CHILEAN JOURNAL of AGRICULTURAL RESEARCH

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RESEARCH ARTICLE

Comparative in vitro screening of carrot cultivars for heavy metal tolerance during germination and early seedling growth

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Received: 20 April 2026; Accepted: 24 June 2026, Available online: 7 September 2026. doi:10.4067/S0718-58392026000600860

ABSTRACT

Heavy metal pollution in agricultural production can negatively affect seedling formation and development, and variety-specific tolerance in carrots (*Daucus carota* L.) during early developmental stages has not been sufficiently characterized. This study aimed to compare the in vitro responses of two carrot cultivars, Esvet and Scarlet Nantes, to Cu, Cd, Pb, Co, and Ni during germination and early seedling growth. Sterilized seeds were cultured in MS medium supplemented with 0, 100, 300, or 500 µM of each metal, and germination percentage, mean germination time, germination speed index, shoot and root length, and fresh and dry weights were evaluated. Heavy metal stress significantly affected most germination and growth characteristics, and responses varied depending on the cultivar and dose ($p < 0.05$ or $p < 0.001$). 'Esvet' generally showed higher tolerance than 'Scarlet Nantes'. Under Cu stress, the average germination speed index was 42.62 in 'Esvet' and 16.14 in 'Scarlet Nantes', while the average shoot length was 34.29 and 19.83 mm, respectively. Increasing metal concentrations significantly reduced seedling development. Under Cu, the average root length decreased from 68.55 mm in the control group to 8.47 mm at 500 µM, while under Ni, shoot and root lengths decreased to 5.85 and 2.15 mm, respectively, at the highest dose. Low Pb and Co doses increased the germination percentage to 72.50% and 71.00%, respectively, but seedling performance remained lower than the control group. Overall, Ni showed the strongest inhibitory effect on early growth, and 'Esvet' was the more tolerant cultivar.

Key words: Cultivar tolerance, *Daucus carota*, germination, heavy metal stress, in vitro screening, seedling growth.

INTRODUCTION

Carrot (*Daucus carota* L.) is an important root vegetable cultivated widely worldwide and valued for its high nutritional quality. Owing to its rich vitamin content, particularly provitamin A in the form of beta carotene, and its mineral composition, carrot is widely consumed fresh and cooked and ranks among the leading vegetables in terms of production. However, heavy metal contamination has become a major limiting factor in carrot cultivation. Toxic metals introduced into soil reduce plant growth and yield and may also accumulate in the edible root tissue, thereby threatening human health through transfer along the food chain. Metals such as Cd can be taken up and stored relatively readily by carrot, resulting in high metal residues in carrots grown in contaminated soils. Indeed, some studies have reported that carrot, together with spinach, is among the vegetables that accumulate the highest amounts of Cd from soil (Ghani et al., 2021; Novák et al., 2023; Zhao et al., 2024). This situation increases the risk of both reduced crop production and compromised safety of the harvested food in heavy metal contaminated soils. Consequently, examining the responses and tolerance doses of carrot to different heavy metals is important for both agricultural productivity and food safety.

Today, heavy metal pollution has become a serious problem in agricultural soils because of modern industrial and agricultural activities. Mining, metal processing, industrial waste, and the use of metal containing fertilizers and pesticides cause heavy metals such as Cu, Co, Ni, Pb, and Cd to enter soils across wide areas and to accumulate as environmental contaminants (Rashid et al., 2023; Hou et al., 2025). From the perspective of plant biology, these metals are considered in two main categories. Some metals, including Cu, Ni, and Co, are required in trace amounts for the activity of many enzymes, yet they cause severe toxicity at elevated concentrations. By contrast, Pb and Cd, which have no known beneficial function in plants, are regarded among the most hazardous heavy metals because they exert toxic effects even at very low doses (Rashid et al., 2023; El-Sappah et al., 2024). When these heavy metals exceed their normal doses in soil because of various activities, they induce phytotoxicity and adversely affect essential processes such as seed germination, seedling development, and root and shoot elongation (Elazab et al., 2023; Rashid et al., 2023; El-Sappah et al., 2024). Reduced germination and slower growth in young seedlings are frequently observed under heavy metal stress. Such growth inhibition is associated with physiological disturbances including oxidative damage, accumulation of harmful metabolites, membrane injury, hormonal imbalance, and impaired mineral nutrition (Elazab et al., 2023; El-Sappah et al., 2024). Global assessments have shown that contamination with Cd, Co, Cu, Ni, and Pb reduces agricultural productivity and threatens food security (Kara et al., 2022; Hou et al., 2025). Therefore, understanding the effects of these heavy metals, which are commonly encountered in agricultural production, on plant growth and development is of major importance.

In vitro germination and seedling growth assays are widely used to investigate the effects of heavy metal stress in plants. Under controlled tissue culture conditions, for example on Murashige and Skoog (MS) medium, seeds can be exposed to defined heavy metal concentrations, and their germination behavior and seedling development can be monitored. This approach eliminates external factors such as heterogeneity in soil conditions and interactions with microorganisms and thus allows the direct effects of metals on plants to be evaluated. Seed germination and early seedling development are known to be among the most sensitive stages of the plant life cycle to heavy metal stress (Elazab et al., 2023; Parera et al., 2023). Because defense mechanisms are not yet fully developed during these early stages, toxic metals can readily inhibit or delay germination. For this reason, in vitro germination tests are used as a rapid and reliable screening method to evaluate the effects of different metal types and doses on early plant growth. Under suitable conditions, petri dish germination trials can enable the identification of heavy metal tolerant or sensitive genotypes within a short period (Elazab et al., 2023; Parera et al., 2023; El-Sappah et al., 2024; Sun et al., 2024).

