Zinc Oxide Nanoparticle Impact to Phytoremediation of Cd-Contaminated Soil Using Brown Mustard (*Brassica juncea* L.)

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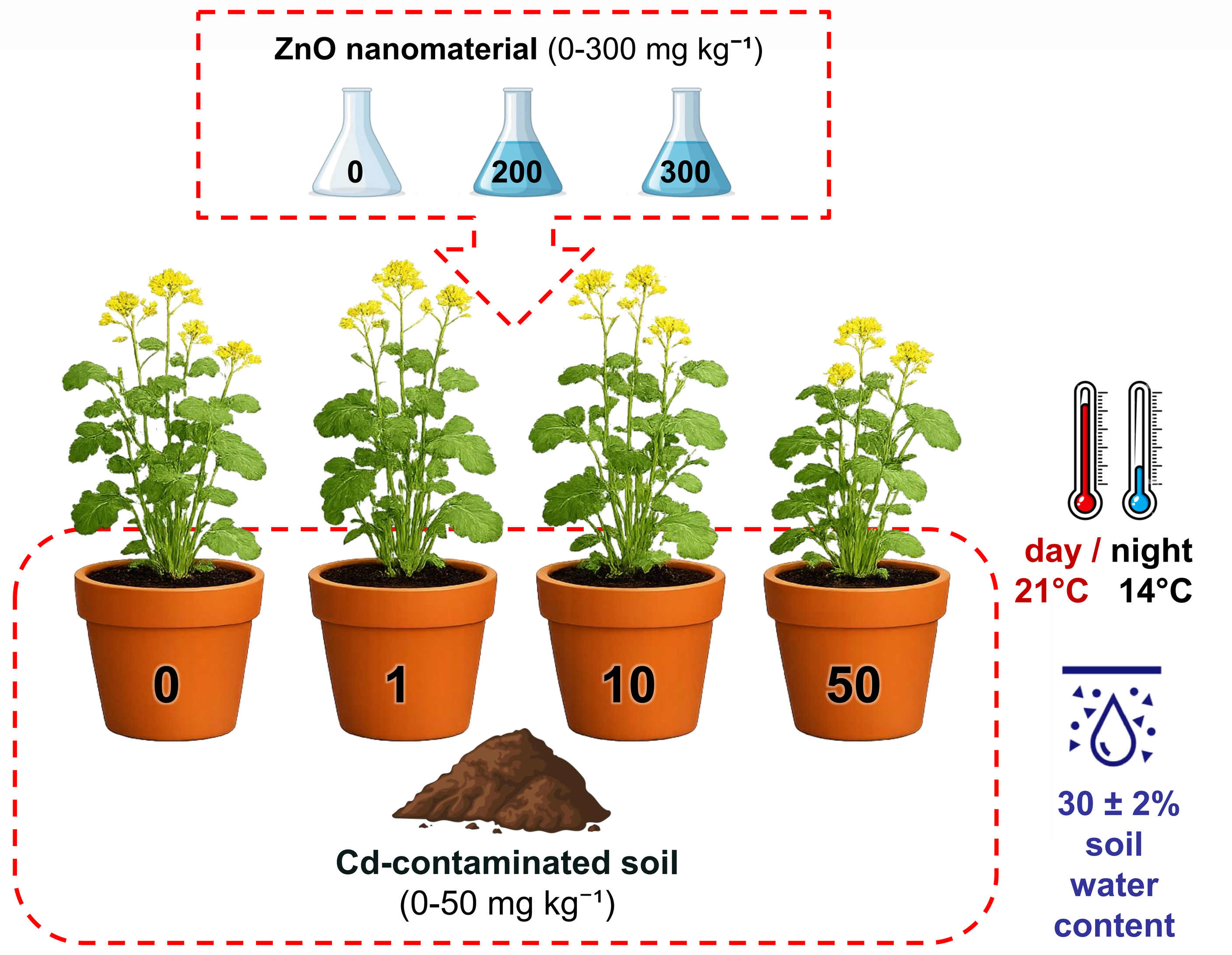
Introduction

