




ISSN 2709–3662 (Print)  
ISSN 2709–3670 (Online)  
<https://doi.org/10.52587/JAF050203>  
*Journal of Agriculture and Food*  
2024, Volume 5, No.2, pp. 37-54

## Insights into morphological, physio-biochemical, and phytoremediation alterations in ornamental plants under nickel stress

Amir Hameed<sup>a</sup>, Gulzar Akhtar<sup>a\*</sup>, Nazar Faried<sup>a</sup>, Sami Ullah<sup>a</sup>, Muhammad Asif  
Shehzad<sup>b</sup> , Ishtiaq A. Rajwana<sup>a</sup>, Kashif Razzaq<sup>a</sup>, Tahir Saeed<sup>c</sup>, Iftikhar Ahmad<sup>c</sup>,  
Muhammad Rizwan Shah<sup>a</sup>

### Abstract

Nickel (Ni) is an essential metal that causes soil toxicity at higher levels and reduces crop growth and quality. Therefore, the present study was planned to explore the soil phytoremediation potential of different ornamental plants (stock, snapdragon, and gladiolus) in Ni-contaminated soil. Stock, snapdragon, and gladiolus plants were grown in pots supplemented with different levels of Ni (0, 20, 40, 60, 80  $\mu\text{M}$ ) to analyze different growth, physiological, biochemical, and phytoremediation parameters. Results showed that a higher level of Ni (80  $\mu\text{M}$ ) significantly decreased growth and physio-chemical attributes of stock, snapdragon, and gladiolus. Maximum shoot length (15.40%), root length (16.00%), shoot fresh weight (6.75%), root fresh weight (15.19%), shoot dry weight (19.80%), root dry weight (27.52%), relative water content (12.29%), membrane stability index (10.64%) and total chlorophyll content (4.33%) were recorded in stock flower at 20  $\mu\text{M}$ . Moreover, higher values for photosynthetic rate (27.81%), transpiration rate (9.23%), stomatal conductance (19.89%), and sub-stomatal conductance (44.19%) were noted in stock flower at 40  $\mu\text{M}$ . Whereas, the maximum activities of catalase (20.41%), peroxidase

---

<sup>a</sup>Department of Horticulture, Muhammad Nawaz Shareef University of Agriculture Multan, Pakistan; <sup>b</sup>Institute of Plant Breeding and Biotechnology, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan; <sup>c</sup>Horticultural Research Sub-Station for Floriculture and Landscaping, Multan, Pakistan; \* Corresponding author: Gulzar Akhtar ([gulzar.akhtar@mnsuam.edu.pk](mailto:gulzar.akhtar@mnsuam.edu.pk))

(66.17%), and superoxide dismutase (64.48%) were measured in stock flower at 60  $\mu\text{M}$ . Stock plants showed more tolerance against Ni toxicity than snapdragon and gladiolus based on a higher bio concentration factor (70.70%) and lesser translocation factor (46.44%) at 60  $\mu\text{M}$  and 40  $\mu\text{M}$  respectively. Conclusively, stock has performed better than snapdragon and gladiolus for phytoremediation of Ni-polluted soil.

**Keywords:** Soil pollution, Crop productivity, Soil remediation, Phytoextraction, *Mathiola incana*

Article History: 12<sup>th</sup> December, 2024 **Received:** 13<sup>th</sup> January, 2025; **Revised:** Accepted: 14<sup>th</sup> January, 2025

## Introduction

Soil pollution is one of the major global issues that affect the quality of soil, water, and food (Massa et al. 2010). One of the key factors contributing to soil degradation is the addition of heavy metals (HMs) in the soil. These metals have densities higher than 5  $\text{gcm}^{-3}$  with poisonous, cancer-causing effects for humans, plants, and animals (Ali and Khan, 2018). These metals are added to the soil through different natural (weathering of rocks) and anthropogenic (excessive use of fertilizers, pesticides, coal burning, sewage sludge and composted manures, etc.) activities (Damodaran et al. 2014; Hagmann et al. 2015; Gupta et al. 2016). Cadmium, zinc, mercury, nickel, lead, and cobalt are common HMs causing soil pollution (Zhang et al. 2015).

Nickel (Ni) is 5<sup>th</sup> most abundant HM on earth with atomic number 28 and 3% concentration in the earth's crust (Cempel and Nikel, 2006; Harasim and Filipek, 2015). Globally it is produced at a rate of around 130,000 metric tons per year with China, Australia, Canada, Philippines, Indonesia, Cuba, and Russia as major producing nations. Its toxicity adversely affects plant growth and development by affecting their physiological and biochemical attributes (Amjad et al. 2020; Rai et al. 2019). Leaf chlorosis, stunted growth, distortion of plant components, and the production of free radicals are common symptoms of Ni toxicity in plants (Smialowicz et al. 1984; Subhashini and Swamy, 2013).

For the reclamation of HM-polluted soil different methods could be used like thermal treatment, acid leaching, electro-reclamation, excavation, solarization, and landfilling of contaminated places (Kamran et al. 2014; Mishra et al. 2017). However, these approaches are expensive and time-consuming. Besides the high cost, these technologies also affect the fertility of the soil and harm the natural environment (Brack et al. 2015). On the other hand, soil phytoremediation is a safe, efficient, and economically viable approach to mitigate soil HM pollution (Zhang et al. 2013). Chaney first time in 1983 introduced the term phytoremediation by which plant variety could be used to remove (organic) carbon-based and (inorganic) non-carbon contaminants from the soil. Phytoremediation includes different mechanisms like phytodegradation, phytovolatilization, phytostabilization, and phytoextraction to remove HMs from the soil (Trapp et al. 2001; Cristaldi et al. 2017; García-Sánchez et al. 2018). Previously, different food crops were used for phytoremediation of HMs from the soil like. Consumption of these HMs polluted foods causes harmful effects on human health. Whereas, ornamental plants are used for aesthetic beautification and could be an efficient source to mitigate soil HM pollution (Gupta et al. 2013).

Snapdragon (*Antirrhinum majus*) is an important herbaceous perennial flower belonging to the family Plantaginaceae and is commonly used as a potted and bedded plant (Seo et

al. 2020). Stock (*Mathiola incana*) is another flowering plant of the winter season that belongs to the family Brassicaceae and is commonly used as a cut flower and bedded plant (Miceli et al. 2019). Gladiolus (*Gladiolus grandiflora*) is commonly known as a sward lily due to its sward-like flowers of different colors that is the most popular cut flowers of the local floriculture industry. It is also known as the "queen of bulbous flowers" because of its flower spikes, which can be up to 3 feet tall and bear large, colorful florets. It is also rated as being the second most popular cut flower that is grown all over the world including Pakistan (Khan et al. 2021).

Although few reports are available on the phytoremediation potential of ornamental plants still knowledge about the combined response of *Antirrhinum majus*, *Mathiola incana*, and *Gladiolus grandiflora* in Ni-contaminated soils needs to be explored. therefore, this study was planned to assess the morphological, physiological, and biochemical potential of *Antirrhinum majus*, *Mathiola incana*, and *Gladiolus grandiflora* in Ni-contaminated soil.

