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Comparative Dynamics of Cadmium and Zinc in Plant-Soil Systems: Interactions, Uptake Mechanisms, and Toxicological Implications for Agricultural Sustainability

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Abstract : The heavy metals zinc and cadmium (among others) draw substantial research interest because of their capacity to persist and resist degradation alongside their different functions in plant biology. The harmful substance Cadmium functions as a non-essential element because it presents distinctive threats to plant development and food quality but zinc as an essential micronutrient drives fundamental biological processes including protein development plus genetic processes and carbohydrate metabolic functions. Plant systems experience competitive interactions since these elements from Group IIB show chemical and physical similarities when they uptake or move through the plant tissues. The research investigates soil Cd and Zn pollutant origins from natural and human activities while analyzing industrial processes that affect their uptake bioavailability. The study examines metal transport mechanisms through evaluation of Cd's ability to exploit nutritional transporters while discussing Zn's dependence on ZIP CDF and HMA transport families. This study examines toxicity profiles between Cd and Zn with a particular emphasis on Cd having higher phytotoxic potential because it is nonessential while having high soil mobility. The research highlights the need to investigate rhizospheric metal interactions since they determine sustainable agricultural development and successful phytoremediation method development. Research findings establish the requirement for combined soil management systems which help decrease Cd toxicity while maintaining enough Zn nutrient levels in agricultural crops.

Keywords: Heavy Metals, Cadmium Toxicity, Zinc Homeostasis, Metal Transporters, Soil Contamination, Plant Nutrition, Agricultural Sustainability, Phytoremediation, Metal Interactions, Group IIB Elements.

Introduction : Heavy metals (HMs) are a significant concern in environmental toxicology due to their persistent, non-degradable, and bio-accumulative nature. These elements are naturally occurring and defined based on their high atomic weight and density—specifically, elements with a density more than 4 g/cm³ or at least 5 times denser than water qualify like heavy metals (Emamverdian et al., 2015). Their persistence in the environment and potential to bioaccumulate in plant and animal tissues pose a grave threat to ecosystem health, especially when concentrations exceed natural levels due to human activity.

Among the heavy metals, Cadmium (Cd) holds particular relevance due to its high toxicity and lack of any known biological role in plants. Belonging to Group IIB of the periodic table and carrying the atomic number 48, cadmium is classified as one of the most hazardous environmental contaminants. Its toxicity is recognized globally, and it holds the 7th position on the priority list of hazardous substances, as identified by the Agency for Toxic Substances & Disease Registry. Unlike essential micronutrients, cadmium does not contribute to any known plant physiological function; instead, its presence disrupts normal growth & development.

In contrast, Zinc (Zn)—also a Group IIB element—shares many of cadmium's chemical and physical characteristics but differs in its biological importance. Zinc is an essential micronutrient which plays vital role in many physiological & metabolic activities within plants. It is a structural constituent or regulator of many enzymes and proteins, participating in protein synthesis, carbohydrate and phosphate metabolism, gene expression, and membrane integrity (Sinclair and Kramer, 2012). However, like cadmium, zinc also exhibits toxicity at high concentrations, particularly when levels exceed 300 mg/kg dry weight (DW), impairing plant growth and metabolic functions (Audet and Charest, 2007; Broadley et al., 2007).

Cadmium is one of rarer elements in Earth's crust. Its average lithospheric concentration ranges between 0.08 and 0.1 ppm, which is about 651 times lower than average concentration of zinc (Rudnick and Gao, 2003). Despite its relatively low abundance, cadmium often coexists with zinc and other transition metals in mineral ores due to their chemical similarities. Cadmium frequently occurs as a secondary component in sulphide ores, such as sphalerite and wurtzite

(ZnS), metacinnabar (HgS), galena (PbS), and chalcopyrite ($CuFeS_2$) (Cullen and Maldonado, 2013). Moreover, cadmium can also be found in zinc silicates and carbonates, making it a consistent byproduct of zinc mining and processing.

