dry weights of stems and roots. Although, at all the three harvest intervals a steep decline in all these parameters was observed with increasing concentration of nickel in rooting medium, the maximum decline over control was observed at the highest concentration of Ni application (Table 1). Overall, variety M-1 performed better compared with M-6. On the comparison between the harvest intervals it was revealed that the relative reduction in growth in all the four treatments was greater at the third harvest interval compared with the second and first harvest intervals. As evident from our study, this gradual decrease in vegetative parameters was due the increased accumulation of nickel in leaves, stem and roots (Fig. 3). Accelerated accumulation of nickel in underground and aerial parts produced symptoms of leaf necrosis and chlorosis, and thus decreased the chlorophyll contents in the aerial parts of the stressed plants (Fig. 1). This situation ultimately reduced photosynthetic area of leaves, disturbed normal metabolism of cells and subsequently decreased the photosynthate supply to the non-photosynthetic regions of plants (roots and shoots). In addition, energy depletion also decreased the nutrient uptake and supply to the aerial parts (Figs. 2, 3) and ultimately suppressed growth and development of plants growing under nickel stress (Gajewska et al. 2006; Seregin and Kozhevnikova 2006).

Similarly, nickel application also significantly reduced the photosynthetic pigments (chlorophyll *a*, *b* and total chlorophyll) of the stressed plants (Fig. 1). Nickel toxicity on pigment concentration increased with the increasing concentration of nickel in the soil. Both the cultivars also behave differently to all the four levels of nickel applied in the rooting medium. Overall the response of cultivar M-1 was better compared with M-6. In our findings, sever chlorosis on older leaves and scarce appearance on younger leaves suggested that decline in chlorophyll content in shoots of nickel treated plants result mostly from its enhanced degradation. Increase in chlorophyll *a*/*b* ratio under nickel stress (Fig. 1) indicated that chlorophyll *b* is more sensitive to Ni toxicity than chlorophyll *a* (Gopal et al. 2002). These results suggested that nickel might have

Fig. 2 Na^+ and K^+ content in leaves, shoots and roots of mungbean [*Vigna radiata* (L.) Wilczek] when 30-day old plants were subjected to varying levels of nickel. [*, **, *** significant at 0.05, 0.01 and 0.001 levels, respectively, *ns* non-significant (*df*, T = 4, G = 1, TxG = 4, Error = 40)]



oxidative damaged to membranous system of chloroplast (Baccouch et al. 1998) and thus had an inhibitory affects on chlorophyll synthesis (Stobart et al. 1985) or degraded the active photosynthetic pigments (Somashekaraiah et al. 1992).

Concentrations of different cations in leaves, shoots and roots were analyzed to draw relationships between growth and accumulation of cations in different organs under Ni stress. It was observed that Na^+, K^+ and Ca^{2+} decreased with the increasing concentration of Ni in the rooting medium. Cultivar M-1 performed better compared with M-6 and showed less reduction in cation accumulation at all the four levels applied. The concentration of all the three cations was higher in shoots and leaves compared with roots (Figs. 2, 3). It is now well established that cations (Na^+, K^+ and Ca^{2+} etc) are essentially required for the activities of enzymes, protein synthesis, integrity of cell wall and plasma membrane, and as components of proteins, photosynthetic protein complexes, photosynthetic pigments, RNA and DNA (Taiz and Zeiger 2002). It seems plausible to propose that toxicity of nickel is due to deficiency of cations as well as the direct effect of high concentration of Ni^{2+} under nickel stress conditions.

Nickel treatment also had a significant effect on yield parameters including number of seeds/pod, 100-seed weight and seed yield per plant. Cultivar M-1 performed better compared with M-6 and showed less reduction in yield parameters under nickel stress. This reduction was greater in higher concentration of nickel in the soil compared with the lower one (Fig. 1). This reduction in yield parameters might be attributed to poor plant development and reduced photosynthesis as a consequence of reduction in photosynthetic pigments in the leaves of the Ni-treated plants which resulted in suppressed supply of nutrients and photosynthates to the reproductive parts that ultimately affected yield (Tripathy et al. 1981).