## Materials and Methods

### *Planting materials and Research area*

Present research work was carried out during the winter of 2022 in B-Block MNS University of Agriculture Multan. Healthy and uniform seedlings of *Mathiola incana* (stock) and *Antirrhinum majus* (snapdragon) were purchased from Hammad Nursery Farm Multan and acclimatized for 15 days before transplanting. *Gladiolus grandiflorus* (gladiolus) bulbs were purchased from Lahore and disinfected in Topsin-M (active ingredient thiophanate-methyl 70%) for 30 minutes followed by air-drying. Loam soil was used having 0.77% organic matter, pH 8.0, electrical conductivity 1.59  $\mu\text{S}/\text{cm}$ , saturation percentage 33%, available phosphorus 9.46 mg/kg, and available potassium 186 mg/kg. Soil particles larger than 2 mm were removed by sieving with 2 mm mesh. The soil was filled in 9-inch clay pots; each pot contained 3 kg of soil. Two seedlings were transplanted in each pot while two bulbs of gladiolus were sown in each pot. Different doses of Ni ( ) were applied to the plant through soil application at four

### *Determination of growth parameters*

Experimental plants were harvested from each pot. After 50 to 70 days of exposure to heavy metals. Stock plants were harvested when one-third to one-half of the florets on the stem were open and gladiolus was harvested when the lowermost florets had just shown colure. Snapdragon was harvested when the first three florets were open. The measuring scale was used to record the shoot length (SL) and root length (RL) from base to tip of shoots and root whereas, scientific weight balance (Sanyo-model DY-728) was used to record the shoot fresh weight (SFW) and root fresh weight (RFW) while samples were oven dried (model SLN.15) at 60 °C for 24 hours for to determine shoot dry weight (SDW) and root dry weight (RDW).

### *Relative water content and membrane stability index*

Young and healthy leaves of gladiolus, stock, and snapdragon were excised in the morning to measure fresh weight (Wf) using scientific balance and oven dried for 24 hours at 60 °C to measure dry weight (Wd). RWC was calculated by using the equation given by (Barrs, 1968).

$$\text{RWC}\% = \frac{(\text{Wf} - \text{Wd})}{\text{Wf}} \times 100$$

*Chlorophyll contents*

Chlorophyll contents were measured with a SPAD-502 chlorophyll meter (Konica Minolta, Europe) from leaves of gladiolus, stock.

*Measurement of gaseous attributes*

Gaseous exchange parameters like photosynthetic rate ( $A$ ), transpiration ( $E$ ), stomatal conductance ( $g_s$ ), and sub-stomatal conductance ( $C_i$ ) were determined from healthy leaves of the gladiolus, stock, and snapdragon under full sunlight using CIRAS-3 SW portable photosynthetic system (manufactured by PP system, MA, USA serial no c3F0255) at 12 to 2 pm.

*Determination of enzymatic activity*

Catalase (CAT) activity was determined from the leaves using the procedure of Liu *et al.* (2007) whereas, peroxidase (POX) and superoxide dismutase (SOD) activities were also determined from leaf samples by using the procedure of Zhang *et al.* (2015) and respectively.

*Estimation of bio-concentration factor and translocation factor*

Bio-concentration factor (BCF) and translocation factor (TF) were determined from the plants by using the procedure of Amin *et al.* (2021) and following equations were used to calculate the BCF and TF.

$$\text{BCF} = \text{Ni in plant} / \text{Ni in soil}$$

$$\text{TF} = \text{Ni in shoot} / \text{Ni in root}$$

The data was analyzed using Statistix 8.1, and a linear model was selected to examine the relationships between variables. Subsequently, ANOVA was used to evaluate treatment means. The Completely Randomized Design (CRD) with a two-factor factorial arrangement was utilized for the experimental design and the LSD (Least Significant Difference) test was applied to compare the means (Steel *et al.* 2019).

**Results***Growth attributes*

A marked increase in SL (15.4%) and RL (16%) of stock plants was observed at 20  $\mu\text{M}$  (Ni) in parallel to control. Under 80  $\mu\text{M}$  Ni, a maximum reduction of 14.7% and 4.7% in SL and RL respectively was recorded than control. In Snapdragon, a significant increase of 13.5% SL and 6.4% RL was recorded at Ni (20  $\mu\text{M}$ ); while the maximum reduction by 17.53% (SL) and 26.4% (RL) was noted at Ni (80  $\mu\text{M}$ ) in comparison to control. Gladiolus supplemented with 20  $\mu\text{M}$  Ni exhibited a notable increase in SL and RL by 4.96% and 5.5% respectively, whereas, a marked decrease in SL (18.1%) and RL (31.4%) was recorded at 80  $\mu\text{M}$  Ni as compared to control (Table 1). A marked increase in SFW and RFW of 6% and 15.2%, respectively were noted in stock plants exposed to 20  $\mu\text{M}$  (Ni) than control (no Ni). Whereas, 80  $\mu\text{M}$  (Ni) revealed the highest reduction in SFW and RFW by 20.3% and 7.6%, respectively in parallel to the control. Likewise, snapdragon plants supplemented with Ni (20  $\mu\text{M}$ ) exhibited a notable increase in SFW (10%) and RFW (10.3%), However, Ni (80  $\mu\text{M}$ ) considerably reduced SFW and RFW by 30% and 39% respectively the control. Gladiolus exhibited a significant increase in SFW and RFW by 5% each at Ni (20  $\mu\text{M}$ ) compared to control. While, Ni (80  $\mu\text{M}$ ) notably decreased SFW and RFW by 27% and 39%, respectively, in comparison with the control (Table 1). Maximum increased SDW and RDW by 9.8% and 27.48% respectively in stock plants were observed at 20  $\mu\text{M}$  Ni in parallel to control whereas, 29.75% and 18.95% reduction in RDW and SDW was recorded at 80  $\mu\text{M}$  Ni. In snapdragon, a significant increase of

15.4% SDW and 47.3% RDW was recorded at Ni (20  $\mu\text{M}$ ) while, the maximum reduction in SDW (51.52%) and RDW (34%) was noted at Ni (80  $\mu\text{M}$ ) in comparison to control. Gladiolus supplemented with 20  $\mu\text{M}$  (Ni) exhibited a notable increase in SDW and RDW (7.99% and 10.3% respectively) whereas, marked decrease of 39% (SDW) and 33.68% (RDW) was recorded at 80  $\mu\text{M}$  (Ni) as compared to control (Table 1).

*Relative water contents and Membrane stability index*

Stock plants supplemented with Ni (20  $\mu\text{M}$ ) markedly improved RWC and MSI by 15% and 5% respectively in parallel to control (Table 2). However, a higher level of Ni (80  $\mu\text{M}$ ) resulted in a maximum reduction in RWC (7%) and MSI (10%) than control. Supplementation of snapdragons with 20  $\mu\text{M}$  of Ni demonstrated an enhancement in RWC and MSI by 15% and 5% respectively; whereas, Ni at 80  $\mu\text{M}$  elicited a significant reduction in RWC (10%) and MSI (12%) in comparison with control conditions. Gladiolus supplemented with Ni at 20  $\mu\text{M}$  exhibited a significant increase in RWC (10%) and MSI (8%); however, a marked decrease in RWC and MSI by 12% and 15% respectively was observed at 80  $\mu\text{M}$  (Ni) compared to the control conditions (Table 2).

**Table 1.** Shoot and root length, fresh and dry weight of stock, snapdragon, and gladiolus grown in the Ni-contaminated soil (0, 20, 40, 60, 80  $\mu\text{M}$ ).