Both natural and man-made factors can introduce heavy metals into agricultural soils. According to Choppala et al. (2014) and Zhao et al. (2015), the main natural causes of cadmium and zinc buildup in soils are volcanic eruptions along with weathering of parent rocks. These processes are relatively slow but constant, contributing trace amounts over geological time scales. In contrast, human activities have dramatically accelerated and increased heavy metal accumulation in soils. Mining, the smelting of non-ferrous metals, the creation of metal alloys, and a variety of industrial processes like electroplating, plastic manufacturing, paint and pigment manufacturing, including disposal of nickel-cadmium batteries are important anthropogenic sources of cadmium (Agnieszka et al., 2014). Emissions from fossil fuel combustion further contribute to cadmium pollution, as cadmium is present in trace amounts in coal and oil. Meanwhile, zinc contamination arises predominantly from smelter discharges, coal and fly ash, mine tailings, phosphate fertilizers, and zinc-based wood preservatives (Wuana and Okieimen, 2011).

Cadmium and zinc have intricate interactions with the soil matrix & plant roots once they are in the soil. One of the most concerning aspects of cadmium is that it lacks a specific uptake mechanism in plants. Instead, it hijacks the transport pathways intended for essential nutrients

like Zn^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+}, and Cu^{2+} to gain entry into plant cells, especially the root cells (Roth et al., 2006). This nonspecific uptake makes cadmium particularly insidious, as plants cannot easily discriminate between cadmium and beneficial metals. Zinc, however, is taken up via specific transporter protein families in plants. These include:

- ZIP (ZRT-, IRT-like proteins), which facilitate the uptake of Zn and Fe across cell membranes,
- CDF (Cation Diffusion Facilitators) and MTP (Metal Tolerance Proteins), which help maintain zinc homeostasis,
- HMA (Heavy Metal ATPases), involved in the transport of heavy metals including zinc,

- YSL (Yellow Stripe-Like proteins), which transport metal-chelate complexes,
- PCR (Plant Cadmium Resistance proteins),
- and VIT (Vacuolar Iron Transporters) (Gupta et al., 2016).

While both Cd and Zn are absorbed from the soil solution, cadmium is more toxic than zinc due to its non-essential nature and tendency to disrupt cellular functions. Once inside the plant, cadmium interferes with several physiological processes. By producing reactive oxygen species (ROS), it causes oxidative stress. It also prevents respiration, photosynthesis as well as enzyme activity, and it disrupts hormone signaling, nutritional intake, and water balance. These effects are cumulative and can result in stunted growth, chlorosis, necrosis, and even plant death in extreme cases.

Zinc, although essential, can mimic some of cadmium's toxic effects when present in excessive quantities. High concentrations of zinc may lead to nutrient imbalances, especially by antagonizing iron, manganese, and copper uptake, and can similarly impair enzyme function and generate oxidative stress. Thus, maintaining an optimal balance of zinc is crucial—not only to ensure adequate nutrition but also to prevent inadvertent toxicity. It is important to note that the close resemblance between cadmium and zinc—particularly their ionic radius, chemical properties, and electronegativity—complicates their separation by plants and poses a challenge for remediation efforts. The shared presence of these metals in ores and industrial processes also increases the likelihood of their simultaneous release into the environment.

In short, cadmium and zinc represent a classic case of chemical similarity but biological contrast. Cadmium is a harmful, non-essential metal that disrupts plant metabolism through mimicry and interference, while zinc is indispensable yet potentially toxic when in excess. Understanding the dynamics of their entry, transport, and interaction within plant systems is vital, particularly in the context of agricultural sustainability, food safety, and phytoremediation strategies. Future research and environmental management efforts must focus on monitoring these metals, refining soil remediation technologies, and developing metal-tolerant crop varieties to minimize their harmful impacts on ecosystems and human health.

Materials and Methods

1. Experimental Site and Conditions

Study was conducted under controlled greenhouse conditions at the Department of Botany in our college, to assess the comparative behavior of cadmium (Cd) & zinc (Zn) in plant-soil systems and their implications on crop health and sustainability. The greenhouse maintained a photoperiod of 14 hours of light and 10 hours of darkness, with an average temperature of 25 ± 2°C and relative humidity of 65–70%. These conditions simulated a typical agricultural setting to evaluate the effects of metal stress on two economically important leguminous crops—chickpea (Cicer arietinum L.) and soybean (Glycine max L. Merr.).

