



Toxicity and tolerance of nickel in sunflower (*Helianthus annuus* L.)

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Received: 16 June 2022 / Accepted: 30 January 2023 / Published online: 16 February 2023
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Abstract

This study aimed at exploration of nickel (Ni) application (0, 10, 20, 30, and 40 mg L⁻¹) on physiological and biochemical attributes of sunflower cultivars (Hysun-33 and SF-187) grown in sand culture. Results revealed a significant decrease in vegetative parameters in both sunflower cultivars by increasing Ni concentration, although low levels of Ni (10 mg L⁻¹) improved growth attributes to some extent. Among photosynthetic attributes, 30 and 40 mg L⁻¹ Ni application severely reduced the photosynthetic rate (*A*), stomatal conductance (*g_s*), water use efficiency (WUE), and *Ci/Ca* ratio but improved the transpiration rate (*E*) in both sunflower cultivars. The same level of Ni application also reduced leaf water potential, osmotic potentials, and relative water contents but increased leaf turgor potential and membrane permeability. At low level (10 and 20 mg L⁻¹), Ni improved the soluble proteins, while high Ni concentration decreased it. The opposite was true for total free amino acids and soluble sugars. To conclude, the high Ni concentration in various plant organs had a strong impact with the changes in vegetative growth, physiological and biochemical attributes. A positive correlation of growth, physiological, water relations, and gas exchange parameters at low levels of Ni and negative correlation at higher Ni level confirmed that the supplementation of low Ni levels greatly modulated studied attributes. Based on observed attributes, Hysun-33 showed high tolerance to Ni stress as compared to SF-187.

Keywords Growth · Nickel · Photosynthesis · Proteins · Sugars · Water relations

Introduction

Nickel is the 22nd most abundant element and is a natural constituent of the earth crust, forming about 0.008% of total elemental composition (Hedfi et al. 2007; Hussain et al. 2013). Among industrial sources, burning of oil, coal, and various fossil fuels releases significant quantities of Ni to the environment. Some other industrial sources contributing toward Ni emission include mining and refining processes, synthesis of Ni alloy, electroplating, and combustion of municipal trashes. Collectively, these sources cause accumulation of toxic levels of Ni in soil and water reserves and

put several limitations on plant growth and survival (Ahmad and Ashraf 2011).

Nickel has a significant importance due to its involvement in a number of biological functions in plants. The life cycle of some plants may remain incomplete without proper availability of Ni (Kumar and Trivedi 2016; Shahzad et al. 2018). It is a part of many metallo-enzymes, like urease in plant cells (Fabiano et al. 2015). At low concentration, Ni improves plant water relation attributes viz. water potential (ψ_w), osmotic potential (ψ_s), turgor potential (ψ_p), and relative water content (Chris 2012; Jagetiya et al. 2013). It also participates in different vital metabolic activities viz. hydrogen metabolism, biogenesis of methane, and acetogenesis. It has a significant contribution in the synthesis of phytoalexin and defensive mechanism of plants to different biotic stresses (Fabiano et al. 2015). Therefore, the absence of Ni has many effects on metabolic activities that regulate plant growth and development. These wide range effects include reduction in plant growth, senescence of plants, chlorosis of young leaves, and iron uptake (Wood et al. 2006). Nickel deficiency decreases urease activity and disturbs nitrogen assimilation (González-Guerrero et al. 2014).

Responsible Editor: Gangrong Shi

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Although low Ni concentration functions as micro-nutrient in some cases, its high concentration has several phyto-toxic effects on growth and development of plants (Amari et al. 2014; Shahzad et al. 2018). The common symptoms linked with excess of Ni concentration in plants involve reduction in shoot and root length and fresh and dry weight. Other effects include reduced development of branches, deformation of different plant organs, irregular flower shape, reduced biomass, spotting of leaves, mitotic root tip disorders, reduced germination, and chlorosis following foliar necrosis (Ahmad and Ashraf 2011; Shahzad et al. 2018). Moreover, Ni toxicity causes reduction in nutrient absorption by roots as a result of poor root development (Wood 2013). High concentration of Ni can also replace cobalt and various other heavy metals, positioned at active sites in metallo-enzymes and disturb their functions (Shanying et al. 2012; Stoikou et al. 2017; Moghaddam 2018). Nickel can also have drastic effects on membrane integrity that reduces its permeability function. Other physiological phenomena affected by Ni toxicity include changes in the concentration of photosynthetic pigments resulting in reduction in net photosynthetic rate (Batool 2018), stomatal conductance, transpiration rate, and water-use efficiency (Hassan et al. 2019). Excessive Ni contents have also been shown to alter the concentration of physiologically important organic molecules such as soluble sugars (Nie et al. 2015), free amino acids (Bai et al. 2006), and soluble proteins (Duman and Ozturk 2010).

Previously, the application of higher levels of Ni has been shown to have inhibitory effects on growth and yield of many plants. In our previous paper of same series of Ni tolerance potential of sunflower, we have studied the toxicity of Ni on seed germination and growth (Ahmad et al. 2009; Ashraf et al. 2011), as well as yield and macro and micronutrient contents in sunflower cultivars (Ahmad et al. 2011). In the current paper, we have focused on the Ni-induced physiological bases of growth maintenance, photosynthetic pigments, and gas exchange attributes of two elite sunflower cultivars at vegetative stage. In view of above facts, it was hypothesized that, despite of several beneficial roles of Ni in low concentration, the presence of Ni in higher quantities should impose certain limitations on growth, photosynthetic, water relation, gas exchange attributes, and imbalance in secondary osmolytes. The objectives of the present study were (i) to determine the Ni tolerance limits of the selected sunflower cultivars, (ii) to establish whether lower levels of Ni have beneficial effects on sunflower as reported for other crops, and (iii) to investigate the possible toxicity mechanisms induced by higher Ni doses on growth, physiological and biochemical processes.

Materials and methods

Plan of experiment and treatment application

A preliminary experiment was conducted earlier to establish the tolerance of five sunflower cultivars, Nstt-160, Mehran-II, Hysun-33, M-3260, and SF-187 at seedling stage. Based on differences in seed germinability, seedling growth and nutrient acquisition under 7 different Ni levels ($0\text{--}60\text{ mg L}^{-1}$), two sunflower cultivars, i.e., Hysun-33 (tolerant) and SF-187 (sensitive) and four levels of Ni (i.e., 0, 10, 20, 30, and 40 mg L^{-1}) were selected for further experimentation in this work. Please see Ahmad et al. (2009) for details.

Ten surface-sterilized (0.1% HgCl_2) achenes of two sunflower (*Helianthus annuus* L.) cultivars (Hysun-33 and SF-187) were sown in plastic pots containing 10 kg sand. Various Ni concentrations (0, 10, 20, 30, and 40 mg L^{-1}) were administrated in Hoagland's nutrient solution using $\text{NiCl}_2 \cdot 7\text{H}_2\text{O}$. Then 2 L of each solution was added in every pot before sowing. After seed germination, plants were thinned to keep two plants per pot. The solution in each pot was drained on alternate days by adding sufficient amount of distilled water. After half an hour, pots were saturated with new solutions to maintain constant level of Ni. The pots were kept in natural environment in the Botanical Garden of the Department of Botany, University of Agriculture, Faisalabad. Through the course of the experiment, the meteorological conditions were as follows: mean day temperature, $31.4 \pm 5\text{ }^\circ\text{C}$; night temperature, $16.9 \pm 6.7\text{ }^\circ\text{C}$; relative humidity (RH), 35.1 ± 8.1 ; and the day length was 9.5 to 10.5 h. The phyto-toxic effects of Ni on seed germination (Suppl. Figure 1) have already been reported in Ahmad et al. (2009). This manuscript reports the effects of Ni stress on growth, physiological and biochemical attributes of sunflower at vegetative stage (Suppl. Figure 2). All data reported in the present manuscript were recorded after 5 weeks of germination.

Growth attributes

After careful uprooting from every pot, plants were washed thoroughly with distilled water. Their roots and shoots were detached and weighed. Separated roots and shoots were then wrapped in paper bags, dried at $70\text{ }^\circ\text{C}$ in an oven for a period of 72 h, and, then, dried weights were measured.

Determination of Ni contents

The concentration of Ni (mg g^{-1} d. wt.) in roots, stems, and leaves was measured in acid-digested material according to the method of Wolf (1982). The quantitative determination of Ni was performed by Atomic Absorption

Spectrophotometer (AAAnalyst-300, Perkin Elmer, Germany) and expressed as mg L^{-1} dry weight of plant tissue.

Concentration of photosynthetic pigments

For the estimation of Chl *a* and *b*, the procedure given by Arnon (1949) was followed, while carotenoid contents were found by following Davis (1976). Fresh leaves (0.2 g) were ground and extracted in acetone (80%), followed by centrifugation at 10,000 g for 5 min at 4 °C. The supernatant was separated and absorbance was noted at 645, 663, and 480 nm for Chl *a*, *b*, and carotenoids, respectively, by using a spectrophotometer Hitachi 220 (Japan). The chlorophylls and carotenoid contents were determined by using formulas proposed by Arnon (1949) and Davis (1976), respectively.

Gas exchange attributes

For the determination of gas exchange attributes, data were collected at 10.30 a.m. to 1:30 p.m. The measurements for net CO_2 assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), and sub-stomatal CO_2 concentration (C_i) were done on a fully extended youngest top leaf with the help of an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). The specification adjustments of IRGA were as follows: leaf surface area, 11.35 cm^2 ; ambient CO_2 concentration (C_{ref}), $342.12 \mu\text{mol mol}^{-1}$; temperature of leaf chamber (T_{ch}) varied from 39.2 to 43.9 °C; leaf chamber volume gas flow rate (v), 396 mL min^{-1} ; leaf chamber molar gas flow rate (*U*), $251 \mu\text{mol s}^{-1}$; ambient pressure (*P*), 99.95 k Pa; molar flow of air per unit leaf area (U_s), $221.06 \text{ mol m}^{-2} \text{ s}^{-1}$; maximum PAR (*Q* leaf) at leaf surface was $918 \mu\text{mol m}^{-2}$.

Leaf water relation

Leaf water potential was determined at 6:00 to 8:00 a.m. A completely extended smallest leaf was removed from each plant, and water potential was measured with a Scholander-type pressure chamber (Arimad-2, Japan). The same leaf was frozen at 70 °C in an ultra-low freezer for 2 weeks (Boyer 1967). Then, the leaf was thawed and extracted by using glass rod for the measurement of osmotic potential. The supernatant (10 mL) was put in a vapor pressure osmometer (Vapro-5520, Wescor, Inc., Logan, UT), and the values were calculated in MPa. The leaf turgor potential was found by the following equation:

$$\psi_p = \psi_w - \psi_s$$

Relative water content

Leaves sampled at a similar level, other than those used for the determination of leaf water potential, were used to

estimate relative water content. Twenty circles of 5-mm diameter were separated with the help of cork borer, and the fresh weight was measured. The circles then were put in distilled water for 2 h at room temperature, and saturated weight was determined. The leaf disks were then wrapped in paper bags, dried in an oven for 72 h at 70 °C, and dry weights were measured. The relative water content (RWC) was calculated with the following formula:

$$\text{Relative water content} = (\text{Fresh weight} - \text{Dry weight}) / (\text{Saturated weight} - \text{Dry weight}) \times 100.$$

Relative membrane permeability/electrolyte leakage

The electrolyte leakage was determined by following Yang et al. (1996). A completely mature small leaf from each plant was excised into small circles. Then, 0.5-g material was placed into test tubes having 20-mL deionized distilled water, following vortexing for 3 s, and initial electrical conductivity (EC_0) of each plant sample was measured. The plant samples were then kept at 4 °C for 24 h, and electrical conductivity (EC_1) was measured. The sample material was autoclaved at 121 °C for 15 min, and then cooled to room temperature, and electrical conductivity (EC_2) was determined. The relative membrane permeability was measured by the following formula:

$$\text{Relative permeability}(\%) = \frac{(EC_1 - EC_0)}{(EC_2 - EC_0)} \times 100$$

Total soluble sugars

The estimation of reducing and non-reducing sugars was carried out by following Riazi et al. (1985). Fresh leaves were harvested and immediately cooled at 0 °C. The leaves were stored at 40 °C for further use. The leaf (1 g) was grounded in 10 mL of 80% ethanol (v/v). The grinded leaf material was centrifuged at 10,000 g, and the supernatant was collected for the determination of sugars. Reducing sugars were determined from ethanolic extract of leaves. One mL of the extract and 5 mL of *O*-tolidine were put in a test tube. This solution was shaken well and heated at 97 °C for 15 min and cooled in ice-cold water. The absorbance was read at 630 nm on a spectrophotometer Hitachi 220 (Japan). The concentrations of total reducing sugars were determined from a standard curve, made by using known concentrations of glucose.