Treatments Ni $\mu\text{M}$	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weigh t (g)	Shoot dry weigh t (g)	Root dry weigh t (g)
<b>Stock + Control</b>	58.08 $\pm 1.44^b$ c	42.00 $\pm 1.05^c$ d	50.67 $\pm 1.00^b$	13.00 $\pm 0.48^b$	10.53 $\pm 0.63^b$	3.17 $\pm 0.15^{ef}$
<b>Stock + 20</b>	68.67 $\pm 0.733^a$	50.00 $\pm 0.94^a$	54.33 $\pm 0.73^a$	15.33 $\pm 0.73^{ab}$	13.13 $\pm 0.39^a$	4.37 $\pm 0.19^d$
<b>Stock + 40</b>	60.75 $\pm 1.08^{bc}$	44.33 $\pm 0.35^b$	49.67 $\pm 1.00^b$	12.33 $\pm 0.73^{bc}$	12.60 $\pm 0.24^a$	3.23 $\pm 0.10^e$
<b>Stock + 60</b>	54.35 $\pm 1.26^c$	43.92 $\pm 0.35^{bc}$	46.33 $\pm 0.73^c$	11.00 $\pm 0.96^{cd}$	8.70 $\pm 0.19^{cd}$	3.10 $\pm 0.10^{ef}$
<b>Stock + 80</b>	49.50 $\pm 0.66^{de}$	40.00 $\pm 0.72^d$	40.33 $\pm 0.73^d$	12.00 $\pm 0.48^{b-d}$	7.40 $\pm 0.34^{d-f}$	2.57 $\pm 0.12^{fg}$
<b>SD + Control</b>	35.17 $\pm 0.49^{hi}$	34.00 $\pm 0.48^{fg}$	28.00 $\pm 0.96^f$	4.57 $\pm 0.20^g$	5.50 $\pm 0.29^{gh}$	3.37 $\pm 0.24^e$
<b>SD + 20</b>	40.67 $\pm 2.46^{fg}$	36.33 $\pm 0.84^e$	31.33 $\pm 0.73^f$	8.67 $\pm 0.37^{ab}$	6.50 $\pm 0.52^b$	4.27 $\pm 0.18^a$

<b>SD + 40</b>	34.70 ± 0.67 <sup>hi</sup>	33.40 ± 1.13 <sup>g</sup>	25.00 ±0.48 <sup>fg</sup>	6.83 ± 0.77 <sup>f</sup>	4.80 ± 0.14 <sup>hi</sup>	2.00 ± 0.10 <sup>gh</sup>
<b>SD + 60</b>	31.53 ± 0.72 <sup>ij</sup>	27.47 ± 0.65 <sup>h</sup>	23.00 ±1.27 <sup>gh</sup>	4.10 ± 0.53 <sup>g</sup>	4.10 ± 0.38 <sup>i</sup>	1.57 ± 0.25 <sup>h</sup>
<b>SD + 80</b>	29.00 ± 0.48 <sup>i</sup>	25.00 ± 0.64 <sup>ij</sup>	19.33 ±0.73 <sup>ij</sup>	3.00 ± 0.48 <sup>g</sup>	2.67 ± 0.50 <sup>j</sup>	1.43 ± 0.19 <sup>h</sup>
<b>Glad + Control</b>	44.00 ± 0.72 <sup>ef</sup>	34.00 ± 0.96 <sup>fg</sup>	25.67 ±0.77 <sup>fg</sup>	13.50 ± 0.30 <sup>ab</sup>	9.60 ± 0.25 <sup>bc</sup>	6.43 ± 0.18 <sup>b</sup>
<b>Glad + 20</b>	46.30 ± 0.53 <sup>de</sup>	36.00 ± 0.48 <sup>ef</sup>	27.17 ±0.50 <sup>e</sup>	15.07 ± 0.67 <sup>ef</sup>	10.43 ± 0.67 <sup>e-g</sup>	7.93 ± 0.19 <sup>d</sup>
<b>Glad + 40</b>	42.23 ± 0.36 <sup>fg</sup>	33.63 ± 1.1 <sup>g</sup>	23.33 ±0.73 <sup>gh</sup>	12.23 ± 0.33 <sup>bc</sup>	8.67 ± 0.28 <sup>cd</sup>	5.43 ± 0.19 <sup>c</sup>
<b>Glad + 60</b>	38.17 ± 0.49 <sup>gh</sup>	26.33 ± 0.50 <sup>hi</sup>	21.83 ±0.84 <sup>hi</sup>	10.17 ± 0.50 <sup>de</sup>	7.83 ± 0.73 <sup>de</sup>	4.63 ± 0.26 <sup>d</sup>
<b>Glad + 80</b>	36.00 ± 0.96 <sup>hi</sup>	23.30 ± 0.58 <sup>i</sup>	18.50 ±1.34 <sup>j</sup>	8.17 ± 0.60 <sup>f</sup>	6.37 ± 0.49 <sup>fg</sup>	3.37 ± 0.15 <sup>e</sup>
<b>P-value</b>						
<b>T</b>	0	0	0	0	0	0
<b>V</b>	0	0	0	0	0	0
<b>T x V</b>	0.0077	0.0002	0.3487	0.0343	0.0505	0
<b>CV</b>	4.2	4.5	5.61	12.03	11.56	9.95

### Chlorophyll

Ni (40  $\mu$ M) supply to stock plants revealed the highest increase in chlorophyll by 9.4%; however, Ni at 80  $\mu$ M caused a reduction in chlorophyll by 7% in contrast to control plants. Snapdragon, at 20  $\mu$ M (Ni) caused a significant increase in chlorophyll by 10% in parallel to the control. While the highest reduction in chlorophyll by 12% was recorded in Ni (80  $\mu$ M) exposed snapdragon plants than control. Gladiolus plants supplemented with 20  $\mu$ M Ni increased chlorophyll contents by 8%; whereas, Ni (80  $\mu$ M) caused a maximum reduction of 5.24% in chlorophyll in comparison with the control (Table 2).

### Gaseous attributes

Net photosynthesis rate (*A*) and transpiration rate (*E*) were remarkably increased by 27% and 9.2% respectively in stock plants at Ni 40  $\mu$ M whereas, the minimum increase of 13.34% and 14.12% in *A* and *E* respectively was recorded at Ni 80  $\mu$ M in contrast to control (Figure 1a,b). Likewise, in snapdragon, a significant increase of 5.4% and 56.2% in *A* and *E* respectively was observed at Ni 20  $\mu$ M and 60  $\mu$ M than control. along with

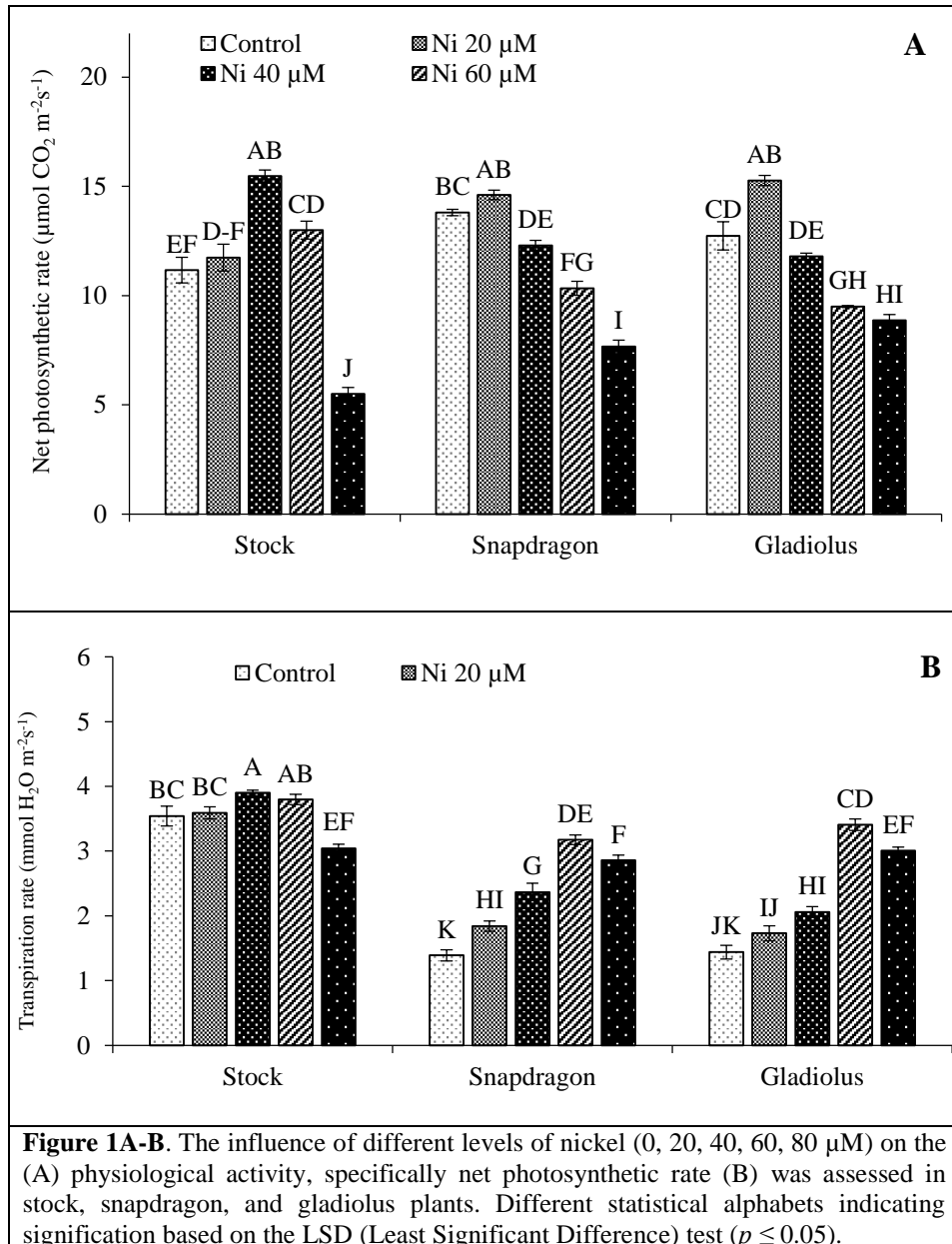
suppression in values of *A* (22.72%) and *E* (24.45%) at 80 and 20  $\mu\text{M}$  respectively was recorded in contrast to control. In gladiolus, a prominent increase of 16.5% at 20  $\mu\text{M}$  and efficient demoted of 18.01% at 80  $\mu\text{M}$  in *A* were observed and a marked increase of 57% in *t* under Ni 60  $\mu\text{M}$  along with significant retardation by 24.45% at 20  $\mu\text{M}$  was recorded in parallel to control.

