



## STRESS RESPONSES AND EVALUATION OF PHYTOREMEDIATION POTENTIAL IN ORNAMENTAL KALE SEEDLINGS EXPOSED TO NICKEL NICKEL STRESS AND PHYTOREMEDIATION POTENTIAL IN ORNAMENTAL KALE SEEDLINGS

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**ABSTRACT** - Phytoremediation is an environmental friendly and cost-effective approach that uses plants to remove hazardous chemicals from contaminated soil, air, and water. The effects of nickel (Ni) concentration on Kamome Red, Kamome White and Kamome Pink cultivars of ornamental kale were evaluated in this study. For this purpose, seeds of cultivars were grown in perlite and were irrigated with 0 (control), 25, 50, 100 ppm Ni solution including Hoagland nutrient solution for 40 days. Results showed that the germination percentage was more than 70% in all cultivars and applications. At a Ni concentration of 25 ppm, it had positive effects on seedling growth parameters with 76.1 mm root length, 73.1 mm shoot length, 6.7 g shoot fresh weight, 0.97 g shoot dry weight. However, high Ni concentrations negatively impacted shoot and root lengths, seedling fresh and dry weights. Seedling water content decreased as Ni concentration increased. Proline content increased significantly in response to high Ni concentrations in Kamome Red and Kamome White cultivars. The Ni concentrations of the cultivars were also affected, with the highest Ni content being obtained from 100 ppm Kamome White (89.32 mg/kg). These findings indicate that Ni concentration has an important effect on the growth of ornamental kale cultivars and K. White showed significant Ni accumulation at 100 ppm. Although Ni uptake occurred, the significant biomass reduction at higher doses limited the phytoremediation potential of the cultivars in the seedling stage. It is recommended to evaluate different Ni concentrations at various growth stages to better assess phytoremediation potential in the future studies.

**KEYWORDS:** HEAVY METAL, NICKEL, TOLERANCE INDEX, ORNAMENTAL KALE, PHYTOREMEDIATION

### INTRODUCTION

Plants are exposed to biotic (bacteria, fungi, viruses) and abiotic (drought, salinity, extreme temperatures, heavy metal pollution) stress factors in natural environments. These stress factors can impact plant growth and yield (Nabi et al., 2019). Heavy metal pollution is a significant abiotic stressor, caused by the release of metallic elements and metalloids from various sources, including industrial waste, secondary metal production, energy production, natural sources like volcanic eruptions and dust or forest fires, fertilizer use, and automobiles (Ghori et al., 2019). Heavy metals are defined as metallic

chemical elements and metalloids, with densities greater than 5 g/cm<sup>3</sup>, including toxic elements such as chromium (Cr), cobalt (Co), nickel (Ni), cadmium (Cd), and lead (Pb) (Briffa et al., 2020). Ni is a heavy metal with its 8.9 g/cm<sup>3</sup> density that is widely present in the environment (22nd most abundant element) and is indispensable for plants from germination to yield (Sreekanth et al., 2013; Shahzad et al., 2018; Zhang et al., 2020). Plants need Ni between 0.01 and 5.0 µg/g dry weight (Seregin & Kozhevnikova, 2006; Rizwan et al., 2017; Zaid et al., 2019). Nickel participates in several biological functions and enzymes (Shahzad et al., 2018). Plants display symptoms of Ni deficiency when the Ni levels in their leaves are below 1 mg/kg, according to Rue et al. (2020) and Dotaniya et al. (2022).

However, high concentrations of Ni can have negative impacts on plant anatomy and physiology (Shahzad et al., 2018), such as seed germination, shoot and root growth inhibition (Rahman et al., 2005), iron (Fe) deficiency and chlorosis (Ewais, 1997; Kirkby & Römheld, 2004), photosynthesis and transpiration deterioration (Sheoran et al., 1990; Ahmad et al., 2011). High concentrations of Ni in plant tissues also reduce nutrient uptake and other metabolic processes, cause oxidative damage, alter to membrane stability and change to antioxidant enzyme activities (Borah et al., 2023; Gajewska et al., 2006; Nawaz et al., 2022; Shahzad et al., 2018; Tipu et al., 2021; Yusuf et al., 2011). Nickel contamination is one of the leading sources of heavy metal pollution, coming from industrial discharge, metallurgical and electroplating industries, the use of pesticides, industrial and municipal waste, coal combustion, solid and liquid fuel usage (Borah et al., 2023; Briffa et al., 2020; Chen et al., 2019; Genchi et al., 2020; Ghori et al., 2019; Nabi et al., 2019). Although Ni exist naturally in soil and water, its level is typically lower than 100 mg/kg in soil and 0.005 mg/L in water (Alloway, 2013; Hassan et al., 2019; Shahzad et al., 2018).

Phytoremediation is an eco-friendly and cost-effective approach that uses plants to remove hazardous chemicals from contaminated soil, air, and water (Salt et al., 1998). It exploits the natural ability of some plant species, known as hyperaccumulators, to concentrate pollutants in their tissues (Pilon-Smits, 2005). Phytoremediation has been successfully used worldwide to eliminate contaminants like heavy metals, pesticides, solvents, explosives, and crude oil (Genchi et al., 2020). Phytoremediation employs two main techniques: phytoextraction and phytostabilization (McGrath & Zhao, 2003). Phytoextraction includes removing contaminants with plants from soil, plants absorb and accumulate them in their tissues and are then removed along with harvesting (Pilon-Smits, 2005). Phytostabilization is immobilizing the contaminants through absorption, adsorption in soil, or precipitation in the rhizosphere via plants (Korzeniowska & Stanislawska-Głubiak, 2019).

