



Cadmium Toxicity in Common Beans: Comparative Analysis Germination in Aydıntepe and Ispir Genotypes

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ABSTRACT

Background: Heavy metal contamination in agricultural soils is a significant issue. *Phaseolus vulgaris*, a vital crop, is particularly sensitive to Cd, which impacts germination and early growth stages. This study aims to examine these effects to develop strategies for managing Cd toxicity in agriculture.

Methods: This study was conducted at Kahramanmaraş Sutçu Imam University between June 2023 and September 2023. This study investigates the effects of cadmium (Cd) toxicity on the growth and germination of *Phaseolus vulgaris* L. (common bean) cultivars Aydıntepe and Ispir. Various concentrations of Cd (0, 2, 4, 8, 16, 32, 64 and 128 mg/l) were applied and their impacts on root weight, plumula fresh weight, plumula length, radicle length and germination percentage were evaluated.

Result: The results revealed that higher Cd concentrations significantly reduced root weight, radicle length and germination percentage in both cultivars. The Ispir variety exhibited slightly better tolerance in terms of root weight and plumula fresh weight compared to Aydıntepe. Additionally, Aydıntepe showed longer plumula lengths than Ispir across all Cd concentrations. No significant difference was observed between the two cultivars in terms of radicle length and germination percentage. These findings highlight the detrimental effects of Cd on plant growth and germination, emphasizing the need for selecting Cd-tolerant varieties for cultivation in contaminated soils. The study suggests further research into the genetic and physiological mechanisms underlying Cd tolerance to develop more resilient crop varieties.

Key words: Cadmium toxicity, Cd tolerance, Germination, *Phaseolus vulgaris*.

INTRODUCTION

Heavy metal contamination in agricultural soils is a serious problem today (Kara, 2024; Kara *et al.*, 2024; Kara *et al.*, 2023; Saltali *et al.*, 2023). Heavy metals in the environment can rise naturally or anthropogenically, posing potential health risks directly or indirectly to humans, animals, plants and microorganisms. The most common metals found in polluted areas due to natural sources or human activities are lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd), mercury (Hg) and nickel (Ni) (Adriano, 2001; Nagajyoti *et al.*, 2010; Kabata-Pendias, 2000; Wuana and Okiyeimen, 2011; Alloway, 2013). Negative effects of heavy metals on various living organisms have been reported in numerous studies (Olgunoglu and Olgunoglu, 2016; Olgunoglu *et al.*, 2021; Artar *et al.*, 2024).

Cadmium (Cd) is a highly toxic heavy metal that significantly impacts plant growth and development (Satarug *et al.*, 2003). It can accumulate in agricultural soils through industrial activities such as mining, smelting and the use of phosphate fertilizers, as well as through natural processes like volcanic activity and weathering of rocks (Alloway, 1995). This accumulation leads to detrimental effects on crop health and yield, posing a serious threat to food security and safety (Grant *et al.*, 2008).

Understanding the mechanisms by which Cd affects seed germination and early growth stages is essential for developing strategies to mitigate its impact on agriculture (Kaur *et al.*, 2023; Zhang *et al.*, 2024). Previous research

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has shown that Cd can interfere with water uptake, enzyme activity and hormonal balance, all of which are crucial for seed germination and seedling development (Sanità di Toppi and Gabbrielli, 1999). Furthermore, Cd has been reported to cause oxidative stress by generating reactive oxygen species (ROS) that damage cellular structures, thereby impairing germination and growth (Shah *et al.*, 2001).

Phaseolus vulgaris, commonly known as the common bean, is an important legume crop worldwide (Molina *et al.*, 1975; Ganesan and Xu, 2017; Myers and Kmiecik, 2017; Celmeli *et al.*, 2018; Karavidas *et al.*, 2022; Girgel *et al.*, 2023; Bernardi *et al.*, 2024). It is particularly sensitive

to Cd exposure, which can lead to reduced germination rates, impaired root and shoot development and overall decreased plant vigor (Benavides *et al.*, 2005). Given its agricultural importance, studying the effects of Cd on *Phaseolus vulgaris* can provide valuable insights into the broader implications of Cd toxicity on crop production.