For the determination of non-reducing sugars, ethanolic extracts of leaves (0.1 mL) were mixed with 0.1 mL 5.4 N KOH in test tubes, heated at 97 °C for 10 min and cooled

in ice-cold water. Then, 3 mL of anthrone was added, and the tubes were heated again (97 °C, 5 min), cooled down and kept at room temperature for 20 min. The absorbance was noted at 625 nm by using a spectrophotometer Hitachi 220 (Japan). The concentrations of total non-reducing sugars were determined from a standard curve formed by using known concentrations of glucose. The values of reducing and non-reducing sugars were summed in each treatment to get total soluble sugars.

Total soluble proteins

Total soluble protein contents were determined by following Lowry et al. (1951). A fresh leaf (1 g) was ground in 10 mL of phosphate buffer (0.2 M with pH 7.2). Then, this extract was centrifuged at 5000 g for 5 min, and the supernatant was collected for the determination of total soluble proteins. The supernatant (1 mL) was completely mixed with copper reagent solution (1 mL) in a test tube and kept at room temperature for 30 min. Then, Folin phenol reagent (0.5 mL) (diluted in a ratio of 1:1) was thoroughly mixed to it and kept at room temperature for 30 min. The absorbance of the mixture was measured at 620 nm with the help of a spectrophotometer Hitachi 220 (Japan). A blank containing the phosphate buffer and standards was also run for the

whole procedure of protein determination. Total proteins were calculated from the standard curve developed from known concentrations of bovine serum albumin (BSA).

$$\text{Proteins (mg/g)} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times \text{Concentration of standard} \times \text{dilution factor}$$

Total free amino acids

For the determination of total free amino acids, the procedure proposed by Hamilton and Van-Slyke (1943) was followed. The fresh leaf (1 g) was ground in 10 mL of phosphate buffer (0.2 M with pH 7.2). After centrifugation at 5000 g for 5 min, the supernatant was collected for the determination of total free amino acids. The extract (1 mL) was shaken well with 1 mL each of pyridine (10%) and ninhydrin (2%) in test tubes. The test tubes were heated in water bath at 100 °C for 30 min and allowed to stand at room temperature. The volume was maintained to 50 mL, and the absorbance was measured at 570 nm with the help of a spectrophotometer Hitachi 220 (Japan). A standard curve was developed with known concentration of leucine. The total free amino acids were calculated by the following formula:

$$\text{Total amino acid} = \frac{\text{Graph reading of sample} \times \text{volume of sample} \times \text{dilution factor} \times 1000}{\text{Weight of the tissue}}$$

Statistical analysis

The study was conducted in a completely randomized design (CRD) with three replicates. Two-way ANOVA was used for the statistical analysis of the measured traits by considering cultivars and treatments as independent factors (CoStat software v 6.303). LSD values computed at 5% level were used for post-hoc multiple comparisons of significant effects (Steel et al. 1996). Microsoft excel was used for the representation of data in graphical form. Clustered heatmaps, correlogram, and PCA plot were performed with R studio (Version 1.1.463, RStudio, Inc.) to draw inferences regarding response of various vegetative and physiological attributes toward different Ni application levels. The clustered heatmaps were constructed separately for both cultivars' (a) morphological, water relation, and organic osmotica (b) photosynthetic pigments and gas exchange attributes. The performance of each attribute was plotted against different Ni treatments as colored rectangles, and their correlation was expressed on a color scale from red (most positive) to blue (most negative). Different groups of plant attributes and Ni treatments were then clustered based on hierarchical clustering (hcluster), and branches were colored at first branching

level using a custom R code. A combined correlogram for all morphological, water relation, organic osmotica, photosynthetic pigments, and gas exchange attributes of both cultivars was made. The principal component analysis (PCA) was performed to visualize the influence of different Ni treatments on specific parameters of both cultivars used in this study. Before running the PCA analysis, the data was standardized in order to reduce the larger variation in numerical values of the dataset. The strength of different variables was expressed on a color scale where the bright red color expressed the highest contributory variables, while the light red color showed less contributory traits in PCA biplot.

Results

Growth attributes

Statistical analysis of the data revealed that application of Ni significantly affected shoot and root lengths and fresh and dry weights. Moreover, the ANOVA revealed that both cultivars responded differently to Ni treatment (Table 1). Results

Table 1 Analysis of variance (ANOVA) for growth and photosynthetic attributes of sunflower cultivars exposed to varying levels of nickel

SoV	df	Root length	Root f. wt	Root d. wt	Shoot length
Cultivars (C)	1	15.84 **	9.75 ***	1.01 ***	1035.47 ***
Treatments (T)	4	6.06**	0.85 ***	0.25 ***	273.68 ***
C x T interaction	4	0.56 ns	0.02 ns	0.01 ns	7.34 ns
Error	20	1.36	0.09	0.03	14.98
		Shoot f. wt	Shoot d. wt	Chl. <i>a</i>	Chl. <i>b</i>
Cultivars (C)	1	113.41 ***	1.01 ***	0.20 ***	0.12 ***
Treatments (T)	4	32.04 ***	0.25 ***	0.12 ***	0.02 ***
C×T interaction	4	0.51 ns	0.01 ns	0.02 *	0.00 **
Error	20	1.36	0.03	0.01	0.00
		Total Chl	Chl. <i>alb</i> ratio	Carotenoids	Chl/Carot. ratio
Cultivars (C)	1	0.63 ***	6.13 ***	0.75 **	0.06 ns
Treatments (T)	4	0.23 ***	0.20 ns	0.00 ns	0.22 **
C×T interaction	4	0.04 **	0.08 ns	0.06 *	0.01 ns
Error	20	0.01	0.15	0.02	0.04

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively. *ns*, non-significant

revealed cultivar × treatment (C × T) interactive effect was non-significant for various studied growth attributes. Shoot length was affected to a lesser extent, but its fresh and dry weight was consistently reduced with elevating Ni contents in the growing medium (Fig. 1; Suppl. Figure 2). Shoot length slightly improved at 10 mg L⁻¹ level of Ni application (Fig. 1a; Suppl. Figure 3). Cultivar Hysun-33 exhibited less reduction in various studied growth parameters under different levels of Ni application.

Nickel contents

The concentration of Ni in roots, stems, and leaves of sunflower plants highly increased upon exposure to Ni stress. Statistical analysis revealed highly significant C × T interaction for both cultivars exposed to different treatments (Table 2). SF-187 accumulated more Ni in all studied plant organs than Hysun-33 (Fig. 2).

Photosynthetic attributes

Results showed that the application of Ni highly affected the concentration of photosynthetic attributes including chlorophyll *a*, *b*, and carotenoids, chlorophyll *alb*, and chlorophyll/carotenoid ratio in both sunflower genotypes. The C × T interaction term was significant for chlorophyll *a*, *b*, total chlorophyll, and carotenoids (Table 1). The concentration of chlorophyll *a*, *b*, and total chlorophyll decreased steadily with increasing Ni contents in growing media, and the maximum reduction was found at 40 mg L⁻¹ Ni application. The most deleterious effects of Ni on concentration of chlorophyll *a*, *b*, and total chlorophyll were observed particularly in cultivar SF-187 (Fig. 3a, b, c). The chlorophyll/carotenoid ratio showed a consistent decrease in both cultivars by increasing Ni concentration (Fig. 3f).

Gas exchange attributes

A significant inhibitory effect of Ni (40 mg/L) was observed on photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (*g_s*), water use efficiency, and Ci/Ca ratio. Statistical analysis revealed that C × T interactive effect was non-significant for various gas exchange attributes (Table 3). A consistent decrease in *A* was observed by elevating Ni contents in growing media. The cultivar Hysun-33 showed a little decrease at higher levels of Ni as compared to that of SF-187 (Fig. 4a). The transpiration rate (*E*) increased with increasing Ni level in the growth medium. The cultivar Hysun-33 exhibited greater increase in transpiration rate as compared to that of SF-187 where *E* remained almost unaffected (Fig. 4b). However, the elevation of *E* in Hysun-33 was accompanied by a non-significant reduction in *g_s* (Fig. 4c). Both cultivars exhibited almost parallel decrease in water-use efficiency (*A/E*) and Ci/Ca ratio (Fig. 4d, e).

Water relation attributes

Leaf water relations including water potential (ψ_w), osmotic potential (ψ_s), turgor potential (ψ_p), relative water contents (RWC), and relative membrane permeability (RMP) were also affected by Ni application. Analysis of variance revealed that the C × T interaction term was not statistically significant for most of the attributes studied (Table 3). Leaf ψ_w mostly remained unaffected as no significant effects for the cultivar and treatment C × T interaction terms were observed for this trait. The ψ_s improved steadily with the elevating Ni level in the root environment. Cultivar Hysun-33 showed greater increase in both these parameters as compared to SF-187. A rise in ψ_p was found by increasing the Ni level in

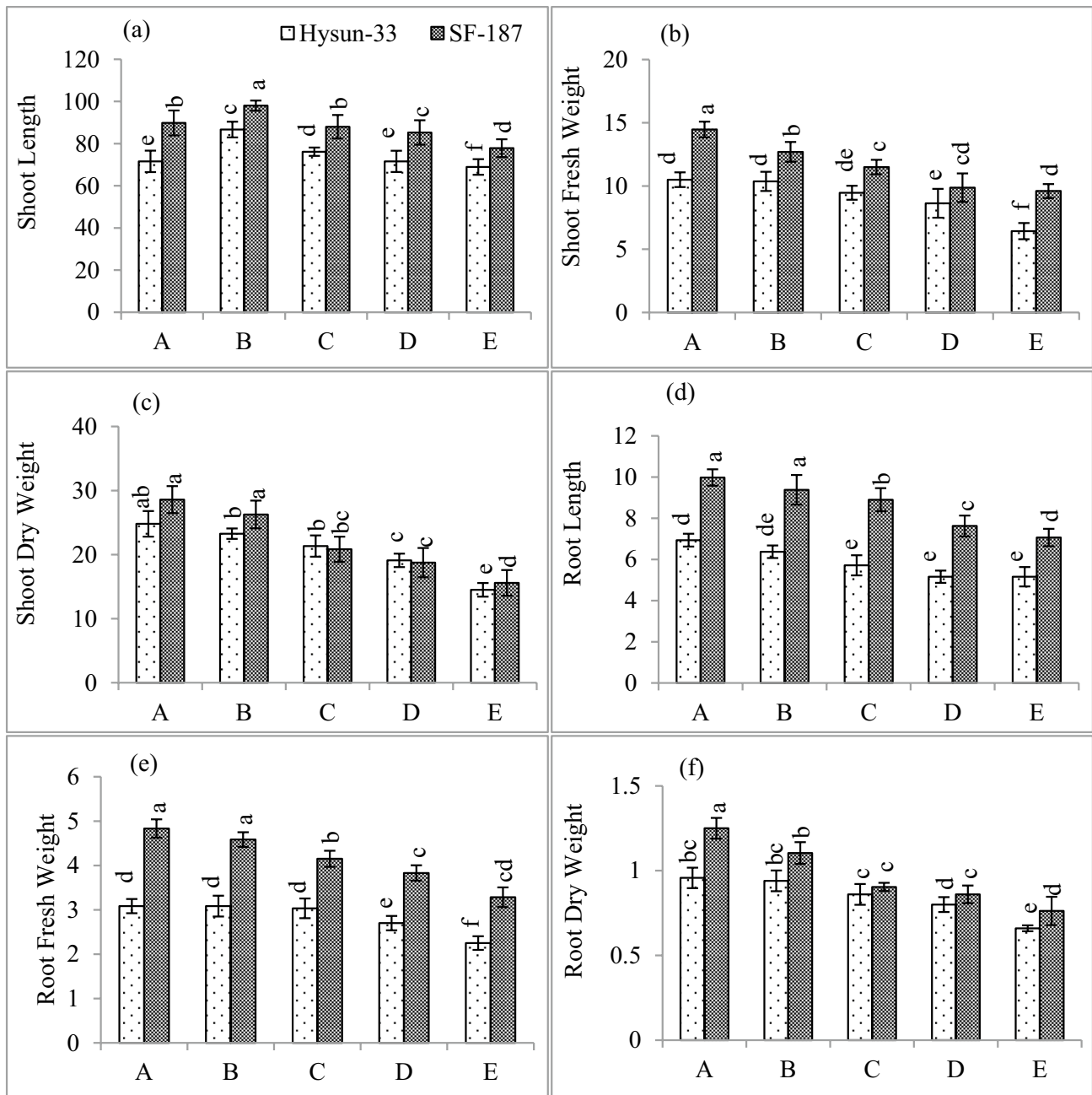


Fig. 1 Effect of different levels of nickel (A=0 mg/L, B=10 mg/L, C=20 mg/L, D=30 mg/L, E=40 mg/L) on shoot length (cm) (a), shoot fresh weight (g) (b), shoot dry weight (g) (c), root length (cm)