Phytoremediation potential of plants can be assessed with different parameters, including translocation factor (TF) and tolerance index (TI), as noted by Usman et al. (2019). The TF indicates the ability of the plant to transport heavy metals from the roots to the aerial parts of a plant (Pilon-Smits, 2005). When the TF value is greater than one, it suggests that the plant has an efficient metal transport system, resulting in effective metals translocation from the roots to the aerial parts. However, when the TF value is less than one, it indicates that the plant has an ineffective metal transfer process, leading to metal accumulation in the roots instead of the aerial parts (Korzeniowska & Stanislawska-Głubiak, 2019; Samreen et al., 2021; Yoon et al., 2006).

Ornamental plants can play a role in phytoremediation as they

can exhibit characteristics of hyperaccumulation, accumulation, and exclusion of heavy metals (Rocha et al., 2022). It is preferred to use non-edible plant species for phytoremediation purposes. For example, *Cosmos bipinnatus* has been identified as a cadmium hyperaccumulator (Huang et al., 2017), while *Euphorbia milli* has been recognized as a chromium accumulator (Ramana et al., 2015) and *Acacia pycnantha* as a lead excluder (Khan et al., 2021; Nirola et al., 2016). Because it is not preferred to accumulate heavy metals in edible plant species, ornamental kale has potential for phytoremediation as they are not consumed as food or feed. Ornamental kale (*Brassica oleracea* var. *acephala*) is a cultivated ornamental plant that has high commercial value and is widely grown due to its colorful lace-like leaves. It is commonly used as a horticultural decoration due to its biennial growth habit and long-lasting foliage. The leaves of the plant have distinctive shapes and margins that can be lobed, serrated, or entire, with attractive colors (Ari et al., 2021; Chen et al., 2019; Karagöz & Dursun, 2021; Ren et al., 2019). Planting ornamental kale during the autumn and winter months is optimal for growth. Ornamental kale belongs to the Brassicaceae family and is considered one of the oldest vegetable forms, with a long history of cultivation in Europe (Dixon, 2017). Brassica plants can accumulate heavy metals (da Mota Gonçalves et al., 2022; Vannucchi et al., 2021). Research has indicated that ornamental kale can be used as hyperaccumulator plants for Cd, Pb, Zn, and Cu in soils (Haghighi et al., 2016; Nasiri et al., 2022). *Brassica oleracea* var. *acephala*, a subspecies of *Brassica oleracea* and considered an ornamental plant, was found to be a potential hyperaccumulator plant for the improvement of boron-contaminated areas (Gökseven et al., 2021).

The present study had two main objectives: (1) to examine the impact of Ni stress on the germination (germination rate, germination index and mean germination time), growth (weight, length and water content), and physiological parameters (proline and Ni content) of ornamental *Brassica oleracea* seedlings during the vegetative stage; (2) to assess the phytoremediation potential of ornamental kale.

## MATERIALS AND METHODS

### Plant material

Ornamental Kale (*Brassica oleracea* L. var. *acephala*) Kamome Pink (K.Pink), Kamome White (K.White), and Kamome Red (K.Red) cultivars were utilized as the plant material in this study. The seeds were sourced from a local seed distributor (Tasaco Farm, Antalya, Turkey) and underwent disinfection with commercial bleach for

1 minute (Taghizadeh et al., 2018) prior to being sown in plastic pots filled with perlite. Each pot was treated with a solution containing one of the four nickel concentrations (0 (Control), 25, 50, and 100 ppm), prepared from  $\text{Ni}(\text{NO}_3)_2$ , alongside the standard Hoagland nutrient solution. Each pot contained five seeds, and the experiment was initiated on December 30, 2021, in a controlled climate room with a day/night temperature of 22/15 °C, humidity of 55-65%, photosynthetically active radiation of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and a day/night photoperiod of 14/10 (Pathirana et al., 2017). Plants were irrigated at two-day intervals with 50 ml of the solution. The experiment was conducted at the Suluova Vocational School of Amasya University in Turkey and was terminated on 27 January 2022.

### Germination parameters

Seed germination was assessed on the fourth day of the experiment by counting the number of seeds that had germinated. The germination rate, index, and mean germination time were calculated using the following formulas which described by (Çarpıcı et al., 2009) and (Sadeghi et al., 2011):

$$\text{Germination rate (\%)} = (\text{Number of germinated seeds} / \text{Number of total seeds}) * 100 \quad (1)$$

$$\text{Germination index} = \sum(\text{Gt} / \text{Tt}) \quad (2)$$

(Gt represents the number of seeds germinated on a given day; Tt is the number of days from the beginning of the experiment until that day.)

$$\text{Mean germination time (days)} = \sum \text{Dn} / \sum \text{n} \quad (3)$$

(n represents the germinated seeds number on day D; D is the days number which counted from first germination)

### Seedling growth parameters

Three randomly selected seedlings from each pot were harvested for plant part analysis 40 days after sowing. Shoot and root length were measured with a ruler. The shoot and root sections were separated and their total fresh weight and length (shoot height) were measured.

