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Alteration of macronutrients, metal translocation and bioaccumulation as potential indicators of nickel tolerance in three *Vigna* species

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Abstract. Macronutrients (Na^+, K^+, Ca^{2+}, Mg^{2+}), yield and yield components, bioaccumulation and translocation of metal in plant parts of three *Vigna* species (*V. cylindrica*, *V. mungo*, *V. radiata*) were evaluated at 0, 50, 100 and 150 mg kg^{-1} soil of Nickel (Ni). A marked inhibition ($p < 0.001$) in the distribution of various macronutrients was noticed in these *Vigna* species except for Mg^{2+} content of the shoot and leaves. Similarly, all species retained more Ca^{2+} in their roots ($p < 0.05$) as compared to the aerial tissues. Ni induced a drastic decline ($p < 0.001$) for various yield and yield attributes except for 100 seed weight. Toxicity and accumulation of Ni in plant tissues considerably increased in a concentration dependent manner. *Vigna* species signify an exclusion approach for Ni tolerance as both bioaccumulation factor (BF) and translocation factor (TF) were less than 1.0. The Ni content of plants being root > shoot > leaves > seeds. Scoring for percentage stimulation and inhibition (respective to control) at varying levels of Ni revealed tolerance of the species in an order of *V. radiata* > *V. cylindrica* > *V. mungo*. The acquisition of Ni tolerance in *V. radiata* seems to occur through an integrated mechanism of metal tolerance that includes sustainable macronutrients uptake, stronger roots due to greater deposition of Ca^{2+}in the roots, restricted transfer of Ni to above ground tissues and seeds as well as exclusion capacity of the roots to bind appreciable amount of metal to them. Thus, metal tolerant potential of *V. radiata* could be of great significance to remediate metal contaminated soil owing lesser impact of Ni on macro-nutrients, hence the yield.

Keywords: macronutrients; yield; metal excluders; nickel tolerance; *Vigna* species

1. Introduction

Natural ecosystems suffer from the damaging effects of heavy metal pollution which can cause permanent negative ecological effects (Mengoni *et al*. 2012). Among heavy metals, nickel (Ni) is ubiquitously distributed in nature and constitutes 0.008% of the earth crust (Tian *et al*. 2012),

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however, naturally occurring concentrations of Ni in soil and water are up to 100 and 0.005 ppm, respectively (Chen *et al*. 2009). In the recent years, rapid industrialization and high anthropogenic pressures in the developing countries have caused 20-30 times increase in Ni in the environment (Naaz and Pandey 2010) and as such its levels may reach up to 26000 ppm and 0.2 mg L^{-1} in polluted soil and water, respectively (Kunhikrishnan *et al*. 2012, Rajapaksha *et al*. 2012). Ni is mainly released into the environment through metallurgical processes and from various industrial sources. Electroplating, chemical and food, electrical batteries, spray, paints, ink, jewellery, stainless steel, textile dyes, varnish, welding and ceramic industries are important contributor of Ni pollution (Bermudez *et al*. 2012). In addition, untreated municipal wastewater, sludge disposal, fossils fuel combustion, pesticides and phosphate fertilizers also cause Ni contamination (Ensink *et al*. 2007).

Ni is an essential micro-element for plant growth (Brown *et al*. 1987) which is nutritionally required in an optimal concentration for various metabolic activities (Yusuf *et al*. 2011). However, the optimal requirement varies in different plant species. Ni deficiency hampers plant growth and reproduction by producing non-viable seeds (Houshmandfar and Moraghebi 2011). Similarly, excessive concentrations of Ni are toxic and can alter soil properties as well as impede metabolic processes in plants (Wani *et al*. 2008). Visible phytotoxicity symptoms associated with excessive Ni are stunted growth, chlorosis, necrosis and deformation of various plant parts (Pandey and Singh 2011). High concentrations of Ni have been reported to cause decrease biomass production and yield (Khan and Khan 2010). Excessive Ni in the growth medium induces deleterious effects on photosynthesis, respiration, nitrogen metabolism and protein biosynthesis which eventually lead to crop losses (Altinozlu *et al*. 2012, Gautam and Pandey 2008).

High concentrations of Ni also affects absorption, translocation and subsequent utilization of essential macronutrients (Na^+, K^+, Ca^{2+}, Mg^{2+}) within plants because Ni ions have a tendency to compete with these macronutrients ions thus causing nutritional imbalance (Matraszek and Hawrylak-Nowak 2010). The alterations of mineral nutritional status greatly influence the ability of plants to cope with several abiotic environmental stresses because accumulating evidences suggested that deficiencies of mineral nutrients enhance the sensitivity of plants to oxidative damage to biological membranes and macro molecules (Rajkumar and Freitas 2008). Consequently, integrity of cell, chloroplast, DNA and RNA is disrupted in the presence of high concentrations of Ni (Hansch and Mendel 2009).

Ni is highly mobile element, absorbed by the roots and then translocated to different plant parts including tissues of fruits and seeds (Naik *et al*. 2010). Hyper accumulation of Ni in edible plant parts is also of hazardous consequences for health as metal enters the food chain via consumption of contaminated plant material by animals and human (Duman and Ozturk 2010; Pande *et al*. 2012).

A significant proportion of population in Pakistan has to rely on pulses as a source of low cost vegetable proteins because of socio-economic situation of the country. In addition, scarcity of fresh water, low soil fertility and high cost of fertilizers result in the use of untreated municipal wastes and sewerage water for crop production in many cultivated areas of the country (Mushtaq and Khan 2010) causing metal contamination which rendered agricultural soils less productive (Khan *et al*. 2011). Under these situations, selection of high yielding pulse crop species which have a better potential for metal tolerance would be of great significance owing to lesser impact metal pollution on their growth and hence the yield.

Our previous study (Ishtiaq and Mahmood 2011) revealed potential of three *Vigna* species (*V. cylindrica*, *V. mungo* and *V. radiata*) for excessive Ni at their early growth phases. Therefore, in

the present study we aimed to evaluate the effects of Ni on yield and yield components of these species. Ni induced changes in the distribution of macroelements (Na^+, K^+, Ca^{2+}, Mg^{2+}) within plant tissues and their impact on the performance of the species was also evaluated. The study also aimed to reveal metal tolerant strategy of the species based on capacity of roots or aerial tissues to accumulate Ni. In addition, Ni contents of seeds were determined to suggest food safety when grown under metal contaminated soils.