- Soil contamination with heavy metals (HM) remains a persistent environmental challenge due to their non-biodegradable nature and high toxicity. Phytoremediation is a promising bioremediation technology for the restoration of HM sites by utilizing harvestable plants and posing no adverse effect to environment. However, its effectiveness is often limited by metal-induced stress, which restricts plant growth and overall remediation capacity.
- Zinc oxide nanoparticles (ZnO NPs) are increasingly explored as soil amendments due to their ability to influence plant growth, stress tolerance, and metal uptake. Still, the role of nanoparticles in phytoremediation is not yet fully understood, particularly in terms of their interactions with metals and the identification of safe and effective application rates.
- ➢ The aim of this study was to evaluate the effects of ZnO NPs (0, 200, 300 mg kg^{-1}) on the phytoremediation potential of brown mustard (*Brassica juncea* L.) grown in Cd-contaminated soil (0–50 mg kg^{-1}) under controlled conditions.

Materials & methods

➢ **Brown mustard (*Brassica juncea* L.)** was grown for 45 days in growth chamber under controled conditions.



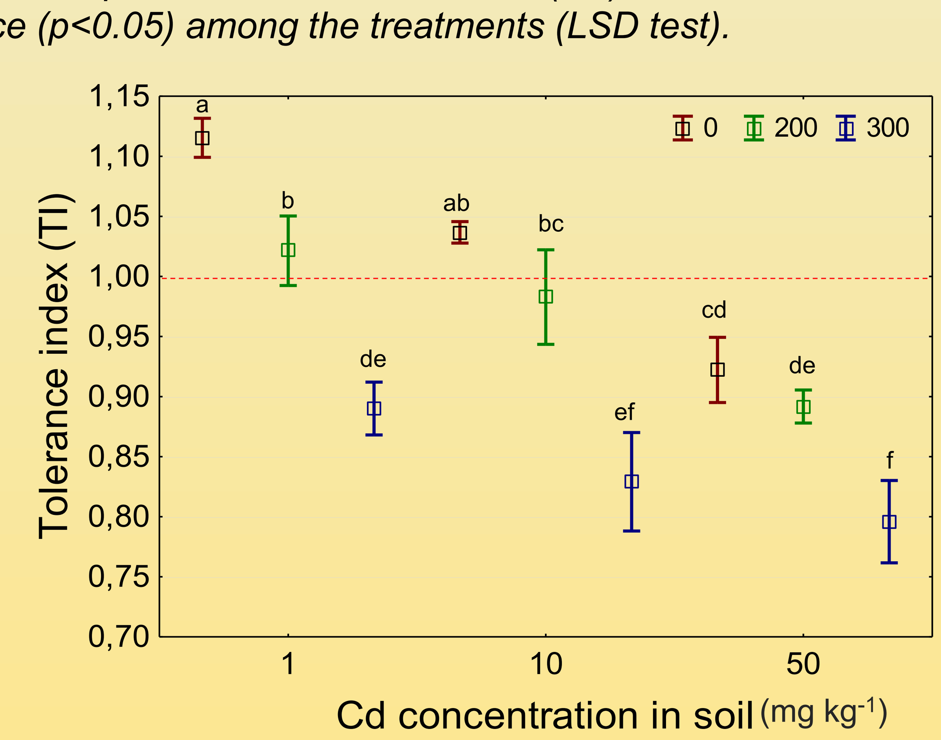
- ➢ The following parameters were measured: chlorophyll content, shoot height, root length, shoot and root dry biomass (DW), and plant tolerance to contamination.
- ➢ Chlorophyll content was determined with an Apogee MC-100.
- ➢ Tolerance index (TI) was calculated as the ratio of plant shoot dry biomass (DW) in contaminated soil to that in control soil.
- ➢ The statistical analysis was performed using Statistica software.

Table 1. Levels of significance of variance analysis (factorial ANOVA) of shoot and root DW, shoot height, root length, chlorophyl content, tolerance index (TI) in *Brassica juncea* grown in Cd polluted soil under different NPs ZnO doses.