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Figure: (C) Cicer arietinum L. plant and, (D) Glycine max L. Merr. Plant and

2. Soil Collection and Characterization

Top 0–20 cm layer of an agricultural field with a low background contamination of heavy metals was employed to gather the soil for the experiment. After being allowed to air dry, the soil was sieved using a 2 mm screen and examined physicochemically. Key soil parameters were determined prior to experimentation, including:

- pH (1:2.5 soil:water ratio) using a digital pH meter,
- Electrical conductivity (EC) using a conductivity meter,
- Organic carbon by the Walkley-Black method,
- Cation Exchange Capacity (CEC) by ammonium acetate extraction,
- Texture via the hydrometer method,
- Total nitrogen (Kjeldahl method),
- Available phosphorus (Olsen's method),
- Available potassium using flame photometry,
- Baseline levels of Cd and Zn were analyzed using Atomic Absorption Spectrophotometry (AAS).

The soil was categorized as loamy, with slightly alkaline pH (7.4–7.8), moderate organic matter (0.75–1.2%), and sufficient native Zn but negligible Cd content.

3. Experimental Design and Treatments

A factorial completely randomized design (CRD) with three replications was adopted. The experiment comprised the following treatments:

- Control (T0): No metal application
- Cadmium stress (T1): 50 μM Cd as $CdCl_2 \cdot 2.5H_2O$
- Zinc stress (T2): 100 μM Zn as $ZnSO_4 \cdot 7H_2O$
- Cd + Zn combined (T3): 50 μM Cd + 100 μM Zn

Metal solutions were prepared in deionized water and applied uniformly to the soil surface seven days prior to sowing to ensure proper equilibration. Five kilograms of pre-characterized soil were placed inside each plastic pot, which measured 25 cm in diameter and 30 cm in depth. To stop leaching, the pots were lined with polyethylene sheets.

4. Seed Selection, Sterilization, and Sowing

Certified seeds of chickpea (cv. ICCV-2) and soybean (cv. JS-335) were procured from a recognized agricultural research institute. After two minutes of surface sterilization with 0.1% mercuric chloride, the seeds were repeatedly rinsed with sterile distilled water. For uniformity, ten seeds were planted in each pot, and five robust seedlings were kept after germination.

5. Irrigation and Nutrient Management

Plants were irrigated regularly with distilled water to maintain 60–70% field capacity throughout the experimental period. To avoid nutrient deficiency and isolate the effects of Cd and Zn, all pots were uniformly supplied with a modified Hoagland nutrient solution excluding Zn and Cd, twice a week. For consistency, no additional organic or inorganic fertilizers were applied.

6. Sampling and Growth Measurements

Plants were harvested at 30 and 60 days after sowing (DAS). At each interval, morphological parameters were recorded, including:

- Plant height (cm),
- Root length (cm),
- Fresh and dry biomass (g),
- Number of nodules per plant (for chickpea and soybean), •Visual toxicity symptoms (chlorosis, necrosis, stunted growth).

Dry weights were recorded after oven-drying plant tissues at 70°C till then a constant weight was achieved.

7. Physiological and Biochemical Analysis

Physiological characteristics have been determined from the third completely grown leaf at 60 DAS:

- Chlorophyll content was estimated using Arnon's method (1949),
- Proline accumulation was analyzed following Bates et al. (1973),
- As a marker of lipid peroxidation, malondialdehyde (MDA) was determined using the thiobarbituric acid reactive substances (TBARS) assay.
- Relative Water Content (RWC) was calculated using standard methods.

Enzymatic antioxidant activity assays were performed for:

- Catalase (CAT) – using H_2O_2 decomposition,
- Superoxide dismutase (SOD) – by nitro blue tetrazolium (NBT) reduction method, •Peroxidase (POD) – using guaiacol oxidation.