2. Materials and methods

Seeds of *Vigna cylindrica* Skeel var. cp-386, *Vigna mungo* L. var. 6036-7 and *Vigna radiata* L. var. 97003 were obtained from Pulses Crop Division, Ayub Agriculture Research Institute, Faisalabad, Pakistan. Air-dried sandy loam soil (pH 7.90) sieved through a 2 mm sieve was thoroughly mixed with $NiCl_2.6H_2O$ (Merck, Germany) at concentrations of 50, 100 and 150 mg kg^{-1} substrate singly at the beginning of experiment, while control plants were without Ni. Since Ni salt was hexa-hydrated therefore actual concentration factor of Ni (4.05 g) was used to maintain Ni levels used in the study. The selected concentrations were used on the basis of nickel content reported in agriculture soils of Pakistan present in the vicinities of large cities and industries (Ensink *et al*. 2007).

The experiments were arranged in a Complete Randomized manner. To simulate field conditions, plants were grown in a wire netting house under natural conditions (Temperature 28 $\pm$ 5°C, day-length 12 h and relative humidity 38%). Eight (pre-germinated) 6 days old seedlings were transplanted into each of 36 earthen pots (height 45 cm and internal diameter 30 cm) which were filled with 8.0 kg of soil containing Ni. Seedlings were acclimatized for a week then thinned out to four in each pot. Watering was carried out by gentle spraying using a spray gun to avoid leaching. Plants were allowed to grow until maturity. Pods were collected before their dehiscence and data records were made for number of flowers, number of pods, the number of seeds per pod and for hundred seeds weight during the course of experiment. Plants were then harvested carefully and thoroughly washed with distilled deionised water. Plants parts (roots shoots and leaves) were separated, oven dried and were used for determination of macronutrients and Ni concentrations.

Determination of macronutrients (Na^+, K^+, Ca^{2+}, Mg^{2+}) and Ni content in plant tissues (roots, shoots, leaves and seeds) by a wet digestion of the dried samples was done with a solution of 3:1 HNO_3:$HClO_4$ (v / v) (Allen *et al*. 1986). The digestion was carried out at 100°C using a Microwave Digestion System (MDS 2000, Canada). The concentrations of Na^+, K^+ and Ca^{2+} were determined with a flame photometer (Jenway, PFP-7, UK), double beam and deuterium background correction while Mg^{2+} and Ni were determined with an atomic absorption spectrophotometer (Varian AAS, 1475, California, USA). A blank digestion solution was made for comparison. The detection limit was 0.01 μg/l. The recovery rate ranged from 96-98%, with variability index 3.5-10%. The accuracy of the analytical method was confirmed by the determination of the content by AA Standards (Camlab, U.K.).

Bioaccumulation factor (BF) and translocation factor (TF) of Ni in three *Vigna* species after exposure to varying levels of Ni in the soil were calculated by following equation:

$$BF = \frac{\text{Metal concentration in the plant}}{\text{Metal concentration in the soil}}$$

$$TF = \frac{\text{Metal concentration in the shoots}}{\text{Metal concentration in the roots}}$$

Hyperaccumulating plants are those that have a BF > 1.0 and TF > 1.0.

2.1 Statistical analysis

Data presented as means (± S.E) for each parameter were subjected to a two-way analysis of variance using MS Excel 2007 in order to determine significant effects of different Ni levels as well as to determine inter-specific variability. Least Significant Differences (LSD) between means for species and Ni levels were calculated by employing a multiple range test following Snedecor and Cochran (1989).

3. Results and discussion

The presence of elevated Ni level in the soil can pose serious threat to the sustainable agriculture. Ni inhibited the growth of important pulse crop species studied. However, each species had shown its own response in terms of tolerance and sensitivity in the presence of Ni. The results clearly depicted that application of Ni induced inhibitory effects on macronutrient (Na^+, K^+, Ca^{2+}, Mg^{2+}) in root, stem and leaves of plants, yield and yield components in *Vigna* species studied. Accumulation of Ni in plant tissues considerably increased in a concentration dependent manner. The statistical analysis also revealed differential influence of varying Ni levels and variable response of three *Vigna* species for various attributes studied.

A consistent decline in Na^+ content was observed in tissues of Ni treated plants of *Vigna* species but the highest level of Ni (150 mg kg^{-1}) caused more profound decline in Na^+ content in all species. More Na^+ content was observed for leaves than roots and shoots at varying Ni levels (Fig. 1). Among species, *V. cylindrica* exhibited 27%, 48% and 57% decline in root Na^+ at 50, 100 and 150 mg kg^{-1} Ni respectively, over their controls. The most elevated level of Ni induced 74% decline in shoot Na^+ in *V. mungo* followed by 70% and 57% reduction *V. radiata* and *V. cylindrica*, respectively. However, Na^+ content of leaves increased by 3% in *V. radiata* at 50 mg kg^{-1} but at 150 mg kg^{-1} Ni 49% decline in Na^+ content in leaves was noticed but the species exhibited the lowest reduction of Na^+ in the leaves as compared to other species. *V. mungo* consistently had lower Na^+ content at all Ni levels in all plant tissue. Ni caused highly significant influence on Na^+ contents of root and shoot ($p < 0.001$) and leaves ($p < 0.01$). However, the species exhibited a marked contrast ($p < 0.01$) only for Na^+ content of roots (Fig. 1).

Na^+ is the key factors for maintaining turgor within the plant tissues but application of Ni induced changes in the distribution of Na^+ ions in plant parts. Since, roots are directly exposed to Ni therefore Na^+ contents were more influenced than Na^+ concentration in the above ground plant parts. The decline of Na^+ from different plant parts supports the earlier findings in other leguminous species. The possible reason for this decline could be that Ni disrupts a number of physiological functions particularly nutrient imbalance and even change the concentrations of basic nutrients (Yusuf *et al*. 2012). Moreover, the decrease in Na^+ content as a result of heavy metal treatment might be a consequence of deterioration in the physiological state of the plant, which in turn resulted in a reduction in its uptake and these findings are in close conformity with Manivasagaperumal *et al*. (2011) and Tezotto *et al*. (2012).