* < 0.05; ** < 0.01; *** < 0.001; ns > 0.05

	Factors		
	Cd	**NPs ZnO**	**Cd x NPs ZnO**
Shoot DW	***	*	*
Root DW	*	*	ns
Shoot height	***	ns	ns
Root length	ns	ns	ns
CCI	ns	ns	*
TI	***	***	ns

Fig. 2. Tolerance index (TI) of *B. juncea* grown in Cd polluted soil (1-50 mg kg^{-1}) under different ZnO-NPs doses (0-300 mg kg^{-1}). Values of TI ≥ 1 indicate high plant tolerance under contaminated conditions.
Error bars represent standard errors (SE). Different letters indicate significant difference (p<0.05) among the treatments (LSD test).



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Results & discussions

- ❖ Cd soil concentration significantly affected shoot and root DW, shoot height and tolerance to Cd contamination (Table 1), confirming strong concentration-dependent phytotoxicity.
- ❖ ZnO-NPs significantly influenced only shoot and root DW, tolerance to Cd contamination (Table 1), indicating that nanoparticle amendments primarily affected biomass-related traits.
  - Low-dose ZnO-NPs amendment (200 mg kg^{-1}) partially alleviated Cd-induced stress by enhancing shoot and root biomass by up to 20 % relative to ZnO-free plants grown at the same Cd contamination (Fig. 1A,B), suggesting stimulation of plant growth under moderate contamination.
  - In contrast, the highest ZnO-NPs dose (300 mg kg^{-1}) consistently reduced shoot biomass and height (up to up to 11 %), CCI (up to 16 %), and TI (up to 21 %) relative to ZnO-free plants grown at the same Cd contamination, indicating a shift from stimulatory to phytotoxic effects at elevated nanoparticle concentrations (Fig. 1).
- ❖ A significant effect of interaction between Cd concentration and ZnO-NPs dose in soil (Cd × ZnO-NPs) was observed for shoot DW and chlorophyll content (Table 1), demonstrating that nanoparticle effects depended on contamination level.
- ❖ Both Cd concentration and ZnO-NPs dosage independently and significantly reduced the Tolerance Index (TI) of *B. juncea* ($p < 0.001$), but their interaction was not statistically significant ($p > 0.05$) (Table 1). *Brassica juncea* tolerance to Cd contamination decreased with increasing soil Cd concentration, while at each contamination level, the highest ZnO-NPs dose resulted in the lowest TI (Fig. 2).

