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Phytotoxic effects of nickel on yield and concentration of macro- and micro-nutrients in sunflower (*Helianthus annuus* L.) achenes

Muhammad Sajid Aqeel Ahmad[a,*], Muhammad Ashraf[a,b], Mumtaz Hussain[a]

[a] *Department of Botany, University of Agriculture, Faisalabad, Jail Road, Faisalabad 38040, Pakistan*
[b] *Department of Botany and Microbiology, King-Saud University, Riaydh, Saudi Arabia*

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ABSTRACT

The phytotoxic effects of varying levels of nickel (0, 10, 20, 30, and 40 mg L^{-1}) on growth, yield and accumulation of macro- and micro-nutrients in leaves and achenes of sunflower (*Helianthus annuus* L.) were appraised in this study. A marked reduction in root and shoot fresh biomass was recorded at higher Ni levels. Nickel stress also caused a substantial decrease in all macro- and micro-nutrients in leaves and achenes. The lower level of Ni (10 mg L^{-1}) had a non-significant effect on various yield attributes, but higher Ni levels considerably decreased these parameters. Higher Ni levels decreased the concentrations of Ca, Mn and Fe in achenes. In contrast, achene N, K, Zn, Mn and Cu decreased consistently with increasing level of Ni, even at lower level (10 mg L^{-1}). Sunflower hybrid Hysun-33 had better yield and higher most of the nutrients in achenes as compared with SF-187. The maximum reduction in all parameters was observed at the maximum level of nickel (40 mg L^{-1}) where almost all parameters were reduced more than 50% of those of control plants. In conclusion, the pattern of uptake and accumulation of different nutrients in sunflower plants were nutrient- and cultivar-specific under Ni-stress.

1. Introduction

Nickel is one of the 23 metals that are of great concern to environment and human health [1,2]. Nickel is the 24th most abundant elements (twice as Cu) forming about 0.008% of the earth crust, and hence, it is a natural constituent of soil (parent material) and water [3,4]. Nickel is emitted in the environment from a variety of natural and anthropogenic sources including industries [5]. It is among the most commonly encountered heavy metals that cause toxicity in plants and animals [3]. This metal (Ni) can be easily taken up by plants and tend to bioaccumulate in different organs [6]. It can ultimately enter the human body through ingestion of food, use of metal contaminated water or breathing in air containing toxic levels of Ni [2,7].

In plants, the high concentration of Ni has been reported to impose deleterious effects on plant growth and metabolism and produce visible signs of toxicity. The general signs associated with Ni toxicity in plants include inhibition of germination [8], reduced shoot and root growth [9], poor development of branching system [10], deformation of various plant parts [11], abnormal flower shape [12,13], decreased biomass production [9,14], leaf spotting [15], mitotic root tip disturbances [13], Fe deficiency that induces chlorosis [16,17] and foliar necrosis [18]. Excess Ni also affects nutrient absorption by roots [9,12], impairs plant metabolism [14], and inhibits photosynthesis and transpiration [19,20]. Ultimately, all these processes lead to reduced yield of agricultural crops [21,22].

Nickel has been reported to be translocated through phloem to the aerial parts including stem and leaves and it ultimately accumulates in fruits and seeds [10,13,23]. However, its uptake and distribution within the plant tissues (root, stem, leaves) and parts of a seed varies greatly depending upon plant species and other factors such as interaction with other soil nutrients, soil physico-chemical properties and pathogenic or insect infection [24]. Nickel is highly mobile in plants and can be easily retranslocated from old to young leaves [25,26]. Evidence shows that it also translocates to the neonatal parts of plants such as buds, fruits and seeds through the phloem [23,27]. For example, in seeds of *Stackhousia tryonii*, a metal hyperaccumulator species, Ni has been reported to be partitioned to the pericarp (fruit wall), while very little amount of Ni was partitioned within endospermic and cotyledonary tissues [28]. Thus, if high Ni containing vegetative parts or seeds are consumed by animals or humans, it can cause severe toxicity in these organisms.

Keeping in view these aspects, the present study was conducted to assess the effect of nickel on growth, yield and yield components of sunflower. In addition, changes in accumulation of macro- (K, Ca and Mg), micro-nutrients (Zn, Mn, Fe, Cu) as well as of Ni in different

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[*] Corresponding author. Tel.: +92 41 9200312; fax: +92 41 9200764.
E-mail address: sajidakeel@yahoo.com (M.S.A. Ahmad).

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plant parts mainly achenes in sunflower plants were determined in this study.

2. Materials and methods

The achenes (seeds) of five most widely cultivated sunflower hybrids, Nstt-160, Mehran-II, Hyssun-33, M-3260 and SF-187 were obtained from the Plant Genetic Resource Institute (PGRI) of the National Agricultural Research Centre (NARC), Islamabad. The seeds were surface sterilized with mercuric chloride (0.1%) for two min and sown in Petri-plates double lined with filter paper. Different levels of nickel (0, 10, 20, 30, 40, 50 and 60 mg L^{-1}) were prepared in Hoagland's nutrient solution [29] using $NiSO_4.6H_2O$ as a source of Ni metal and 10 mL of solution from each treatment were applied to each Petri-plate. The solutions were changed every-day to ensure constant level of Ni. The Petri-plates were placed in a growth room under continuous white fluorescent light (*PAR* 300 μmol m^{-2} s^{-1}) at 25 °C ± 3 °C. The number of seeds germinated were counted daily and used to calculate germination percentage (GP), seed emergence index (EI) and time to achieve 50% germination (t_{50}). Plumule and radicle lengths and concentration of different nutrients were also determined. On the basis of data obtained, a tolerant (Hysun-33) and a sensitive (SF-187) cultivar and four levels (up to 50% decrease in seed germinability parameters) of Ni (10, 20, 30 and 40 mg L^{-1}) were selected for further studies. The details of selection procedure are already reported in one of our previous publications [30].