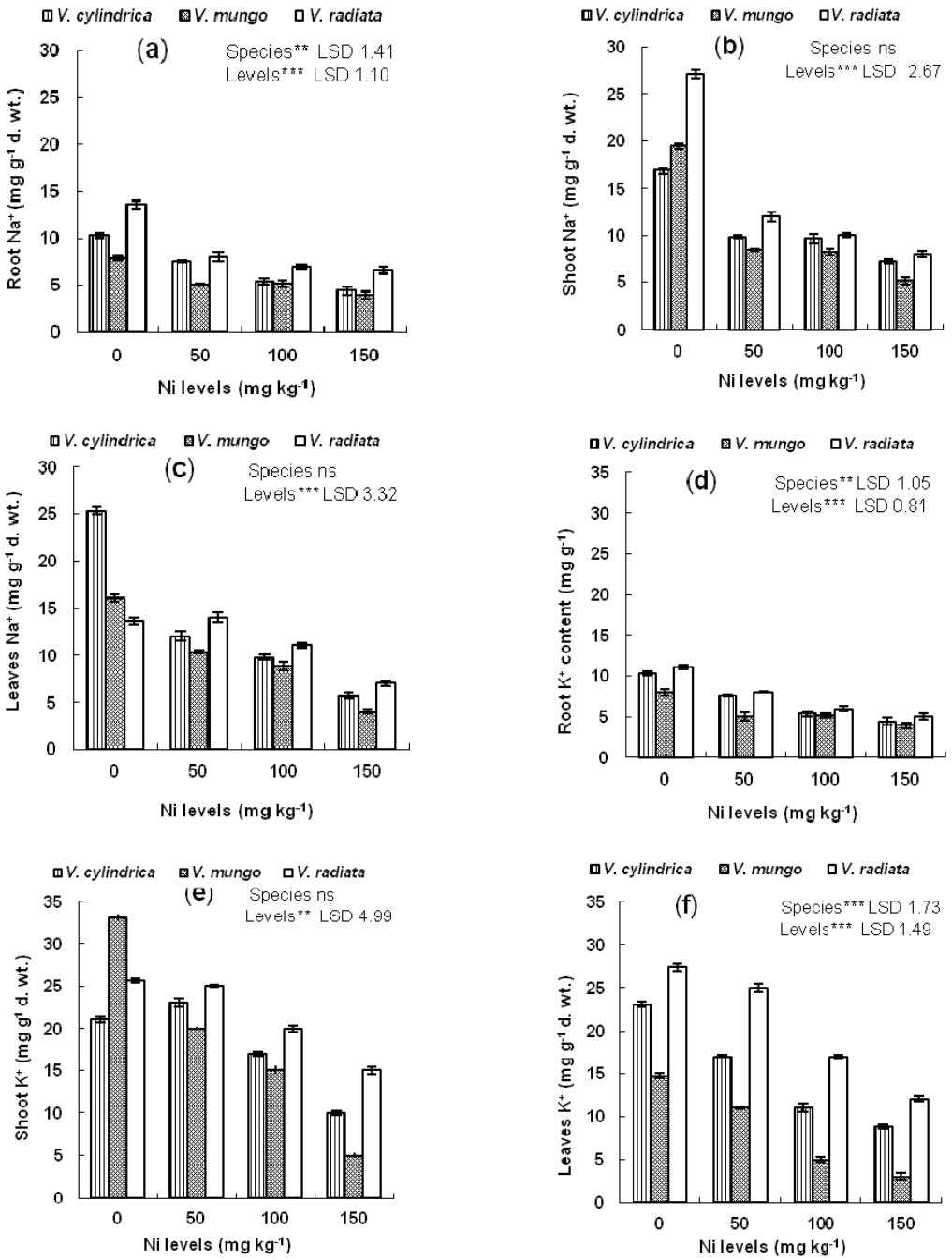

Fig. 1 Effect of varying levels of Ni on macronutrient contents: Na^+ content in roots (a), shoots (b), and leaves (c), K^+ content in roots (d), shoots (e) and leaves (f) in three *Vigna* species. Values presented are means across three replicates. Vertical lines indicate $\pm$ S.E. Statistical analysis: Two Way Analysis of Variance, degree of freedom with df = 3 (levels), df = 2 (species), df = 6 (interaction), LSD = least significant difference. *, **, ***, at 0.05, 0.01 and 0.001% level of probability, respectively, ns = non-significant

Ni levels in the rooting medium considerably ($p < 0.001$) influence K^+ content but distinct responses of the species were observed for root ($p < 0.01$) and leaf K^+ ($p < 0.001$) only (Fig. 1). The pattern of reduction was similar for all tissues (root, shoot and leaves) at 100 and 150 mg kg^{-1} which caused more pronounced decline in K^+ content in all species (Fig. 1). *V. cylindrica*, *V. radiata* and *V. mungo* exhibited 57%, 55% and 51% decline in root K^+, respectively at 150 mg kg^{-1}. 50 mg kg^{-1} Ni enhanced shoot K^+ by 9% but 150 mg kg^{-1} Ni induced 53% decline in shoot K^+ in *V. cylindrica* as compared to 85% in *V. mungo* at 150 mg kg^{-1} Ni. The decline in leaf K^+ was up to 80% (*V. mungo*), 56% (*V.radiata*) and 62% (*V. cylindrica*) at 150 mg kg^{-1}.