In addition to its direct toxic effects, Cd can also alter the uptake and transport of essential nutrients, further exacerbating its impact on plant growth (Clemens, 2006). The dual challenge of Cd toxicity and nutrient imbalance necessitates a comprehensive approach to understanding and mitigating Cd's effects on plants (Kabata-Pendias, 2000; Wuana and Okeimen, 2011; Alloway, 2013; Pireh *et al.* 2017; Aslam *et al.*, 2023; Baruah *et al.*, 2023).

This study aims to investigate the specific impacts of various concentrations of Cd on the germination process and early growth stages of *Phaseolus vulgaris* seeds. By elucidating these effects, we hope to contribute to the development of effective strategies for managing Cd contamination in agricultural soils and ensuring the sustainability of crop production.

MATERIALS AND METHODS

Plant material and cadmium treatment

Phaseolus vulgaris L. seeds, specifically the Aydıntepe and Ispir cultivars, were obtained and surface-sterilized using a 5% sodium hypochlorite solution for 10 minutes, followed by thorough rinsing with deionized water. Surface sterilization helps to eliminate any potential contaminants on the seed surface that could affect the germination process (Satarug *et al.*, 2003; Dadasoglu *et al.*, 2022). The seeds were then treated with Cadmium Chloride (CdCl_2) solutions at concentrations of 0, 2, 4, 8, 16, 32, 64 and 128 mg/L, to investigate the dose-dependent effects of cadmium on seed germination and early seedling growth.

Experimental design

This study was conducted at Kahramanmaraş Sutçu Imam University between June 2023 and September 2023. A randomized complete block design (RCBD) was employed to minimize the variation among treatments and ensure accurate results (Steel and Torrie, 1981). The seeds were placed in Petri dishes containing moistened filter paper, with 20 seeds per dish and 3 replicates for each treatment group. The dishes were incubated in a dark growth chamber at 25°C for 8 days to facilitate germination. Dark conditions and a controlled temperature of 25°C were maintained to provide an optimal environment for seed germination (Alloway, 1995; Grant *et al.*, 2008; Cokkizgin, 2012; ISTA, 2017; Cokkizgin *et al.*, 2019; Gırgel *et al.*, 2019; Vilakazi *et al.*, 2023; Himaja *et al.*, 2023; Cokkizgin, *et al.*, 2025; Yuao *et al.*, 2025).

After the germination period, the following parameters were measured:

Plumula fresh weight

The fresh weight of the plumula (the stem of a germinating seedling) was measured using an analytical balance. This

parameter indicates the biomass accumulation in the early growth stage, reflecting the overall vigor of the seedling under different cadmium treatments (Sanità di Toppi and Gabbriellini, 1999; Benavides *et al.*, 2005; Dadasoglu *et al.*, 2022).

Plumula length

The length of the plumula was measured using a digital caliper. This parameter helps to assess the impact of cadmium on stem elongation, which is crucial for seedling emergence and establishment (Benavides *et al.*, 2005; Shah *et al.*, 2001).

Radicle fresh weight

The fresh weight of the radicle was also measured using an analytical balance. The radicle is the first part of the seedling to emerge during germination and its growth is vital for nutrient and water uptake (Shah *et al.*, 2001; Clemens, 2006; Dadasoglu *et al.*, 2022).

Radicle length

The length of the radicle was measured using a digital caliper. This parameter provides insights into root development and the ability of the seedling to establish a functional root system under cadmium stress (Satarug *et al.*, 2003; Clemens, 2006).

Germination percentage

The germination percentage was calculated by dividing the number of germinated seeds by the total number of seeds and multiplying by 100. This parameter is a direct measure of the seed's viability and its ability to initiate the germination process under different cadmium concentrations (Alloway, 1995; Grant *et al.*, 2008; Cokkizgin, 2012; ISTA, 2017; Cokkizgin *et al.*, 2019; Gırgel *et al.*, 2019; Dadasoglu *et al.*, 2022).