Stomatal conductance (*gs*) and sub-stomatal conductance (*Ci*) remarkably increased by 19.8% and 44% correspondingly in stock at Ni 40  $\mu\text{M}$ . In contrast maximum decline was noted in *gs* (0.6%) and *Ci* (31.46%) when stock was subjected to Ni 80  $\mu\text{M}$  in contrast to control (Fig 1c, d). Likewise, in snapdragon, a remarkable increase of 4.5% in *gs* and 6.1%

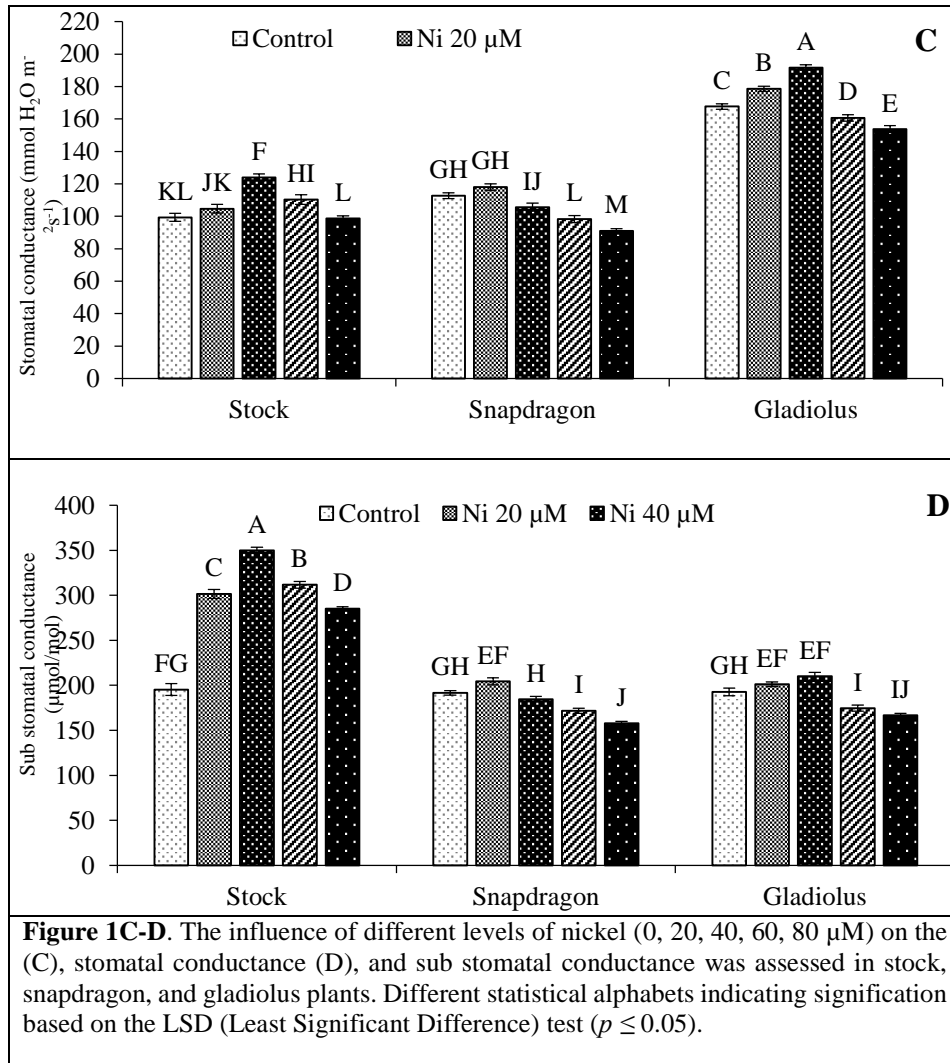
**Table 2.** Relative water content, membrane stability index, and chlorophyll content of stock, snapdragon, and gladiolus grown in the Ni-contaminated soil (0, 20, 40, 60, 80  $\mu\text{M}$ ).