In this study, the effects of Cu, Co, Ni, Pb, and Cd, which are common contaminants in agricultural soils, on carrot germination and seedling development were investigated. In this context, the objective of this study was to compare the responses of 'Esvet' and 'Scarlet Nantes' carrots to five heavy metals applied at four different concentrations (0, 100, 300, and 500 µM), to determine their effects on germination and seedling growth characteristics, and to identify variety-specific differences in tolerance during the early developmental stage. Heavy metal treatments were conducted under in vitro conditions. It was hypothesized that increasing heavy metal concentrations would reduce germination performance and seedling growth, and that the two cultivars would show distinct tolerance patterns.

MATERIALS AND METHODS

This study was conducted in the Plant Tissue Culture Laboratory of the Faculty of Agricultural Sciences and Technology at Sivas University of Science and Technology (Sivas, Türkiye). Two carrot (*Daucus carota* L.) cultivars, Esvet and Scarlet Nantes, were used as plant material. 'Esvet' is an open-pollinated purple carrot; it is described as a purple/black carrot with dark purple internal and external coloration, cylindrical roots with a pointed tip, and vigorous plant growth. 'Scarlet Nantes' is a widely adapted open-pollinated Nantes-type carrot cultivar characterized by smooth orange roots, a cylindrical to cylindroconical shape with a blunt tip, good flavor, and slow bolting.

Heavy metal treatments and in vitro culture conditions

Heavy metal treatments were established by supplementing MS (Murashige and Skoog, 1962) medium with five heavy metals: Cu, Cd, Co, Pb, and Ni. For each heavy metal, three doses were applied: 100, 300, and 500 µM. The culture medium was prepared by adding 4.4 g MS, 30 g sugar, 7 g agar, and different concentrations of metals. The pH of the medium was adjusted to 5.8. The control treatment consisted of MS medium without heavy metal addition. Prepared media were sterilized by autoclaving at 121 °C and 1.2 atm for 15 min. After sterilization, the media were poured into Petri dishes at predetermined volumes under aseptic conditions and allowed to solidify at room temperature.

The seeds were surface sterilized by treatment with 15% sodium hypochlorite (NaOCl) solution for 15 min. Following sterilization, the seeds were rinsed three times with sterile distilled water to ensure aseptic conditions prior to sowing. Sterilized seeds were then transferred onto the culture media under aseptic conditions using sterile forceps. Seed incubation was carried out at 25 ± 2 °C under a light intensity of 3000 lux with a 16:8 h photoperiod.

Statistical analysis

The experiment was designed as a completely randomized factorial design. For each cultivar × heavy metal × dose combination, a total of 100 seeds were used, consisting of four replicates, with five Petri dishes per replicate and five seeds per Petri dish. Germination parameters, including germination percentage (%) (Kumar et al., 2012), germination time (d), and germination speed index, were recorded (Kaya et al., 2006). After germination, early seedling growth was evaluated by measuring shoot length (mm), root length (mm), and shoot and root fresh and dry weights (Uçar et al., 2026). Data was analyzed using the JMP 13.0.1 statistical software package (JMP Statistical Discovery, Cary, North Carolina, USA). When treatment effects were significant, mean separations were performed using the LSD test and results were grouped by lettering. Correlation analysis was also conducted for the measured parameters.

RESULTS AND DISCUSSION

Heavy metal stress significantly affected germination behavior, seedling growth, and biomass accumulation in both carrot cultivars, and the magnitude of these effects depended on metal type, concentration, and genotype. Across treatments, 'Esvet' generally exhibited faster germination, higher germination speed index, and better maintenance of shoot and root growth than 'Scarlet Nantes', indicating greater tolerance during early seedling establishment. In general, increasing metal dose reduced seedling performance, whereas some low-dose treatments, particularly Pb and Co, produced partial stimulatory responses in germination-related traits. These findings confirm that early developmental responses of carrots to heavy metal stress are both dose-dependent and genotype-dependent.

The inhibitory effects observed in the present study are consistent with previous in vitro studies reporting that heavy metal toxicity primarily restricts root and shoot development by disrupting basic physiological and metabolic processes. Baktemur (2023) reported that increasing concentrations of Cd, Co, Ni, Cu, Pb, Cr, and Al reduced leaf and root development in garlic under in vitro conditions, and that root formation was completely inhibited at high doses of Cd, Co, Ni, and Cu. Similarly, Mancak and Baktemur (2023) found that increasing heavy metal concentrations reduced root number, root length, stem length, and leaf number in squash seedlings grown under in vitro conditions. These findings support the present results, in which increasing Cu, Cd, Pb, Co, and Ni concentrations generally reduced germination vigor, shoot and root elongation, and biomass accumulation in carrot seedlings.

The stronger inhibition of root growth compared with final germination percentage suggests that early root development is one of the most sensitive indicators of heavy metal toxicity. These physiological disturbances may reduce photosynthetic capacity and DM accumulation, thereby limiting seedling establishment. Thus, the reductions in root length, shoot length, and fresh and dry weight in the present study may be associated with oxidative damage, enzyme inhibition, disturbed mineral nutrition, and impaired growth regulation.

Effects of Cu

Copper significantly affected most germination and seedling growth traits in both cultivars (Table 1). Germination percentage declined only moderately with increasing Cu concentration, but germination speed index decreased sharply relative to the control. Morphological and biomass traits were also strongly reduced as Cu dose increased. In cultivar means, 'Esvet' performed better than 'Scarlet Nantes', with greater shoot length, root length, shoot fresh weight, and shoot dry weight. At the highest Cu dose, seedling growth was severely inhibited, and both shoot and root elongation approached minimum values, indicating that Cu strongly restricted early development.