(d), root fresh weight (g) (e), and root dry weight (g) (f) of two sunflower (*Helianthus annuus* L.) cultivars. Standard error is presented as vertical line on bars

Hysun-33 (Fig. 5c). Relative water contents (RWC) consistently decreased with increasing concentration of Ni, where the highest reduction was observed at 40 mg L⁻¹. Though the inter-cultivar differences for RWC were statistically non-significant (Table 1), the effect of Ni treatments was highly significant, where the cultivar Hysun-33 showed slightly better RWC at all Ni levels (Fig. 5d). The relative membrane permeability increased by elevating Ni contents in the rooting zone. The cultivar Hysun-33 showed less electrolyte leakage,

indicating greater membrane stability, while electrolyte leakage increased in SF-187, indicating greater membrane damage at higher Ni concentration (Fig. 5e).

Primary osmolytes

Statistical analysis showed a significant effect on the concentration of various biochemical molecules including soluble proteins, amino acids, and soluble sugars by

Table 2 Analysis of variance (ANOVA) for concentration of Ni (mg g^{-1} d. wt.) in roots, stems, and leaves of sunflower cultivars exposed to varying levels of nickel

SoV	df	Leaf Ni	Shoot Ni	Root Ni
Cultivars (C)	1	0.03 ***	0.03 ***	0.04 ***
Treatments (T)	4	0.07 **	0.08 ***	0.12 ***
C×T interaction	4	0.00 ns	0.00 ns	0.00 *
Error	20	0.00	0.00	0.00

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively. ns, non-significant

imposition of Ni stress. The interactive effect of Ni on cultivars was non-significant for concentration of all studied osmolytes in sunflower plants (Table 3). The concentration of soluble protein was slightly increased at low levels of Ni (10 mg L^{-1}), while the differences were non-significant between 20 mg L^{-1} Ni application and control plants (Fig. 6a). The opposite trend was observed for amino acids in both cultivars, which slightly decreased at 10 mg L^{-1} Ni application but increased thereafter. The

maximum increase was observed at the highest concentration (40 mg L^{-1}) of Ni application (Fig. 6b). Changes in concentration of soluble sugars showed a mixed trend through different treatment levels. Firstly, it decreased at low level (10 mg L^{-1}) but increased consistently thereafter (Fig. 6c). SF-187 exhibited higher concentration of soluble protein, soluble sugars, and total free amino acid as compared to Hysun-33. Moreover, the total soluble protein contents were decreased by increasing Ni concentration, while soluble sugar and total free amino acids were increased by increasing Ni concentration (Fig. 6).

Heatmap clustering

The two-way clustered heatmaps were constructed to visualize the influence of different Ni levels on plant attributes in both cultivars used in this study. In clustered heatmap, the parameters were grouped depending on their similarity in response (hierarchical clustering) at specific treatment levels, and their correlation with treatments was indicated

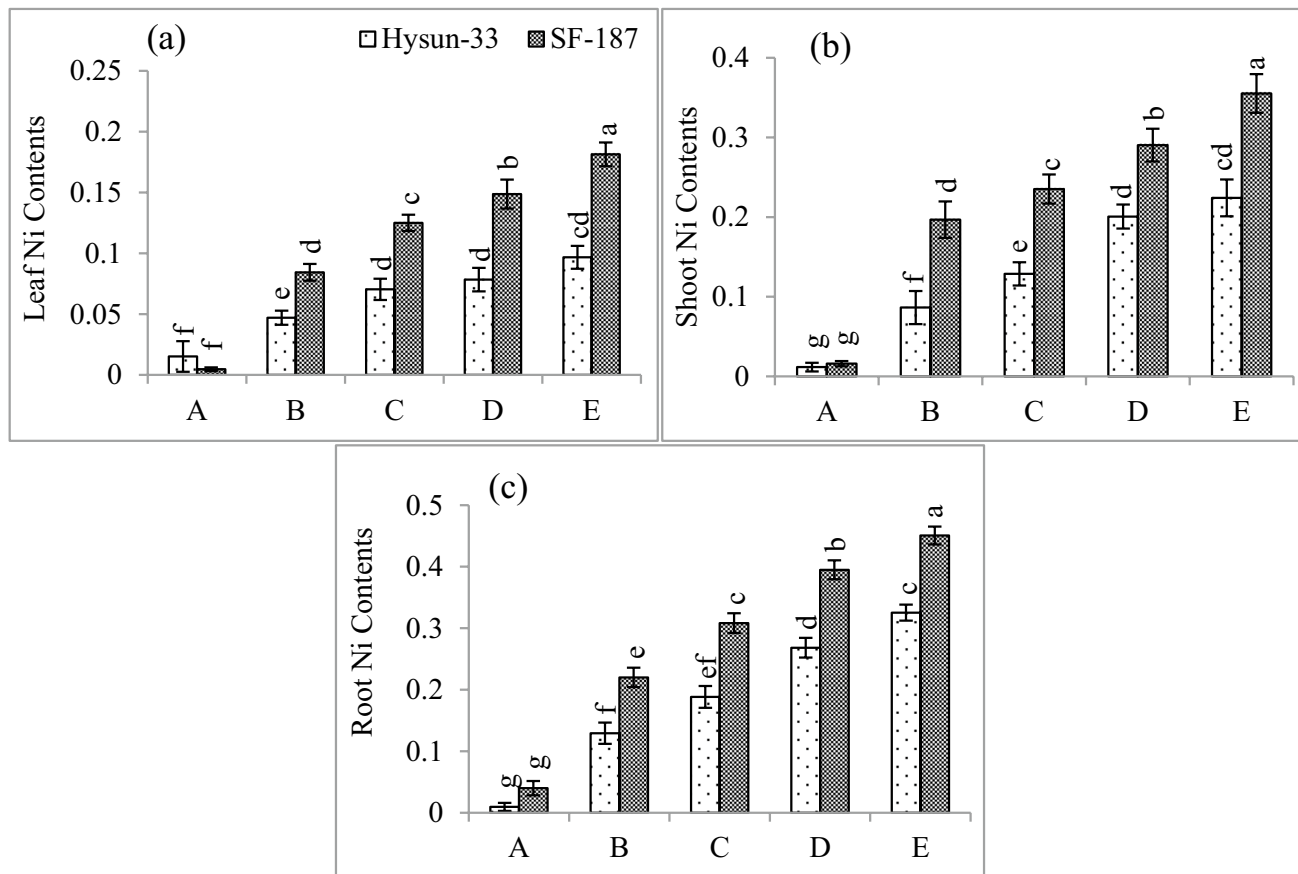


Fig. 2 Effect of different levels of nickel (A=0 mg/L, B=10 mg/L, C=20 mg/L, D=30 mg/L, E=40 mg/L) on leaf Ni contents (mg g^{-1}) (a), shoot Ni contents (mg g^{-1}) (b), and root Ni contents (mg g^{-1}) (c)

of two sunflower (*Helianthus annuus* L.) cultivars. Standard error is presented as vertical line on bars

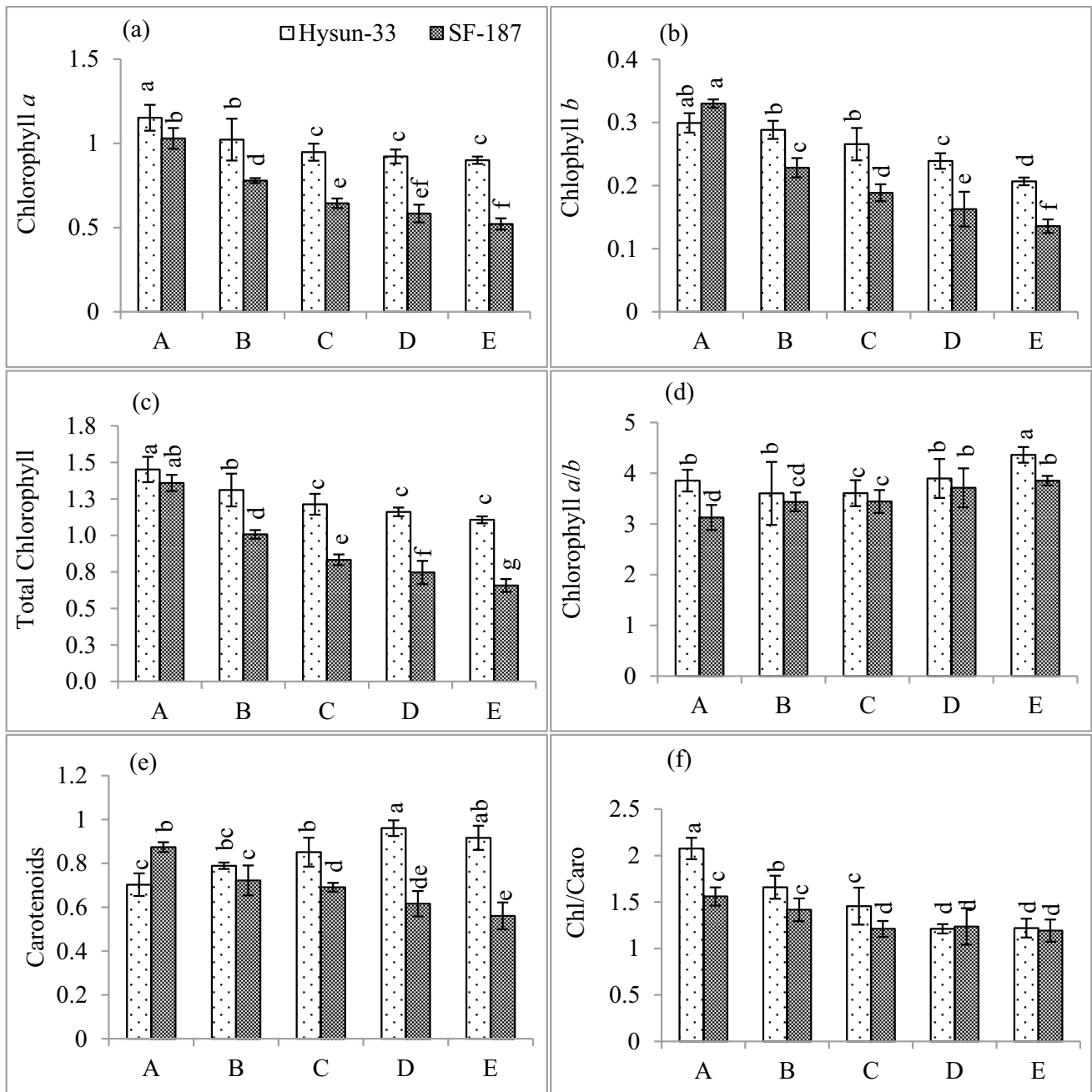


Fig. 3 Effect of different levels of nickel (A=0 mg/L, B=10 mg/L, C=20 mg/L, D=30 mg/L, E=40 mg/L) on chlorophyll a (mg g⁻¹ fresh weight) (a), chlorophyll b (mg g⁻¹ fresh weight) (b), total chlorophyll (mg g⁻¹ fresh weight) (c), chlorophyll a/b ratio (d), carotenoid (mg/g fresh weight) (e), and chlorophyll/carotenoid ratio (f) of two sunflower (*Helianthus annuus* L.) cultivars. Standard error is presented as vertical line on bars

as colored rectangles. The red color indicated the positive, and blue indicated the negative correlation of observed plant traits with specific Ni treatment level.