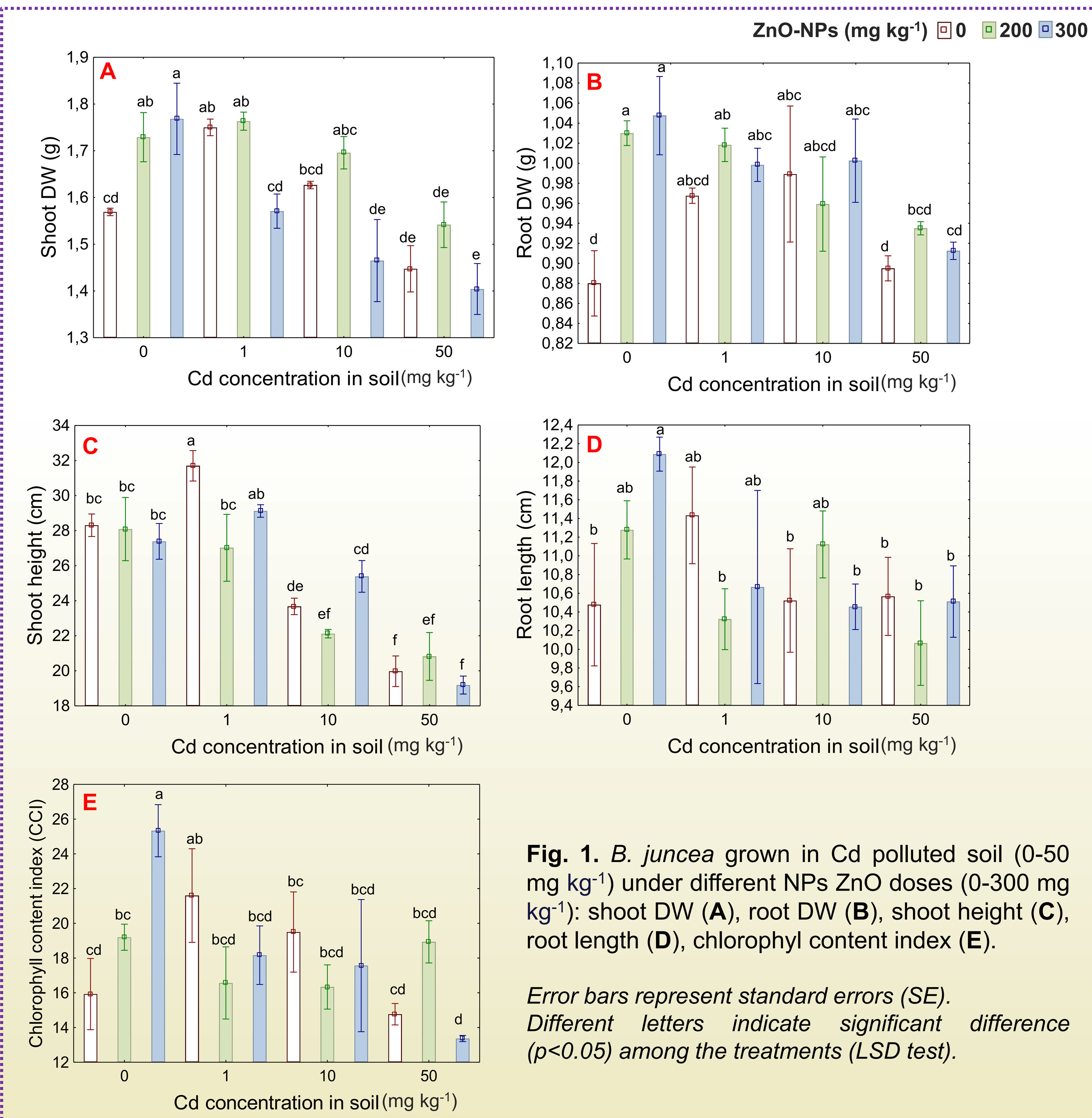

Fig. 1. *B. juncea* grown in Cd polluted soil (0-50 mg kg^{-1}) under different NPs ZnO doses (0-300 mg kg^{-1}): shoot DW (**A**), root DW (**B**), shoot height (**C**), root length (**D**), chlorophyl content index (**E**).

Error bars represent standard errors (SE). Different letters indicate significant difference (p<0.05) among the treatments (LSD test).

Concluding remarks

- Cd significantly affected shoot and root DW, shtoot height, tolerance to Cd contamination, confirming strong concentration-dependent phytotoxicity and suggesting that *B. juncea* is more suitable for phytoremediation of low–moderate Cd-contaminated soils.
- ZnO NPs had a significant effect on biomass production and a clear dose-dependent response: low dose (200 mg kg^{-1}) partially mitigated Cd stress, while the highest dose (300 mg kg^{-1}) intensified toxicity at some cases.
- Significant Cd concentration in soil and ZnO-NPs interaction effect on shoot biomass and chlorophyll content, indicating contamination-level-dependent nanoparticle effects.
- Both Cd concentration in soil and ZnO-NPs independently reduced plant tolerance index (ANOVA, $p < 0.001$).
- Altogether, these findings demonstrate that modest applications of ZnO NPs can modulate plant stress physiology, promote biomass production, and enhance cadmium uptake—ultimately improving phytoremediation efficiency in contaminated soils. Meanwhile excessive nanoparticle application may aggravate stress and reduce phytoremediation efficiency under Cd contamination.