The achenes (seeds) of two selected sunflower (*Helianthus annuus* L.) hybrids, Hyssun-33 and SF-187, were surface sterilized with 0.1% $HgCl_2$ and sown in separate plastic pots containing 10 kg thoroughly washed river sand. Different levels of Ni (0, 10, 20, 30 and 40 mg L^{-1}) were prepared in Hoagland's nutrient solution [29] using $NiSO_4.6H_2O$ and one L of the solution of an appropriate treatment was applied to each pot prior to sowing. The treatment solutions were applied every-day to ensure constant level of Ni by leaching of the previously contained solution in sand through a hole in the bottom of each pot. The pots were placed in a wire-house under natural conditions (mean day temperature 29.9 ± 5 °C, night temperature 21.1 ± 7.3 °C, relative humidity (RH) 36.1 ± 7.1%, and the day-length varied from 8 to 11 h). The experiment was laid down in a completely randomized fashion with three replicates. The achenes were allowed to germinate for a week and thinning was practiced to maintain four plants in each pot. The plants were grown to maturity (110 days) under the above-mentioned conditions and data for various growth and yield parameters, and concentration of macro- and micro-nutrients in leaves and achenes were recorded. Plants were harvested carefully, washed with tap water followed by distilled water and separated into shoots and roots. Fresh weights of roots and shoots, and root lengths were measured. At maturity, the capitula were carefully separated and dried to a constant dry weight. Data for number of achenes $capitulum^{-1}$, achene yield $capitulum^{-1}$ and 100 achene weight (g) were recorded. The concentrations of macro- and micro-nutrients in leaves and achenes were determined by the acid digestion method. The well-dried leaves and achenes were ground and 0.5 g well-ground material was digested by the wet digestion method (H_2SO_4 and H_2O_2) following Wolf [31]. The concentrations of K and Ca were determined with a flame photometer (Jenway, PFP-7, UK), whereas those of Mg, Zn, Mn, Fe, Cu and Ni determined with an atomic absorption spectrophotometer (AAnalyst 300, Perkin-Elmer, Germany). The concentration of N was estimated by the Kjeldahl method as described by Bremner [32] using a micro-Kjeldahl ammonia distillation unit (Behr Labor-Technik GmbH, Behrotest® InKjel, Germany). The concentration of P was estimated following Jackson [33]. Two milliliters of Barton reagent were added to 5 mL of the acid-digested extract and total volume was made up to 50 mL using distilled H_2O. The samples were kept for half an hour and then run through spectrophotometer (Hitachi, 2001, Japan) at 460 nm. Actual amount of phosphorus was calculated using a standard curve developed using the known standards of K_2PO_4. The data so obtained were subjected to a two-way analysis of variance (ANOVA) keeping treatments (T) and hybrids (H) as main factors, using a COSTAT computer package (CoHort Software, 2003, Monterey, California). Least significance difference (LSD) values (at 5% probability) were also calculated to compare the mean values. The Pearson's correlation coefficient between different parameters was calculated using the above-mentioned statistical package.

3. Results

Application of Ni-stress had a significant adverse effect on growth, yield and concentrations of macro- (N, P, K, Ca and Mg) and micro-nutrients (Zn, Mn, Fe and Cu) in the leaves and achenes of both sunflower hybrids. Statistical analysis revealed highly significant differences for hybrid (H) and treatment (T) terms, whereas H × T interaction term was non-significant for shoot and root fresh weights, as well as root length recorded in this study (Table 1). It was observed that root and shoot fresh weights, and root length decreased consistently with increase in Ni concentration in the rooting medium, the lowest Ni concentration (10 mg L^{-1}) being the least, while the highest (40 mg L^{-1}) the most effective in causing reduction in these parameters. Hybrid Hysun-33 showed less reduction in fresh biomass and concentrations of macro- and micro-nutrients analyzed in this study as compared to that in SF-187 (Fig. 1).

Nickel stress had a significant effect on all yield attributes observed in this study. Analysis of variance of the data showed highly significant differences for both hybrid (H) and treatment (T) terms for number of achenes $capitulum^{-1}$, achene yield $capitulum^{-1}$ and 100 achene weight. However, the H × T interaction term was significant only for number of achenes $capitulum^{-1}$ (Table 1). Generally, the application of Ni significantly reduced the number of achenes $capitulum^{-1}$, achene yield $capitulum^{-1}$ and 100 achene weight of both sunflower hybrids. However, reduction in yield was non-significant at lower level of Ni application (10 mg L^{-1}), thereafter all yield parameters decreased consistently in both sunflower hybrids. The maximum reduction in yield was observed at the highest level of Ni (40 mg L^{-1}) at which all yield parameters reduced more than 50% as compared to those of control plants (Fig. 1). The calculation of correlation coefficients revealed that reduction in all these yield parameters under Ni stress was positively correlated with reduction in growth and concentration of macro- and micro-nutrients. In addition, the reduction in yield was found to be associated with high accumulation of Ni in both cultivars (Table 2).