In both cultivars, all growth attributes like shoot and root fresh and dry weights and lengths along with total proteins were clustered in one group, exhibiting mostly positive correlation with control and 10 mg L⁻¹ Ni application level. The same attributes were strongly negatively

correlated with the highest Ni level, i.e., 40 mg L⁻¹, reflecting severe growth inhibition. A major difference was noted at 30 mg L⁻¹ where the growth attributes were negatively correlated in SF-187 while weakly negative correlated in Hysun-33, indicating growth retardation at this treatment level in SF-187 than Hysun-33 (Fig. 7a). In comparison, turgor, water and osmotic potentials and relative membrane permeability were positively correlated

correlated with the highest Ni level, i.e., 40 mg L⁻¹, reflecting severe growth inhibition. A major difference was noted at 30 mg L⁻¹ where the growth attributes were negatively correlated in SF-187 while weakly negative correlated in Hysun-33, indicating growth retardation at this treatment level in SF-187 than Hysun-33 (Fig. 7a). In comparison, turgor, water and osmotic potentials and relative membrane permeability were positively correlated

to control plants. This could be explained as a strong link between relative membrane permeability and water relation attributes. The total soluble sugars and amino acids were positively correlated with 10 mg L^{-1} Ni treatment level. At highest level of Ni application, all these attributes were negatively correlated (at 30 mg L^{-1} in Hysun-33 and at 40 mg L^{-1} Ni level in SF-187). The changes in the concentration of soluble sugars and amino acids more likely reflect metabolic alterations rather than membrane permeability dysfunctions, and changes in these compounds may clearly alter osmotic potentials (and, hence, tissue water relationships, turgor pressure, etc.) (Fig. 7b).

In Hysun-33, all photosynthetic pigments (Chl. *a*, Chl. *b*, total chlorophyll, carotenoids, and Chl/Carot ratio) showed a positive correlation with photosynthetic rate (*A*), transpiration rate (*E*), and water use efficiency (WUE) at control level (0 mg L^{-1}) except for chlorophyll *a/b* and *E* being negatively correlated. The same attributes were weakly correlated with lower levels (10 and 20 mg L^{-1}), and most of them were strong negatively correlated with higher levels (30 and 40 mg L^{-1}) (Fig. 8a). Almost similar clustering was observed in SF-187 for photosynthetic pigments and gas exchange attributes. These attributes were positively correlated with control and 10 mg L^{-1} Ni level, but carotenoids and *E* were strong negatively correlated with 10 mg L^{-1} Ni. At higher levels (20 – 40 mg L^{-1}), pigments and gas exchange attributes were weakly negatively correlated (Fig. 8b). These findings showed those photosynthetic rates (*A*) were severely inhibited at higher Ni levels possibly due to reduction in photosynthetic pigments recorded in this study. The WUE was most possibly linked to changes in *g_s* rather than *E* as *E* showed opposite correlation pattern at the higher Ni levels.

Correlogram

The correlogram showed that most of growth attributes (i.e., shoot and root fresh and dry weights and shoot and root lengths) were positively correlated with photosynthetic pigments (Chl. *a*, Chl. *b*, total Chl., and carotenoid), except for Chl *a/b* ratio that was negatively correlated. These growth attributes were positively correlated with photosynthetic attributes *A*, *g_s*, *A/E*, while weakly correlated with *C_i/C_a* ratio and non-significantly with *E*. Turgor potential had a strong positive, osmotic potential a weakly positive and water potential a non-significant correlation with growth attributes. Relative water contents and proteins had a positive, while relative membrane permeability had a negative correlation with growth. Amino acids and soluble sugars had a non-significant correlation with growth attributes (Fig. 9).

Principal component analysis (PCA)

The principal component analysis was performed to visualize the influence of various Ni treatments on plant attributes of tolerant (Hysun-33) and sensitive (SF-187) sunflower cultivars. The PCA plot showed 65.4% variability covered by PC1 and 28.3% by PC2 axis. Most of the attributes were plotted toward cultivar Hysun-33, indicating a strong linking of growth attributes in this genotype to Ni levels. The relative membrane permeability, Chl *a*, *C_i/C_a*, and Chl/carotenoids ratio were the only attributes plotted with SF-187 cultivar. This showed that the membrane permeability was correlated to reduced Chl *a* in this genotype upon exposure to Ni stress (20 to 40 mg L^{-1}). Amino acids and soluble sugars were plotted with water relation components (osmotic, turgor, and water potentials), indicating their significant contribution in regulating water relation components at 30 - and 40-mg L^{-1} Ni levels. Photosynthetic pigments (except carotenoids) and other gas exchange attributes were also plotted toward growth attributes of cultivar Hysun-33 at 10-mg L^{-1} Ni level, perhaps due to higher tolerance of this genotype at a lower Ni application level. The *C_i/C_a* and Chl./Carot ratios were plotted with SF-187 at 10-mg L^{-1} Ni level. Relative membrane permeability and chlorophyll *a/b* ratio were plotted at 20 - and 30-mg L^{-1} Ni levels in same cultivar. The 40-mg L^{-1} Ni level applied to SF-187 was plotted at distance, indicating no specific relationship with any of the studied plant attributes (Fig. 10).

Discussion

In this experiment, a noticeable reduction in various studied growth attributes was observed by increasing Ni concentration in both sunflower cultivars. Lower levels of Ni (10 mg L^{-1}) exhibited a slight improvement in shoot length; however, root length, shoot and root, and fresh and dry weight were highly decreased by Ni application. Although low levels of Ni have been shown to enhance growth of different crops to some extent in previous studies (Hassan et al. 2019), its high concentration has been reported to reduce different growth attributes such as plant height and fresh and dry weight in various crop plants (Gupta et al. 2017). The Ni concentration in all the analyzed tissues of cv SF-187 was significantly higher than in cv Hysun-33 at all Ni levels tested (Fig. 2). This may explain why growth (and related physiological) traits were more negatively affected by Ni supply in the former genotype. A more efficient exclusion of Ni ions (i.e., a reduction of net Ni uptake) seems to be the main reason that can explain the greater tolerance of cv Hysun-33 to Ni treatments. Only when comparing genotypes containing similar endogenous Ni levels it is possible

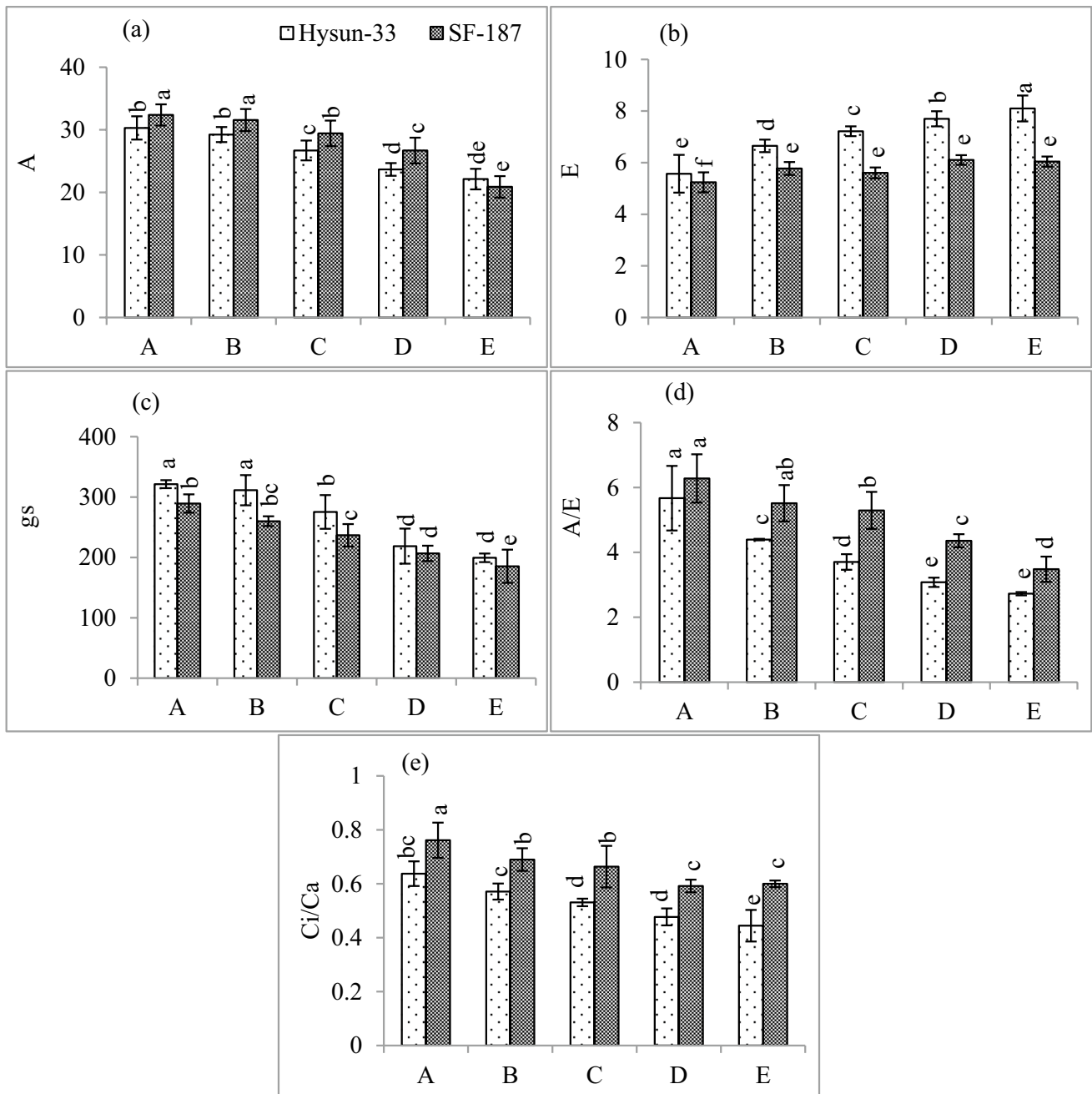


Fig. 4 Effect of different levels of nickel (A=0 mg/L, B=10 mg/L, C=20 mg/L, D=30 mg/L, E=40 mg/L) on photosynthetic rate (A) ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (a), transpiration rate (E) ($\text{mmol m}^{-2} \text{s}^{-1}$) (b), stomatal conductance (g_s) ($\text{mmol m}^{-2} \text{s}^{-1}$) (c), water use efficiency (A/E)

($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$) (d), and Ci/Ca ratio (e) of two sunflower (*Helianthus annuus* L.) cultivars. Standard error is presented as vertical line on bars

to speculate whether the maintenance of higher growth rate, photosynthetic capacity, Chl levels, etc., in one genotype (Hysun-33) may be related to an inherent genetic potential to reduce the toxic effects of Ni.