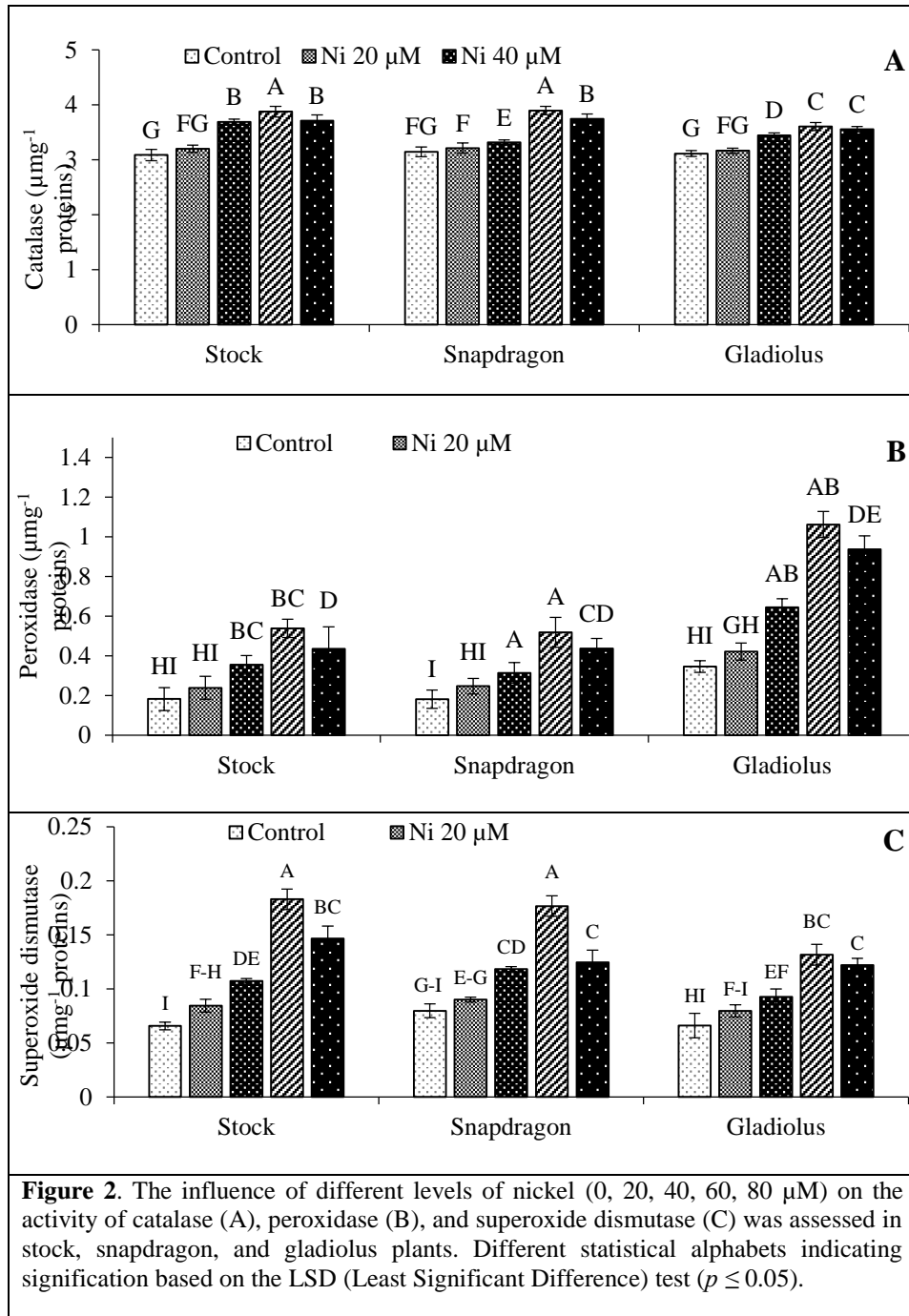
Treatments Ni $\mu\text{M}$	Relative water content (%)	Membrane stability index (%)	Chlorophyll content (SPADE)
Stock + Control	53.50 $\pm$ 1.34 <sup>c</sup>	58.53 $\pm$ 0.91 <sup>ef</sup>	67.45 $\pm$ 1.14 <sup>c</sup>
Stock + 20	61.00 $\pm$ 0.87 <sup>a</sup>	65.50 $\pm$ 1.14 <sup>bc</sup>	70.52 $\pm$ 0.84 <sup>b</sup>
Stock + 40	55.17 $\pm$ 0.50 <sup>bc</sup>	53.73 $\pm$ 1.59 <sup>g</sup>	74.45 $\pm$ 1.03 <sup>a</sup>
Stock + 60	46.00 $\pm$ 0.87 <sup>d</sup>	49.10 $\pm$ 1.01 <sup>h</sup>	67.30 $\pm$ 1.04 <sup>c</sup>
Stock + 80	41.17 $\pm$ 0.84 <sup>de</sup>	43.83 $\pm$ 1.08 <sup>i</sup>	58.45 $\pm$ 1.20 <sup>f</sup>
SD + Control	52.83 $\pm$ 1.21 <sup>c</sup>	58.83 $\pm$ 0.69 <sup>d-f</sup>	59.53 $\pm$ 0.88 <sup>ef</sup>
SD + 20	59.33 $\pm$ 0.84 <sup>a</sup>	62.17 $\pm$ 1.69 <sup>a</sup>	63.37 $\pm$ 1.03 <sup>b</sup>
SD + 40	45.17 $\pm$ 2.59 <sup>de</sup>	53.50 $\pm$ 1.18 <sup>g</sup>	59.00 $\pm$ 0.96 <sup>ef</sup>
SD + 60	41.83 $\pm$ 1.13 <sup>de</sup>	43.80 $\pm$ 1.47 <sup>i</sup>	52.33 $\pm$ 0.73 <sup>h</sup>
SD + 80	31.67 $\pm$ 1.60 <sup>f</sup>	31.67 $\pm$ 1.73 <sup>j</sup>	46.00 $\pm$ 0.94 <sup>i</sup>
Glad + Control	57.67 $\pm$ 0.97 <sup>a-c</sup>	66.23 $\pm$ 1.23 <sup>b</sup>	68.07 $\pm$ 0.87 <sup>c</sup>
Glad + 20	60.67 $\pm$ 0.84 <sup>ab</sup>	72.57 $\pm$ 2.35 <sup>cd</sup>	71.83 $\pm$ 1.00 <sup>d</sup>
Glad + 40	52.67 $\pm$ 1.08 <sup>c</sup>	59.37 $\pm$ 0.60 <sup>de</sup>	65.87 $\pm$ 1.01 <sup>c</sup>
Glad + 60	44.33 $\pm$ 2.41 <sup>de</sup>	55.67 $\pm$ 0.67 <sup>fg</sup>	60.77 $\pm$ 0.87 <sup>ef</sup>
Glad + 80	40.67 $\pm$ 1.70 <sup>e</sup>	49.20 $\pm$ 1.81 <sup>h</sup>	55.80 $\pm$ 0.73 <sup>g</sup>
<i>P</i> -value			
T	0000	0	0
V	0000	0	0
T $\times$ V	0.0575	0.012	0.0014
CV	5.79	5.18	3.19

in *Ci* was observed when supplemented with Ni (20  $\mu\text{M}$ ). In contrast, maximum decline was noted by 19.2% for *gs* and 17.7% for *Ci* when snapdragon was exposed to Ni 80  $\mu\text{M}$  in contrast to control. In gladiolus, a prominent increase of 12.5% in *gs* and 8.2% in *Ci* was observed when supplemented with Ni 40  $\mu\text{M}$ . In contrast, the maximum decline was noted by 8.3% in *gs* and 13.4% in *Ci* when gladiolus was polluted to Ni 80  $\mu\text{M}$  in parallel to control (Figure 1c,d)