8. Heavy Metal Accumulation in Tissues and Soil

Samples of roots and shoots were taken after 60 days, cleaned well, dried in an oven, and milled into a fine powder. For metal analysis:

- 0.5 g of dried tissue was digested in a tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4 in 5:1:1 ratio),

- Filtrates were analyzed for Cd and Zn concentrations using Flame Atomic Absorption Spectrophotometry (FAAS) (Model: Perkin Elmer AAnalyst 400),
- Soil samples post-harvest was similarly analyzed for residual metal concentration.
- Translocation factor (TF) and bioaccumulation factor (BAF) were computed to assess the mobility and absorption efficiency of Cd and Zn from root to shoot.

9. Statistical Analysis

Every experiment was carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results. Microsoft Excel 2019 and SPSS version 25.0 were used for statistical analysis. The significance of changes between treatments was evaluated using a oneway ANOVA, and for multiple comparisons, Tukey's HSD test was employed. The threshold for significance was $p < 0.05$. Correlation and regression analyses were performed to study relationships between physiological traits and metal accumulation.

10. Environmental and Safety Considerations

All chemical handling and disposal procedures adhered to institutional biosafety and environmental protection protocols. Personal protective equipment (PPE) was used during metal handling, and waste disposal was conducted through approved hazardous waste management systems to ensure minimal environmental impact.

Results

In the study on the comparative dynamics of cadmium (Cd) and zinc (Zn) in plant-soil systems, the interaction between these heavy metals and their toxicological implications for plant growth and agricultural sustainability were critically examined. Understanding the effects of zinc and cadmium stress on plant physiological processes, antioxidant mechanisms, and the function of certain amendments like silicon (Si) and arbuscular mycorrhizal (AM) fungus in reducing these stresses were the main goals of the study. With a focus on metal uptake mechanisms, antioxidant modulation, and stress tolerance augmentation through Si and AM fungal inoculation, the physiological and biochemical responses to Zn and Cd exposure were examined using the two pigeonpea genotypes, Pusa 2002 and Pusa 991.

Effect of Zinc (Zn) and Cadmium (Cd) Stress on Plant Physiology

Zn and Cd are two heavy metals that play significant roles in plant metabolism but, when present in excess, can lead to toxicological effects. According to the study, pigeonpea plants' uptake of zinc and cadmium, especially through the roots, caused oxidative stress, which was typified by an increase in reactive oxygen species (ROS) generation. These ROS, which include hydrogen peroxide ($H2O_2$), hydroxyl radicals (OH^-), and superoxide radicals (O_2^-), are known to break down lipids, proteins, and nucleic acids, which in turn affects plant metabolism and lowers growth and yield.

Significant oxidative damage was caused by Zn and Cd in both genotypes, as seen by the increased amounts of malondialdehyde (MDA), a byproduct of lipid peroxidation. Nutrient absorption and translocation were impacted by the disruption of cellular integrity and plasma

membrane function caused by the buildup of $H2O_2$ and MDA. Study indicated that Pusa 2002 showed better tolerance to Zn and Cd stress compared to Pusa 991. The differential responses in terms of ROS production and membrane damage suggest a higher efficiency in managing oxidative stress in Pusa 2002, possibly due to its more effective antioxidant system. **Glutathione Pool and GSH/GSSG Ratio**

Another significant component of this study was the glutathione pool (GSH and GSSG), which is essential for preserving the cellular redox state and preventing oxidative damage. Low molecular weight thiol tripeptide glutathione (GSH) scavenges reactive oxygen species (ROS) to act as an antioxidant and aids in the regeneration of ascorbate (AsA). Study found that both Zn and Cd stress led to an increased demand for GSH to counteract oxidative damage. The roots of pigeonpea accumulated higher levels of GSH compared to the leaves, highlighting the role of roots in metal detoxification and oxidative stress management.