The concentrations of macro-nutrients (N, P, K, Ca and Mg) in the leaves and achenes of both sunflower hybrids were also significantly affected by the application of Ni to the rooting medium. Both sunflower hybrids (H) differed significantly for concentrations of N, P, K and Ca in the leaves as well as achenes. However, Mg content differed significantly only in the leaves of both hybrids and non-significant difference was observed for achene Mg contents. The treatment (T) term was significant for N, K and Ca contents in achenes as well as leaves, whereas, it was non-significant for P and Mg contents in achenes. The interaction (T × H) term was non-significant for all macro-nutrients (N, P, K, Ca and Mg) determined in this study (Table 1). It was observed that the concentrations of N and K in the leaves as well as achenes decreased consistently in both hybrids with increase in external Ni regimes. Although, leaf P contents were decreased markedly due to Ni-stress, the achene P content was not affected by the metal stress. Leaf Ca con-

Table 1
Mean sum of squares from analyses of variance (ANOVA) for data for growth, yield and concentrations of macro- and micro-nutrients in two sunflower hybrids subjected to different levels of nickel in sand culture.

Sources of variation	d.f.	Shoot f. wt.	Root f. wt.	Root length	# of achenes/capitulum
Hybrids (H)	1	1073.96***	140.29***	170.65	929.63***
Treatments (T)	4	557.16***	20.38***	44.74	1226.42***
H × T interaction	4	3.32 ns	0.59 ns	0.82	133.38*
Error	20	30.78	2.03	3.58	31.53

Sources of variation	d.f.	Achene yield/capitulum	100 achene weight	Leaf N	Achene N
Hybrids (H)	1	2.87***	10.01***	51.17***	83.86***
Treatments (T)	4	8.09***	9.48***	46.75***	132.28***
H × T interaction	4	0.32 ns	0.52 ns	6.10 ns	1.57 ns
Error	20	0.19	0.27	2.94	7.60

Sources of variation	d.f.	Leaf P	Achene P	Leaf K	Achene K
Hybrids (H)	1	1.43*	0.717**	54.67***	25.91***
Treatments (T)	4	1.63**	0.099 ns	62.45***	34.48***
H × T interaction	4	0.28 ns	0.149 ns	2.22 ns	0.80 ns
Error	20	0.28	0.057	3.29	1.57

Sources of variation	d.f.	Leaf Ca	Achene Ca	Leaf Mg	Achene Mg
Hybrids (H)	1	6.17*	1.75*	0.35989**	0.0070 ns
Treatments (T)	4	3.09*	2.44**	0.31386***	0.0014 ns
H × T interaction	4	0.34 ns	0.10 ns	0.01612 ns	0.0020 ns
Error	20	0.85	0.40	0.03656	0.1368

Sources of variation	d.f.	Leaf Zn	Achene Zn	Leaf Mn	Achene Mn
Hybrids (H)	1	0.0093***	0.016 ns	0.14214***	0.0574***
Treatments (T)	4	0.0028***	0.231***	0.04027***	0.1050***
H × T interaction	4	0.0001 ns	0.012 ns	0.00106 ns	0.0041 ns
Error	20	0.0002	0.031	0.00246	0.0025

Sources of variation	d.f.	Leaf Fe	Achene Fe	Leaf Cu	Achene Cu
Hybrids (H)	1	2.14936*	0.3652**	0.00303*	0.0000 ns
Treatments (T)	4	2.56661***	0.3003***	0.00446***	0.0249***
H × T interaction	4	0.06334 ns	0.0040 ns	0.00013 ns	0.0009 ns
Error	20	0.28402	0.0397	0.00038	0.0012

Sources of variation	d.f.	Leaf Ni	Achene Ni
Hybrids (H)	1	0.03363***	0.0439***
Treatments (T)	4	0.12113***	0.3756***
H × T interaction	4	0.00401**	0.0124**
Error	20	0.00077	0.0020

ns = non-significant.
* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

Table 2
Pearson correlation coefficient (r) for growth and leaf nutrient concentration with achene yield and achene nutrient concentration of two sunflower hybrids subjected to Ni stress.

	Shoot f. wt.	Root f. wt.	Root length	Leaf N	Leaf P	Leaf K	Leaf Ca	Leaf Mg	Leaf Zn	Leaf Mn	Leaf Fe	Leaf Cu	Leaf Ni
# of achene	0.79***	0.68***	0.78***	0.84***	0.63***	0.84***	0.65***	0.75***	0.78***	0.74***	0.75***	0.77***	−0.84***
Achene yield	0.82***	0.72***	0.79***	0.78***	0.64***	0.84***	0.67***	0.71***	0.74***	0.72***	0.72***	0.72***	−0.89***
100 Achene wt.	0.86***	0.77***	0.77***	0.79***	0.57***	0.86***	0.73***	0.73***	0.83***	0.75***	0.68***	0.69***	−0.81***
Achene N	0.85***	0.67***	0.67***	0.80***	0.70***	0.72***	0.66***	0.78***	0.77***	0.79***	0.83***	0.76***	−0.85***
Achene P	0.61***	0.73***	0.65***	0.60***	0.59***	0.59***	0.55**	0.54**	0.57***	0.54**	0.52**	0.43*	−0.52**
Achene K	0.82***	0.80***	0.76***	0.76***	0.66***	0.82***	0.62***	0.76***	0.81***	0.79***	0.82***	0.69***	−0.87***
Achene Ca	0.63***	0.59***	0.62***	0.69***	0.50**	0.63***	0.36 ns	0.56**	0.62***	0.55**	0.51**	0.67***	−0.67***
Achene Mg	0.04 ns	0.19 ns	0.06 ns	0.09 ns	0.08 ns	−0.06 ns	−0.11 ns	−0.07 ns	0.12 ns	0.08 ns	0.03 ns	0.01 ns	−0.01 ns
Achene Zn	0.57**	0.36 ns	0.36 ns	0.58***	0.46*	0.52**	0.27 ns	0.47**	0.43*	0.45*	0.61***	0.61***	−0.65***
Achene Mn	0.80***	0.68***	0.72***	0.80***	0.54**	0.84***	0.72***	0.81***	0.80***	0.74***	0.72***	0.71***	−0.83***
Achene Fe	0.80***	0.65***	0.69***	0.69***	0.60***	0.69***	0.61***	0.58***	0.66***	0.68***	0.70***	0.44*	−0.65***
Achene Cu	0.62***	0.45*	0.54**	0.62***	0.56**	0.64***	0.46*	0.56**	0.59***	0.66***	0.75***	0.68***	−0.76***
Achene Ni	−0.81***	−0.66***	−0.73***	−0.84***	−0.72***	−0.84***	−0.66***	−0.77***	−0.74***	−0.76***	−0.82***	−0.79***	0.95***