The reduction in shoot and root growth had been most widely attributed to reduction in concentration of photosynthetic pigments, leading to impaired photosynthetic and

gas exchange attributes, alterations in water relations, and imbalanced uptake and transport of essential nutrients such as that of iron and magnesium (Hussain et al. 2013). Thus, the supply of photo-assimilates required for normal growth and development is reduced, leading to suppressed growth under Ni stress. Moreover, roots are directly exposed to excess concentration of Ni that can lead to reduction in their

Table 3 Analysis of variance (ANOVA) for gas exchange and water relation attributes of sunflower cultivars exposed to varying levels of nickel

SoV	df	<i>A</i>	<i>E</i>	g_s	<i>A/E</i>	<i>Ci/Ca</i>	Ψ_w	Ψ_s
Cultivars (C)	1	405.24 ***	6.84 ***	36,192.13 ***	1.49 ns	0.01 ns	0.15 **	0.16 **
Treatments (T)	4	71.52 **	1.84 ns	9164.33 ***	6.38 **	0.04 ***	0.02 ns	0.22 ***
C×T interaction	4	10.29 ns	0.35 ns	103.13 ns	0.10 ns	0.00 ns	0.01 ns	0.01 ns
Error	20	9.06	0.35	1128.13	0.70	0.01	0.01	0.01
		Ψ_p	RWC	RPM	TSP	TFA	TSS	
Cultivars (C)	1	0.00 ns	135.98 ns	778.97 **	10.01 **	30.20 ***	970.15 ***	
Treatments (T)	4	0.11 ***	226.94 **	136.15 ns	3.53 **	13.00 ***	268.73 **	
C×T interaction	4	0.00 ns	4.96 ns	18.44 ns	0.30 ns	0.46 ns	16.07 ns	
Error	20	0.00	33.59	57.58	0.71	1.05	57.27	

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively. *ns*, non-significant

Abbreviations: *A*, photosynthetic rate; *E*, transpiration rate; g_s , stomatal conductance; *A/E*, water use efficiency; *Ci/Ca*, internal and ambient carbon dioxide ratio; Ψ_w , water potential; Ψ_s , solute potential; Ψ_p , turgor potential; *TSP*, total soluble proteins; *TFA*, total free amino acids; *TSS*, total soluble sugars

growth and proliferation (Demchenko et al. 2010). It might be due to the reason that Ni can efficiently penetrate endodermal layer and gather in the pericycle cells, thus affecting cell division and cell multiplication (D'Antò et al. 2012). Thus, reduced root growth can have a direct influence on development of aerial parts of plants. Overall, these vegetative growth parameters were affected to a lesser extent by application of Ni in cultivar Hysun-33 as compared to that of SF-187. This could be due to the fact that Hysun-33 exhibited less decrease in various photosynthetic pigments, photosynthetic attributes, and nutrient concentration, thereby directly enhancing Ni-tolerance potential of this cultivar. Such maintenance of higher nutrient and pigment levels, as well as higher photosynthetic activity in Hysun-33 than the other hybrid, contributed to a better maintenance of growth rate under nickel stress (Kozłmińska et al. 2018).

Application of Ni-stress severely reduced chlorophyll *a*, *b* and total chlorophyll. In contrast, the concentration of carotenoids was slightly increased in Hysun-33 but severely reduced in SF-187. The high Ni concentration has been found to change the photosynthetic pigments like chlorophyll *a*, *b* and carotenoids in many crop plants (Batool 2018). At high concentration, Ni directly damages photosynthetic apparatus of leaf in several ways. For instance, surplus Ni led to reduction in the photosynthetic pigments, mostly chlorophyll *a*, *b*, carotenoids, and xanthophylls (Younis et al. 2020). Moreover, Ni also interacts with essential nutrients and reduces their uptake and translocation, thus causing nutrient deficiency (Velikova et al. 2011). It has been recognized that magnesium is an essential constituent of heme group of chlorophyll; moreover, other nutrients like iron and manganese are needed for metabolic activities of chlorophyll. So their lower absorption rates can reduce synthesis of chlorophyll at high Ni concentration (Batool 2018). In addition, Ni at high level breakdowns remaining

chlorophyll in chloroplast that causes chlorosis of leaves and ultimately necrosis of plant parts (Rucińska-Sobkowiak et al. 2013). In our earlier findings, we have reported constantly higher level of major nutrients in Hysun-33 than SF-187 (Ahmad et al. 2011). The maintenance of relatively consistent levels of carotenoids in Hysun-33 in current study was linked to its ability to accumulate essential nutrients required for chlorophyll synthesis and metabolism such as that of magnesium, iron, and manganese that could help plants to withstand Ni-stress (Ahmad and Ashraf 2011). Despite the concentration of chlorophyll *a* and *b* was reduced under Ni-stress conditions, the chlorophyll *a/b* ratio was not affected in Hysun-33. This showed that, in Hysun-33, both pigments (chlorophyll *a* and *b*) were equally sensitive to Ni-toxicity. In contrast, chlorophyll *a/b* increased in SF-187, indicating greater sensitivity of chlorophyll *b* to Ni-toxicity than that of chlorophyll *a*. In contrast, chlorophyll/carotenoid ratio severely decreased in both cultivars under Ni-stress, showing that chlorophylls were more sensitive to Ni stress than that of carotenoids. In this study, it was revealed that application of Ni at high concentration significantly reduced *A*, g_s , WUE, and *Ci/Ca* ratio. It is in accordance to previous studies showing that application of high concentration of Ni causes reduction in *A*, g_s , and WUE of plants (Vernay et al. 2007; Kachenko et al. 2011). However, transpiration rate (*E*) increased with elevation of external Ni despite g_s was slightly decreased. This could have been linked to the damages to leaf epidermis caused by high Ni concentrations in the leaf tissues (Hassan et al. 2019).

Results revealed that application of Ni at the highest concentration significantly affected plant water relation attributes. Overall, leaf ψ_w and ψ_s decreased, while leaf ψ_p increased in both sunflower cultivars with elevating external Ni level. However, the differences between both cultivars were significant only for ψ_w and ψ_s , while both

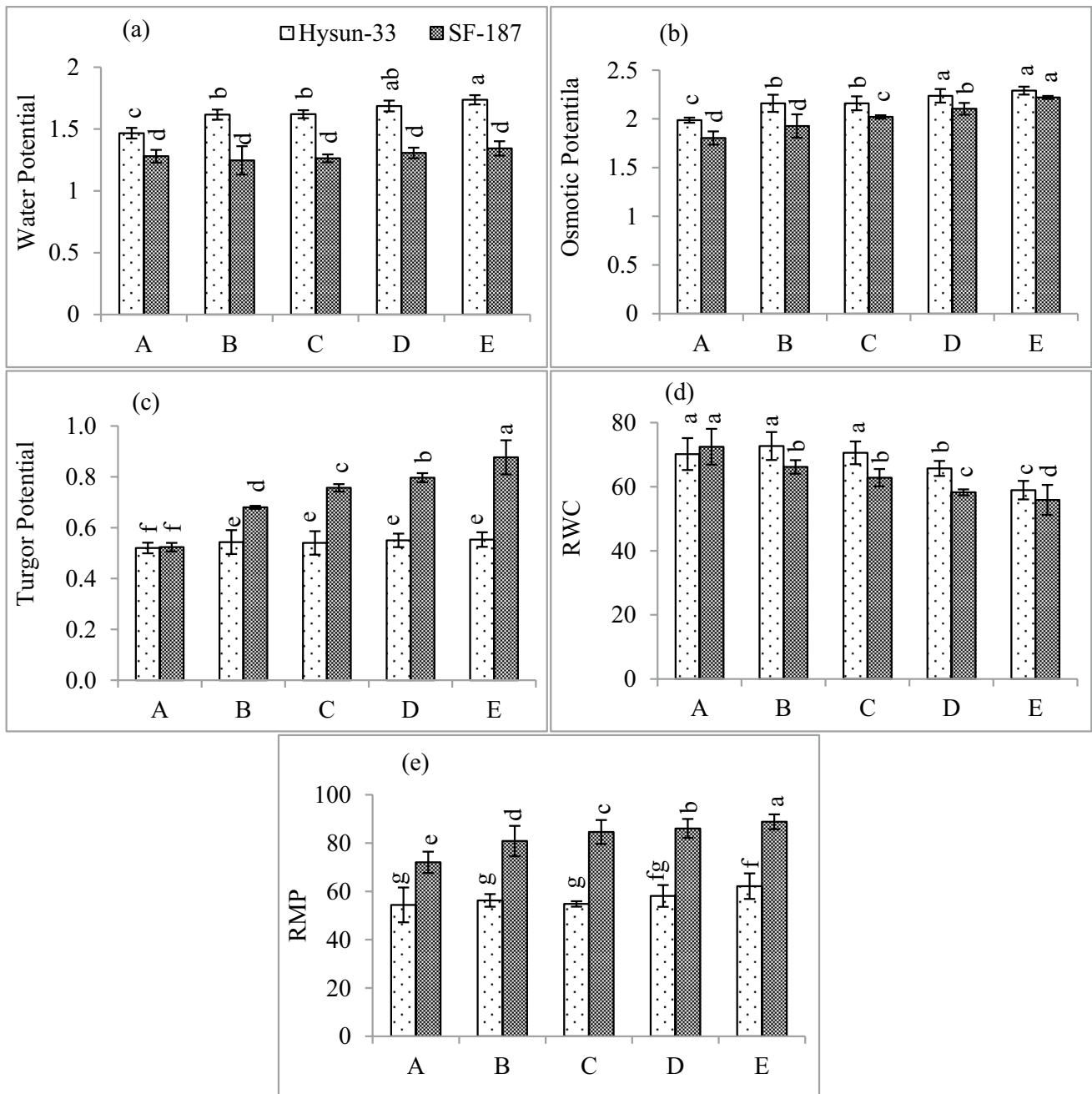


Fig. 5 Effect of different levels of nickel (A=0 mg/L, B=10 mg/L, C=20 mg/L, D=30 mg/L, E=40 mg/L) on water potential (-M Pa) (a), osmotic potential (-M Pa) (b), turgor potential (M Pa) (c), relative

water content (%) (d), and relative membrane permeability (%) (e) of two sunflower (*Helianthus annuus* L.) cultivars. Standard error is presented as vertical line on bars

cultivars showed almost similar increasing trend for leaf ψ_p . Nickel has significant impact on water relation components of stressed plants. For example, Hassan et al. (2019) reported that plants exposed to excess Ni showed a substantial rise in water potential of leaves. Such changes in plant water relations are possibly a result of accumulation of compatible solutes/osmotic under metal stressed conditions. For example, there is strong evidence that

soluble sugars and other free amino acids gather in metal-stressed plants. These solutes can considerably contribute in decreasing the water and solute potential of plants under metal stress (Rucińska-Sobkowiak 2016). Consistent with this, the changes in water-relation components of both cultivars showed a parallel change with increased concentration of compatible solutes such as total sugars and amino acids of Ni-stressed plants.

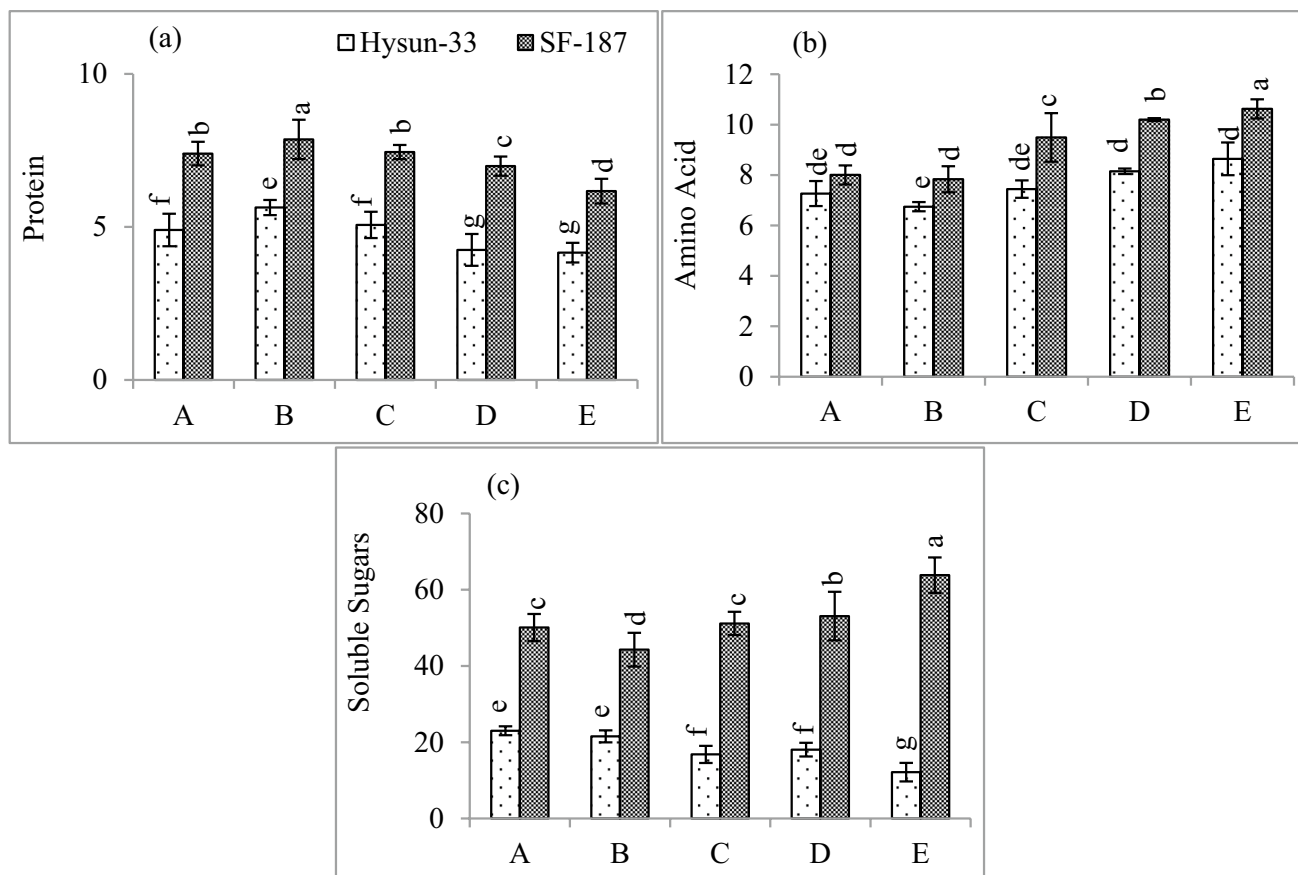


Fig. 6 Effect of different levels of nickel (A=0 mg/L, B=10 mg/L, C=20 mg/L, D=30 mg/L, E=40 mg/L) on protein (mg g⁻¹ fresh weight) (a), amino acid (mg g⁻¹ fresh weight) (b), and total soluble

sugar (mg g⁻¹ fresh weight) (c) of two sunflower (*Helianthus annuus* L.) cultivars. Standard error is presented as vertical line on bars