A decline in K^+ content in roots may occur due to reduced K^+ uptake, or increased K^+ transport to aerial tissues in the presence of Ni as proposed by Tezotto *et al*. (2012). Thus, negative effects on K^+ content in the roots of *Vigna* species can be attributed to Ni contamination which may alter or disturb the soil properties which influence K^+ uptake (Ashley *et al*. 2006). The decrease in K^+ content of *Vigna* species due to elevated level of Ni can be ascribed to the reason that Ni has the ability to compete with other ions including K^+ which in turn exercised a regulatory control on its uptake (Riesen and Feller 2005). Pande *et al*. (2012) also suggested that Ni contamination hampers K^+ uptake and distribution in plant tissues but tolerant and sensitive crops respond differentially for K^+. In addition, Kurtyka *et al*. (2008) and Manivasagaperumal *et al*. (2011) emphasized the physiological significance of leaf K^+ as more K^+ content in the leaves was found in tolerant lines of spring rape and oats than K^+ content of shoots and roots. The results of the study are parallel to the findings of these workers as greater K^+ in the leaves was observed than other tissues. The stimulating effects of Ni when present in low concentration can be attributed to the fact that several enzymes become active in the presence of Ni thus it promotes plant growth and development but excessive ions of Ni are able to displace other micronutrients from some other metaloenzymes thus higher concentrations found to be deleterious (Khoshgoftarmanesh and Bahmanziari 2012).

The species possessed a marked contrast for Ca^{2+} content ($p < 0.01$ for root and $p < 0.001$ for shoots and leaves). Likewise, a highly significant ($p < 0.001$) influence of Ni levels became evident for shoot and leaf Ca^{2+} (Fig. 2). Ca^{2+} content decreased with ascending Ni levels in tissues and all the species exhibited greater Ca^{2+} in the roots as compared to the aerial tissues at all Ni levels. *V. mungo* showed the highest reduction (49%), while *V. cylindrica* and *V. radiata* had 47% and 29% decline in root Ca^{2+} content, respectively at 150 mg kg^{-1} Ni. The reduction of Ca^{2+} in shoots was more pronounced (82%) in *V. cylindrica* but the most negative effect (92% decline) was noticed for *V. mungo* at the highest Ni level (Fig. 2). Similarly, 150 mg kg^{-1} Ni strongly influenced leaf Ca^{2+} content as 89%, 80% and 71% reduction was observed for *V. mungo*, *V. cylindrica* and *V. radiata*, respectively.

Among macronutrients, Ca^{2+} plays a crucial role in plant growth and development as it is a basic component of plant cell wall thus provides strength and thickness to cells and tissues (Kurtyka *et al*. 2008). In addition to structural integrity of plant tissues, quality of flower, fruit and seeds is strongly coupled to Ca^{2+} availability (Yang *et al*. 2012). Several other workers (Gautam and Pandey 2008, Yusuf *et al*. 2011) have reported a sharp decline in Ca^{2+} content of plant tissues when Ni was applied in a higher concentration. Although, the underlying mechanism responsible for this process remains to be elucidated but Chen *et al*. (2009) reported that heavy metal ions tend to displace Ca^{2+} from exchange sites or Ca^{2+} remains strongly bound in root free space. Reduction of Ca^{2+} content in tissues of *Vigna* species due to high levels of Ni is also in consonance with the earlier reports of Tezotto *et al*. (2012).

In our study, *Vigna* species had more Ca^{2+} in the roots as compared to aerial parts. More Ca^{2+} in