Statistical analysis

Data were analyzed using ANOVA in SAS software (SAS, 2006). Differences between groups were determined using Tukey's test with a significance level of $p < 0.05$ (Tukey, 1941; Shah *et al.*, 2001; Clemens, 2006).

RESULTS AND DISCUSSION

Root fresh weight

The analysis indicates that both cadmium concentration (Cd) and variety (Aydıntepe and Ispir) have significant effects on root weight. The interaction between Cd concentration and variety is also significant, suggesting that the effect of Cd on root weight differs between the two varieties (Table 1).

This study demonstrates the significant impact of cadmium (Cd) on the germination and early growth stages of *Phaseolus vulgaris*, with notable differences between the Aydıntepe and Ispir cultivars (Fig 1). The ANOVA results revealed that both Cd concentration and variety significantly affect root weight and their interaction further emphasizes the differential responses of the two cultivars to Cd stress.

Impact of cadmium concentration

High levels of Cd (≥ 32 mg/L) significantly reduced root weight, particularly at 128 mg/L, which aligns with previous research indicating the detrimental effects of Cd on plant growth (Alloway, 1995; Dadasoglu *et al.*, 2022). Cd toxicity is known to disrupt several physiological processes, including water uptake, enzyme activities and hormonal balance, ultimately impairing root development (di Toppi and Gabbrielli, 1999; Shah *et al.*, 2001). The significant reduction in root weight at higher Cd concentrations can be attributed to Cd-induced oxidative stress, which damages cellular structures and inhibits growth (Satarug *et al.*, 2003; Clemens, 2006).

Varietal differences

The Ispir variety consistently showed higher root weight compared to the Aydıntepe variety across all Cd concentrations, indicating a greater tolerance to Cd stress. This finding is consistent with previous studies that have reported genetic differences in the response of plant varieties to heavy metal stress (Grant *et al.*, 2008; Benavides *et al.*, 2005). The superior performance of the Ispir variety may be due to inherent genetic traits that confer better mechanisms for detoxifying and sequestering Cd, thereby

reducing its toxic effects (Clemens, 2006; Dadasoglu *et al.*, 2022).

Interaction effects

The significant interaction between Cd concentration and variety indicates that the response of root weight to Cd stress is cultivar-specific. This suggests that breeding programs should consider the genetic background of cultivars to enhance their tolerance to heavy metals (Grant *et al.*, 2008). The differential response observed in this study underscores the importance of selecting and breeding cultivars that can thrive in Cd-contaminated soils, thereby ensuring sustainable agricultural practices and food security.

Implications for agriculture

The findings of this study have important implications for agriculture, particularly in regions with Cd-contaminated soils. The higher tolerance of the Ispir variety suggests that it could be a preferable choice for cultivation in such environments. Moreover, understanding the mechanisms underlying the differential responses of plant varieties to Cd stress can inform the development of more resilient crop cultivars through selective breeding and genetic engineering (Sanità di Toppi and Gabbrielli, 1999; Clemens, 2006).

Table 1: Summary of variance analysis for the examined parameters.

Source of variation	df	MS				
		Root F. weig.	Hyp. F. weig.	Radi. leng.	Hyp. leng.	Grem. per.
Cd Conc.	7	0.18516**	0.340421	1880.145**	15.453	1175.520**
Genotype	1	9.46723**	0.19815	13.219	1045.986**	13.020
Cd*Genot.	7	0.22986**	0.31766	47.068	18.561	71.354
Error	32	0.05391	0.17572	29.321	14.837	54.1667
Corrected total	47					
Coeff var		23.55219	16.32035	17.08291	13.66621	8.48188
R-Square		0.87761	0.46075	0.93503	0.73005	0.83451