Table 1. Effects of increasing Cu doses on germination and early seedling growth traits of carrot cultivars. Cu-1, Cu-3, and Cu-5 correspond to 100, 300, and 500 μM Cu, respectively. Control represents the metal-free treatment. The first two rows represent the main effect means of the cultivars, while the following control and Cu dose rows represent the main effect means of the treatments. The remaining rows show the Cultivar × Dose interaction means. Therefore, the data are not repeated; they are presented separately to show cultivar effects, dose effects, and Cultivar × Dose interaction. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ^{ns}Nonsignificant. Means followed by different letters are significantly different according to the LSD test.

Factors		Germination percentage	Mean germination time	Germination speed index	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
		%	d		mm	mm	g	g	g	g
Esvet		65.31^{A}	1.59^{B}	42.62^{A}	34.29^{A}	43.13^{A}	0.0206^{A}	0.0031	0.0030^{A}	0.0010
Scarlet Nantes		55.00^{B}	3.60^{A}	16.14^{B}	19.83^{B}	35.75^{B}	0.0143^{B}	0.0031	0.0023^{B}	0.0009
Control		64.61^{A}	2.78	66.03^{A}	41.02^{A}	68.55^{A}	0.0308^{A}	0.0053^{A}	0.0035^{A}	0.0018^{A}
Cu-1		60.00^{B}	2.44	19.39^{B}	29.68^{B}	53.19^{B}	0.0202^{B}	0.0032^{B}	0.0028^{B}	0.0010^{B}
Cu-3		60.00^{B}	2.56	16.30^{C}	25.90^{C}	27.55^{C}	0.0111^{C}	0.0029^{B}	0.0027^{B}	0.0007^{C}
Cu-5		56.00^{C}	2.61	15.82^{C}	11.65D	8.47^{D}	0.0076^{D}	0.0008^{C}	0.0016^{C}	0.0003^{D}
Esvet	0%	73.22^{a}	1.28^{d}	98.37^{a}	56.48^{a}	77.80^{a}	0.0377^{a}	0.0059^{a}	0.0043^{a}	0.0022^{a}
	Cu-1	68.00^{b}	1.41^{d}	29.25^{c}	35.40^{b}	53.77^{bc}	0.0245^{b}	0.0026^{d}	0.0031^{b}	0.0010^{c}
	Cu-3	60.00^{c}	1.95^{bcd}	20.17^{e}	33.80^{b}	34.00^{d}	0.0104^{d}	0.0030^{cd}	0.0029^{b}	0.0005^{d}
	Cu-5	60.00^{c}	1.73^{cd}	22.70^{d}	11.50^{e}	6.93^{f}	0.0099^{d}	0.0008^{e}	0.0016^{c}	0.0004^{de}
Scarlet Nantes	0%	56.00^{d}	4.27^{a}	33.68^{b}	25.56^{c}	59.30^{b}	0.0240^{b}	0.0047^{b}	0.0027^{b}	0.0013^{b}
	Cu-1	52.00^{e}	3.46^{ab}	9.52^{g}	23.97^{c}	52.60^{c}	0.0161^{c}	0.0039^{bc}	0.0025^{b}	0.0011^{c}
	Cu-3	60.00^{c}	3.17^{abc}	12.42^{f}	18.00^{d}	21.10^{e}	0.0119^{d}	0.0029^{d}	0.0024^{b}	0.0010^{c}
	Cu-5	52.00^{e}	3.50^{ab}	8.93^{g}	11.80^{e}	10.00^{f}	0.0054^{e}	0.0009^{e}	0.0016^{c}	0.0003^{e}
$LSD_{(cultivar)}$		0.84***	0.86***	1.73***	2.63***	3.20***	0.0011***	^{ns}	0.0004*	^{ns}
$LSD_{(dose)}$		1.18***	^{ns}	1.22***	3.72***	4.52***	0.0015***	0.0007***	0.0005**	0.0002**
$LSD_{(c×d)}$		1.67***	1.73*	0.87***	5.27***	6.39**	0.0022***	0.0010*	0.0008*	0.0002**

The relatively moderate effect of Cu on final germination percentage, compared with its strong inhibitory effect on germination speed and seedling vigor, agrees with previous reports showing that excess Cu often affects early seedling performance more severely than final germination itself (Moreira et al., 2020; Siqueira et al., 2020; Chen et al., 2022; Acila et al., 2026). The pronounced inhibition of root growth under Cu stress is also consistent with the view that excess Cu impairs reserve mobilization, disrupts meristematic activity, and promotes oxidative damage in root tissues, thereby limiting elongation and biomass accumulation (Wairich et al., 2022; Jarin et al., 2025). Overall, these results indicate that 'Esvet' is more tolerant to Cu stress, whereas 'Scarlet Nantes' is comparatively more sensitive.

The strong reduction in root growth under Cu stress may be associated with oxidative damage and disruption of enzyme activity, which restrict early seedling development.

Effects of Cd

Cadmium treatments induced significant dose- and genotype-dependent changes in germination and seedling growth (Table 2). Although cultivar means did not differ significantly for germination percentage, 'Esvet' exhibited a shorter mean germination time and a higher germination speed index than 'Scarlet Nantes', indicating a faster and more vigorous germination pattern. Cd exposure markedly reduced germination speed index and progressively decreased shoot length, root length, and biomass traits. The Cultivar × Dose interaction was significant for most growth parameters. 'Esvet' maintained higher morphological and biomass values than 'Scarlet Nantes' across Cd treatments, although both cultivars were negatively affected as the Cd dose increased.