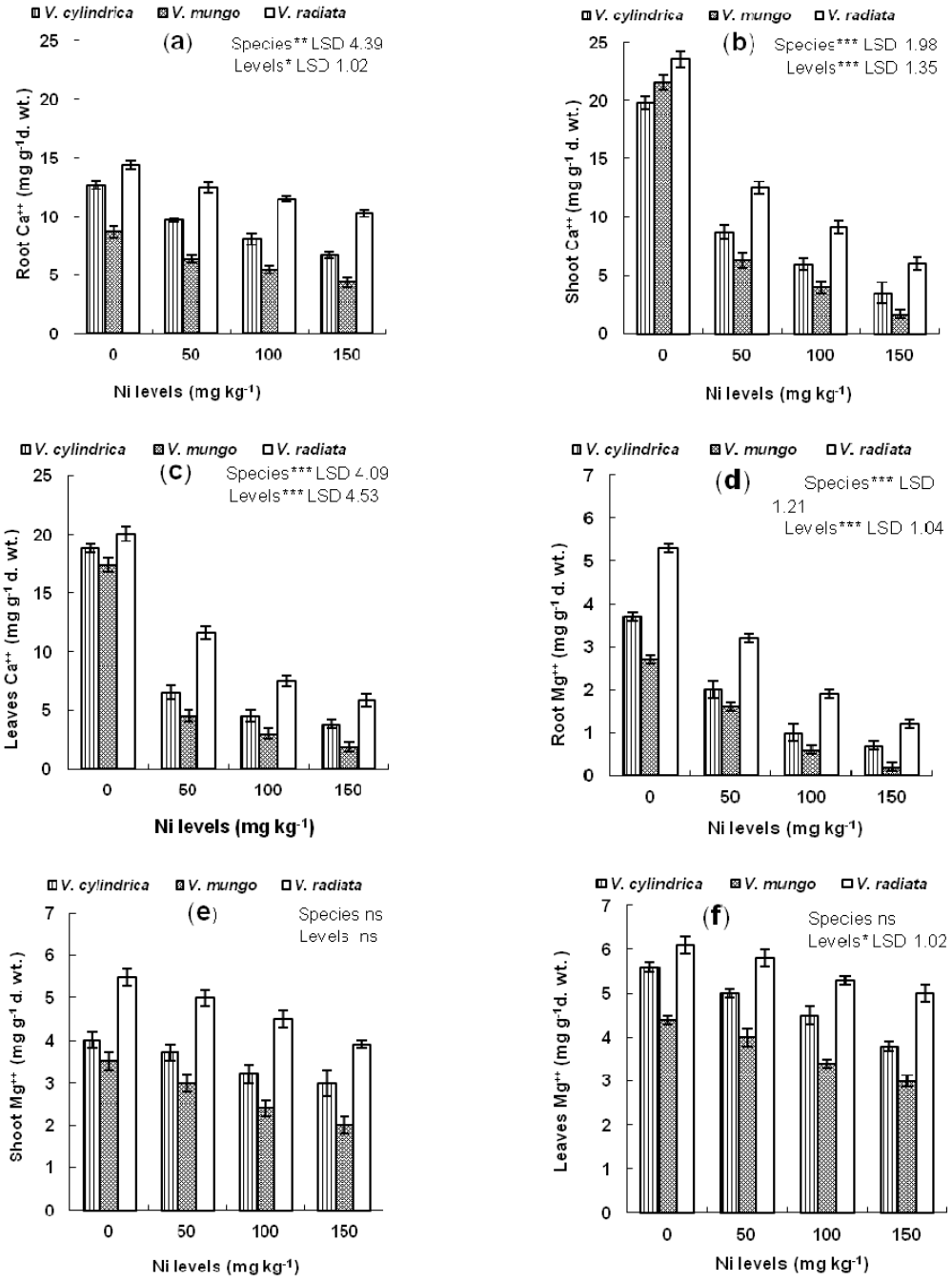

Fig. 2 Effect of varying levels of Ni on macronutrient contents: Ca^{2+} content in roots (a), shoots (b) and leaves (c), Mg^{2+} content in roots (d), shoots (e) and leaves (f) in three *Vigna* species. Values presented are means across three replicates. Vertical lines indicate $\pm$ S.E. Statistical analysis: Two Way Analysis of Variance, degree of freedom with df = 3 (levels), df = 2 (species), df = 6 (interaction), LSD = least significant difference

*, **, ***, at 0.05, 0.01 and 0.001% level of probability, respectively, ns = non-significant

plant roots seems to provide thickness and strength to cell wall because Ca^{2+} is an integral part of Ca^{2+} pectate which gives strong structural rigidity by forming cross-links within the pectin polysaccharide matrix. Thus, these results suggested that strong protective barriers against penetration of Ni existed in the roots and may prevent its movement to the aerial parts of plants.

Although species had differential accumulation of Mg^{2+} in their tissues but Ni had a significant influence on root ($p < 0.001$) and leaf Mg^{2+} ($p < 0.05$) content. Similarly, distinct responses ($p < 0.001$) of the species for root Mg^{2+} became evident (Fig. 2). The Mg^{2+} content of the roots declined drastically in all species as compared to shoot and leaves. Among species, *V. mungo* had consistently lower Mg^{2+} content in the tissues of tested plants at all levels of Ni while, *V. radiata* had a supreme approach of Mg^{2+} in its tissues in response to varying levels of Ni. *V. mungo* had the highest reduction (93%) of root Mg^{2+} while *V. cylindrica* and *V. radiata* showed 81% and 77% decline, respectively at 150 mg kg^{-1} Ni. Among species, the extent of decline in Mg^{2+} content in above ground parts was lower in *V. radiata* as the species had shown 29% reduction for shoot Mg^{2+} content while 18% decline was noticed in the leaves at the highest levels of Ni. *V. cylindrica* and *V. mungo* also had greater Mg^{2+} content of the shoot and leaves as compared to the roots (Fig. 2).

Mg^{2+} is one of the mobile elements which can easily move within and between tissues in plants. Mg^{2+} is a component of chlorophyll molecule and is necessary for the formation of fuel molecules as well as it regulates the uptake of other nutrients especially phosphorus (Kleiber *et al*. 2012).

Qurainy (2009) demonstrated that excess Ni in the soil influenced the uptake and distribution of Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} in a similar manner but other workers, Chen *et al*. (2009) and Yusuf *et al*. (2012) have reported that displacement of Mg^{2+} by Ni occurs more frequently owing to similar characteristics of these two elements and is considered as a primary functional effect of Ni toxicity. Since, Mg^{2+} is an integral component of chlorophyll thus disturbance in the absorption and translocation of Mg^{2+} may lead to reduced photosynthesis (Wyszkowski 2002). For this study, ascending trends were observed for uptake and distribution of Mg^{2+} in plant parts. Such results were also obtained by Bose *et al*. (2011), Gautam and Pandey (2008) and Molas (2002) who reported a steady maintenance of Mg^{2+} in plant parts regardless of metal concentration in the soil.

Fig. 3 indicated a greater reduction in yield (number of flowers, pods and seeds) at the highest level of Ni (150 mg kg^{-1}) in all *Vigna* species. Flowering, fruiting and seed formation in *V. cylindrica* was adversely affected by increasing Ni levels (Fig. 3). At 50 mg kg^{-1} Ni, *V. radiata* had shown 45% and 50% increase for flowers and pods development, respectively. However, the most elevated Ni level had caused 18% and 20% reduction of flowers and fruits in the species. Although, 100 mg kg^{-1} Ni did not influence flowering and fruiting in *V. mungo* but the highest Ni level induced 44% and 37% reduction of these two attributes, respectively. *V. radiata* also had the lowest decline (20%) for seed weight as compared with *V. cylindrica* and *V. mungo* in which reduction in seed weight was 25% and 56%, respectively at 150 mg kg^{-1} Ni. Highly significant ($p < 0.001$) effects of Ni were observed for number of flowers, pods and seeds but no marked variability was observed for seed weight. Likewise, the species were significantly variable for yield parameters; number of flowers ($p < 0.01$) and for pods and seeds ($p < 0.001$ for both).

Accumulating evidences suggested that Ni is an important component of enzymes involved in flowering, fruiting and seed development but excessive Ni ions strongly compete with other essential ions by blocking their transport pumps thus influence yield components (Altinozlu *et al*. 2012, Roy and Prasad 2010). Ni contamination induces deficiencies of essential macro and micro nutrient in plants via competition and anatagonism (Khoshgoftarmanesh and Bahmanziari 2012). Ni induced reduction in growth and yield of agricultural crops owing to alteration of enzymes,