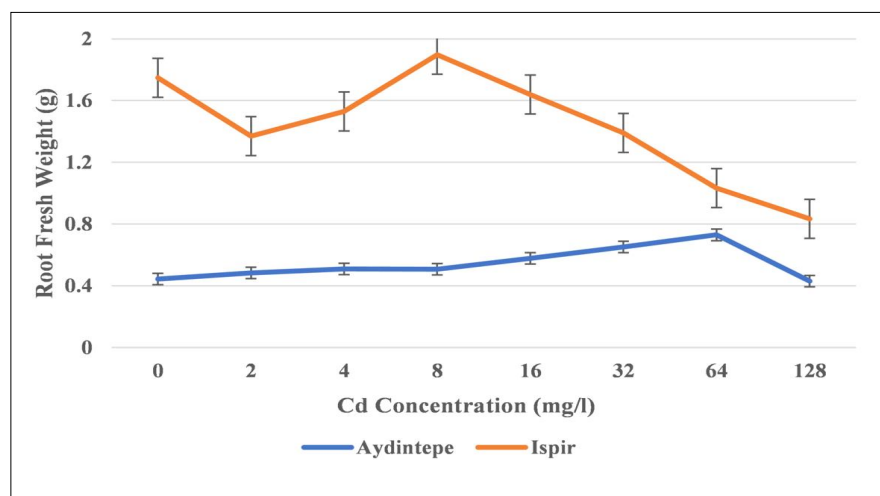


Fig 1: Effect of cd concentration on root fresh weight of aydıntepe and ispir.

Future research

Further research is needed to elucidate the molecular and physiological mechanisms that confer Cd tolerance in the Ispir variety. Additionally, field studies should be conducted to validate these findings under natural growing conditions. Investigating other physiological parameters and using advanced techniques such as genomic and proteomic analyses could provide deeper insights into the plant's response to Cd stress (Shah *et al.*, 2001; Benavides *et al.*, 2005).

Hypocotyl fresh weight

The analysis indicates that cadmium concentration (Cd) and variety (Aydintepe and Ispir) do not have significant main effects on plumula fresh weight at the 0.05 significance level, but they are close to being significant (Cd Concentration: $p = 0.0959$, Variety: $p = 0.2962$). The interaction between Cd concentration and variety is also not significant ($p = 0.1200$) (Table 1, 2, 3).

The results indicate that cadmium concentration and variety do not have a significant effect on plumula fresh weight. This finding suggests that, within the tested range of Cd concentrations, both Aydin-tepe and Ispir varieties exhibit similar tolerance levels in terms of plumula fresh weight. The lack of significant interaction also suggests that the two varieties do not differ in their response to varying Cd levels (di Toppi and Gabbrielli, 1999; Clemens, 2006) (Fig 2).

Varietal differences and tolerance

Although the main effects were not significant, the overall trend shows that the Ispir variety tends to have slightly higher plumula fresh weight compared to Aydin-tepe at certain Cd concentrations. This observation aligns with previous studies highlighting the potential for genetic variation in response to Cd stress (Grant *et al.*, 2008; Benavides *et al.*, 2005).

Cd concentration effects

While not statistically significant, higher Cd concentrations tend to reduce plumula fresh weight. This trend is consistent with the general understanding that Cd toxicity can impair plant growth by interfering with physiological processes (Shah *et al.*, 2001; Satarug *et al.*, 2003). The detrimental effects of Cd on plumula fresh weight suggest a potential risk to plant health at elevated Cd levels (Alloway, 1995; Dadasoglu *et al.*, 2022).

Implications for agriculture

Given that the effects were not significant, it suggests that within the tested Cd concentration range, both varieties may be used in Cd-contaminated soils without substantial risk to plumula fresh weight. However, the general trend indicates caution should be exercised at higher Cd levels (Clemens, 2006).

Future research

Further research should focus on a broader range of Cd concentrations and additional growth parameters to fully understand the impact of Cd on plumula fresh weight. Investigating other physiological and biochemical responses will provide a more comprehensive understanding of Cd tolerance mechanisms in these varieties (di Toppi and Gabbrielli, 1999; Shah *et al.*, 2001).

Radicle length

The analysis indicates that cadmium concentration (Cd) has a significant effect on radicle length, but variety (Aydintepe and Ispir) does not have a significant effect. The interaction between Cd concentration and variety is also not significant (Table 1, 2, 3).