Table 2. Effects of increasing Cd doses on germination and early seedling growth traits of carrot cultivars. Cd-1, Cd-3, and Cd-5 correspond to 100, 300, and 500 µM Cd, respectively. Control represents the metal-free treatment. The first two rows represent the main effect means of the cultivars, while the following control and Cd dose rows represent the main effect means of the treatments. The remaining rows show the Cultivar × Dose interaction means. Therefore, the data are not repeated; they are presented separately to show cultivar effects, dose effects, and Cultivar × Dose interaction. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ^{ns}Nonsignificant. Means followed by different letters are significantly different according to the LSD test.

Factors		Germination percentage	Mean germination time	Germination speed index	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
		%	d		mm	mm)	g	g	g	g
Esvet		57.17	1.75^{B}	38.57^{A}	42.17^{A}	41.02^{A}	0.0207^{A}	0.0036^{A}	0.0028^{A}	0.0011^{A}
Scarlet Nantes		58.00	3.32^{A}	16.26^{B}	19.27^{B}	29.61^{B}	0.0158^{B}	0.0029^{B}	0.0020^{B}	0.0007^{B}
Control		64.34^{A}	2.28	65.68^{A}	41.02^{A}	61.75^{A}	0.0300^{A}	0.0043^{A}	0.0035^{A}	0.0018^{A}
Cd-1		49.00^{D}	2.61	13.55^{B}	31.71^{B}	40.59^{B}	0.0188^{B}	0.0041^{A}	0.0025^{B}	0.0009^{B}
Cd-3		57.00^{C}	2.53	14.90^{B}	26.10^{C}	24.42^{C}	0.0135^{C}	0.0026^{B}	0.0019^{C}	0.0006^{C}
Cd-5		60.00^{B}	2.72	15.54^{B}	24.06^{C}	14.51^{D}	0.0107^{C}	0.0021^{B}	0.0016^{C}	0.0003^{D}
Esvet	0%	72.67^{a}	1.28^{b}	98.12^{a}	56.48^{a}	77.80^{a}	0.0360^{a}	0.0038^{ab}	0.0043^{a}	0.0022^{a}
	Cd-1	44.00^{f}	2.00^{ab}	15.04^{d}	44.20^{b}	43.29^{bc}	0.0191^{c}	0.0051^{a}	0.0028^{b}	0.0009^{c}
	Cd-3	56.00^{d}	1.75^{ab}	21.07^{c}	34.60^{c}	25.74^{d}	0.0150^{cd}	0.0030^{bc}	0.0021^{c}	0.0008^{c}
	Cd-5	56.00^{d}	1.96^{ab}	20.04^{c}	33.40^{c}	17.27^{ef}	0.0127^{de}	0.0025^{bc}	0.0017^{cd}	0.0002^{d}
Scarlet Nantes	0%	56.00^{d}	3.27^{a}	33.23^{b}	25.56^{d}	45.70^{b}	0.0240^{b}	0.0047^{a}	0.0027^{b}	0.0013^{b}
	Cd-1	54.00^{e}	3.22^{a}	12.06^{de}	19.21^{e}	37.90^{c}	0.0185^{c}	0.0030^{bc}	0.0022^{c}	0.0008^{c}
	Cd-3	58.00^{c}	3.31^{a}	8.72^{e}	17.59^{e}	23.10^{de}	0.0120^{de}	0.0022^{c}	0.0017^{cd}	0.0003^{d}
	Cd-5	64.00^{b}	3.47^{a}	11.03^{de}	14.72^{e}	11.76^{f}	0.0087^{e}	0.0017^{c}	0.0014^{d}	0.0003^{d}
$LSD_{(cultivar)}$		^{ns}	1.73**	4.41***	2.27***	3.03***	0.0021**	0.0006*	0.0003**	0.0002**
$LSD_{(dose)}$		1.22***	^{ns}	3.12***	1.51***	4.29***	0.0030*	0.0010***	0.0004**	0.0002**
$LSD_{(c×d)}$		1.73*	0.87*	2.21***	4.54**	6.07***	0.0042***	0.0014*	0.0005*	0.0002*

These findings are consistent with studies reporting that Cd inhibits germination and early seedling development by impairing metabolic activity and restricting elongation growth (Huybrechts et al., 2019; Kara et al., 2022). In the present study, Cd caused a gradual reduction in seedling performance rather than a complete arrest of growth, even at the highest dose. This pattern suggests that carrot seedlings are clearly sensitive to Cd, yet their response is somewhat less abrupt than the response observed under Cu or Ni stress. The stronger performance of 'Esvet' under Cd exposure further supports the conclusion that heavy metal tolerance during early development is genotype specific.

Cd-induced reductions in seedling growth may be explained by its negative effects on nutrient uptake, water balance, and cellular metabolism.

Effects of Pb

Lead significantly affected germination percentage and germination speed index, whereas mean germination time was not significantly altered by dose (Table 3). In contrast to most other treatments, low and moderate Pb doses increased germination percentage relative to the control, while germination speed index declined at all Pb levels. This indicates that Pb may stimulate final germination under some conditions without improving germination uniformity or early vigor. For seedling growth traits, 'Esvet' consistently outperformed 'Scarlet Nantes'. Notably, 'Esvet' showed unusually high root length and shoot dry weight at 500 µM Pb, whereas 'Scarlet Nantes' displayed a more typical dose-dependent reduction in growth and biomass.

Table 3. Effects of increasing Pb doses on germination and early seedling growth traits of carrot cultivars. Pb-1, Pb-3, and Pb-5 correspond to 100, 300, and 500 µM Pb, respectively. Control represents the metal-free treatment. The first two rows represent the main effect means of the cultivars, while the following control and Pb dose rows represent the main effect means of the treatments. The remaining rows show the Cultivar × Dose interaction means. Therefore, the data are not repeated; they are presented separately to show cultivar effects, dose effects, and Cultivar × Dose interaction. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ^{ns}Nonsignificant. Means followed by different letters are significantly different according to the LSD test.