The water content of the seedlings was calculated using the following formula (Fradera-Soler et al., 2021):

$$\text{Seedling Water Content (\%)} = (\text{Seedling fresh weight} - \text{seedling dry weight}) / \text{Seedling fresh weight} * 100 \quad (4)$$

### Tolerance index

By subtracting the biomass content of the control plants from that of the treated plants, the tolerance index (TI) can be determined. If the TI values are greater than 1 or less than

1, it indicates an increase or decrease in biomass content, respectively, for the plant cultivars in question. The equation used to calculate TI is provided in Eq. 5, as described by Wilkins (1978) and Borah et al. (2023):

$$\text{TI (tolerance index)} = \text{Biomass of the treated plants (g)} / \text{Biomass of the control plants (g)} \quad (5)$$

### Proline content

The proline content of the plant material was determined using a modified method of Bates et al. (1973). For this, 100 mg of dried plant material was homogenized with 10 ml of 3% sulfosalicylic acid and filtered through a blue band filter paper. Then, 2 ml of the filtrate was added to a glass tube and mixed with 2 ml of ninhydrin solution (which contains ninhydrin, orthophosphoric acid, and acetic acid) and 2 ml of acetic acid. The mixture was vortexed and then incubated in a 100 °C water bath for one hour, followed by being transferred to an ice bath. Finally, 4 ml of toluene was added to the samples, which were then vortexed and analyzed spectrophotometrically at 520 nm. The proline content was calculated using commercial L-proline as a reference standard.

### Nickel concentration

Dried plant shoots (0.2 g) were taken and burned in the muffle furnace at 550 °C for 5 hours, 2 ml of HCl (37%) was added and slowly heated on a hot plate. After the acid was removed from the medium 2 ml of 1/3 HCl was added and filtered through a blue band filter paper. The filtrates were made up of 40 ml of distilled water. Prepared samples were analyzed by ICP OES, and the results were given as mg/kg (Miller, 1997; Pequerul et al., 1993).

### Translocation factor (TF)

Translocation factor (TF) was determined for 25 ppm Ni application by the following equation (Korzeniowska & Stanislawski-Glubiak, 2019):

$$\text{TF} = \text{metal concentration in shoots (mg/kg)} / \text{metal concentration in roots (mg/kg)} \quad (6)$$

### Statistical analysis

The study was conducted using a completely randomized design with two factors (cultivar and Ni concentration). The treatments had four replications with five plants per replication. The homogeneity of the data was verified using the Levene's test. The quantitative data, expressed as percentages, were transformed using the arcsine transformation. To analyze the data, two-way Analysis of Variance (ANOVA) was used, and the Least Significant Difference (LSD) multiple range test at a significance level

of  $p \leq 0.05$  was used to compare the means. The coefficient of variation (CV) was determined for determining reliability. Statistical analyses were performed using JMP Software (Version 14.3, SAS Institute Inc., Cary, NC, 1989-2021). Correlation analysis with heatmap and principal component analysis were performed with Python 3.11.0. GraphPad-Prism 8.3.0 was used to design graphics.

## RESULTS

### Germination parameters

The interaction between Ni and cultivar significantly affected the germination parameters, as shown by statistical analysis (Table 1). Overall, the germination percentage was found to be up to 70%. The highest germination percentage was found in the interactions of 0 ppm Ni + K.Pink, 25 ppm Ni + K.Red, and 0 ppm Ni + K.White. The K.Pink cultivar had the longest germination time in the 50 ppm Ni application. On the other hand, Ni shortened the germination time in K.Red and K.White. The germination index was lowest in K.Pink +50 ppm (2.96) and K.Red + 0 ppm (3.63).

### Plant Growth Parameters

In the present study, the impact of cultivars on ornamental kale's shoot length and seedling dry weights was significant, statistically, but not for root length and seedling fresh weight (as shown in Table 2).

The shoot length was the highest in the K.Red cultivar (49.8 mm). On the other hand, the seedling dry weight was the lowest in the K.Pink cultivar (0.35 g).

The effect of Ni stress on the Ni concentrations, shoot and root length, seedling fresh and dry weights was statistically significant (Fig. 1).

Shoot length (Fig. 1A), root length (Fig. 1B), seedling fresh weight (Fig. 1C), and seedling dry weight (Fig. 1D) increased under 25 ppm Ni treatment. These seedling growth parameters dramatically decreased under 50 and 100 ppm Ni treatments.

The results of the study revealed that the interaction between cultivar and Ni concentration had a significant impact on the seedling growth parameters of Kamome cultivars (K. Pink, K.Red, and K.White), with the exception of shoot fresh weight (Table 3).

The longest shoot length was found in the 0 ppm Ni and K.Red cultivar (84.2 mm). A 25 ppm concentration of Ni resulted in an increase in the shoot length of both K.Pink (76.3 mm) and K.White (67.0 mm). The highest root length

(90.4 mm) was found in K. Red and 25 ppm interaction. In all cultivars, 25 ppm induced the root length under 25 ppm Ni. As Ni content increased root length decreased in all cultivars. Seedling dry weight was higher in K.White cultivar under 25 ppm Ni (1.11 g).

### Seedling water, proline and nickel Content

The results indicated a significant difference ( $p < 0.05$ ) between cultivars in terms of seedling water, proline and nickel content was significant (Table 4).

Seedling water (79%) and proline content (0.245) were lower in the K.White cultivar. Proline content was the highest in K.Pink (0.245). Additionally, Ni content was highest in K.White (30.69), followed by K.Pink (18.38) and K.Red (15.11), respectively.

The effect of Ni stress on the seedling water, proline and Ni content was important and statistically significant (Fig. 2).

Seedling water content decreased with increasing Ni concentration (Fig. 2A). On the other hand, the proline content increased in response to Ni treatment (Fig. 2B). Results showed that there was slightly Ni uptake with 25 ppm Ni treatment (1.44 mg/kg). When the Ni concentration was 50 and 100 ppm, the Ni concentrations in the seedling also increased, with the highest Ni concentrations observed in the 100 ppm Ni treatment (60.20 mg/kg) (Fig. 2C).