ns = non-significant.
* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

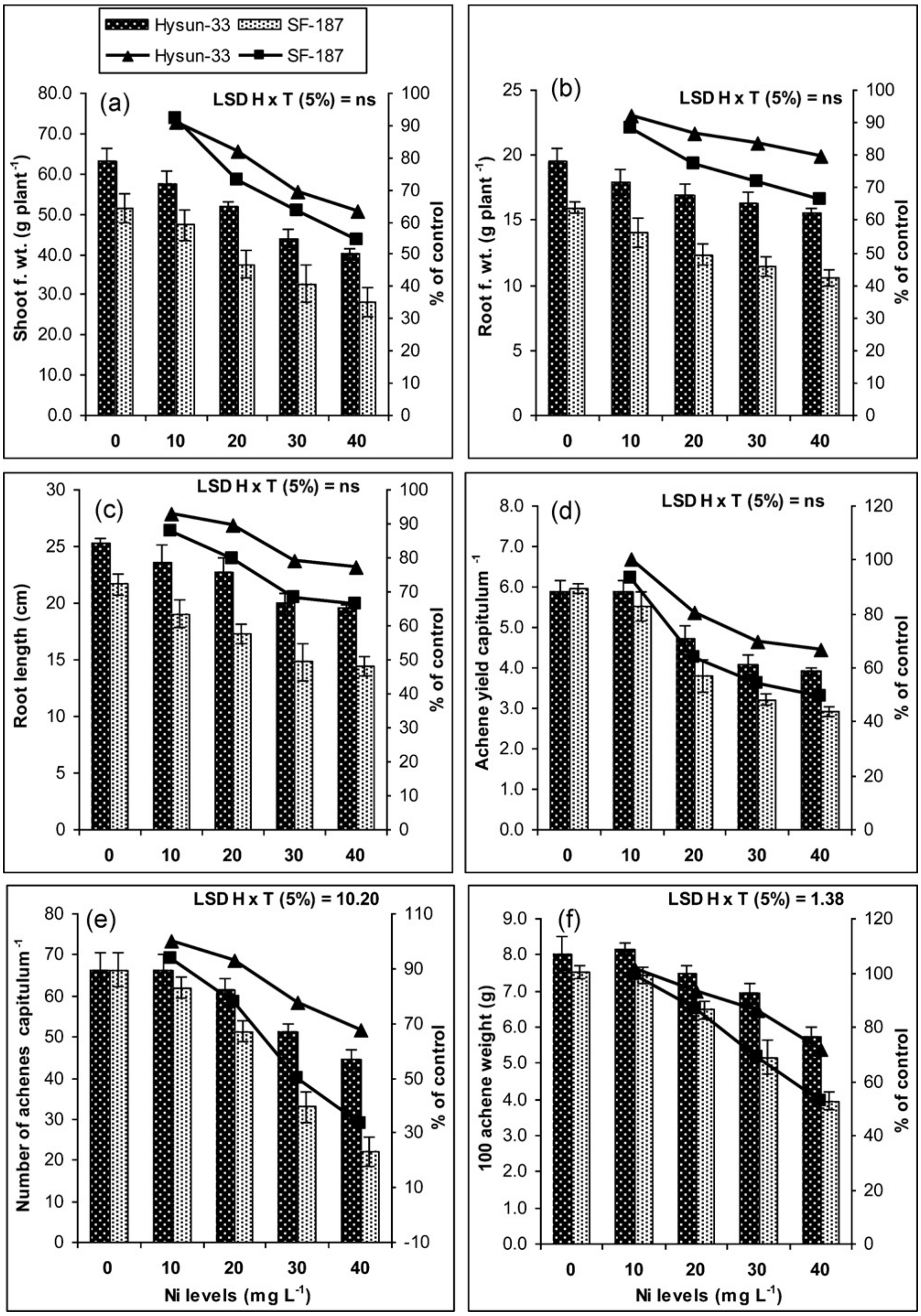

Fig. 1. Growth and yield attributes of two sunflower hybrids subjected to different levels of nickel in sand culture. Lines on each bar represent SE values.

tents were not much affected in cultivar Hysun-33 at lower level of nickel application ($10\,\mathrm{mg\,L^{-1}}$), but they decreased consistently with a further increase in external Ni concentration. In contrast, a marked reduction in leaf Ca contents was observed in cultivar SF-187. Achene Ca content increased at $10\,\mathrm{mg\,L^{-1}}$ in both cultivars, but it decreased with a further increase in external Ni level (Figs. 2 and 3). The correlation coefficient (r) revealed that decrease in achene N, P, K, Ca and Mg in both cultivars was highly correlated with reduced growth accompanied by a decrease in concentrations of these macro-nutrients as well as increase in concentration of Ni in leaf tissue. Cultivar Hysun-33 was superior in Ni tolerance as it showed less decrease in concentrations of macro-nutrients recorded in this study (Table 2).