Factors		Germination percentage	Mean germination time	Germination speed index	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
		%	d		mm	mm	g	g	g	g
Esvet		72.64^{A}	1.55^{B}	46.10^{A}	45.02^{A}	79.13^{A}	0.029^{A}	0.0080^{A}	0.0044^{A}	0.0012^{A}
Scarlet Nantes		62.00^{B}	3.53^{A}	17.72^{B}	22.08^{B}	36.39^{B}	0.0137^{B}	0.0022^{B}	0.0023^{B}	0.0005^{B}
Control		64.78^{B}	2.28	66.03^{A}	41.02^{A}	68.55^{A}	0.030^{A}	0.0053^{B}	0.0035^{A}	0.0018^{A}
Pb-1		72.50^{A}	2.68	23.88^{B}	29.35^{C}	52.72^{B}	0.0155^{C}	0.0120^{A}	0.0034^{A}	0.0007^{B}
Pb-3		72.00^{A}	2.74	19.98^{C}	29.55^{C}	46.45^{B}	0.0169^{C}	0.0019^{C}	0.0026^{B}	0.0004^{C}
Pb-5		60.00^{C}	2.47	17.77^{D}	34.28^{B}	63.32^{A}	0.0223^{B}	0.0080^{C}	0.0036^{A}	0.0006^{B}
Esvet	0%	73.56^{b}	1.28^{c}	98.37^{a}	56.48^{a}	77.80^{b}	0.0377^{a}	0.0059^{b}	0.0046^{b}	0.0022^{a}
	Pb-1	85.00^{a}	1.35^{c}	37.17^{b}	35.80^{c}	72.43^{bc}	0.0173^{c}	0.0202^{a}	0.0044^{b}	0.0009^{cd}
	Pb-3	72.00^{b}	1.81^{bc}	26.07^{d}	38.60^{c}	64.40^{c}	0.0240^{b}	0.0022^{d}	0.0031^{c}	0.0007^{de}
	Pb-5	72.00^{b}	1.77^{bc}	22.78^{e}	49.20^{b}	101.90^{a}	0.0373^{a}	0.0063^{c}	0.0056^{a}	0.0011^{bc}
Scarlet Nantes	0%	56.00^{d}	3.27^{ab}	33.68^{c}	25.56^{d}	59.30^{d}	0.0240^{b}	0.0047^{bc}	0.0027^{cd}	0.0013^{b}
	Pb-1	60.00^{c}	4.00^{a}	10.58^{g}	23.30^{de}	33.02^{e}	0.0138^{cd}	0.0022^{d}	0.0024^{cd}	0.0005^{e}
	Pb-3	72.00^{b}	3.67^{a}	13.88^{f}	20.10^{e}	28.50^{e}	0.0099^{de}	0.0016^{de}	0.0022^{de}	0.0001^{f}
	Pb-5	60.00^{c}	3.17^{ab}	12.75^{f}	19.36^{e}	24.73^{e}	0.0073^{e}	0.0004^{e}	0.0016^{e}	0.0002^{f}
$LSD_{(cultivar)}$		0.90^{***}	0.78^{***}	0.87^{***}	2.54^{***}	5.14^{***}	0.0021^{***}	0.0007^{***}	0.0004^{**}	0.0001^{**}
$LSD_{(dose)}$		1.27^{***}	^{ns}	1.22^{***}	$3.^{60***}$	7.28^{***}	0.0003^{***}	0.0009^{***}	0.0005^{**}	0.0002^{*}
$LSD_{(c\times d)}$		1.79^{***}	1.56^{***}	1.73^{***}	5.09^{***}	10.29^{**}	0.0042^{***}	0.0013^{***}	0.0007^{**}	0.0002^{*}

Similar reductions in seedling growth under Pb stress were reported by Baktemur (2022) in arugula under in vitro conditions, where control plants showed better shoot and root development than Pb-treated plants. In the present study, Pb also reduced seedling performance, particularly in 'Scarlet Nantes', while 'Esvet' maintained better growth. This suggests that Pb tolerance in carrot may vary depending on the genotype. The inhibitory effect of Pb may be mainly associated with reduced root development and lower biomass accumulation during early seedling growth. The increase in germination percentage at low Pb doses suggests a hormetic or trait-specific stimulatory response, whereas the concurrent decline in germination speed index indicates that germination still proceeded more slowly and less synchronously under Pb exposure (Salinitro et al., 2021; Wang et al., 2025). The contrasting cultivar responses under Pb are among the clearest examples of genotype dependence in the present study. 'Scarlet Nantes' behaved as a sensitive genotype, whereas 'Esvet' maintained substantial growth even at the highest Pb concentration. This divergence agrees with previous reports showing that Pb toxicity may vary considerably among plant genotypes and that growth inhibition is not always uniform across traits or cultivars (García de la Torre et al., 2021; Abdolmalaki et al., 2024). Therefore, Pb responses in carrot appear to depend not only on dose, but also strongly on genetic background.

The different responses of the two cultivars to Pb indicate that Pb tolerance may be genotype-dependent.

Effects of Co

Cobalt significantly affected all germination traits as well as morphological and biomass parameters, and the response varied with both dose and cultivar (Table 4). 'Esvet' had higher germination percentage, shorter mean germination time, and higher germination speed index than 'Scarlet Nantes', indicating better tolerance to Co stress. Increasing Co concentration reduced shoot length, root length, and biomass traits, especially at 500 µM Co. However, low-dose Co produced a partial stimulatory response in some traits, particularly in 'Esvet', where biomass values at 100 µM Co were comparable to or even exceeded the control.