Relative water contents of both cultivars decreased under Ni-stress. A putative explanation for such decrease in RWC despite the significant increment in turgor pressure and the more negative osmotic and water potentials as recorded in this study could be linked to the reduction of mesophyll cell size and thickness with a parallel reduction in midrib thickness in expanding leaves under Ni stress (Khaliq et al. 2016). Other reasons that might account for the observed trends in RWC might include severe reduction in leaf cortical region thickness and area accompanied by a reduction in vascular bundle thickness (both xylem and phloem). These anatomical modifications caused a significant change in specific leaf area (SLA) of the nickel stressed plants (this data is not reported in this manuscript and can be found in the PhD dissertation of first author Ahmad 2011), which significantly hampered the plant water relation attributes.

Similarly, the fact that *E* increased despite g_s decreased could result in damages to the leaf epidermis that increases cuticular transpiration. These results are in accordance with Jagetiya et al. (2013), who reported that green gram seedlings exposed to 1- μ M Ni increased RWC of leaves.

However, RWC significantly decreased at 10- to 1000- μ M Ni levels. In another study, Singh and Pandey (2011) reported a significant decrease in RWC of metal stressed lettuce plants. Likewise, Llamas et al. (2008) showed that RWC of rice plants were considerably reduced with rise in Ni concentration. This evidence shows that RWC are generally reduced in plants exposed to Ni stress, but further research is needed to determine the underlying mechanisms, causing alterations in relative water contents of Ni-stressed plants.

Electrolyte leakage in leaves of both sunflower cultivars increased with the increment of Ni level, indicating that the membrane permeability was significantly disturbed under Ni-stress. It has been argued that the metal stress causes loss of membrane permeability, which leads to loss of osmolytes and, ultimately, turgor potential (Jogawat 2019). Although Ni cannot directly produce ROS, high production of reactive oxygen species (ROS) has been reported under Ni stress (Boominathan and Doran 2002; Gajewska and Skłodowska 2007). These facts show that production of ROS is an indirect consequence of Ni stress that might damage micro- and

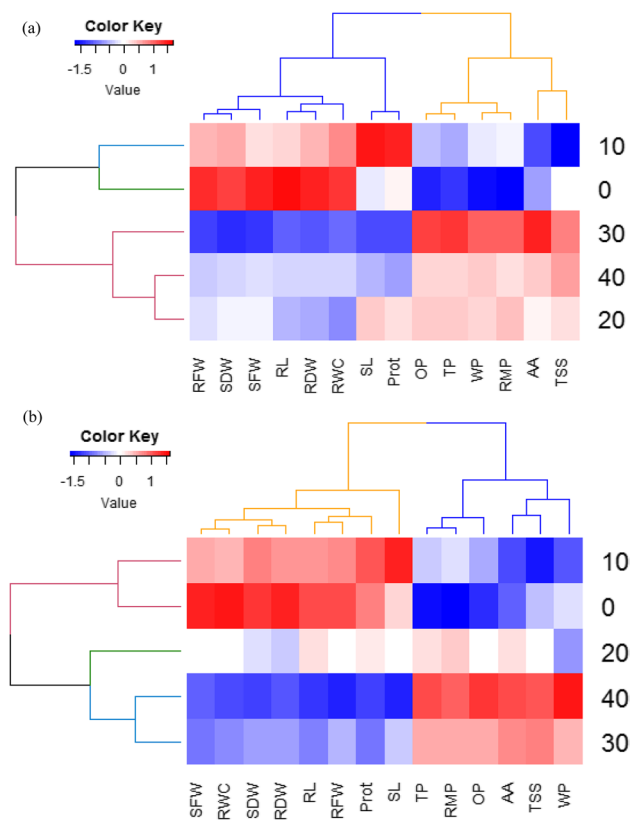


Fig. 7 A heatmap constructed for various growths and physiological attributes of Hysun-33 (a), SF-187 (b). The numbers on right (0, 10, 20, 30, and 40 mg/L) show Ni concentration. Within heat-plot, red color indicates strong positive correlation, while blue color indicates strong negative correlation with applied Ni levels. Same color in clades and leaves of dendrogram on top and left sides indicates parameters placed in same cluster. Abbreviations: AA, total free amino acids; Prot, total soluble proteins; RDW, root dry weight; RFW, root fresh weight; RL, root length; RMP, relative membrane permeability; RWC, relative water content; SDW, shoot dry weight; SFW, shoot fresh weight; SL, shoot length; TSS, total soluble sugars; OP, leaf osmotic potential; TP, leaf turgor potential; WP, leaf water potential

macro-molecules including biological membranes. Other reports indicate that Ni causes alterations in sterol and phospholipids of the plasma membranes that can cause modifications in the permeability of membranes leading to enhanced electrolyte leakage under Ni-stress (Hassan et al. 2019).

Results revealed that the content of soluble proteins was slightly improved at low level of Ni (10 and 20 mg L⁻¹); however, it was reduced by increasing Ni concentration. Moreover, the opposite was true for the concentration of free amino acids. At high Ni application, the concentration of soluble proteins was severely decreased as an indication of toxic effects of elevated concentration of Ni in the growing environment. Cultivar Hysun-33 exhibited fewer reductions in soluble protein contents but

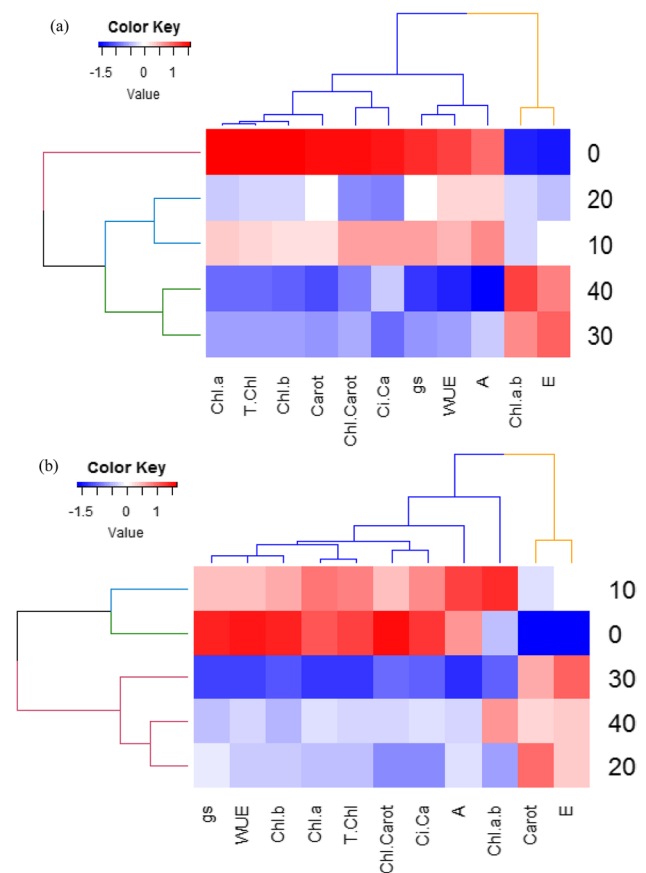
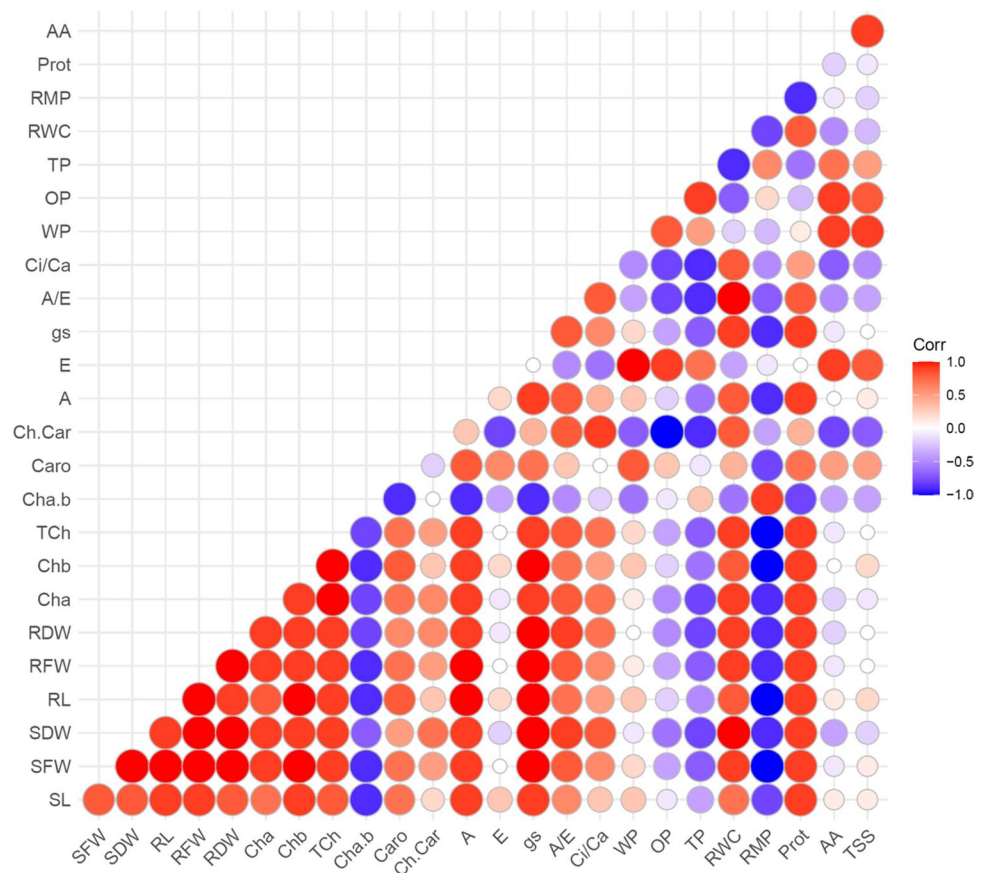


Fig. 8 A heatmap constructed for photosynthetic pigments and gas exchange attributes of Hysun-33 (a), SF-187 (b). The numbers on right (0, 10, 20, 30, and 40 mg/L) show Ni concentration. Within the heat-plot, red color indicates strong positive correlation, while blue color indicates strong negative correlation with applied Ni levels. Same color in clades and leaves of dendrogram on top and left sides indicates parameters placed in the same cluster. Abbreviations: A, net photosynthetic rate; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a/b, chlorophyll a/b ratio; Ci/Ca, Ci/Ca ratio; Chl/Carot, chlorophyll/carotenoids ratio; Carot, carotenoids; T Chl, total chlorophyll; E, transpiration rate; gs, stomatal conductance; WUE, water use efficiency

more increase in free amino acids than that of SF-187. It has been reported that total free amino acids might accumulate in various crop plants under metal stress. The higher total free amino acids may be the result of increased synthesis of protective osmolytes (e.g., proline) or enhanced protein degradation due to Ni stress (Ahmad and Ashraf 2011). Proline performs protective function, while asparagine acts as chelating agent for Ni, Cd, Pb, Zn, and other metals by forming a metal–ligand complex for detoxification (Hassan et al. 2019). Such increment in free amino acids is mostly complemented with a reduction in total soluble proteins (Ali et al. 2009).