When considering cultivar and Ni interactions, the highest seedling water content was recorded for the combination of 0 ppm Ni and K.Red cultivar (91%) (Table 5).

The lowest water content was noted for 100 ppm Ni and K.White cultivar (69%). The highest proline value was found in the combination of 100 ppm Ni and K.Red cultivar (0.37 mM), while the lowest value was observed in both 0 ppm Ni and K.White cultivar and K.Red cultivar (0.02 mM). The increasing percentage of proline was high in K.Red and K.White under Ni treatments, but it was low in K.Pink.

Roots and shoots of the Kamome cultivars couldn't grow well under 50 and 100 ppm Ni treatments, at least, we could not have enough samples separately as root and shoot, to perform Ni analysis. For this reason, plantlets could not be divided into roots and shoots in these concentrations. The Ni concentration of the roots and shoots belongs to Kamome cultivars under 0 and 25 ppm was shown in Fig. 3A. The K.Red and K.White accumulated low amount of Ni in the roots but not in the shoots. When considering all Ni concentrations for all Kamome cultivars, the impact of Ni treatments on the Ni content of the cultivars was found to be statistically significant (Fig. 3B). The Ni content increased with Ni applications, with the highest Ni concentrations found in the 100 ppm K.White cultivar (89.32 mg/kg).

**Table 1.** Effects of Ni and cultivar interaction on germination parameters of K.Pink, K.Red and K.White.

Kamome Cultivars	Ni (ppm)	Germination (%)	Germination time	Germination index
K.Pink	0	100 (90)±0a	2.66±0.1cde	8.33±1.0ab
	25	95 (83) ±10ab	2.77±0.1c	7.02±1.3abc
	50	70 (57) ±12c	3.19±0.2a	2.96±0.7d
	100	95 (83) ±10ab	2.69±0.2cde	7.81±2.0abc
K.Red	0	70 (57) ±11.5c	2.95±0.1b	3.63±1.1d
	25	100 (90) ±0a	2.75±0.1cd	7.54±0.6abc
	50	95 (83) ±10ab	2.79±0.1bc	6.40±1.1bc
	100	75 (60) ±10c	2.73±0.2cde	5.94±1.7c
K.White	0	100 (90) ±0a	2.71±0.2cde	8.17±2.1abc
	25	95 (83) ±10ab	2.57±0.1de	9.27±2.3a
	50	80 (70) ±23bc	2.55±0.1e	7.83±2.5abc
	100	95 (83) ±10ab	2.56±0.1e	9.06±1.4a
<i>LSD</i>		16.266***	0.182***	2.303**
<i>CV (%)</i>		12.1	4.6	22

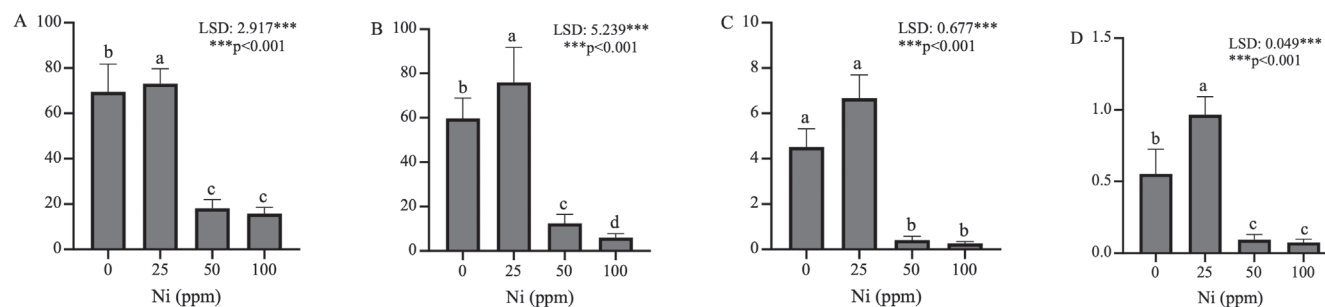
\*\*p<0.01, \*\*\*p<0.001, CV: coefficient of variation.

Values in parenthesis were transformed with arcsine. Measurements are presented as mean ± standard deviation. The differences between the averages were indicated by separate letters.

**Table 2.** Effects of cultivars on seedling growth parameters.

Cultivars	Shoot Length (mm)	Root length (mm)	Seedling Fresh Weight (g)	Seedling Dry Weight (g)
K.Pink	41,6b	36,7	2,81	0,35b
K.Red	49,8a	41,5	2,91	0,44a
K.White	41,0b	37,6	3,17	0,49a
<i>LSD</i>	2.527***	---NS	---NS	0.049***
<i>CV (%)</i>	10	20	23	13,9

\*\*\*p<0.001, ---NS: nonsignificant, CV: coefficient of variation, Measurements are presented as mean. The differences between the averages were indicated by separate letters.

**Figure 1.** Effects of Ni concentrations on seedling growth parameters. The differences between the averages were indicated by separate letters.

### Tolerance Index and Translocation Factor

Tolerance index and translocation factor were evaluated by heatmap graphics. Tolerance index was higher in 25 ppm Ni than 50 and 100 ppm Ni. Tolerance index was  $>1$  in 25 ppm Ni in three cultivars and  $<1$  under 50 and 100 ppm Ni (Fig. 4A). While translocation factor of K.White was calculated as 0.00048, it was 0.00045 for K.Red (Fig. 4B). Translocation factor was less than 1 in these cultivars, meanly they could not transfer the Ni from roots to the shoots under 25 ppm Ni concentration.