Table 4. Effects of increasing Co doses on germination and early seedling growth traits of carrot cultivars. Co-1, Co-3, and Co-5 correspond to 100, 300, and 500 µM Co, respectively. Control represents the metal-free treatment. The first two rows represent the main effect means of the cultivars, while the following control and Co dose rows represent the main effect means of the treatments. The remaining rows show the Cultivar × Dose interaction means. Therefore, the data are not repeated; they are presented separately to show cultivar effects, dose effects, and Cultivar × Dose interaction. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Means followed by different letters are significantly different according to the LSD test.

Factors		Germination percentage	Mean germination time	Germination speed index	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
		%	d		mm	mm	g	g	g	g
Esvet		69.17^{A}	1.83^{B}	44.01^{A}	42.75^{A}	49.56^{A}	0.0321^{A}	0.0048^{A}	0.0042^{A}	0.0014^{A}
Scarlet Nantes		59.50^{B}	3.34^{A}	17.26^{B}	19.37^{B}	32.50^{B}	0.0158^{B}	0.0028^{B}	0.0022^{B}	0.0010^{B}
Control		64.34^{B}	2.28	66.03^{A}	41.02^{a}	63.70^{a}	0.0300^{a}	0.0043^{ab}	0.0037^{a}	0.0019^{a}
Co-1		71.00^{A}	2.50	22.25^{B}	37.12^{a}	47.17^{b}	0.0306^{a}	0.0048^{a}	0.0038^{a}	0.0013^{b}
Co-3		60.00^{D}	2.40	18.29^{C}	28.82^{b}	29.00^{c}	0.0207^{b}	0.0035^{bc}	0.0029^{b}	0.0010^{c}
Co-5		62.00^{C}	3.17	15.97^{D}	17.30^{c}	24.25^{c}	0.0147^{c}	0.0027^{c}	0.0024^{c}	0.0006^{d}
Esvet	0%	72.67^{b}	1.28^{b}	98.37^{a}	56.48^{a}	77.80^{a}	0.0360^{ab}	0.0047^{b}	0.0043^{b}	0.0022^{a}
	Co-1	80.00^{a}	1.65^{ab}	32.12^{b}	50.00^{b}	45.63^{bc}	0.0409^{a}	0.0069^{a}	0.0053^{a}	0.0015^{b}
	Co-3	66.00^{c}	1.64^{ab}	25.53^{c}	41.93^{c}	39.10^{cd}	0.0316^{b}	0.0046^{b}	0.0040^{b}	0.0011^{c}
	Co-5	58.00^{e}	2.76^{ab}	11.93^{e}	22.60^{d}	35.70^{d}	0.0203^{c}	0.0038^{bc}	0.0032^{c}	0.0008c^{d}
Scarlet Nantes	0%	56.00^{f}	3.27^{ab}	33.68^{b}	25.56^{d}	49.60^{b}	0.0240^{c}	0.0047^{b}	0.0031^{c}	0.0016^{b}
	Co-1	62.00^{d}	3.35^{ab}	12.37^{e}	24.23^{d}	48.70^{b}	0.0204^{c}	0.0027^{cd}	0.0024^{d}	0.0011^{c}
	Co-3	54.00^{g}	3.15^{ab}	11.05^{e}	15.70^{e}	18.90^{e}	0.0098^{d}	0.0023^{de}	0.0018^{de}	0.0008^{cd}
	Co-5	66.00^{c}	3.58^{a}	11.93^{e}	12.00^{e}	12.80^{e}	0.0091^{d}	0.0015^{e}	0.0017^{e}	0.0005^{d}
$LSD_{(cultivar)}$		0.87***	1.14***	0.87***	2.76***	4.51***	0.0030***	0.0006***	0.0003**	0.0002**
$LSD_{(dose)}$		1.22***	1.62***	1.22***	3.90***	6.38***	0.0042***	0.0008**	0.0004**	0.0003**
$LSD_{(c \times d)}$		1.73***	2.29***	1.73***	5.52*	9.03**	0.0060**	0.0011***	0.0006*	0.0004*

This response pattern suggests that Co may induce hormetic-like effects at low concentrations but become clearly phytotoxic at higher doses. Similar low-dose stimulation followed by growth inhibition has been described as a characteristic hormetic pattern in plants exposed to trace metals (Carvalho et al., 2020; Hu et al., 2021; Salinitro et al., 2021; Radi et al., 2025). In the present study, the beneficial effect of Co was limited and did not persist at higher concentrations. Instead, severe reductions at 300 and 500 µM Co confirmed that excess Co restricts seedling development, particularly in the more sensitive 'Scarlet Nantes'. Thus, Co behaved as a threshold-dependent stress factor in carrot, with 'Esvet' showing the more stable tolerance pattern.

The slight stimulation observed at low Co doses suggests a limited positive response, while higher doses caused clear growth inhibition.

Effects of Ni

Nickel caused the most severe inhibition of early seedling development among the tested metals (Table 5). Although germination percentage increased at 100 µM Ni, it declined at higher doses, and germination speed index was sharply reduced across all Ni treatments. This indicates that even when final germination was not completely suppressed, the germination process became slower and less uniform under Ni stress. Morphological and biomass traits were strongly affected by Ni concentration, and the Cultivar × Dose interaction was significant for most parameters. At 300 µM Ni, growth in 'Scarlet Nantes' was strongly inhibited, whereas at 500 µM Ni complete growth inhibition was observed and no seedling formation occurred. In contrast, 'Esvet' maintained limited but measurable growth under 500 µM Ni, indicating greater tolerance under severe Ni stress.