Nickel stress significantly reduced the concentrations of micro-nutrients (Zn, Mn, Fe and Cu) in the leaves as well as achenes of both sunflower hybrids. For hybrid (H) term, the reduction was significant for Zn, Mn, Fe and Cu contents in leaves while only Mn and Fe contents differed significantly in achenes of the sunflower plants. These differences were non-significant for achene Zn and Cu concentrations. The treatment (T) term was significantly different for concentrations of all these micro-nutrients (Zn, Mn, Fe and Cu) in leaves as well as achenes. The H × T interaction term was non-significant for all these micro-nutrients (Table 1). The reduction in concentrations of these nutrients in achenes was highly correlated with their reduction as well as multifold increase in Ni concentration in leaf tissues (Table 2). Generally, cultivar Hysun-33 showed

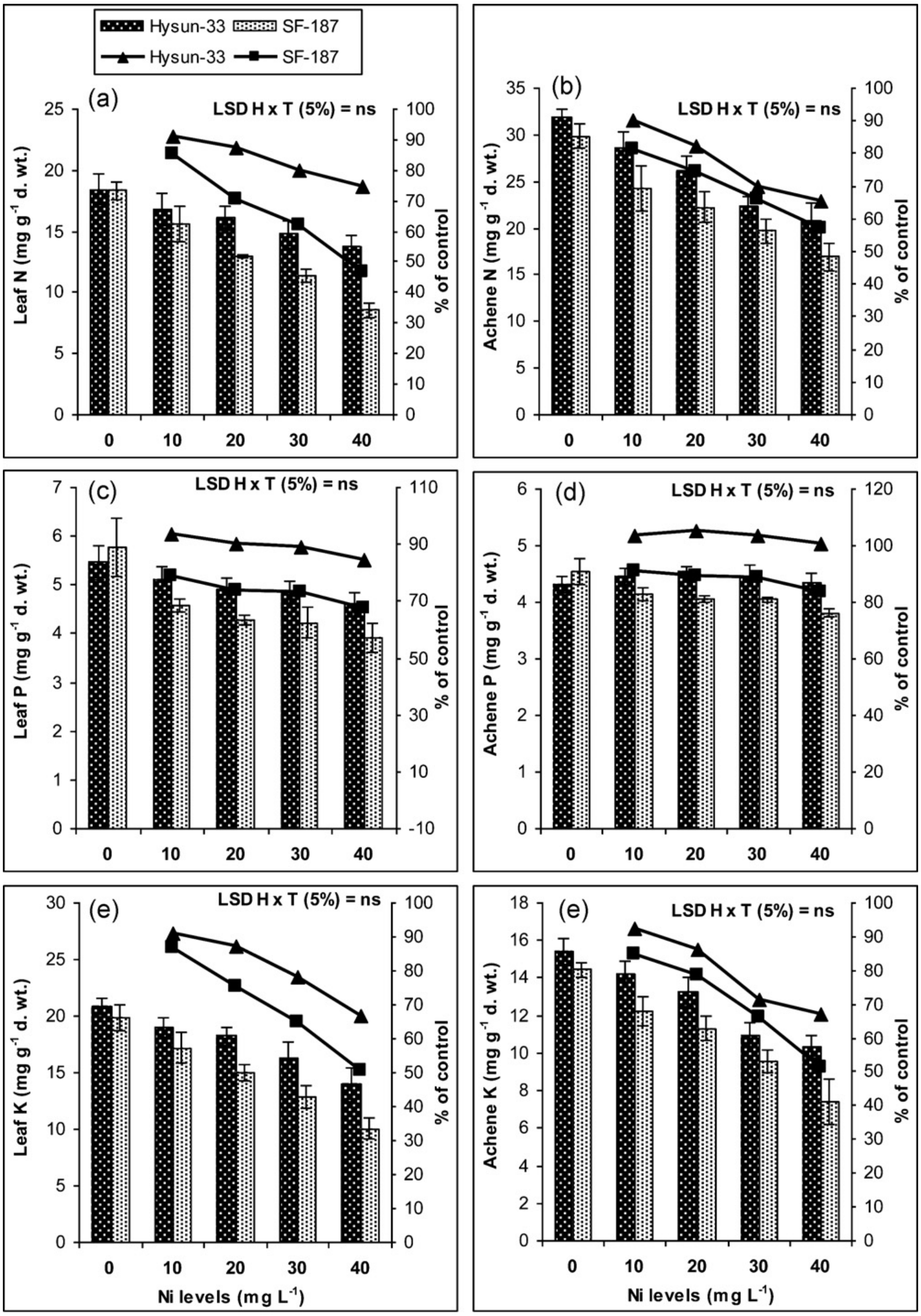

Fig. 2. Concentrations of N, P and K in leaves and achenes of two sunflower hybrids subjected to different levels of nickel in sand culture. Lines on each bar represent SE value.

less decrease in concentration of all the micro-nutrients (Zn, Mn, Fe and Cu) in leaves as well as achenes with increase in external Ni concentration. However, this was not true for achene Zn concentration which was higher in cultivar SF-187 at all levels of Ni application (Figs. 3 and 4). It was noted that reduction in achene N, P, K and Ca was highly associated with reduction in concentrations of these nutrients in leaf tissue. However, achene Mg contents did not show a significant correlation with growth or concentration of any of these nutrients recorded in this study (Table 2).