An important observation in our studies was that despite M-1 accumulated higher nickel in root and leaves, this cultivar showed more stable growth and less reduction in photosynthetic and yield attributes as compared the M-6 (Fig. 3). These findings suggested the compartmentalization of nickel in central vacuole as low solubility complexes with organic acids as tolerance mechanism in M-1 (Boyd and Martens 1998; Kupper et al. 2001; Seregin and Kozhevnikova 2006). Similarly, lesser accumulation in leaves followed by stems of both the cultivars suggested

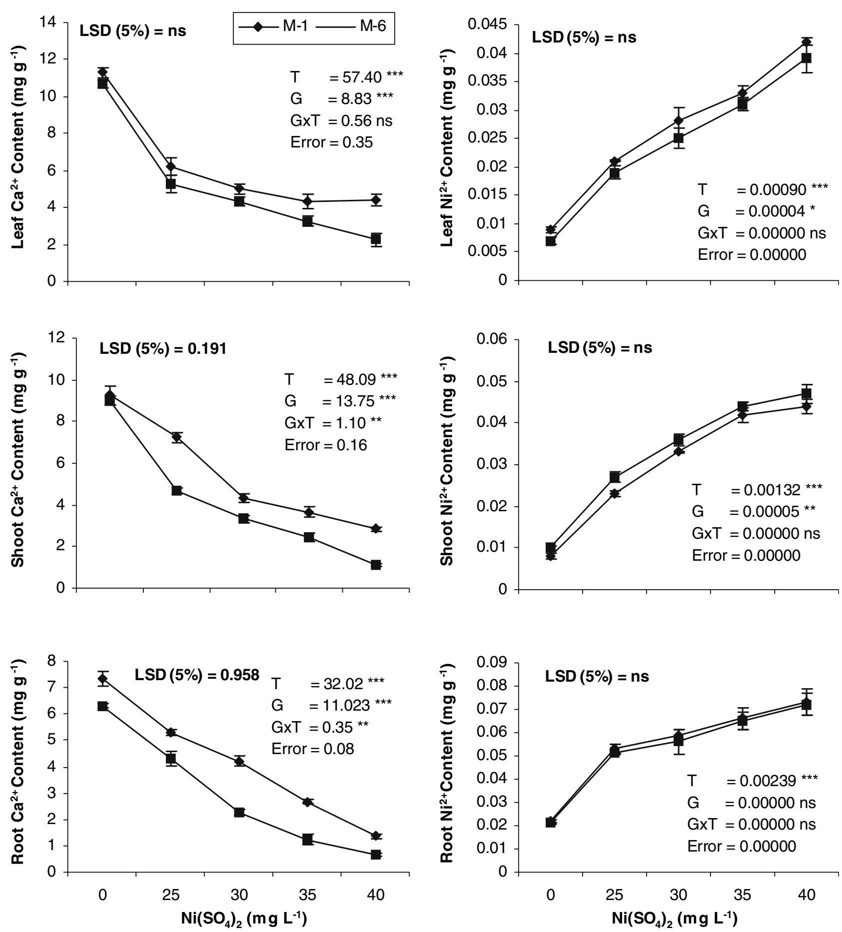


Fig. 3 Ca^{2+} and Ni^{2+} content in different parts of mung bean [*Vigna radiata* (L.) Wilczek] when 30-day old plants were subjected to varying levels of nickel. ([*, **, *** significant at 0.05, 0.01 and 0.001 levels, respectively, *ns* non-significant (*df*, T = 4, G = 1, TxG = 4, Error = 40)]

that the mungbean plants proffered to store nickel in roots and avoided its transportation to aerial parts which can help plant to significantly reduce damage in aerial parts. Thus mungbean can be placed among metal excluders that restrict the transport of nickel to the aerial parts (Baker 1981; Antosiewicz 1992).

In conclusion, nickel decreased growth, photosynthetic pigments, yield attributes and cations (Na^{+}, K^{+} and Ca^{2+}) accumulation in mungbean. This reduction was less at 25 mg L^{-1} application while was greater at 40 mg L^{-1} nickel application. On the basis of our findings, it was conclude that reduction in these parameters was majorly due to damage to photosynthetic pigments that ultimately reduced photosynthesis and nutrient supply to the actively growing plant parts. Moreover, nickel compartmentalization seemed to be tolerance mechanism operative in nickel tolerant cultivars. In addition, storage of nickel mainly in roots and restricted transfer to the aerial parts (shoot and least by leaves) by both mungbean cultivars suggested the placement of mungbean among nickel excluders.

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