### Heatmap correlation and principal component analysis

Correlation coefficients-heatmap analysis of germination parameters, seedling growth parameters, Ni and proline results in response to different cultivar and Ni applications are shown in Fig. 5A. Positive correlation was determined between the germination percentage and germination index. Germination time had negative correlation with them. Additionally, the seedling water content was positively correlated with the seedling growth parameters and tolerance index. On the other hand, there were significant negative correlations between seedling water content and Ni and proline content. There was not significant correlation between Ni content and the germination parameters. Ni and the seedling growth parameters had negative correlation, while Ni and proline content had positive correlation. Positive and negative correlations between parameters was also showed with the principal component analysis (Fig. 5B).

### DISCUSSION

Nickel is a vital microelement for growth and is essential in enzyme synthesis in plants. Ni is beneficial to plants at low concentrations, but high concentrations can be toxic (Das et al., 1978; Hassan et al., 2019). Ni toxicity can reduce the metabolic processes, cell division, cell wall elasticity, and protein synthesis. It can also decrease the activities of essential enzymes such as  $\alpha$ -amylase, protease, and nuclease which are important for germination and growth (Ashraf et al., 2011; Bishnoi et al., 1993; Hassan et al., 2019; Seregin & Kozhevnikova, 2006; Sethy & Ghosh, 2013; Walker et al., 1985). In this study, Ni treatment decreased germination time in K.Red and K.White, while germination percentage increased with low Ni levels in K.Red. K.White's germination percentage slightly decreased, but Ni extended the germination time and reduced the germination in K.Pink. Lower level Ni promoted germination rate and seedling growth but high levels inhibited them in rice (Das et al., 1978). Sunflower cultivars showed a significant decrease in their germination rates with the addition of inorganic Ni, except for control seedlings that had the highest germination rate (Zhang et al., 2020). Increasing concentrations of Ni also decreased the germination percentage and rate in rice and lentil seeds (Khan et al., 2020; Shaikat et al., 2021). Barley seeds have also been negatively affected by Ni toxicity (Vasić et al., 2020).

The impact of Ni on plant growth varies depending on the species and level of soil contamination. Korzeniowska & Stanislawska-Glubiak, (2019) found that Ni reduced the

**Table 3.** Effects of Ni and cultivar interaction on seedling growth parameters.

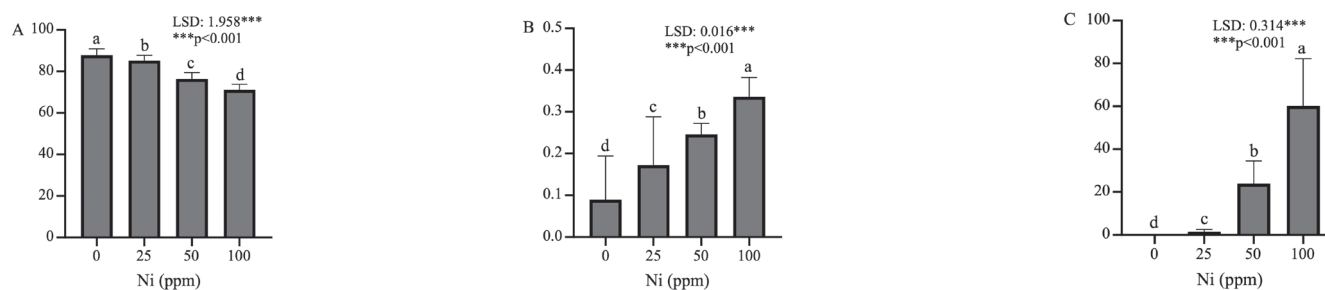
Cultivars	Ni (ppm)	SL (mm)	RL (mm)	SFW (g)	SDW (g)
K.Pink	0	63.0 $\pm$ 7.3cd	56.2 $\pm$ 8.8d	4,03 $\pm$ 0.84	0,35 $\pm$ 0.12d
	25	76.3 $\pm$ 6.1b	72.2 $\pm$ 7.4b	6,55 $\pm$ 0.47	0,85 $\pm$ 0.09b
	50	13.3 $\pm$ 2.6g	13.3 $\pm$ 6.8e	0,31 $\pm$ 0.18	0,09 $\pm$ 0.05e
	100	13.6 $\pm$ 2.2g	5.0 $\pm$ 1.2e	0,34 $\pm$ 0.04	0,10 $\pm$ 0.01e
K.Red	0	84.2 $\pm$ 4.9a	58.0 $\pm$ 9.8cd	4,42 $\pm$ 0.86	0,63 $\pm$ 0.05c
	25	76.0 $\pm$ 6.2b	90.4 $\pm$ 16.9a	6,42 $\pm$ 0.80	0,95 $\pm$ 0.06b
	50	20.8 $\pm$ 1.3e	11.5 $\pm$ 2.3e	0,55 $\pm$ 0.06	0,11 $\pm$ 0.01e
	100	18.3 $\pm$ 2.6efg	6.0 $\pm$ 1.3e	0,25 $\pm$ 0.07	0,07 $\pm$ 0.02e
K.White	0	61.5 $\pm$ 6.9d	65.2 $\pm$ 8.5bcd	5,07 $\pm$ 0.60	0,68 $\pm$ 0.08c
	25	67.0 $\pm$ 2.4c	65.7 $\pm$ 10.2bc	7,03 $\pm$ 1.75	1,11 $\pm$ 0.02a
	50	20.2 $\pm$ 0.9ef	12.5 $\pm$ 0.8e	0,38 $\pm$ 0.19	0,09 $\pm$ 0.04e
	100	15.3 $\pm$ 0.8fg	7.0 $\pm$ 2.4e	0,19 $\pm$ 0.04	0,06 $\pm$ 0.01e
LSD		5.053***	9.076***	---NS	0.099***

\*\*\* $p < 0.001$  ---NS: nonsignificant, Measurements are presented as mean  $\pm$  standard deviation. The differences between the averages were indicated by separate letters.