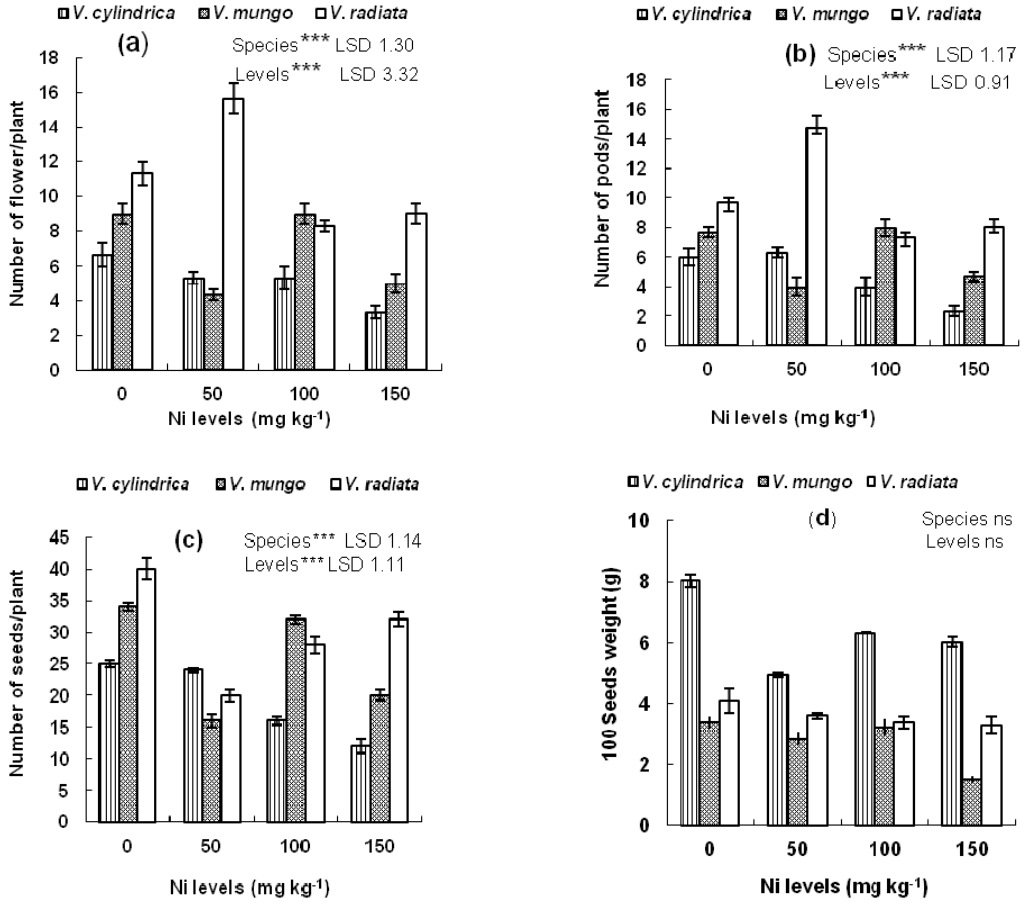

Fig. 3 Effect of varying levels of Ni on various yield and yield components: Number of flowers (a), number of pods (b), number of seeds (c) and 100 seed weight (d), in three *Vigna* species. Values presented are means across three replicates. Vertical lines indicate ± S.E. Statistical analysis: Two Way Analysis of Variance, degree of freedom with df = 3(levels), df = 2 (species), df = 6 (interaction), LSD = least significant difference
*, **, ***, at 0.05, 0.01 and 0.001% level of probability, respectively, ns = non-significant

metabolic disorders, oxidative stress and disruption of photosynthesis are well documented (Namdjoyan *et al*. 2012, Talukdar 2011).

The reductions in plant yield can be attributed to reduced supply of nutrients which are strongly coupled with vegetative and reproductive growth because Ni may lead to reduced biosynthesis of several metalloenzymes due to deficiency of mineral nutrients (Chen *et al*. 2009). Thus Ni stressed plants may show suppressed vegetative and reproductive performance that ultimately result in poor yield. However, reproductive success is associated with optimal Ni levels. An induction of flowering and fruiting in *V. radiata* at lower level of Ni can be a manifestation of role of Ni for the development of reproductive structures (Yusuf *et al*. 2012).The accumulation of Ni in plant tissues of *Vigna* species occurred in a concentration dependent manner. More Ni content (0.02-0.06 mg

g^{-1}) was observed in the roots followed by shoot (0.01-0.04 mg g^{-1}), leaves (0.005-0.028 mg g^{-1}) and seeds (0.001-0.008 mg g^{-1}) of the species (Fig. 4). A consistent pattern of metal accumulation was observed in all species therefore they did not differ markedly for the amount of Ni in their plant parts. At 150 mg kg^{-1} Ni, 63%, 58% and 67% of the metal translocated to the shoots from the

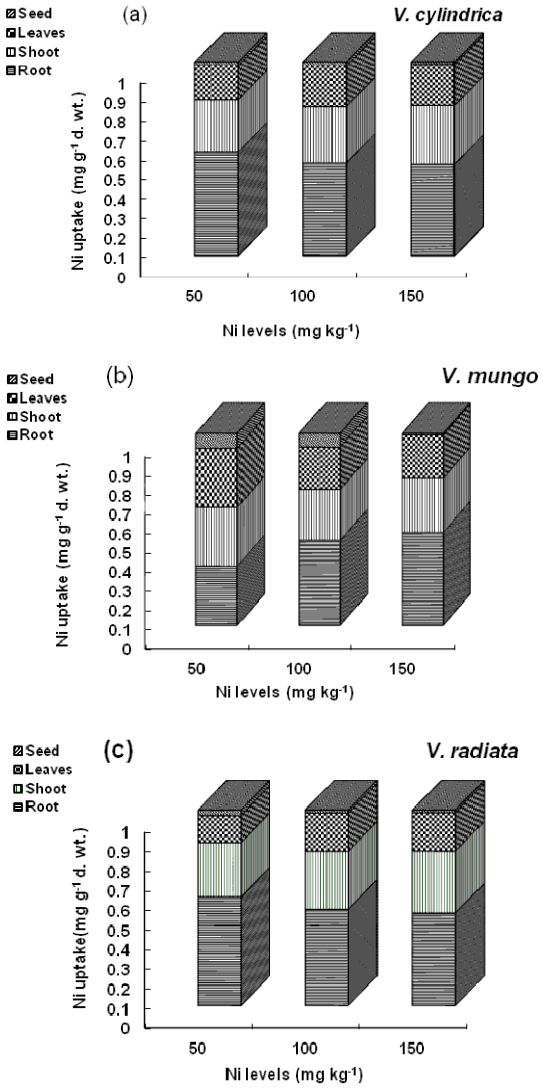

Fig. 4 Metal content in various plant parts in *V. cylindrica* (a), *V. mungo* (b) and *V. radiata* (c) after exposure to varying levels of nickel. Values presented are means across three replicates
Statistical analysis: Two Way Analysis of Variance, degree of freedom with df = 3(levels), df = 2 (species), df = 6 (interaction), Root, Shoot, and Leaves Nickel Content (mg g^{-1}) for Species = ns and Levels*** with LSD 0.01, 0.005, 0.005, respectively and Seed Nickel Content (mg g^{-1}) for Species and Levels = ns
LSD = least significant difference, ***, at 0.001% level of probability, ns = non-significant