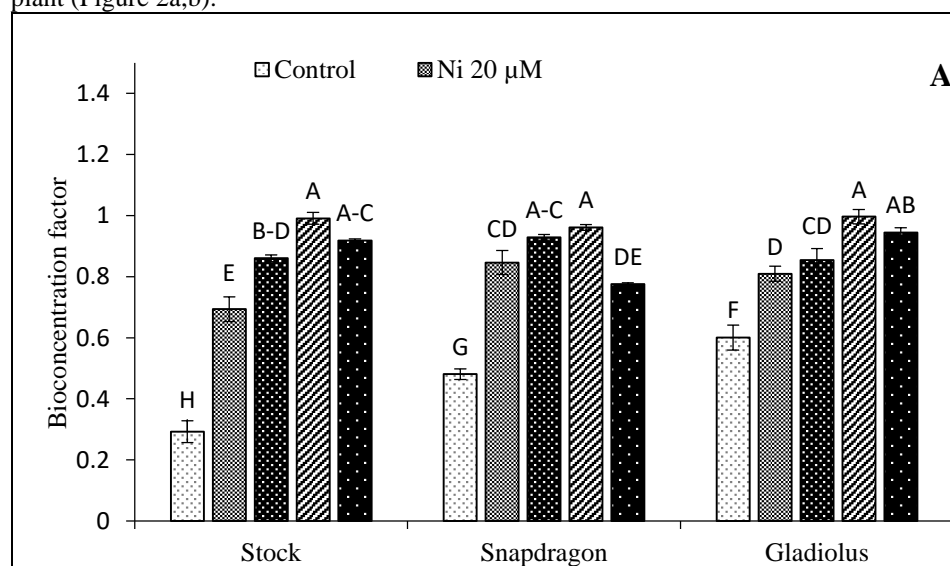


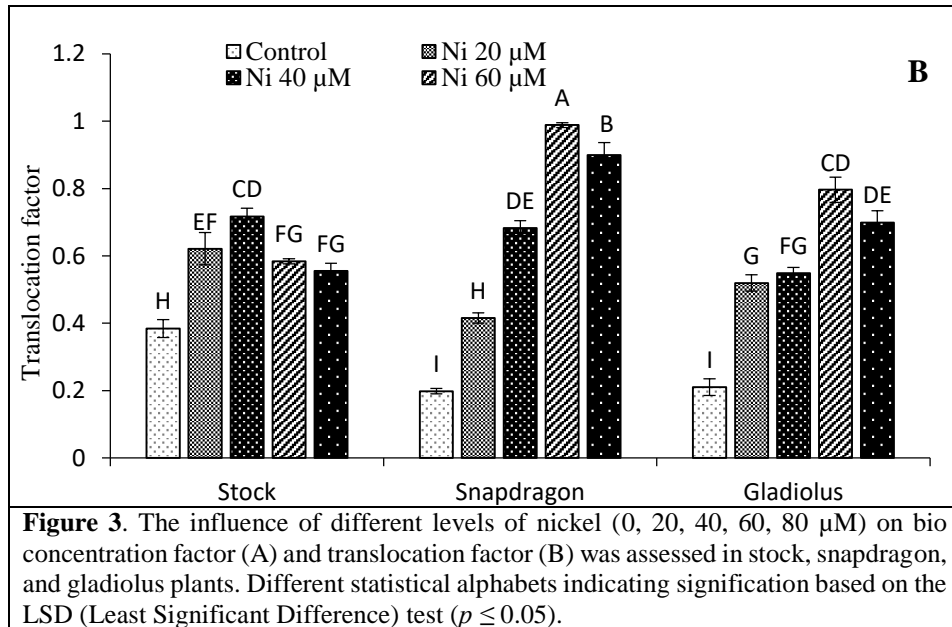
### Antioxidant activities

Nickel application significantly increased the CAT, POX, and SOD activity of stock plant by 20.3%, 66%, and 64% respectively at 60  $\mu\text{M}$  Ni in contrast to control (Figure 2a,b,c). However, minimum CAT (3.75%), POX (21.73%), and SOD (25%) were recorded in leaves of stock plant at 20  $\mu\text{M}$  than control. A significant increase of CAT (19.2%), POX (65%), and SOD (54%) was recorded in snapdragon plants at 60  $\mu\text{M}$  Ni; whereas, a minimum increment of 2.1%, 25%, and 22.22% in CAT, POX, and SOD respectively was noted at 20  $\mu\text{M}$  in comparison with control. Gladiolus plants exhibited 13.8%, 67%, and 49% increase in CAT, POX, and SOD activities respectively at 60  $\mu\text{M}$  Ni, and minimum increase of 1.61%, 17.81%, and 16.45% for CAT, POX, and SOD respectively was noted at 20  $\mu\text{M}$  of Ni in comparison to control.

### Phytoremediation

A remarkable increase in BCF (70.50%) by stock was observed when Ni reached a concentration of 60  $\mu\text{M}$ . While improvement in BCF (57.86%) was noted at 20  $\mu\text{M}$  with respect to control (Fig 3a). Snapdragon plant showed less improvement for BCF than stock. Maximum improvement (50.05%) was noted at 60  $\mu\text{M}$ . On the other hand, the lowest value for BCF was measured (43.95%) when plants were subjected to 20  $\mu\text{M}$  compared to control. Whereas, gladiolus showed maximum and minimum values for BCF by 39.75% and 25.83% at 80 and 20  $\mu\text{M}$  of Ni respectively. The value of TF was significantly higher for snapdragon as compared to gladiolus and stock, maximum TF was 79.26% at 60  $\mu\text{M}$  while the minimum value of TF was 52.40% (Fig 3b). In gladiolus the TF value was increased with the increase of Ni concentration, the maximum was 73.61% and 69.53% under 60  $\mu\text{M}$  and 20  $\mu\text{M}$  compared to the control. Under higher (40  $\mu\text{M}$ ) and lower (20  $\mu\text{M}$ ) concentrations of Ni, TF was 46.44% and 38.16% respectively in stock plant (Figure 2a,b).





## DISCUSSION

Heavy metal pollution in soil poses a significant environmental challenge due to its adverse effects on ecosystems and human health (Ameen et al. 2019a). Ni toxicity causes hindrance in plant growth, yield, and quality due to disruption in water relations, photosynthetic activity, and antioxidative defense system (Parvez et al. 2020). To mitigate soil Ni pollution, the present study explored the potential of different ornamental plants (*Mathiola incana*, *Antirrhinum majus*, *Gladiolus grandiflorus*) to absorb Ni from the soil with minimum effects on plant growth and development. According to the results, lower doses of Ni improved the growth attributes of stock, snapdragon, and gladiolus whereas, higher Ni doses adversely affected the growth of plants in stock plants and showed an increase in growth parameters at 40 µM (Ni). But when the level of Ni increased to 80 µM, a significant decrease in the growth parameters was recorded in plants of stock, snapdragon, and gladiolus.

The significant decrease in growth attributes at lower doses (20–40 µM Ni) might be due to the essentiality of Ni as a micro-nutrient and its contribution in different biological activities. Nickel perfume has its duties as a cofactor in the urease enzyme which helps in the recycling of nitrogen (conversion of organic urea into ammonia and carbon dioxide) (Goyal et al. 2020). Moreover, the availability of Ni helps to avoid cold injury, and dwarfing of leaves, it also helps to maintain the size of the bud and the distance between nodes. Furthermore, building a strong root system (Wood et al. 2006). The possible reason for the decline of growth parameters and chlorophyll contents at higher Ni doses (80 µM) may be that Ni phytotoxicity leads to a decrease in growth (Shahid et al. 2014; Ameen et al. 2019b). Ni toxicity alters plants' growth directly by affecting cell membrane plasticity, distressing the function of plasma membrane, and lipid peroxidation and ultimately

causing inequity in ion exchange between membranes and disturbing membrane molten/fluid. Furthermore, triggered depletion of protein (LHW), failure in gene expression, and enzyme inhibition by the SH- group (Rubisco & nitrate reductase). While, indirect effects include decreased photosynthesis, Calvin cycle enzyme inhibition, electron transportation affected PS II, Thylakoid membrane disruption. Modulate gene expression, competitive inhibition, and disturbed absorption of iron, zinc, and magnesium (Ahmad et al. 2007).

Higher doses of Ni significantly decreased the gaseous exchange (*A*, *E*, *G<sub>s</sub>*, *C<sub>i</sub>*) attributes in stock, snapdragon, and gladiolus plants might be due to the reason that Ni toxicity is associated with the closing of stomata which causes a decline of transpiration rate that affects photosynthesis process (Seregin and Ivanov, 2001). Previously, Lin and Kao (2007) also observed a reduction in stomatal conductance, photosynthesis, and chlorophyll contents in plants subjected to higher Ni doses. Similarly, Wheeler et al. (2001) found that Ni concentration (0.025 mM) caused a reduction in leaf number and chlorophyll content (24-47%). Similarly, Ahmad and Rasool (2014) studied *Vigna radiata* under nickel stress and observed the higher Ni concentration chlorophyll contents of the plant. Another study was conducted on *Vigna mungo L.* and the results showed a significant decline in photosynthetic pigments when the plants were subjected to Ni stress and further concluded that accumulation of Ni has a significant relation with physiological attributes of plants (Dubey and Pandey, 2011). Likewise, stomatal conductance in the developing leaves of *Populus nigra L.* dropped to the extent of 0.40-0.03 mol when the plant was exposed to a higher concentration of Ni (200 µM). The decrease in stomatal conductance is responsible for the inhibition of photosynthesis (Velikova et al. 2011) Previously, Masidur Alam et al. (2007) also recorded photosynthetic pigments and photosynthetic activity in *Brassica junca* plants subjected to Ni toxicity.

Enzymatic activity (CAT, POX, and SOD) of stock, snapdragon, and gladiolus plants was significantly increased in response to the application of different Ni doses that might be due to the activation of the antioxidative defense system to protect the cells from oxidative damage (Shao et al. 2018). Similarly, higher enzymatic activity was recorded in plants of *Pisum sativum* and *Zea mays* grown under Ni stress (Baccouch et al. 2001). However, enzymatic activity adversely affected the plants of stock, snapdragon, and gladiolus with higher Ni doses which may be due to reduced water relation and photosynthetic activity. Previously, Gomes-Junior et al. (2006) also recorded higher ROS generation in *Coffea arabica* subjected to higher Ni dose.