The soluble sugar contents first decreased at low Ni concentration (10 mg L⁻¹) and then increased with rise in Ni level. Most of the metal ions including Ni cause alteration

Fig. 9 A correlogram constructed for various growths and physiological attributes of the two sunflower hybrids. (red = significantly positive, blue = significantly negative). Abbreviations: AA, total free amino acids; Prot, total soluble proteins; RDW, root dry weight; RFW, root fresh weight; RL, root length; RMP, relative membrane permeability; RWC, relative water content; SDW, shoot dry weight; SFW, shoot fresh weight; SL, shoot length; TSS, total soluble sugars; OP, leaf osmotic potential; TP, leaf turgor potential; WP, leaf water potential; A, net photosynthetic rate; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; Chl *a/b*, chlorophyll *a/b* ratio; *Ci/Ca*, *Ci/Ca* ratio; Chl/Carot, chlorophyll/carotenoids ratio; Carot, carotenoids; T Chl, total chlorophyll; *E*, transpiration rate; *gs*, stomatal conductance; WUE, water use efficiency



in carbohydrate metabolism when they are in abundance in the soil. This is due to a negative effect on the functioning of the main metabolic enzymes of Calvin cycle, causing changes in the levels of essential bio-molecules like sugars, proteins, amino acids, and nucleotides in growing plants (Jha and Dubey 2005). In addition, excess Ni concentration caused a marked reduction in the concentration of photosynthetic pigments, thereby affecting photosynthetic rate as observed in this study. Thus, reduced photosynthetic rate can reduce the concentration of soluble sugars under Ni-stress, which affects plant growth. The maintenance of higher soluble sugar content in the tolerant genotype (Hysun-33) can be, in part, related to the maintenance of higher photosynthetic rates, supported by maintenance of higher photosynthetic rates supported by higher photosynthetic pigments under nickel stress (Rosa et al. 2009).

The heatmap clustering to draw the response of different plant attributes to specific Ni treatment levels in both cultivars showed that low levels of Ni (10 mg L^{-1}) were positively correlated with growth, physiological, photosynthetic, and gas exchange attributes, while higher levels ($20\text{--}40 \text{ mg L}^{-1}$) of Ni showed a negative effect on most of these attributes. These findings showed that low level of Ni (10 mg L^{-1}) may have some potential in improving sunflower growth, physiological and

biochemical attributes, probably because of its growth stimulatory effects as reported in some earlier studies (Ahmad and Ashraf 2011; Patra et al. 2020). These findings were further strengthened by PCA plot where all growth attributes, photosynthetic pigments, and gas exchange attributes were plotted with lower Ni levels (10 mg L^{-1}) in cultivar Hysun-33. Water relation attributes, carotenoids, amino acids, and total soluble sugars were plotted with 30- and 40-mg L^{-1} Ni levels in the same cultivars. These findings clearly indicated that lower levels had a significant growth promotory effects mainly because of maintenance of photosynthetic capacity in this cultivar as indicated in some earlier studies (Freitas et al. 2018). Comparably, only a few physiological attributes, i.e., relative membrane permeability (RMP), Chl *a/b* ratio (at 20- to 40-mg L^{-1} Ni levels), and C_i/C_a and chlorophyll/carotenoid ratios (at 10-mg L^{-1} Ni level) were affected. These findings suggest that the sensitivity of this genotype was partly related to enhanced electrolyte leakage from membranes (reduced RMP) and higher sensitivity of chlorophyll *a* over *b*. Chlorophyll *a* is the main component of the reaction center, and any damages to Chl. *a* can significantly reduce photosynthetic capacity under Ni stress (Szymaska and Matraszek 2005; Singh and Pandey 2011).

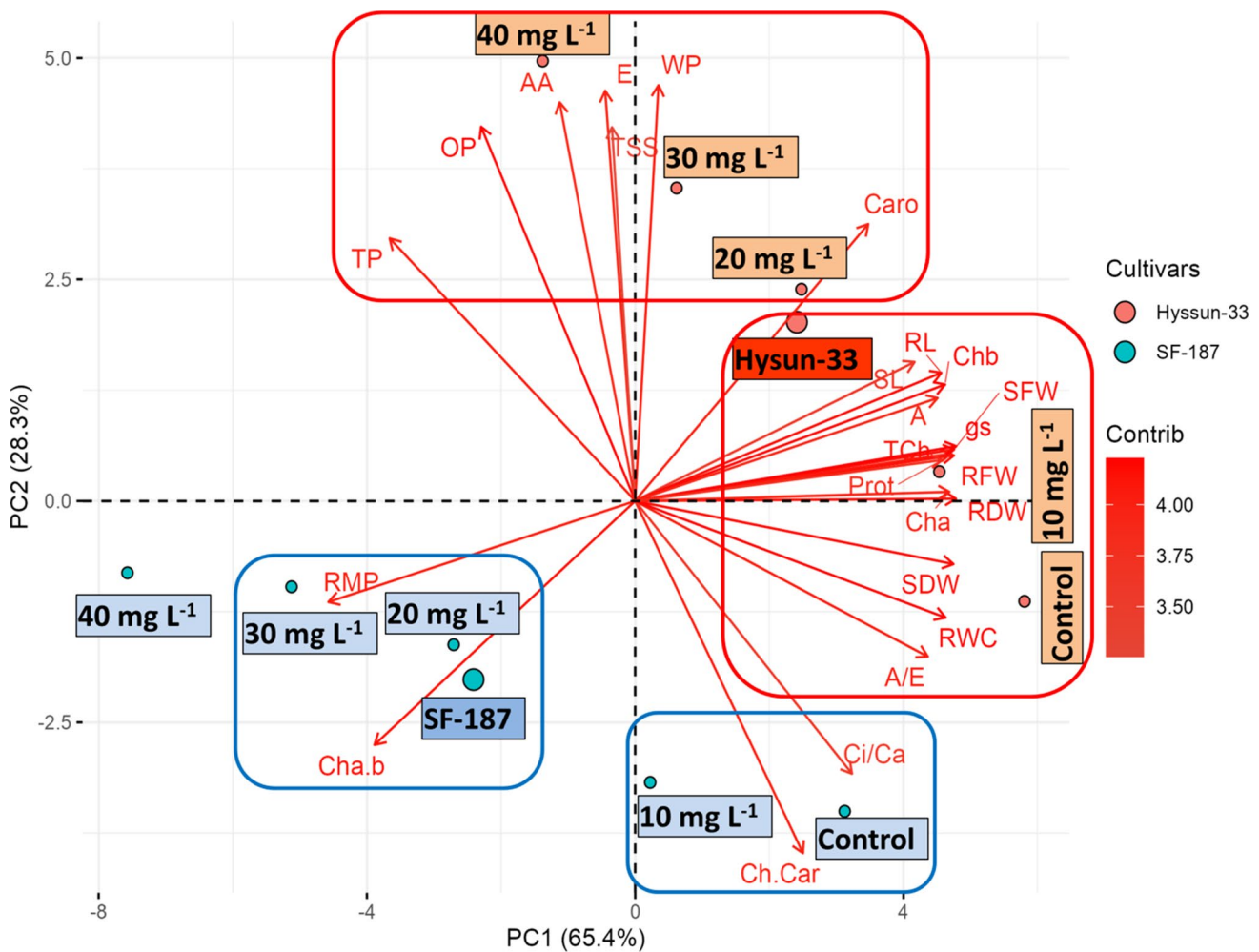


Fig. 10 A principal component analysis (PCA) plot for various growths and physiological attributes of the two sunflower hybrids. Ellipses are drawn to group different attributes with sunflower hybrids, where most of the growth and physiology were attributed toward lower Ni level (10 mg L^{-1}) in Hysun-33. Abbreviations: AA, total free amino acids; Prot, total soluble proteins; RDW, root dry weight; RFW, root fresh weight; RL, root length; RMP, relative membrane permeability; RWC, relative water content; SDW, shoot dry

weight; SFW, shoot fresh weight; SL, shoot length; TSS, total soluble sugars; OP, leaf osmotic potential; TP, leaf turgor potential; WP, leaf water potential; A, net photosynthetic rate; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a/b, chlorophyll a/b ratio; Ci/Ca, Ci/Ca ratio; Chl/Carot, chlorophyll/carotenoids ratio; Carot, carotenoids; T Chl, total chlorophyll; E, transpiration rate; gs, stomatal conductance; WUE, water use efficiency

Conclusion

Application of high levels of Ni showed a marked reduction in growth due to changes in various biochemical and physiological parameters of sunflower. High concentration of Ni severely reduced plant growth and development because of negative effects on photosynthetic pigments and water relation attributes that inhibited photosynthetic capacity of Ni-stressed plants. However, low concentration of Ni (10 mg/L) showed some improvements in shoot length, transpiration rate, leaf turgor potential, membrane permeability, and soluble proteins, indicating that the low levels of Ni can potentially benefit sunflower growth. The lower accumulation of Ni across different treatments in

one of the hybrids (Hysun-33) may account for the better performance shown by this hybrid for most of the traits measured. Cultivar SF-187 was apparently more sensitive to high Ni level as compared to Hysun-33. This was apparently due to a lower capability of SF-187 to decrease net Ni accumulation in both underground and aerial tissues which significantly hindered key physiological processes.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11356-023-25705-2>.

Acknowledgements This paper is a part of Ph.D. studies of Muhammad Sajid Aqeel Ahmad, Department of Botany, University of Agriculture, Faisalabad.

Author contribution Muhammad Sajid Aqeel Ahmad has performed statistical analysis of this paper. Alia Riffat has edited, corrected, and proofread the manuscript. Mumtaz Hussain and Mansoor Hameed read and finalized the manuscript. Ambreen Khadija Alvi participated in manuscript editing and language correction.

Data availability The raw data can be taken from the author on request.

Declarations

Ethical approval and consent to participate The manuscript was submitted solely to Environmental Science and Pollution Research, and no part was published or submitted elsewhere. All ethical guidelines set by parent institution(s) were observed during sampling and analysis.

Consent to publication All the authors completely agreed to publish the manuscript.

Competing interests The authors declare no competing interests.