A multifold increase in Ni contents in leaves as well as achenes was observed in both hybrids with increase in Ni level of the growth medium. Statistical analysis revealed that hybrid (H), treatment (T) as well as H × T interaction terms were significantly different for accumulation of Ni in leaves as well as achenes (Table 1). Cultivar SF-187 accumulated considerably higher amount of Ni in leaves as well as achenes as compared to that of Hysun-33 (Fig. 5). It was observed that all yield and nutrient accumulation parameters were negatively correlated with high nickel accumulation in leaf tissue, except for increase in achene Ni which was positively associated ($r = 0.95$) with increase in Ni concentration in leaves of Ni-stressed sunflower plants (Table 2).

4. Discussion

Nickel stress had a significant adverse effect on all yield attributes of sunflower plants in this study. This is parallel to

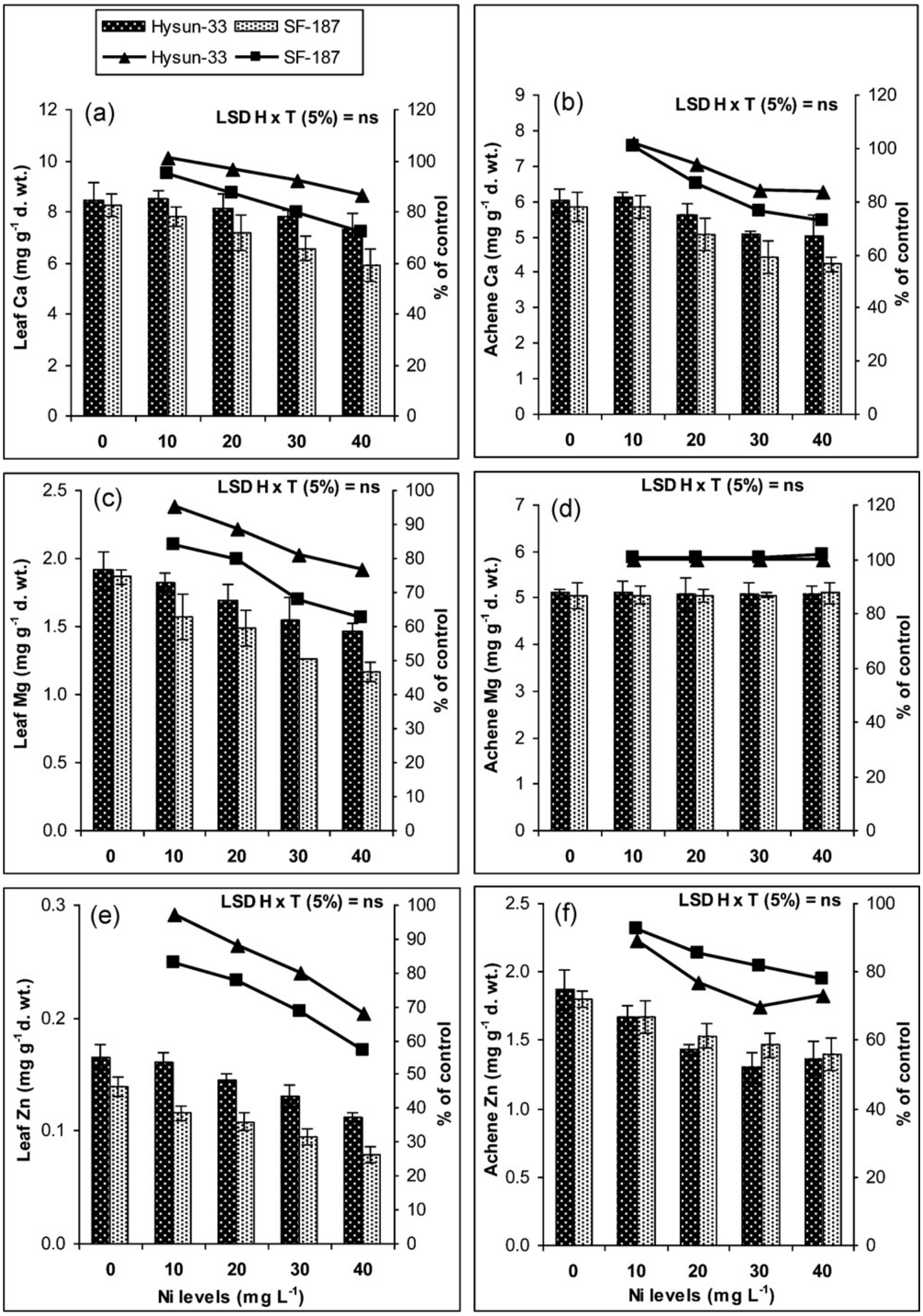

Fig. 3. Concentrations of Ca, Mg and Zn in leaves and achenes of two sunflower hybrids subjected to different levels of nickel in sand culture. Lines on each bar represent SE values.

what has been earlier observed in different studies with different crops, e.g. mungbean [22], tomato [21], cucumber [34,35] and sunflower [36]. In our study, the reduction in number of achene capitulum^{-1}, achene yield, and 100 achene weight was found to be highly correlated with reduction in shoot and root fresh biomass as well as concentrations of macro- and micro-nutrient in the leaves of Ni-stressed sunflower plants. In addition, the reduction in yield was also strongly correlated with high nickel accumulation in leaves ($r = -0.84$) (Table 2). The root system of Ni-treated sunflower plants was found to be less developed than that of control plants. The Ni-induced retardation of root growth resulted in reduced shoot growth and disturbed mineral nutrition in sunflower plants.