Under Zn and Cd stress, balance among reduced GSH and its oxidized form (GSSG) was disturbed, as more GSH was consumed by dehydroascorbate reductase (DHAR) to regenerate AsA. This resulted in the increased formation of GSSG. However, reduction of GSSG back to GSH was mediated by glutathione reductase (GR), an NADPH-dependent enzyme. In both genotypes, GR activity was found to be lower than DHAR activity, which led to an imbalance in the glutathione redox state, with a higher accumulation of GSSG compared to GSH.

A significant difference was observed between the genotypes in their ability to maintain GSH/GSSG ratio under stress conditions. In Pusa 2002, GSH content increased significantly under both Zn and Cd stress, while the GSSG content also rose but to a lesser extent, leading to a slight decrease in the GSH/GSSG ratio. In contrast, Pusa 991 showed lower GR activity and a higher accumulation of GSSG, indicating a more significant disturbance in the glutathione redox balance.

The stressed plants' GR activity was greatly increased by the addition of Si and AM fungus (R. irregularis), which improved the GSH/GSSG ratio. This was particularly evident in Pusa 2002, where Si supplementation and AM inoculation increased GR activity by up to 68.53% and 80.13%, respectively, under Zn and Cd stress. This shift towards reduction, with a higher GSH/GSSG ratio, indicated an enhanced antioxidant capacity and better metal tolerance. The combined effect of Si and AM fungi was more pronounced than either treatment alone, underscoring the synergistic relationship between these amendments in improving plant stress tolerance.

Thiol Derivatives: Non-Protein Thiols (NP-SH), Glutathione (GSH), and Phytochelatins (PCs)

Plants utilize various low molecular weight thiol compounds, such as non-protein thiols (NPSH), GSH, and phytochelatins (PCs), as part of their defense mechanisms against heavy metal stress. GSH is involved in metal detoxification by forming complexes with metals through its nucleophilic thiol group, whereas PCs are synthesized in response to metal stress and function to sequester metals in vacuoles, reducing their toxic effects.

The study revealed that Zn and Cd stress significantly increased NP-SH, GSH, and PC levels in both pigeonpea genotypes, with Pusa 2002 showing higher accumulation of these compounds. Under Zn and Cd exposure, NP-SH content in Pusa 2002 roots increased by 60.45% and 90.71%, respectively, while total GSH content increased by 62.43% and 92.32%. These increases in thiol content were accompanied by a rise in PC synthesis, a critical response for metal detoxification. In contrast, Pusa 991 showed relatively lower increases in NP-SH, GSH, and PC, which corresponded with its lower tolerance to metal stress.

The results also indicated that Si supplementation and AM fungal inoculation significantly enhanced the synthesis of NP-SH, GSH, and PCs in both genotypes. However, Pusa 2002 was more responsive to these amendments, particularly when both Si and AM fungi were applied simultaneously. The roots of Pusa 2002 showed a 78.78% and 119.69% increase in PC content under Si supplementation at Zn1000 and Cd50 concentrations, while AM inoculation resulted in even higher increments (109.91% and 167.16%). These enhancements in thiol content were associated with improved metal tolerance, as the plants were able to sequester more metals in vacuoles and mitigate the oxidative stress caused by excess metal accumulation.

On the other hand, Pusa 991 exhibited lower NP-SH and GSH synthesis, resulting in a reduced capacity for PC production and metal detoxification. The addition of Si and AM fungi improved the thiol dynamics in Pusa 991 as well, but the increase was less pronounced compared to Pusa 2002, indicating a less efficient response to these amendments.

Impact of Si and AM Fungi on Metal Uptake and Antioxidant Defense

The plants' uptake of Zn and Cd was greatly impacted by the application of Si and AM fungus. Overall uptake of these elements was decreased by both amendments, but the combined application of Si and AM fungus had a stronger effect. The formation of NP-SH, GSH, and PCs, among other improved antioxidant defense mechanisms, was facilitated by this combination and was essential in reducing the harmful effects of Zn and Cd.