Table 5. Effects of increasing nickel doses on germination and early seedling growth traits of carrot cultivars. Values of 0.00 indicate complete growth inhibition, with no seedling formation under this treatment. Ni-1, Ni-3, and Ni-5 correspond to 100, 300, and 500 µM Ni, respectively. Control represents the metal-free treatment. The first two rows represent the main effect means of the cultivars, while the following control and Ni dose rows represent the main effect means of the treatments. The remaining rows show the Cultivar × Dose interaction means. Therefore, the data are not repeated; they are presented separately to show cultivar effects, dose effects, and Cultivar × Dose interaction. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ^{ns}Nonsignificant. Means followed by different letters are significantly different according to the LSD test.

Factors		Germination percentage	Mean germination time	Germination speed index	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
		%	d		mm	mm	g	g	g	g
Esvet		62.39^{B}	1.68^{B}	40.60^{A}	32.50^{A}	39.86^{A}	0.0225^{a}	0.0037^{A}	0.0032^{A}	0.0012^{A}
Scarlet Nantes		66.00^{A}	3.10^{A}	18.40^{B}	14.35^{B}	27.70^{B}	0.0124^{b}	0.0023^{B}	0.0015^{B}	0.0007^{B}
Control		64.28^{B}	2.28	66.03^{A}	41.02^{A}	65.20^{A}	0.0308^{A}	0.0053^{A}	0.0035^{A}	0.0018^{A}
Ni-1		73.00^{A}	2.46	19.60^{B}	29.22^{B}	49.41^{B}	0.0253^{B}	0.0035^{B}	0.0030^{B}	0.0014^{B}
Ni-3		61.00^{C}	1.98	18.81^{B}	17.60^{C}	18.35^{C}	0.0104^{C}	0.0024^{C}	0.0018^{C}	0.0005^{C}
Ni-5		58.50^{D}	2.85	13.57^{C}	5.85^{D}	2.15^{D}	0.0033^{D}	0.0009^{D}	0.0010^{D}	0.0002^{D}
Esvet	0%	72.56^{b}	1.28^{a}	98.37^{a}	56.48^{a}	77.80^{a}	0.0377^{a}	0.0059^{a}	0.0043^{a}	0.0022^{a}
	Ni-1	66.00^{c}	1.73^{bc}	24.25^{c}	37.40^{b}	53.93^{b}	0.0316^{b}	0.0038^{bc}	0.0039^{a}	0.0018^{b}
	Ni-3	58.00^{e}	1.52^{c}	22.92^{c}	24.40^{cd}	23.40^{d}	0.0142^{e}	0.0033^{c}	0.0026^{bc}	0.0005^{e}
	Ni-5	53.00^{g}	2.19^{abc}	16.87^{d}	11.70^{e}	4.30^{f}	0.0066^{f}	0.0018^{d}	0.0020^{c}	0.0004^{e}
Scarlet Nantes	0%	56.00^{f}	3.27^{ab}	33.68^{b}	25.56^{c}	52.60^{b}	0.0240^{c}	0.0047^{b}	0.0027^{b}	0.0013^{c}
	Ni-1	80.00^{a}	3.20^{ab}	14.95^{e}	21.04^{d}	44.90^{c}	0.0190^{d}	0.0031^{c}	0.0021^{c}	0.0009^{d}
	Ni-3	58.00^{e}	2.45^{abc}	14.70^{e}	10.80^{e}	13.30^{e}	0.0065^{f}	0.0015^{d}	0.0011^{d}	0.0004^{e}
	Ni-5	64.00^{d}	3.50^{a}	10.27^{f}	0.00	0.00	0.0000	0.0000	0.0000	0.0000
$LSD_{(cultivar)}$		0.082***	0.79*	0.87***	2.18***	2.86***	0.0016***	0.0005***	0.0003**	0.0002**
$LSD_{(dose)}$		1.17***	^{ns}	1.22***	3.08***	4.04***	0.0022***	0.0008***	0.0004**	0.0001**
$LSD_{(c \times d)}$		1.65***	1.58**	1.73***	4.36***	5.71**	0.0031*	0.0011*	0.0006*	0.0002*

The strong suppression of seedling growth under Ni exposure agrees with studies reporting that excess Ni often affects post-germination growth more severely than final germination percentage (Nouri et al., 2019; Tipu et al., 2021; El-Sappah et al., 2024). In this study, Ni was the most aggressive toxicant, particularly with respect to shoot and root elongation. The sharp decline in biomass and the complete growth inhibition observed in 'Scarlet Nantes' at high Ni dose indicate that carrot seedlings are highly sensitive to Ni during establishment. The contrast between the two cultivars further emphasizes that tolerance screening based only on final germination percentage may be misleading, because germination speed and early seedling growth were much more discriminative indicators of stress response.

The severe inhibition under Ni stress suggests that excess Ni strongly disrupts early metabolic processes, particularly root and shoot formation.

Correlation analysis

Correlation analysis further supported the close relationship between germination vigor and seedling development under heavy metal stress (Figure 1). Across Cu, Cd, Pb, Co, and Ni treatments, morphological and biomass traits were generally strongly and positively correlated. Shoot length, root length, fresh weights, and dry weights tended to increase together, indicating coordinated early seedling growth. Germination speed index also showed clear positive associations with growth traits in several treatments, suggesting that faster germination was linked to more vigorous seedling establishment.