The concentrations of macro-nutrients (N, P, K and Ca) except Mg in achenes of both sunflower hybrids were found to be consistently reduced with increase in Ni level of the rooting medium. The pattern of reduction in the concentration of these nutrients in achenes was found to be similar to that of leaves. It clearly shows that Ni-stress adversely affected the uptake of these nutrients into both leaves and achenes. However, in contrast, achene Mg content was not affected in sunflower, although leaf Mg content decreased consistently with increase in external Ni level. Despite the fact that Mg is one of the mobile elements [37], so it can easily move from one part to the other, the lack of relationship of leaf Mg with achene Mg is not easy to explain. During seed development, seed is the main sink to which assimilates as well as mineral nutrients are

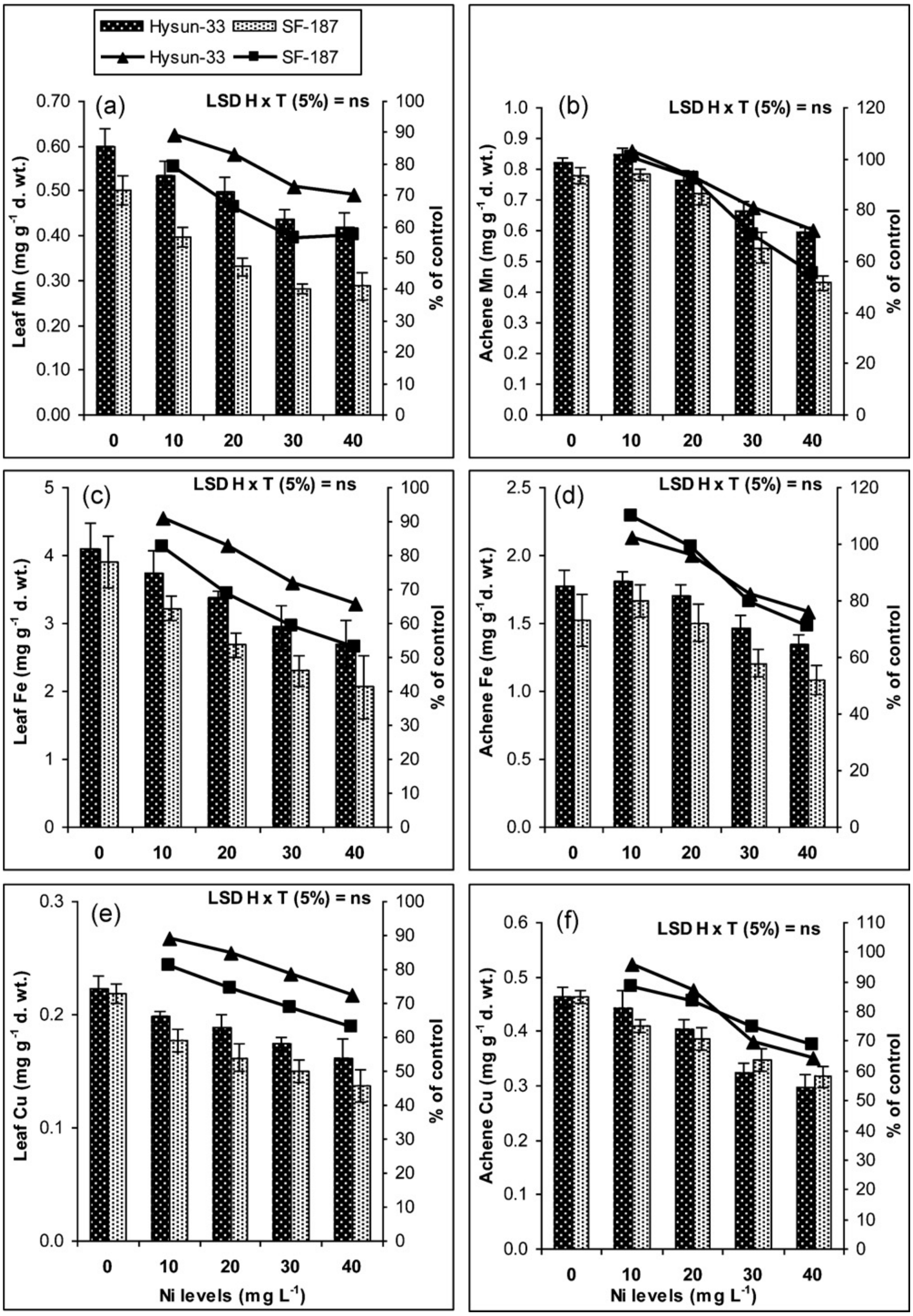

Fig. 4. Concentrations of Mn, Fe and Cu in leaves and achenes of two sunflower hybrids subjected to different levels of nickel in sand culture. Lines on each bar represent SE values.

transported from different sources, mainly leaves [38]. However, it has been reported that nutrient movement is not always coupled to assimilate movement to develop seeds [39]. Thus, maintenance of almost consistent levels of Mg in sunflower achene might have been affected due to processes other than those of leaves.