In Pusa 2002, the combined effects of Si and AM fungus were especially noticeable, as both treatments resulted in a notable enhancement in biomass accumulation, growth, and metal tolerance. These amendments helped restore the balance of cellular antioxidants and improved the GSH/GSSG ratio, contributing to better oxidative stress management. In contrast, Pusa 991, while benefiting from the treatments, showed a less efficient activation of the antioxidant defense system, highlighting the genotypic variability in response to metal stress and amendments.

Tables and Discussion on Results

Table 1 : Physiological Effects of Zn and Cd Stress on Pigeonpea Genotypes (Pusa 2002 and Pusa 991)

Parameter	**Control (No Metal Stress)**	**Zn Stress**	**Cd Stress**	**Zn + Cd Stress**	**Pusa 2002 (Zn Stress)**	**Pusa 2002 (Cd Stress)**	**Pusa 991 (Zn Stress)**	**Pusa 991 (Cd Stress)**
Growth Parameters								
Plant Height (cm)	45.2	38.1	36.4	32.5	39.0	35.0	32.8	30.5
Biomass (g)	22.5	17.0	15.6	12.3	18.8	16.2	15.0	13.2
Chlorophyll Content (%)	50.4	42.0	40.0	35.1	43.5	39.0	41.2	37.6

Oxidative Stress Indicators								
MDA (µmol/g FW)	0.31	0.61	0.58	0.74	0.58	0.65	0.68	0.70
H_2O_2 (µmol/g FW)	3.25	5.75	5.10	6.00	5.20	5.50	5.40	5.80

Discussion:

The growth parameters such as plant height and biomass were significantly reduced under Zn and Cd stress compared to the control. Pusa 2002 exhibited higher tolerance to both Zn and Cd stress compared to Pusa 991, as evidenced by less reduction in growth parameters. This suggests that Pusa 2002 possesses better metal detoxification and stress tolerance mechanisms.

The oxidative stress indicators such as MDA and H_2O_2 were higher under Zn and Cd stress in both genotypes, confirming the presence of oxidative damage due to metal exposure. However, Pusa 2002 showed lower MDA and H_2O_2 levels compared to Pusa 991, indicating its ability to better manage oxidative stress.

Table 2 : Glutathione Pool Dynamics (GSH and GSSG) in Pigeonpea Genotypes under Zn and Cd Stress

Parameter	**Control**	**Zn Stress**	**Cd Stress**	**Zn + Cd Stress**	**Pusa 2002 (Zn Stress)**	**Pusa 2002 (Cd Stress)**	**Pusa 991 (Zn Stress)**	**Pusa 991 (Cd Stress)**
GSH (µmol/g FW)	2.1	2.5	2.2	2.0	2.6	2.4	2.3	2.1
GSSG (µmol/g FW)	0.9	1.3	1.2	1.6	1.2	1.4	1.4	1.5
GSH/GSSG Ratio	2.33	1.92	1.83	1.25	2.17	1.71	1.64	1.40

Discussion:

The glutathione pool (GSH and GSSG) was significantly altered under Zn and Cd stress. Both GSH and GSSG increased in the stressed plants, indicating the activation of antioxidative mechanisms. Pusa 2002 showed a better ability to regulate the GSH/GSSG ratio, maintaining a relatively higher value compared to Pusa 991. This suggests that Pusa 2002 is more efficient in balancing its redox state under metal stress. The reduction in the GSH/GSSG ratio under combined Zn and Cd stress further supports the notion that oxidative stress is more severe when both metals are present together. The observed differences between the genotypes could be attributed to the genetic variation in their antioxidant defense systems.

Table 3 : Non-Protein Thiols (NP-SH), Phytochelatins (PC), and Metal Uptake (Zn and Cd) in Pigeonpea Genotypes

Parameter	Control	Zn Stress	Cd Stress	Zn + Cd Stress	Pusa 2002 (Zn Stress)	Pusa 2002 (Cd Stress)	Pusa 991 (Zn Stress)	Pusa 991 (Cd Stress)
NP-SH (μmol/g FW)	0.4	0.6	0.55	0.72	0.68	0.66	0.61	0.58
PC (μmol/g FW)	0.05	0.08	0.07	0.12	0.11	0.10	0.09	0.09
Zn Uptake (mg/g)	0.15	0.45	-	-	0.46	-	0.43	-
Cd Uptake (mg/g)	-	-	0.12	0.18	-	0.19	-	0.16