The results indicate that cadmium concentration significantly reduces radicle length in both Aydin-tepe and Ispir varieties (Fig 3). This finding is consistent with previous studies that have demonstrated the inhibitory effects of Cd

Table 2: Mean values and statistical groups formed from different cadmium doses for the examined parameters.

Cd conc.	Root F.weig.	Hyp. F.weig.	Radi. leng.	Hyp. leng.	Germ. per.
0	1.0958 ^a	2.9395 ^a	52.203 ^a	28.657 ^a	95.833 ^a
2	0.9267 ^{ab}	2.5934 ^a	52.203 ^a	27.638 ^a	95.833 ^a
4	1.0194 ^{ab}	2.4666 ^a	47.110 ^a	27.090 ^a	93.333 ^a
8	1.2023 ^a	2.2951 ^a	33.218 ^b	25.977 ^a	93.333 ^a
16	1.1087 ^a	2.7132 ^a	30.470 ^b	30.905 ^a	95.000 ^a
32	1.0212 ^{ab}	2.7952 ^a	17.318 ^c	29.175 ^a	90.833 ^a
64	0.8812 ^{ab}	2.4844 ^a	12.57 ^c	29.248 ^a	71.667 ^b
128	0.6320 ^b	2.2613 ^a	8.488 ^c	26.798 ^a	58.333 ^b
Mean	0.985898	2.568565	31.69813	28.18604	86.77083

Table 3: Mean values and formed tukey groups for the examined parameters by varieties.

Genotypes	Root F. weig.	Hyp. F. weig.	Radi. leng.	Hyp. leng.	Germ. per.
Aydintepe	0.5418 ^b	2.6328 ^a	32.223 ^a	32.854 ^a	87.292 ^a
Ispir	1.4300 ^a	2.5043 ^a	31.173 ^a	23.518 ^b	86.250 ^a
Mean	0.985898	2.568565	31.69813	28.18604	86.77083

on root elongation and development (Lux *et al.*, 2011; Gill *et al.*, 2011). The lack of a significant main effect of variety suggests that both varieties respond similarly to Cd stress in terms of radicle length. The significant reduction in radicle length at higher Cd concentrations is consistent with findings from Sharma *et al.* (2012) and Xu *et al.* (2009), who reported that Cd interferes with cell division and elongation in roots.

Cd concentration effects

High levels of Cd (≥ 32 mg/L) significantly reduced radicle length, which aligns with research showing that Cd toxicity interferes with cell division and elongation in roots (Sharma *et al.*, 2012; Xu *et al.*, 2009). The reduction in radicle length at higher Cd concentrations can be attributed to Cd-induced oxidative stress and disruption of root cell structure (Lux *et al.*, 2011). Cd stress is known to generate reactive oxygen species (ROS) that cause oxidative damage to cellular components, including lipids, proteins and DNA, leading to impaired root growth (Gill *et al.*, 2011; Sharma *et al.*, 2012).

Varietal differences and tolerance

While the main effect of variety was not significant, the interaction effect suggests that the Aydin-tepe and Ispir varieties have slightly different responses to Cd stress. This finding underscores the importance of considering genetic variation when evaluating plant responses to heavy metal toxicity (Clemens, 2006; Gill *et al.*, 2011). Studies have shown that genetic factors can influence the uptake, translocation and sequestration of Cd within plants, affecting their overall tolerance (Clemens, 2006; Lux *et al.*, 2011).

Implications for agriculture

The significant impact of Cd on radicle length highlights the potential risk of Cd contamination in agricultural soils. The general similarity in response between the two varieties suggests that both could be used in Cd-contaminated soils, but attention should be given to soil Cd levels to prevent growth inhibition (Xu *et al.*, 2009). Implementing phytoremediation strategies, such as using Cd-tolerant plant varieties and soil