roots while, 45%, 47% and 42% Ni was further trasnlocated to the leaves of *V. cylindrica*, *V. mungo* and *V. radiata*, respectively. The lowest proportion (2-3%) of Ni translocation was observed for seeds in all species. Thus bioaccumulation and subsequent translocation of Ni was in sequence root > shoot > leaves > seeds. Thus, Ni uptake and accumulation in the roots was higher but its translocation to aerial tissues and seeds was comparatively lower. The variations in metal content in various parts of plants can be ascribed to compartmentalization and differential translocation of the metal in plant parts (Jadia and Fulekar 2008). Heavy metal ions are stored by the roots in non toxic form through vacuolar compartmentalization (Mellem *et al*. 2012). Nevertheless, some of the metal ions are also translocated to the more sensitive aerial tissues and organs (Wong and Cobbett 2009). Stem plays the role of transfer organ (Patil and Gaikwad 2012) thus metal is further translocated to the leaves through vascular system. An appreciable amount of Ni present in the roots may signify a better threshold of roots to accumulate more metal content. The roots are considered to be more robust organs of the plants as they may show excessive lignification, proliferation and other modification to avoid metal toxicity (Badr *et al*. 2012). The hyperaccumulation of Ni in the roots can be a manifestation of detoxification mechanism which may involve binding of metal ions with cell wall, pumping of ions to vacuole or formation of non toxic complexes with specific metal binding protein without changing cellular metabolism and alteration of membrane structure thus causing no dramatic effects of metals (Mahmood *et al*. 2007).

Previous studies also demonstrated a negligible proportion of heavy metal uptake by the seed (Fargasova 2012). In this study we have also reported a small fractions of Ni in the seeds which do not pose any potential risk for human consumption owing to negligible quantity which is much lesser than critical Ni level recommended by WHO (2006).

Plant species have an innate variability and potential to grow on metal contaminated soil. This selective advantage is acquired through the existence of two types of mechanisms that include metal exclusion or metal accumulation by the plants. Consequently, plant species are either classified as metal excluders or hyper accumulators. In order to explore one of the above possible mechanisms two important predictors; bioaccumulation factor (BF) and translocation factor (TF) are frequently used to reveal metal tolerance strategy (Hassan and Aarts 2011). The BF is the ratio of metal concentration in the plant to metal concentration in the soil while translocation is the ratio of the metal concentration in the shoots to that in the roots. Hyperaccumulating plants are those that have a BF > 1.0 and TF > 1.0 (Majid *et al*. 2012). The values for BF and TF presented in Table 1 indicated that the mechanism of metal tolerance in these species appeared to be exclusion of metal because the values for both BF and TF are less than 1.0.

Table 1 Bioaccumulation factor (BF) and translocation factor (TF) of Ni in three *Vigna* species after exposure to varying levels of Ni in the soil

Species	Ni levels (mg kg^{-1})					
	50	100	150	50	100	150
	Bioaccumulation Factor (BF)			Translocation Factor (TF)		
V. cylindrica	0.98	0.99	0.85	0.5	0.6	0.63
V. mungo	1.03	1.1	0.83	1.0	0.6	0.58
V. radiata	0.72	1.02	0.85	0.5	0.6	0.67

Table 2 Percentage (%) stimulation and inhibition (relative to control) induced by varying levels of Ni in three *Vigna* species. Each percentage/ value superscript by •••,•• and • indicate tolerant, moderate and sensitive response, respectively