References

- Ahmad MSA, Ashraf M (2011) Essential roles and hazardous effects of nickel in plants. *Rev Environ Contam Toxicol* 214:125–167. https://doi.org/10.1007/978-1-4614-0668-6_6
- Ahmad MSA, Hussain M, Ashraf M, Ahmad R, Ashraf MY (2009) Effect of nickel on seed germinability of some elite sunflower (*Helianthus annuus* L.) cultivars. *Pak J Bot* 41:1871–1882
- Ahmad M, Iqbal J, Kaleem S, Wasaya A, Ishaque M (2011) Qualitative analysis of spring planted sunflower hybrids as influenced by varying nutritional area. *Pak J Nutr* 10:291–295. <https://doi.org/10.3923/PJN.2011.291.295>
- Ahmad MSA (2011) Influence of nickel stress on growth, morpho-physiological and anatomical attributes of sunflower (*Helianthus annuus* L.). PhD Dissertation, Submitted to University of Agriculture, Faisalabad. <http://pr.hec.gov.pk/jspui/handle/123456789/1866>
- Ali MA, Ashraf M, Athar HR (2009) Influence of nickel stress on growth and some important physiological/biochemical attributes in some diverse canola (*Brassica napus* L.) cultivars. *J Hazard Mater* 172:964–969. <https://doi.org/10.1016/j.jhazmat.2009.07.077>
- Amari T, Ghnaya T, Debez A, Taamali M, Lucchini YNB, G, Sacchi GA, Abdely C, (2014) Comparative Ni tolerance and accumulation potentials between *Mesembryanthemum crystallinum* (halophyte) and *Brassica juncea*: metal accumulation, nutrient status and photosynthetic activity. *J Plant Physiol* 171:1634–1644. <https://doi.org/10.1016/j.jplph.2014.06.020>
- Arnon DI (1949) Copper enzymes in isolated chloroplasts Polyphenoloxidase in *Beta Vulgaris*. *Plant Physiol* 24:1–15. <https://doi.org/10.1104/pp.24.1.1>
- Ashraf MY, Sadiq R, Hussain M, Ashraf M, Ahmad MSA (2011) Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of sunflower (*Helianthus annuus* L.). *Biol Trace Elem Res* 143:1695–1703. <https://doi.org/10.1007/s12011-011-8955-7>
- Bai C, Reilly CC, Wood BW (2006) Nickel deficiency disrupts metabolism of ureides, amino acids, and organic acids of young pecan foliage. *Plant Physiol* 140:433–443. <https://doi.org/10.1104/pp.105.072983>
- Batool S (2018) Effect of nickel toxicity on growth, photosynthetic pigments and dry matter yield of *Cicer arietinum* L. varieties. *Asian J Agric Biol* 6:143–148
- Boominathan R, Doran PM (2002) Ni-induced oxidative stress in roots of the Ni hyperaccumulator, *Alyssum bertolonii*. *New Phytol* 156:205–215
- Boyer J (1967) Leaf water potentials measured with a pressure chamber. *Plant Physiol* 42(1):133–7. <https://doi.org/10.1104/pp.42.1.133>
- Chris A (2012) Effect of nickel stress on growth and antioxidants in *Cyanobacterium Cylandrospermum* sp. *Asian J Biol Sci* 7:13–17
- D'Antò V, Valletta R, Amato M, Schweickl H, Simeone M, Paduano S, Rengo S, Spagnuolo G (2012) Effect of nickel chloride on cell proliferation. *Open Dent J* 6:177–181. <https://doi.org/10.2174/1874210601206010177>
- Davis BH (1976) Carotenoids. In: Goodwin TW (ed) *Chemistry and biochemistry of plant pigments*. Academic Press Inc., London, pp 38–165
- Demchenko NP, Kalimova IB, Demchenko KN (2010) Effect of nickel at high concentration on proliferation of quiescent center cells and initiation of lateral root primordia in wheat seedlings. *Russian J Plant Physiol* 57:438–447. <https://doi.org/10.1134/S1021443710030179>
- Duman F, Ozturk F (2010) Nickel accumulation and its effect on biomass, protein content and antioxidative enzymes in roots and leaves of watercress (*Nasturtium officinale* R. Br.). *J Environ Sci* 4:526–532. [https://doi.org/10.1016/s1001-0742\(09\)60137-6](https://doi.org/10.1016/s1001-0742(09)60137-6)
- Fabiano CC, Tezotto T, Favarin JL, Polacco JC, Mazzafera P (2015) Essentiality of nickel in plants: a role in plant stresses. *Front Plant Sci* 6:754. <https://doi.org/10.3389/fpls.2015.00754>
- Freitas DS, Rodak BW, dos Reis AR, Reis FDB, Carvalho TSD, Schulze J, Carneiro MAC, Guilherme LRG (2018) Hidden nickel deficiency? nickel fertilization via soil improves nitrogen metabolism and grain yield in soybean genotypes. *Front Plant Sci* 9:614. <https://doi.org/10.3389/fpls.2018.00614>
- Gajewska E, Skłodowska M (2007) Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. *Biometals* 20:27–36. <https://doi.org/10.1007/s10534-006-9011-5>
- González-Guerrero M, Matthiadiis A, Sáez Á, Long TA (2014) Fixating on metals: new insights into the role of metals in nodulation and symbiotic nitrogen fixation. *Front Plant Sci* 13:5–45. <https://doi.org/10.3389/fpls.2014.00045>
- Gupta V, Jatav PK, Verma R, Kothari SL, Kachhwaha S (2017) Nickel accumulation and its effect on growth, physiological and biochemical parameters in millets and oats. *Environ Sci Pollut Res Int* 24:23915–23925. <https://doi.org/10.1007/s11356-017-0057-4>
- Hamilton PB, Van-Slyke DD (1943) Amino-acid determination with ninhydrine. *J Biol Chem* 150:231–233
- Hassan MU, Chattha MU, Khan I, Chattha MB, Aamer M et al (2019) Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities-a review. *Environ Sci Pollut Res Int* 26:12673–12688. <https://doi.org/10.1007/s11356-019-04892-x>
- Hedfi A, Mahmoudi E, Boufahja F, Beyrem H, Aissa P (2007) Effects of increasing levels of nickel contamination on structure of off-shore nematode communities in experimental microcosms. *Bullet Environ Contam Toxicol* 79:345–349. <https://doi.org/10.1007/s00128-007-9261-0>
- Hussain S, Farooq M, Wahid MA, Wahid A (2013) Seed priming with putrescine improves the drought resistance of maize hybrids. *Int J Agric Biol* 15:1349–1353. <https://doi.org/10.1016/j.plgene.2017.04.004>
- Jagetiya B, Soni A, Yadav S (2013) Effect of nickel on plant water relations and growth in green gram. *Indian J Plant Physiol* 18:372–376. <https://doi.org/10.1007/s40502-013-0053-8>
- Jha AB, Dubey RS (2005) Effect of arsenic on behaviour of enzymes of sugar metabolism in germinating rice seeds. *Acta Physiol Plant* 27:341–347. <https://doi.org/10.1007/s11738-005-0010-x>
- Jogawat A (2019) Osmolytes and their role in abiotic stress tolerance in plants. In: Roychoudhury A, Tripathi D (eds) *Molecular plant abiotic stress: biology and biotechnology*. Wiley, pp 91–104
- Kachenko A, Bhatia N, Singh B (2011) Influence of drought stress on the nickel-hyperaccumulating shrub *Hybanthus floribundus*

- (Lindl.) F. Muell. subsp. floribundus. *Int J Plant Sci* 172:315–322. <https://doi.org/10.1086/658154>
- Khaliq A, Ali S, Hameed A, Farooq MA, Farid M, Shakoob MB, Mahmood K, Ishaque W, Rizwan M (2016) Silicon alleviates nickel toxicity in cotton seedlings through enhancing growth, photosynthesis, and suppressing Ni uptake and oxidative stress. *Arch Agron Soil Sci* 62:633–647. <https://doi.org/10.1080/03650340.2015.1073263>
- Koźmińska A, Wiszniewska A, Hanus-Fajerska E, Muszyńska E (2018) Recent strategies of increasing metal tolerance and phytoremediation potential using genetic transformation of plants. *Plant Biotechnol Rep* 12:1–4. <https://doi.org/10.1007/s11816-017-0467-2>
- Kumar S, Trivedi AV (2016) A review on role of nickel in the biological system. *Int J Curr Microbiol Appl Sci* 5:719–727. <https://doi.org/10.20546/ijcmas.2016.503.084>
- Llamas A, Ullrich CI, Sanz A (2008) Ni²⁺ toxicity in rice: effect on membrane functionality and plant water content. *Plant Physiol Biochem* 46:905–910. <https://doi.org/10.1016/j.plaphy.2008.05.006>
- Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with folin phenol reagent. *J Biol Chem* 193:265–275
- Moghaddam SR (2018) Phytoremediation ability of nickel-contaminated soil using Sunflower (*Helianthus annuus* L.) and Sorghum (*Sorghum bicolor* L.). *J Soil Manag Sus Prod* 6:131–142. <https://doi.org/10.22069/ejsms.2017.10042.1593>
- Nie J, Pan Y SJ, Guo Y, Yan Z, Duan X, Xu M (2015) A comparative study on the uptake and toxicity of nickel added in the form of different salts to maize seedlings. *Int J Environ Res Public Health* 12:15075–15087. <https://doi.org/10.3390/ijerph121214972>
- Patra A, Dutta A, Mohapatra KK, Pradhan S (2020) Nickel the ultra-micronutrient: significant for plant growth and metabolism. *Food Chem Toxicol* 1:35–37
- Riazi A, Matruda K, Arslan A (1985) Water stress induced changes in concentration of proline and other solutes in the growing regions of plant. *J Exp Bot* 36:1716–1725. <https://doi.org/10.1093/jxb/36.11.1716>
- Rosa M, Prado C, Podazza G, Interdonato R, González JA, Hilal M, Prado FE (2009) Soluble sugars—metabolism, sensing and abiotic stress: a complex network in the life of plants. *Plant Signal Behav* 4:388–393. <https://doi.org/10.4161/psb.4.5.8294>
- Rucińska-Sobkowiak R (2016) Water relations in plants subjected to heavy metal stresses. *Acta Physiol Plant* 38:257. <https://doi.org/10.1007/s11738-016-2277-5>
- Rucińska-Sobkowiak R, Nowaczyk G, Krzesłowska M, Rabęda I, Jurga S (2013) Water status and water diffusion transport in lupine roots exposed to lead. *Environ Exp Bot* 87:100–109. <https://doi.org/10.1016/j.envexpbot.2012.09.012>
- Shahzad B, Tanveer M, Rehman A, Cheema SA, Fahad S, Rehman S, Sharma A (2018) Nickel; whether toxic or essential for plants and environment - a review. *Plant Physiol Biochem* 132:641–651. <https://doi.org/10.1016/j.plaphy.2018.10.014>
- Shanying H, Zhenli H, Yang X, Virupax C, Baligar (2012) Mechanisms of nickel uptake and hyperaccumulation by plants and implications for soil remediation. In: Donald L, Sparks (ed) *Advances in Agronomy*. Academic Press, Volume 117, pp117–189
- Singh K, Pandey SN (2011) Effect of nickel-stresses on uptake, pigments and antioxidative responses of water lettuce, *Pistia stratiotes* L. *J Environ Biol* 32:391–394
- Steel RGD, Torrie JH, Dickey DA (1996) *Principles 3rd and procedures of statistics: a biometrical approach*, (Eds) McGraw Hill Co., New York, USA
- Stoikou V, Andrianos V, Stasinou S, Kostakis MG, Attiti S, Thomaidis NS, Zabetakis I (2017) Metal uptake by sunflower (*Helianthus annuus*) irrigated with water polluted with chromium and nickel. *Food* 6:51. <https://doi.org/10.3390/foods6070051>
- Szymaska M, Matraszek R (2005) Reaction of the sunflower (*Helianthus annuus* L.) to nickel conditioned by the way of metal penetration. *Acta Sci Pol Hortorum Cultus* 4:139–152
- Velikova V, Tsonev T, Loreto F, Centritto M (2011) Changes in photosynthesis, mesophyll conductance to CO₂, and isoprenoid emissions in *Populus nigra* plants exposed to excess nickel. *Environ Pollut* 159:1058–1066. <https://doi.org/10.1016/j.envpol.2010.10.032>
- Vernay P, Gauthier-Moussard C, Hitmi A (2007) Interaction of bioaccumulation of heavy metal chromium with water relation, mineral nutrition and photosynthesis in developed leaves of *Lolium perenne* L. *Chemosphere* 68:1563–1575. <https://doi.org/10.1016/j.chemosphere.2007.02.052>
- Wolf B (1982) A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Commun Soil Sci Plant Anal* 13:1035–1059. <https://doi.org/10.1080/00103628209367332>
- Wood BW, Reilly CC, Nyczepir AP (2006) Field deficiency of nickel in trees: symptoms and causes. *Acta Hort* 721:83–98. <https://doi.org/10.17660/ActaHortic.2006.721.10>
- Wood BW (2013) Iron-induced nickel deficiency in pecan. *Hort Sci* 48:1145–1153. <https://doi.org/10.21273/HORTSCI.48.9.1145>
- Yang G, Joly RG, RJ (1996) Effects of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficiency and glycinebetaine-containing maize lines. *Aust J Plant Physiol* 23:437–443. <https://doi.org/10.1071/PP9960437>
- Younis U, Danish S, Malik SA, Ahmed N, Munir TM, Rasheed MK (2020) Role of cotton sticks biochar in immobilization of nickel under induced toxicity condition and growth indices of *Trigonella corniculata* L. *Environ Sci Pollut Res* 27:1752–1761. <https://doi.org/10.1007/s11356-019-06466-3>

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