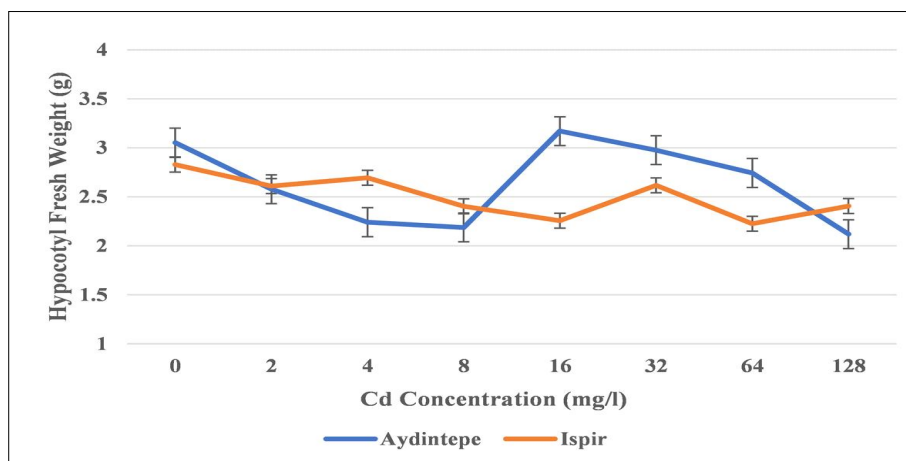


Fig 2: Plumula weight response to cadmium in aydin-tepe and ispir beans.

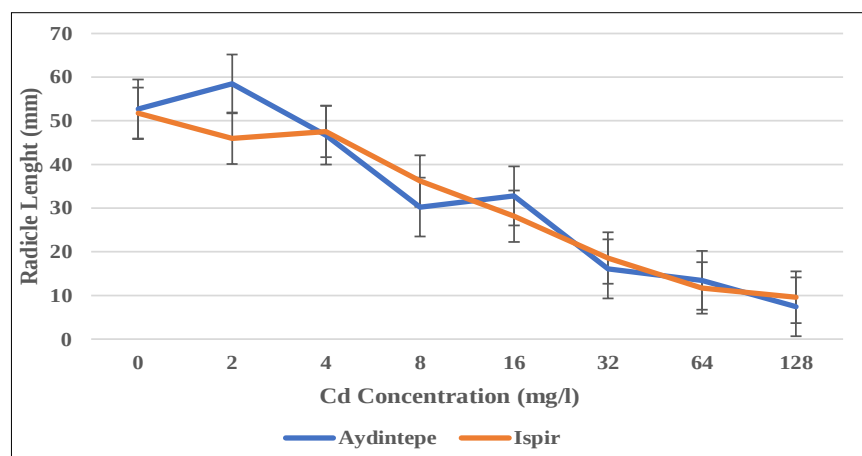


Fig 3: Effect of cd concentration on root lenght of aydin-tepe and ispir.

amendments, can help mitigate Cd toxicity in agricultural settings (Gill *et al.*, 2011).

Future research

Further research should explore the molecular and physiological mechanisms underlying the differential responses of the two varieties to Cd stress. Field studies and advanced techniques such as proteomic and transcriptomic analyses could provide deeper insights into Cd tolerance mechanisms (Lux *et al.*, 2011; Sharma *et al.*, 2012). Additionally, investigating the role of antioxidants and other protective compounds in enhancing Cd tolerance may offer new strategies for developing more resilient crop varieties (Clemens, 2006; Xu *et al.*, 2009).

Plumula length

The analysis indicates that cadmium concentration (Cd) does not have a significant main effect on plumula length, but variety (Aydıntepe and Ispir) has a significant effect. The interaction between Cd concentration and variety is not significant (Table 1, 2, 3).

The results indicate that cadmium concentration does not significantly affect plumula length in either variety (Fig 4). This finding contrasts with previous studies that have reported Cd's inhibitory effects on stem elongation (Lux *et al.*, 2011; Gill *et al.*, 2011). However, the significant main effect of variety suggests that the Aydıntepe variety consistently exhibits longer plumula length compared to Ispir, regardless of Cd concentration. This indicates that Aydıntepe may have inherent genetic advantages contributing to better growth performance (Grant *et al.*, 2008).