Attributes	*V. cylindrica*						*V. mungo*						*V. radiata*					
	Ni levels (mg kg^{-1})						Ni levels (mg kg^{-1})						Ni levels (mg kg^{-1})					
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
	Stimulatory			Inhibitory			Stimulatory			Inhibitory			Stimulatory			Inhibitory		
Root Na^+ content (mg g^{-1})	-	-	-	$27^{•••}$	$48^{••}$	$57^{•}$	-	-	-	$37^{••}$	$34^{•••}$	$51^{•••}$	-	-	-	$41^{•}$	$49^{•}$	$52^{••}$
Shoot Na^+ content (mg g^{-1})	-	-	-	$42^{•••}$	$43^{•••}$	$57^{•••}$	-	-	-	$57^{•}$	$58^{••}$	$74^{•}$	-	-	-	$56^{••}$	$63^{•}$	$70^{••}$
Leaves Na^+ content (mg g^{-1})	-	-	-	$53^{•}$	$61^{•}$	$77^{•}$	-	-	-	$29^{••}$	$45^{••}$	$75^{••}$	$3^{•••}$	-	-	$0^{•••}$	$19^{•••}$	$49^{•••}$
Root K^+ content (mg g^{-1})	-	-	-	$27^{•••}$	$48^{•}$	$57^{•}$	-	-	-	$37^{•}$	$34^{•••}$	$51^{•••}$	-	-	-	$28^{••}$	$46^{••}$	$55^{••}$
Shoot K^+ content (mg g^{-1})	$9^{•••}$	-	-	$0^{•••}$	$19^{•••}$	$53^{••}$	-	-	-	$39^{•}$	$55^{•}$	$85^{•}$	-	-	-	$2^{••}$	$22^{••}$	$41^{•••}$
Leaves K^+ content (mg g^{-1})	-	-	-	$26^{•}$	$52^{••}$	$62^{••}$	-	-	-	$25^{••}$	$66^{•}$	$80^{•}$	-	-	-	$11^{•••}$	$38^{•••}$	$56^{•••}$
Root Ca^{++} content (mg g^{-1})	-	-	-	$24^{••}$	$34^{••}$	$47^{••}$	-	-	-	$27^{•}$	$37^{•}$	$49^{•}$	-	-	-	$13^{•••}$	$20^{•••}$	$29^{•••}$
Shoot Ca^{++} content (mg g^{-1})	-	-	-	$56^{••}$	$70^{••}$	$82^{••}$	-	-	-	$70^{•}$	$81^{•}$	$92^{•}$	-	-	-	$47^{•••}$	$61^{•••}$	$75^{•••}$
Leaves Ca^{++} content (mg g^{-1})	-	-	-	$65^{••}$	$76^{••}$	$80^{••}$	-	-	-	$74^{•}$	$83^{•}$	$89^{•}$	-	-	-	$42^{•••}$	$63^{•••}$	$71^{•••}$
Root Mg^{++} content (mg g^{-1})	-	-	-	$46^{•}$	$73^{••}$	$81^{••}$	-	-	-	$41^{••}$	$78^{•}$	$93^{•}$	-	-	-	$40^{•••}$	$64^{•••}$	$77^{•••}$
Shoot Mg^{++} content (mg g^{-1})	-	-	-	$8^{•••}$	$20^{••}$	$25^{•••}$	-	-	-	$14^{•}$	$31^{•}$	$43^{•}$	-	-	-	$9^{••}$	$18^{•••}$	$29^{••}$
Leaves Mg^{++} content (mgg^{-1})	-	-	-	$11^{•}$	$20^{••}$	$32^{••}$	-	-	-	$9^{••}$	$23^{•}$	$32^{••}$	-	-	-	$5^{•••}$	$13^{•••}$	$18^{•••}$
Number of flowers/ plant	-	-	-	$29^{••}$	$29^{•}$	$57^{•}$	-	-	-	$56^{•}$	$0^{•••}$	$44^{••}$	$45^{•••}$	-	-	$0^{•••}$	$27^{••}$	$18^{•••}$
Number of pods/ plant	-	-	-	$10^{••}$	$33^{•}$	$50^{•}$	-	-	-	$50^{•}$	$0^{•••}$	$37^{••}$	$50^{•••}$	-	-	$0^{•••}$	$30^{••}$	$20^{•••}$
Number of seeds/ plant	-	-	-	$4^{•••}$	$36^{•}$	$52^{•}$	-	-	-	$53^{•}$	$6^{•••}$	$4^{•••}$	-	-	-	$50^{••}$	$30^{••}$	$20^{••}$
100 Seeds weight (g)	-	-	-	$38^{•}$	$21^{•}$	$25^{••}$	-	-	-	$16^{•••}$	$5^{•••}$	$56^{•}$	-	-	-	$18^{••}$	$17^{••}$	$20^{•••}$

Cumulative scoring in three *Vigna* species across three levels of Ni for 16 attributes

Species	Stimulation			Inhibition		
	Tolerant	Moderate••	Sensitive •	Tolerant •••	Moderate ••	Sensitive •
V. cylindrica	1	-	-	10	21	17
V. mungo	-	-	-	10	11	27
V. radiata	3	-	-	28	17	3

Tolerant: •••, Moderate: ••, Sensitive: •

Percentage stimulation and inhibition relative to control at varying levels of Ni for each species is given in Table 2 which clearly demonstrated that *Vigna* species had differential responses for stimulation and inhibition. The stimulating effects were observed at the lowest level of Ni only for leaf Na^+ and flowering and fruiting in *V. radiata* and shoot K^+ in *V. cylindrica*. Higher levels of Ni induced more drastic effects on the performance of species as more inhibition percentages were noticed for all attributes.

The lowest inhibition values may signify a better tolerance of the species while the maximum sensitivity can be ascribed in case of the highest value for inhibition. The percentage for a single attribute for species within each Ni level was scored assuming that the lowest inhibition percentage (indicative of tolerance) can have a maximum score of 3, intermediate value for inhibition (moderate) with 2 scores and the maximum inhibition (sensitive) with a single score. However, each category type (tolerant, moderate or sensitive) was given an equal weightage while taking cumulative score. It was observed that among the species, *V. radiata* performed consistently better at all levels of Ni and has a consistent tolerance for 7 attributes (Table 2). However, the species acquired a cumulative tolerant score of 28 (across all attributes and levels), 17 for moderate and 3 for sensitivity. *V. cylindrica* exhibited an inconsistent degree of tolerance and sensitivity for various attributes at varying Ni levels. However, it had a tolerant score of 10, 21 for moderate and 17 for sensitivity. On the other hand, *V. mungo* had a consistent degree of sensitivity for 5 attributes at all Ni levels having a sensitivity score 27 as it showed the maximum inhibition percentages at different Ni levels. Regarding stimulation percentages, it became evident that among the species only *V. radiata* had shown the maximum stimulation for 3 attributes only at the lowest Ni level followed by *V. cylindrica* which showed stimulation for a single attribute. Thus *V. radiata* had the highest potential while, *V. cylindrica* exhibited a moderate response to Ni stress. Hence, the degree of Ni tolerance in the species appeared in an order *V. radiata* > *V. cylindrica* > *V. mungo*.

4. Conclusions

The present study revealed that Ni induced deleterious effects on the performance of three *Vigna* species for macronutrient, yield and yield attributes in a concentration dependent manner. Ni induced macronutrient changes which can be attributable to poor growth and yield. *Vigna* species had differential responses to varying levels of Ni but the sensitivity was more conspicuous in *V. mungo* to Ni stress. However, in *V. radiata* Ni tolerant potential seems to be achieved by the sustainability of macronutrient uptake particularly leaf K^+ and Mg^{2+}, better yield and yield components. The ability of *V. radiata* plants to restrict more metal in the roots along with improved strength and thickness of cell wall due to greater deposition of Ca^{2+} signify a better threshold of species at elevated Ni levels. Moreover, limited bioaccumulation and translocation of Ni content to above ground parts of plants also seems to be an advantageous metal tolerance approach. An integrated mechanism which includes lesser hindrance for macronutrients uptake, stronger roots and lower translocation of Ni from root to aerial tissues as well exclusion capacity of the roots to bind appreciable amount of metal resulted in improved performance of *V. radiata*. Ni stress may pose lesser impact on plant macronutrients, hence the yield in *V. radiata* thus the species can be a potential choice for Ni contaminated soil.

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