As observed in our study, application of Ni-stress severely affected root proliferation. Thus, reduced root development under high Ni application interfered with the uptake of different nutrients as has earlier been observed in different studies [9,12]. For example, reduced N, P and K contents have been reported in plant tissues under Ni application [40,41]. In addition, some earlier studies on photosynthesis of plant leaves have shown that Ni can competitively remove Ca ion from its binding site in the oxygen evolving complex [42] and replace the Mg ion of chlorophyll pigment [43,44], which eventually inhibits the PSII electron transport. Thus, reduced energy supply for nutrient uptake may result in reduced nutrient uptake and nutrient deficiency in plants tissues as well as achenes under metal stress [22,45–47].

Nickel stress significantly reduced the concentrations of achene Zn, Mn, Fe and Cu. The reduction in concentration of these nutrients in achenes was found to be highly correlated with the reduction of these nutrients in leaves as well as multifold increase in Ni concentration in leaf tissues ($r = 0.95$) (Table 2). This shows that

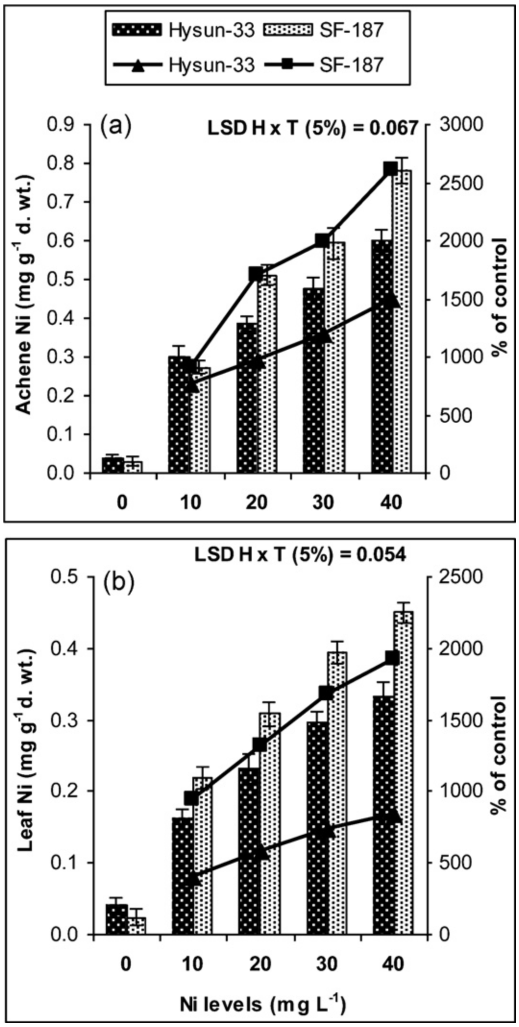

Fig. 5. Concentrations of Ni in leaves and achenes of two sunflower hybrids subjected to different levels of nickel in sand culture. Lines on each bar represent SE values.

Ni was actively transported from leaves to seed through phloem, thereby causing a redaction in concentrations of other nutrients. It has been reported that Ni has similar characteristics (mass to charge ratio) to those of other nutrients such as Ca, Mg, Mn, Fe, Cu and Zn. In addition, in view of some earlier studies, it is evident that Ni is absorbed and transported by the same transport system as that for some other micro-nutrients such as Cu and Zn [43,44,48]. Thus, it can strongly compete with these nutrients in the process of absorption and translocation [9,49–51]. Such an antagonistic effect of Ni may inhibit the absorption of these nutrients under high application of Ni and decrease their concentration to deficiency level in plant tissues. Therefore, Ni-stressed plants are likely to suffer from nutrient deficiency and show clear deficiency symptoms [9,22,52,53].

As all these nutrients are involved in a number of physiological processes, so their deficiency in plants may retard a number of metabolic processes and ultimately, may result in the toxic effects of Ni in plants [15,34,54]. Subsequently, it may result in suppressed growth and development as well as reduced yield of agricultural crops as observed in this study [8,35,55,56]. In addition, some metals such as Fe, Cu, Zn and Mn are integral components of prosthetic groups of a number of metalloenzymes such as superoxide dismutase (SOD) and catalase (CAT). Since in this study, excess concentration of Ni decreased the contents of Fe, Cu and Zn in plant tissues, it is expected that excess Ni may lead to reduced biosynthesis of these metalloenzymes due to deficiency of these mineral nutrients [15,57]. Thus, Ni-stressed plants may suffer from increased oxidative stress that suppresses vegetative growth by damaging bio-molecules, ultimately resulting in poor yield, and nutrient uptake and translocation to the achenes of Ni-stressed plants [15,36,58,59].

5. Conclusion

Application of Ni-stress decreased root and shoot fresh weights, and concentrations of micro- and macro-nutrients in leaf tissues of sunflower plants. Although, achene yield and accumulation of some nutrients such as Ca, Mn and Fe were not much affected at lower level of Ni application (10 mg L^{-1}), considerable reduction in yield and nutrient accumulation was found at higher Ni levels. In contrast, the accumulation of other nutrients such as N, K, Zn, Mn, and Cu showed a consistent reduction in sunflower achenes at all exogenous Ni levels, which was associated with the reduction of these nutrients in the leaves. However, achene P and Mg were not significantly affected by Ni-stress. Thus, it can be concluded that Ni-stress leads to reduction in growth and alteration in nutrient concentrations in the vegetative parts as well as achenes of sunflower plants.

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