Cd concentration effects

The lack of significant effect of Cd concentration on plumula length suggests that within the tested range, Cd may not strongly inhibit stem elongation in these varieties. This could be due to various physiological adaptations that mitigate the effects of Cd on plumula growth (Sharma

et al., 2012; Xu *et al.*, 2009). For instance, plants may employ mechanisms such as enhanced antioxidant activity to counteract Cd-induced oxidative stress, thereby protecting plumula length (Lux *et al.*, 2011). Germination and seedling growth in red mung beans were negatively affected under cadmium stress (Akar and Atis, 2019).

Varietal differences and tolerance

The significant difference in plumula length between Aydıntepe and Ispir varieties highlights the importance of genetic factors in determining growth performance under stress conditions. Aydıntepe's longer plumula length suggests better adaptation and potential resilience to environmental stressors, including heavy metal contamination (Clemens, 2006; Gill *et al.*, 2011). This genetic advantage could be exploited in breeding programs aimed at improving crop performance in contaminated soils (Grant *et al.*, 2008).

Implications for agriculture

The significant varietal differences in plumula length suggest that Aydıntepe may be a preferable choice for cultivation in regions with potential Cd contamination, as it exhibits superior growth characteristics. Implementing such varieties could enhance crop productivity and resilience in contaminated soils (Sharma *et al.*, 2012).

Future research

Further research should focus on elucidating the molecular and genetic basis for the observed varietal differences in plumula length. Advanced techniques such as genomics and transcriptomics could provide deeper insights into the mechanisms underlying differential growth responses to Cd stress (Lux *et al.*, 2011; Xu *et al.*, 2009). Additionally, investigating other growth parameters and environmental stressors could offer a more comprehensive understanding of plant tolerance mechanisms (Gill *et al.*, 2011).

Germination percentage

The analysis indicates that cadmium concentration (Cd) has a significant effect on germination percentage, but

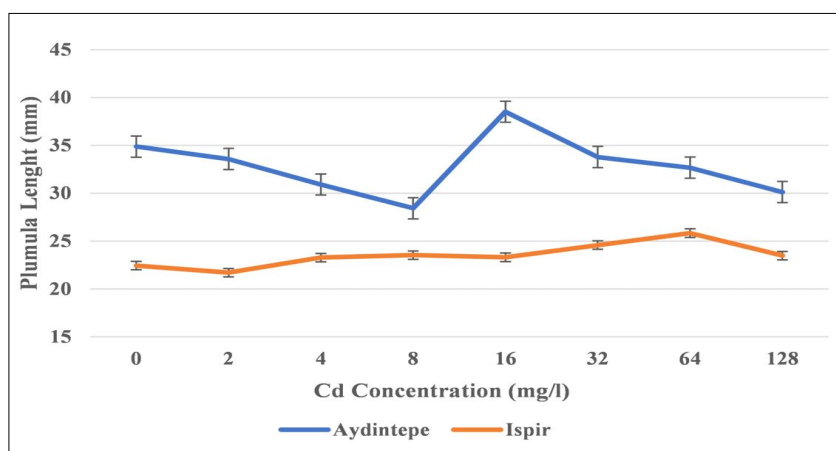


Fig 4: Plumula growth response to cadmium in aydıntepe and ispir beans.