Ornamental plants have the potential to mitigate soil heavy metal pollution due to their higher growth rate and tolerance to contaminants (Liu et al. 2021). Several studies have investigated the potential of ornamental plants for the phytoremediation of heavy metal-contaminated soils. Different plants have different tolerance levels and mechanisms (Havryliuk et al. 2021). Previously, Sajad et al. (2020), identified different ornamental plants (*Xanthium strumarium*, *Filago hurdwarica*, and *Geranium rotundifolium*), as a potential source for phytostabilization and phytoextraction of nickel-contaminated soil. Similarly, *Festuca rubra* was recorded as a potential accumulator of nickel, cobalt, and cadmium (Wyszkowska et al. 2022). The higher phytoremediation potential of *Mirabilis jalapa*, *Eleusine indica*, and *Brassica oleracea* plants was also recorded in Ni-contaminated soils (Gokseven et al. 2021).

## Conclusion

Heavy metal toxicity is a global issue, causing a reduction in the growth and quality of food and ornamental plants. The current study also demonstrated that higher Ni levels significantly decreased plant growth, water relations, photosynthetic pigments, and gaseous exchange in stock, snapdragon, and gladiolus plants. Similarly, antioxidative activity was also reduced in response to Ni stress. However, stock plants showed a higher tolerant level against Ni toxicity than snapdragon and gladiolus. Higher BCF and lower TF values showed that stock has more potential to remediate the Ni-contaminated soil through. Therefore, stock plants could be used to mitigate the Ni toxicity from the polluted soils.

#### **Acknowledgment**

The authors are highly thankful to the Higher Education Commission of Pakistan and Central Lab System, Muhammad Nawaz Shareef University of Agriculture Multan for their financial and infrastructure support.

#### **References**

- Ahmad, P., & Rasool, S. (Eds.). (2014). Emerging technologies and management of crop stress tolerance: volume 1-biological techniques.
- Masidur Alam, M., Hayat, S., Ali, B., & Ahmad, A. (2007). Effect of 28-homobrassinolide treatment on nickel toxicity in *Brassica juncea*. *Photosynthetica*, 45, 139-142.
- Ali, H., & Khan, E. (2018). What are heavy metals? Long-standing controversy over the scientific use of the term 'heavy metals'—proposal of a comprehensive definition. *Toxicological & Environmental Chemistry*, 100(1), 6-19.
- Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, 91(7), 869-881.
- Ameen, N., Amjad, M., Murtaza, B., Abbas, G., Shahid, M., Imran, M., & Niazi, N. K. (2019). Biogeochemical behavior of nickel under different abiotic stresses: toxicity and detoxification mechanisms in plants. *Environmental Science and Pollution Research*, 26, 10496-10514.
- Ameen, N., Amjad, M., Murtaza, B., Abbas, G., Shahid, M., Imran, M., ... & Niazi, N. K. (2019). Biogeochemical behavior of nickel under different abiotic stresses: toxicity and detoxification mechanisms in plants. *Environmental Science and Pollution Research*, 26, 10496-10514.
- Amin, H., Arain, B. A., Jahangir, T. M., Abbasi, A. R., Mangi, J., Abbasi, M. S., & Amin, F. (2021). Copper (Cu) tolerance and accumulation potential in four native plant species: A comparative study for effective phytoextraction technique. *Geology, Ecology, and Landscapes*, 5(1), 53-64.
- Amjad, M., Ameen, N., Murtaza, B., Imran, M., Shahid, M., Abbas, G., ... & Jacobsen, S. E. (2020). Comparative physiological and biochemical evaluation of salt and nickel tolerance mechanisms in two contrasting tomato genotypes. *Physiologia plantarum*, 168(1), 27-37.
- Baccouch, S., Chaoui, A., & El Ferjani, E. (2001). Nickel toxicity induces oxidative damage in *Zea mays* roots. *Journal of plant nutrition*, 24(7), 1085-1097.
- Barrs, H. (1968). Determination of water deficits in plant tissues. *Plant Growth Regulation* 1, 235-368.
- Bharti, R., & Sharma, R. (2022). Effect of heavy metals: An overview. *Materials Today: Proceedings*, 51, 880-885.

- Boros-Lajsner, E., Wyszowska, J., & Kucharski, J. (2021). Phytoremediation of soil contaminated with nickel, cadmium and cobalt. *International journal of phytoremediation*, 23(3), 252-262.
- Cempel, M., & Nikel, G. J. P. J. S. (2006). Nickel: a review of its sources and environmental toxicology. *Polish journal of environmental studies*, 15(3), 37-382.
- Chen, C., Huang, D., & Liu, J. (2009). Functions and toxicity of nickel in plants: recent advances and future prospects. *Clean-soil, air, water*, 37(4-5), 304-313.
- Cristaldi, A., Conti, G. O., Jho, E. H., Zuccarello, P., Grasso, A., Copat, C., & Ferrante, M. (2017). Phytoremediation of contaminated soils by heavy metals and PAHs. A brief review. *Environmental Technology & Innovation*, 8, 309-326.
- Damodaran, D., Shetty, K. V., & Mohan, B. R. (2014). Uptake of certain heavy metals from contaminated soil by mushroom—*Galerina vittiformis*. *Ecotoxicology and Environmental Safety*, 104, 414-422.
- Doumett, S., Lamperi, L., Checchini, L., Azzarello, E., Mugnai, S., Mancuso, S., ... & Del Bubba, M. (2008). Heavy metal distribution between contaminated soil and *Paulownia tomentosa*, in a pilot-scale assisted phytoremediation study: influence of different complexing agents. *Chemosphere*, 72(10), 1481-1490.
- Dubey, D., & Pandey, A. (2011). Effect of nickel (Ni) on chlorophyll, lipid peroxidation and antioxidant enzymes activities in black gram (*Vigna mungo*) leaves. *International Journal of Science Nature* 2(2), 395-401.
- Estefan, G. (2013). Methods of soil, plant, and water analysis: a manual for the West Asia and North Africa region. Beirut, Lebanon: International Center for Agricultural Research in the Dry Areas (ICARDA).
- Gajewska, E., & Skłodowska, M. (2008). Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. *Plant Growth Regulation*, 54, 179-188.
- García-Sánchez, M., Košnář, Z., Mercl, F., Aranda, E., & Tlustoš, P. (2018). A comparative study to evaluate natural attenuation, mycoaugmentation, phytoremediation, and microbial-assisted phytoremediation strategies for the bioremediation of an aged PAH-polluted soil. *Ecotoxicology and Environmental Safety*, 147, 165-174.
- Gökseven, Ş. B. E., Kiran, S., & Ellialtıoğlu, Ş. Ş. (2021). Determination of morphological and physiological changes of ornamental cabbage (*Brassica oleracea* var. *acephala*) against boron toxicity in phytoremediation. *Horticultural Studies*, 38(1), 29-38.
- Gomes-Junior, R. A., Moldes, C. A., Delite, F. S., Gratão, P. L., Mazzafera, P., Lea, P. J., & Azevedo, R. A. (2006). Nickel elicits a fast antioxidant response in *Coffea arabica* cells. *Plant Physiology and Biochemistry*, 44(5-6), 420-429.
- Gupta, A., Joia, J., Sood, A., Sood, R., Sidhu, C., & Kaur, G. (2016). Microbes as potential tool for remediation of heavy metals: a review. *J Microb Biochem Technol*, 8(4), 364-372.
- Gupta, A. K., Verma, S. K., Khan, K., & Verma, R. K. (2013). Phytoremediation using aromatic plants: a sustainable approach for remediation of heavy metals polluted sites. ACS Publications.