**Table 4.** Effects of cultivars on seedling water content (SWC), proline and Ni content.

Cultivars	SWC (%)	Proline (mM)	Ni (mg/kg)
K.Pink	81a	0,245a	18,38b
K.Red	81a	0,220b	15,11c
K.White	79b	0,166c	30,69a
LSD	1.696**	0.014***	0.272***
CV (%)	2,5	7,7	1,5

\*\* $p < 0.01$ , \*\*\* $p < 0.001$ , CV: coefficient of variation. Measurements are presented as mean. The differences between the averages were indicated by separate letters.

**Figure 2.** Effects of Ni concentrations on Seedling Water Content (A), proline content (B) and Ni content (C). The differences between the averages were indicated by separate letters.

biomass of both aboveground and root parts in plants. In our study, we found that 25 ppm Ni stimulated seedling growth, while 50 and 100 ppm Ni inhibited growth drastically. However, Ni at lower concentrations can stimulate growth but inhibit it at higher concentrations, as observed in rice (Das et al., 1978). Additionally, Ni contamination in soil can reduce both aboveground and root growth, except for *S. viminalis* (Korzeniowska & Stanislawska-Glubiak, 2019). The effect of Ni on sunflower cultivars showed that it had a pronounced effect on seedling shoot and root fresh and dry weights and the highest weights were recorded in the control group followed by lower concentrations of Ni (Ashraf et al., 2011). Ni stress can reduce vital processes like the breakdown of starch into sugars and protein synthesis required for germinating seeds to initiate growth and development (Ashraf et al., 2011). Excessive amounts of Ni is negatively affect the plant growth, photosynthesis, and membrane functions (Maheshwari & Dubey, 2007; Sresty & Rao, 2000). Sresty & Rao (2000) found that two pigeonpea cultivars (*Cajanus cajan* (L.) Millspaugh) were studied for the accumulation of Zn and Ni in their seedlings under different concentrations. Both Zn and Ni applications reduced the above and underground parts growth, as well as the relative growth indices of the two cultivars. Plant parts lengths and biomass of rice seedlings (*Oryza sativa* L. cv. Pant-12) reduced, due to Ni (200 and 400  $\mu\text{M}$   $\text{NiSO}_4$ ). The Ni level was higher in the roots compared to aerial parts (Maheshwari & Dubey, 2009). Studies have also reported

negative effects of boron (another heavy metal) on kale growth, specifically in root-shoot fresh and dry weights (Gökseven et al., 2021). Similar results were seen in the case of *Calendula tripterocarpa*, where high Ni concentrations reduced plant growth, including germination and fresh and

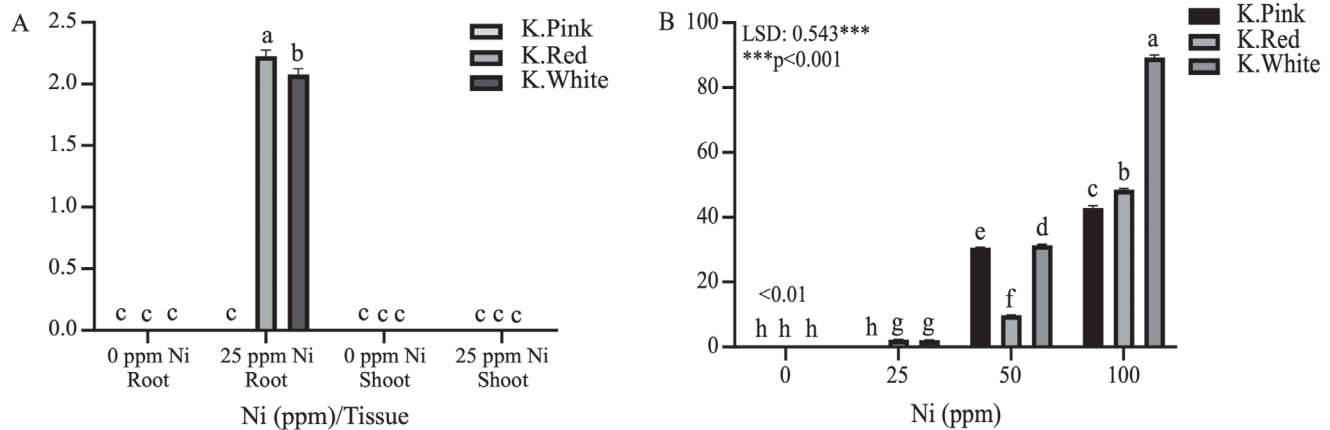
**Table 5.** Effects of Ni and cultivar interaction on seedling water content (SWC) and proline.