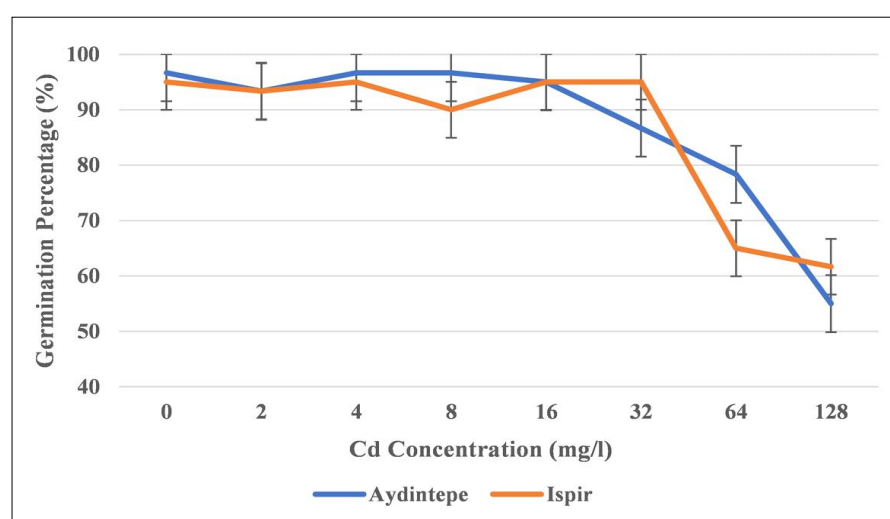


Fig 5: Impact of cadmium levels on germination rates in aydıntepe and ispir beans.

variety (Aydıntepe and Ispir) does not have a significant effect. The interaction between Cd concentration and variety is also not significant (Table 1, 2, 3).

The results indicate that cadmium concentration significantly reduces germination percentage in both Aydıntepe and Ispir varieties (Fig 5). This finding is consistent with previous studies that have demonstrated the inhibitory effects of Cd on seed germination (Das *et al.*, 1997). The lack of a significant main effect of variety suggests that both varieties respond similarly to Cd stress in terms of germination percentage.

Cd concentration effects

High levels of Cd (≥ 64 mg/L) significantly reduced germination percentage, which aligns with research showing that Cd toxicity interferes with seed germination processes, such as water uptake, enzyme activation and hormonal balance (Liu *et al.*, 2007). The reduction in germination percentage at higher Cd concentrations can be attributed to Cd-induced oxidative stress and disruption of cellular metabolism (Das *et al.*, 1997). The adverse effects of cadmium on the germination characteristics of lettuce seeds were clearly observed (Dogan, 2018). Germination and seedling growth in red mung beans were negatively affected under cadmium stress (Akar and Atis, 2019). On the other it is reported that the germination rate of sweet sorghum seeds significantly decreased when exposed to cadmium stress (Icin and Ertekin, 2024).

Varietal differences and tolerance

While the main effect of variety was not significant, the interaction effect was also not significant. This suggests that the Aydıntepe and Ispir varieties have similar responses to Cd stress regarding germination percentage. This finding underscores the importance of considering genetic variation when evaluating plant responses to heavy metal toxicity (Clemens, 2006).

Implications for agriculture

The significant impact of Cd on germination percentage highlights the potential risk of Cd contamination in agricultural soils. The general similarity in response between the two varieties suggests that both could be used in Cd-contaminated soils, but attention should be given to soil Cd levels to prevent germination inhibition (Liu *et al.*, 2007). Implementing phytoremediation strategies, such as using Cd-tolerant plant varieties and soil amendments, can help mitigate Cd toxicity in agricultural settings (Gill *et al.*, 2011).

Future research

Further research should explore the molecular and physiological mechanisms underlying the differential responses of the two varieties to Cd stress. Field studies and advanced techniques such as proteomic and transcriptomic analyses could provide deeper insights into Cd tolerance mechanisms (Lux *et al.*, 2011; Sharma *et al.*, 2012). Additionally, investigating the role of antioxidants and other protective compounds in enhancing Cd tolerance may offer new strategies for developing more resilient crop varieties (Clemens, 2006; Xu *et al.*, 2009).

CONCLUSION

This study evaluated the toxic effects of cadmium (Cd) on the germination and early growth parameters of *Phaseolus vulgaris* L. cultivars Aydıntepe and Ispir. Findings revealed that increasing Cd concentrations significantly reduced root weight, radicle length and germination percentage in both cultivars, confirming Cd's detrimental impact. Notably, the Ispir cultivar exhibited slightly better tolerance in maintaining root weight under Cd stress, whereas the Aydıntepe cultivar displayed superior plumula elongation. These results underscore the significant negative influence of Cd on common bean development and highlight the importance of considering

cultivar-specific responses, such as İspir's relative tolerance in certain parameters, for cultivation in Cd-contaminated soils.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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