- Hagmann, D. F., Goodey, N. M., Mathieu, C., Evans, J., Aronson, M. F., Gallagher, F., & Krumins, J. A. (2015). Effect of metal contamination on microbial enzymatic activity in soil. *Soil Biology and Biochemistry*, *91*, 291-297.
- Harasim, P., & Filipek, T. (2015). *Nickel in the environment*. *Journal of Element 20*: 525–534.
- Hassan, F. A. S., & Fetouh, M. I. (2019). Does moringa leaf extract have preservative effect improving the longevity and postharvest quality of gladiolus cut spikes?. *Scientia Horticulturae*, *250*, 287-293.
- Havryliuk, O. A., Hovorukha, V. M., Gladka, G. V., Bida, I. O., Tashyrev, O. B., & Sachko, A. V. (2021). Bioremoval of hazardous cobalt, nickel, chromium, copper and cadmium compounds from contaminated soil by *Nicotiana tabacum* plants and associated microbiome. *Biosystems Diversity*, *29*(2), 88-93.
- Kamran, M. A., Amna, Mufti, R., Mubariz, N., Syed, J. H., Bano, A., ... & Chaudhary, H. J. (2014). The potential of the flora from different regions of Pakistan in phytoremediation: a review. *Environmental Science and Pollution Research*, *21*, 801-812.
- Kepek, E. S., Severcan, M., Gozen, A. G., & Severcan, F. (2020). Discrimination of heavy metal acclimated environmental strains by chemometric analysis of FTIR spectra. *Ecotoxicology and Environmental Safety*, *202*, 110953.
- Khan, A. H. A., Kiyani, A., Mirza, C. R., Butt, T. A., Barros, R., Ali, B., ... & Yousaf, S. (2021). Ornamental plants for the phytoremediation of heavy metals: Present knowledge and future perspectives. *Environmental Research*, *195*, 110780.
- Lin, Y. C., & Kao, C. H. (2007). Proline accumulation induced by excess nickel in detached rice leaves. *Biologia plantarum*, *51*, 351-354.
- Liu, J. N., Zhou, Q. X., Sun, T., & Wang, X. F. (2007). Feasibility of applying ornamental plants in contaminated soil remediation. *Ying Yong Sheng tai xue bao= The Journal of Applied Ecology*, *18*(7), 1617-1623.
- Liu, Y., Xu, W., Wang, Y., Hao, W., Zhou, Q., & Liu, J. (2021). Growth responses and accumulation characteristics of three ornamental plants to Sn contamination in soil. *Agriculture*, *11*(3), 205.
- Massa, N., Andreucci, F., Poli, M., Aceto, M., Barbato, R., & Berta, G. (2010). Screening for heavy metal accumulators amongst autochthonous plants in a polluted site in Italy. *Ecotoxicology and environmental safety*, *73*(8), 1988-1997.
- Miceli, N., Cavò, E., Ragusa, S., Cacciola, F., Dugo, P., Mondello, L., ... & Taviano, M. F. (2019). Phytochemical characterization and biological activities of a hydroalcoholic extract obtained from the aerial parts of *Matthiola incana* (L.) R. Br. subsp. *incana* (Brassicaceae) growing wild in Sicily (Italy). *Chemistry & biodiversity*, *16*(4), e1800677.
- Mishra, J., Singh, R., & Arora, N. K. (2017). Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. *Frontiers in microbiology*, *8*, 1706.
- Parvez, S., Abbas, G., Shahid, M., Amjad, M., Hussain, M., Asad, S. A., ... & Naeem, M. A. (2020). Effect of salinity on physiological, biochemical and photostabilizing attributes of two genotypes of quinoa (*Chenopodium quinoa* Willd.) exposed to arsenic stress. *Ecotoxicology and environmental safety*, *187*, 109814.



- Patterson, B. D., MacRae, E. A., & Ferguson, I. B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Analytical biochemistry*, *139*(2), 487-492.
- Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K. H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment international*, *125*, 365–385.
- Sajad, M. A., Khan, M. S., Bahadur, S., Shuaib, M., Naeem, A., Zaman, W., & Ali, H. (2020). Nickel phytoremediation potential of some plant species of the Lower Dir, Khyber Pakhtunkhwa, Pakistan. *Limnological Review*, *20*(1), 13–22.
- Seo, J., Lee, J., Yang, H. Y., & Ju, J. (2020). Antirrhinum majus L. flower extract inhibits cell growth and metastatic properties in human colon and lung cancer cell lines. *Food Science & Nutrition*, *8*(11), 6259–6268.
- Seregin, I. V., & Ivanov, V. B. (2001). Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian journal of plant physiology*, *48*, 523-544.
- Shahid, M., Sabir, M., Arif Ali, M., & Ghafoor, A. (2014). Effect of organic amendments on phytoavailability of nickel and growth of berseem (*Trifolium alexandrinum*) under nickel contaminated soil conditions. *Chemical Speciation & Bioavailability*, *26*(1), 37–42.
- Shao, S., Abate, M., Du, X., & Ying, Y. (2018). Exogenous spermidine improves drought tolerance. *Pak. J. Bot*, *50*(3), 921–928.
- Sharma, P., Bhardwaj, R., Arora, N., Arora, H. K., & Kumar, A. (2008). Effects of 28-homobrassinolide on nickel uptake, protein content and antioxidative defence system in *Brassica juncea*. *Biologia Plantarum*, *52*, 767–770.
- Smialowicz, R. J., Rogers, R. R., Riddle, M. M., & Stott, G. A. (1984). Immunologic effects of nickel: I. Suppression of cellular and humoral immunity. *Environmental Research*, *33*(2), 413–427.
- Steel, E. A., Liermann, M., & Guttorp, P. (2019). Beyond calculations: a course in statistical thinking. *The American Statistician*, *73*(sup1), 392–401.
- Subhashini, V., & Swamy, A. V. V. S. (2013). Phytoremediation of Pb and Ni Contaminated Soils Using *Catharanthus roseus* (L.). *Universal Journal of Environmental Research & Technology*, *3*(4).
- Trapp, S., Köhler, A., Larsen, L. C., Zambrano, K. C., & Karlson, U. (2001). Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. *Journal of soils and sediments*, *1*, 71–76.
- Velikova, V., Tsonev, T., Loreto, F., & Centritto, M. (2011). Changes in photosynthesis, mesophyll conductance to CO<sub>2</sub>, and isoprenoid emissions in *Populus nigra* plants exposed to excess nickel. *Environmental Pollution*, *159*(5), 1058-1066.
- Wheeler, C.T., Hughes, L.T., Oldroyd, J., & Pulford, I. D. (2001). Effects of nickel on *Frankia* and its symbiosis with *Alnus glutinosa* (L.) Gaertn. *Plant and Soil*, *231*, 81–90.
- Wyszkowska, J., Boros-Lajsner, E., & Kucharski, J. (2022). Calorific value of *Festuca rubra* biomass in the phytostabilization of soil contaminated with nickel, cobalt and cadmium which disrupt the microbiological and biochemical properties of soil. *Energies*, *15*(9), 3445.

- Zhang, H., Tian, Y., Wang, L., Zhang, L., & Dai, L. (2013). Ecophysiological characteristics and biogas production of cadmium-contaminated crops. *Bioresource technology*, *146*, 628–636.
- Zhang, J., Wang, L. H., Yang, J. C., Liu, H., & Dai, J. L. (2015). Health risk to residents and stimulation to inherent bacteria of various heavy metals in soil. *Science of the total environment*, *508*, 29–36.

**Citation**

- Hameed, A., Akhtar, G., Faried, N., Ullah, S., Shehzad, M.A., Rajwana, I.A., Razaq, K., Saeed, T., Ahmad, I., Shah, M.R. (2024). Insights into morphological, physio-biochemical, and phytoremediation alterations in ornamental plants under nickel stress. *Journal of Agriculture and Food*, *5*(2), 37–54.