Cultivars	Ni (ppm)	SWC (%)	Proline (mM)
K.Pink	0	91±1.5a	0,23±0.001c
	25	87±2.12b	0,24±0.006c
	50	75±3.10d	0,23±0.003c
	100	70±1.06e	0,28±0.003b
K.Red	0	86±1.87b	0,02±0.001d
	25	85±0.92b	0,26±0.025bc
	50	79±1.18c	0,24±0.034c
	100	74±0.64d	0,37±0.007a
K.White	0	87±0.93b	0,02±0.001d
	25	84±3.45b	0,02±0.003d
	50	75±2.59d	0,27±0.002b
	100	69±2.37e	0,36±0.036a
LSD		1.958***	0.027***

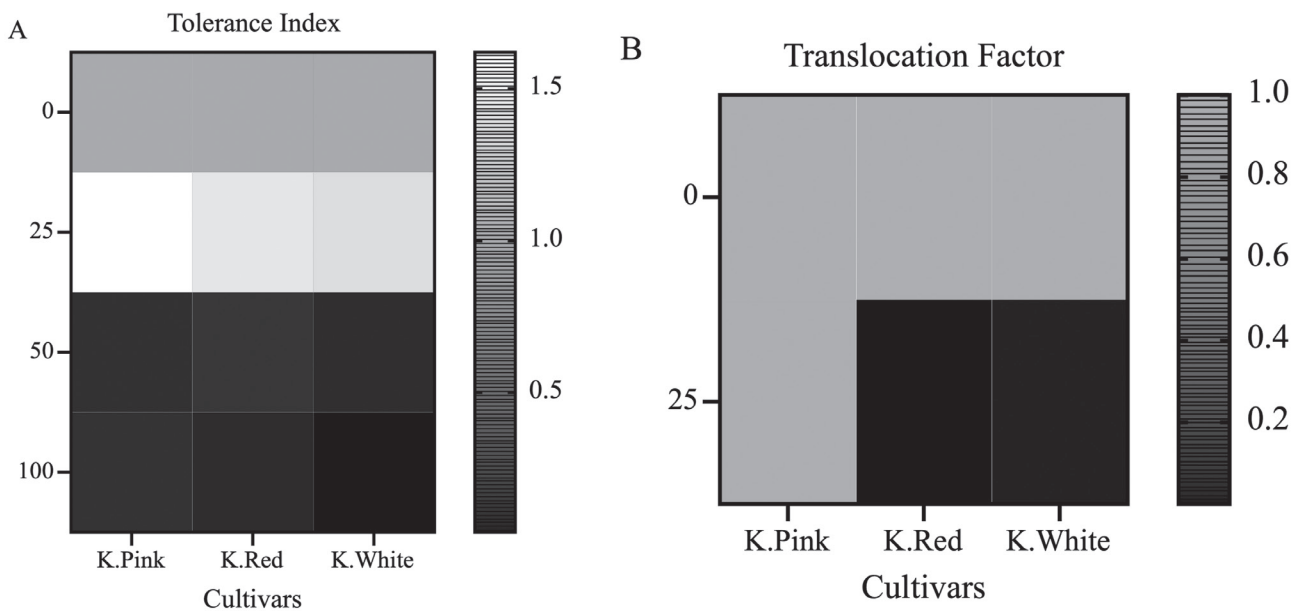
\*\*\* $p < 0.001$  Measurements are presented as mean  $\pm$  standard deviation. The differences between the averages were indicated by separate letters.

dry weights (Heidari et al., 2020). According to Sreekanth et al., (2013) and Valivand et al., (2019), root cell membrane structure changed, water and mineral uptake reduced, and physiological activity affected negatively with high Ni doses. Lešková et al., (2020) also reported that high Ni concentrations reduced auxin transport in roots, damaged the composition of microtubules, affected cell division and, in turn, decreased root growth. Various parameters, including the bioaccumulation factor (BAF), translocation factor (TF), tolerance index (TI), and metal removal efficiency (%), can be used to assess the phytoremediation potential of the plants (Borah et al., 2023; Samreen et al., 2021). Tolerance index was >1 in K.Pink (1.62), K.Red (1.45) and K.White (1.39)

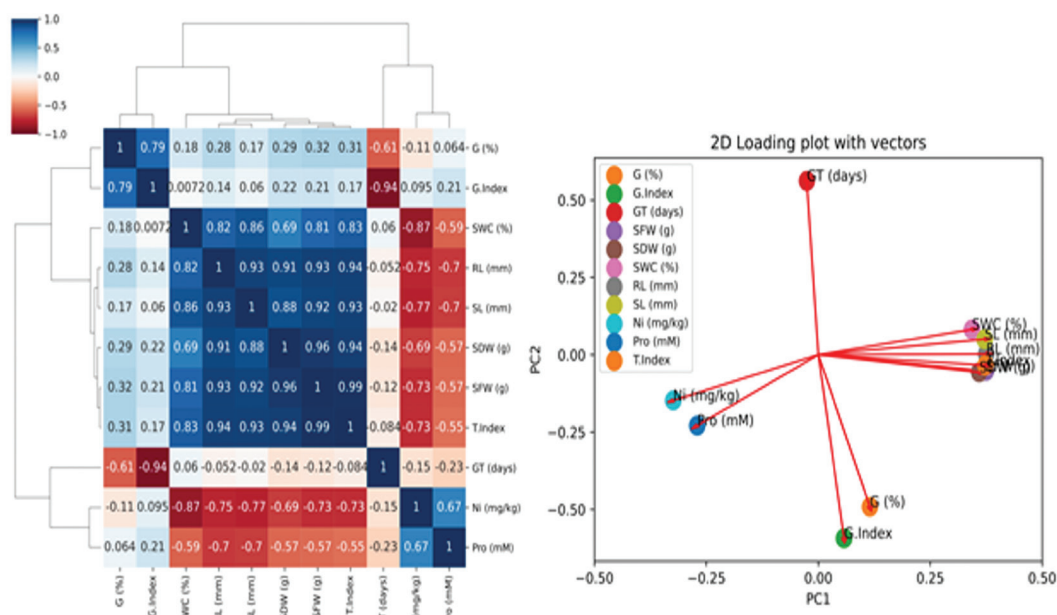
under 25 ppm Ni treatment. When Ni concentration were 50 and 100 ppm, TI decreased. Borah et al., (2023) reported that, to assess phyto-tolerance, TI was calculated for *Jatropha curcas* and *Pongamia pinnata* in different soil combinations. *Pongamia* in PMT25 (municipal landfill 25% : control soil 75%) showed the highest TI value (1.67), indicating greater tolerance than other treatments. *Jatropha* had TI values above 1 (1.02, 7.55, and 1.09 in PT50 [papermill contaminated soil 50% : control soil 50%], PT25 [papermill contaminated soil 25% : control soil 75%], and MT50 (municipal landfill 50%: control soil 50%), respectively), suggesting a robust defense system, which are heavy metal stress tolerance mechanisms. They supposed that lower TI values at high contaminated