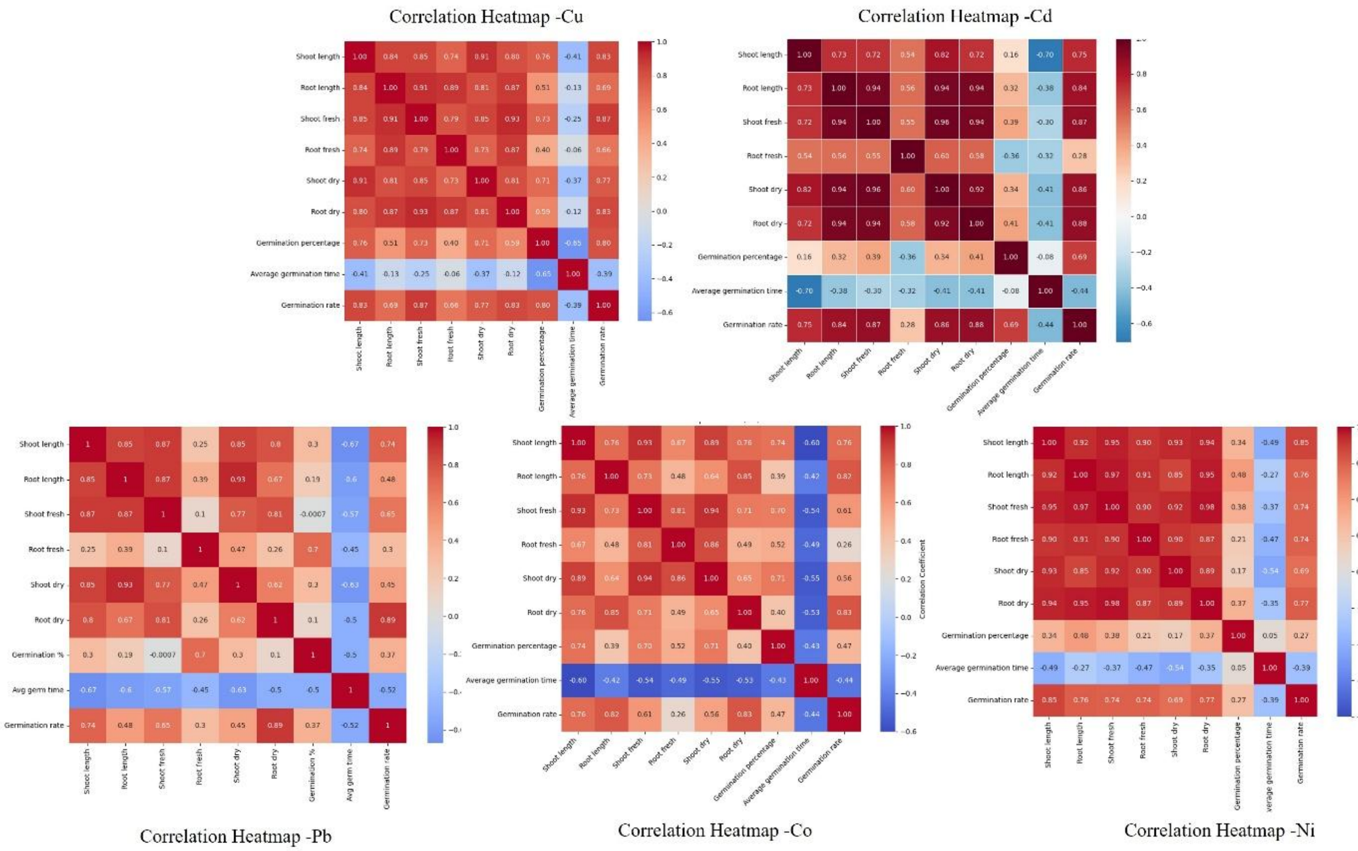

Figure 1. Correlation heatmaps showing the relationships among germination and early seedling growth traits in ‘Esvet’ and ‘Scarlet Nantes’ carrots under Cu, Cd, Pb, Co, and Ni stress conditions.

These correlations indicate that early germination performance is a strong predictor of subsequent seedling growth under heavy metal exposure. In practical terms, this supports the use of germination speed index, together with shoot and root growth, as reliable screening criteria for identifying tolerant genotypes in vitro assays. The correlation patterns also reinforce the broader conclusion of this study: Heavy metal stress impairs carrot establishment not through a single trait, but through a coordinated decline in germination dynamics, elongation growth, and biomass accumulation.

Taken together, the results show that heavy metal toxicity in carrots is not uniform across metals or cultivars. Cu and Ni produced the strongest inhibitory effects on early seedling growth, Cd caused a more gradual reduction, and Pb and Co displayed partial low-dose stimulatory responses in some germination traits before becoming inhibitory at higher concentrations. Across nearly all treatments, ‘Esvet’ maintained better germination dynamics and stronger seedling growth than ‘Scarlet Nantes’, indicating a more robust tolerance profile. These findings demonstrate that early-stage in vitro screening is an effective approach for distinguishing genotype-specific responses to heavy metal stress and for identifying cultivars with greater tolerance potential.

CONCLUSIONS

This study presents a comparative in vitro assessment of early-stage heavy metal tolerance in carrots by exposing two genotypes (‘Esvet’ and ‘Scarlet Nantes’) to Cu, Cd, Pb, Co, and Ni at concentrations ranging from 0 to 500 µM. For all metals, increasing doses consistently disrupted germination dynamics and sharply limited seedling growth and biomass accumulation. This confirms that the establishment stage is highly sensitive to metal stress. Ni, in particular, emerged as the most detrimental metal, with 500 µM Ni completely inhibiting seedling development in ‘Scarlet Nantes’. In contrast, ‘Esvet’ maintained faster germination and relatively stronger shoot and root growth under stress, indicating a more robust tolerance phenotype. Overall, these findings support the hypothesis that increasing concentrations of Cu, Cd, Pb, Co, and Ni disrupt germination and early seedling growth in carrots, and that 'Esvet' and 'Scarlet Nantes' exhibit different tolerance responses to heavy metal stress. Collectively, these findings highlight the narrow safety margin between low-dose responses and pronounced phytotoxicity. The study identifies 'Esvet' as a strong candidate for tolerance screening and breeding programs aimed at improving carrot performance in heavy metal-contaminated systems.

Author contribution

E.K. designed the study and wrote the manuscript.

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