**Figure 3.** A) Root-Shoot Ni content of three different cultivars under control and 25 ppm Ni B) Effects of Ni and cultivar interaction on Ni content. The differences between the averages were indicated by separate letters.



**Figure 4.** A) Tolerance index of the cultivars under different Ni concentrations, B) Translocation factor of cultivars under control and 25 ppm Ni.



**Figure 5.** Heatmap showing the levels of on seedling vegetative parameters, proline and Ni content G (%): germination, G.Index: Germination Index, GT (days): Germination time, SFW (g): Shoot fresh weight, SDW (g): Shoot dry weight, SWC (%): Seedling water content, RL (mm): Root Length, SL: Shoot Length, Ni (mg/kg): Ni content, Pro (mM): proline.

area shows lower Ni and Zn tolerance.

The level of proline accumulation in plants indicates of their heavy metals tolerance. Heavy metals caused to increase the content of proline importantly than the control, indicating their metal tolerance capacity in *Jatropha curcas* and *Pongamia pinnata* (Borah et al., 2023). Proline, a common metabolic compound, was found in plant tissues in response to stress conditions (Gajewska et al., 2006). The K.Red and K.White cultivars showed an important increase in proline content under high Ni concentrations, as indicated in Table 3. Proline was described as a protective compound against various stress, with some properties such as osmoprotectant, a membrane stabilizer, and a scavenger of reactive oxygen species (Bandurska, 2001; Hartzendorf & Rolletschek, 2001; Matsysik et al., 2002; Gajewska et al., 2006). Schat et al. (1997) emphasized that metal-induced proline production was a response of metal-induced water deficit in the *Silene vulgaris* leaf tissues. Similarly, in our study the decrease in water content of kale seedlings and proline accumulation under Ni presence, may reflect this response. Heavy metals can disrupt water transport in plants, leading to shoot dehydration. Transition metals can affect water uptake, movement, and stomatal function at multiple stages of the process (Yusuf et al., 2011).

Ni moves from roots to shoots and leaves via xylem's transpiration stream, facilitated by cation ATPases, ion channels, and cation-proton antiport. In addition, Ni is moved from older leaves, buds, fruits, and seeds to

meristematic parts through the phloem, which is regulated by metal-ligand complexes and specific proteins that bind Ni (Colpas & Hausinger, 2000; Yang et al., 2005). In our own study, we found that the Ni content in K.Pink, K.Red, and K.White ornamental kale seedlings increased with high Ni content, with K.Red and K.White accumulating the most in their roots when exposed to 25 ppm Ni. Similarly, the roots of *Zea mays* contained significantly more Ni compared to shoots, with the plant showing a high ability to accumulate Ni in the roots, as found by Korzeniowska and Stanislawski-Glubiak (2019). In maize, lettuce and field bean plants also showed a much higher Ni accumulation rate in the roots than in the stems as discovered by Antonkiewicz et al. (2016). Additionally, studies have shown that exogenous application of Ni leads to an increase in Ni accumulation in various sunflower tissues (Ahmad et al., 2009; Ashraf et al., 2011). Nonetheless, increasing Ni level in the nutrient solution may have impeded the uptake of essential minerals.

## CONCLUSIONS

In conclusion, this study evaluated the effect of varying Ni concentrations on the growth parameters of three kale cultivars: K.Pink, K.Red, and K.White. The results indicated that 25 ppm Ni significantly enhanced seedling

growth but high Ni levels had a detrimental effect on the growth characteristics of the kale seedlings, including shoot and root length, fresh and dry weight, and water content. Furthermore, the K.Red and K.White cultivars showed a remarkable increase in proline levels in response to high Ni concentrations, suggesting it as a protective mechanism against stress. Additionally, the study observed a statistically significant accumulation of Ni in the seedlings, with the highest concentration found in the 100 ppm K.White cultivar. On the other hand, the 25 ppm K.Pink cultivar exhibited no significant change in Ni content. This study highlights the importance of understanding species-specific responses to nickel stress, which could inform better management practices in cultivating ornamental kale and other plants in contaminated environments.

#### Author Contributions

Conceptualization: [VC, SY, HOB]; Methodology: [VC, SY, HOB]; Formal analysis and investigation: [VC, SY, HOB]; Writing - original draft preparation: [VC, HOB]; Statistical Analysis and Figure preparation [SY], Writing - review and editing: [VC, SY, HOB].

#### DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

#### DECLARATIONS

#### CONFLICT OF INTEREST

The authors state no conflict of interest

#### FUNDING DECLARATION

This research received no external funding.

#### DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work the author(s) used OPENAI in order to English Grammer Check.

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