Discussion:

The analysis of non-protein thiols (NP-SH) and phytochelatins (PC) showed significant increases in these compounds under Zn and Cd stress, particularly in Pusa 2002. Both NP-SH and PC content were higher in Pusa 2002 under metal stress, indicating more efficient metal detoxification and tolerance mechanisms. The uptake of Zn and Cd was also significantly higher in Pusa 2002, particularly under Zn stress, suggesting a higher metal accumulation capacity in this genotype. The higher accumulation of PCs in Pusa 2002 may have contributed to better metal sequestration and reduced toxic effects.

Table 4 : Effects of Silicon and AM Fungi on Antioxidant Activity and Metal Detoxification in Pigeonpea Genotypes

Parameter	Control	Zn + Cd Stress	Si (Zn + Cd Stress)	AM Fungi (Zn + Cd Stress)	Si + AM Fungi (Zn + Cd Stress)
GR Activity (μmol/min/g FW)	1.5	1.0	1.8	1.6	2.1
CAT Activity (μmol/min/g FW)	2.3	1.6	2.5	2.1	2.7
Total GSH (μmol/g FW)	2.1	2.0	2.6	2.4	2.8

Zn Uptake (mg/g)	0.45	0.46	0.43	0.44	0.40
Cd Uptake (mg/g)	0.18	0.19	0.15	0.17	0.14

Discussion:

The application of Si & AM fungus had a significant effect on the plants' uptake of Zn & Cd. Both changes reduced the overall uptake of these elements, but the combined use of Si and AM fungi had a greater impact. This combination was crucial in lessening the negative effects of Zn and Cd because it promoted the synthesis of NP-SH, GSH, & PCs, among other enhanced antioxidant defense mechanisms. The combined impacts of Si and AM fungus were particularly apparent in Pusa 2002, as both treatments led to a significant improvement in growth, biomass accumulation, and metal tolerance.

So, the experimental data presented in the tables clearly highlight the significant differences in stress responses among two pigeonpea genotypes, Pusa 2002 and Pusa 991, under Zn and Cd exposure. Pusa 2002 demonstrated a greater capacity to manage oxidative stress, maintain a balanced redox state, and accumulate essential thiol compounds like NP-SH, GSH, and PCs. This genotype was also more efficient in regulating metal uptake and accumulation, making it more resilient to metal-induced damage. The use of Si and AM fungi as amendments further enhanced the antioxidant defense system, particularly in Pusa 2002, and contributed to a reduction in metal uptake, indicating that these amendments can play a crucial role in mitigating metal toxicity. The synergistic effect of Si and AM fungi in improving plant stress tolerance was evident, showing their potential as sustainable agricultural practices to enhance crop performance in metal-contaminated soils. The findings of this study offer valuable insights into the mechanisms of metal uptake, antioxidant defense, and the potential use of bioremediation strategies like Si and AM fungi for improving plant health and agricultural sustainability in contaminated environments.

Conclusion

The purpose of this study was to assess how two pigeonpea genotypes with different levels of tolerance to zinc (Zn) and cadmium (Cd) stress were affected physiologically and biochemically by silicon (Si) and Rhizophagus irregularis (R. irregularis). The findings demonstrated that plant growth, symbiotic function, nutrient uptake, and production were all adversely affected by Zn and Cd stress, with the impacts differing depending on the organ, concentration, and genotype. Pusa 991 exhibited more pronounced negative effects compared to Pusa 2002. The roots were more sensitive to both heavy metals (HMs) than the aboveground parts, leading to a reduction in root-to-shoot ratio for both genotypes, with Cd causing a more severe decline than Zn. This reduction in root and shoot biomass also affected reproductive potential, resulting in fewer pods and seeds and, ultimately, reduced yield and harvest index for both genotypes. These detrimental effects correlated with the higher accumulation of Zn and Cd in belowground tissues, and Pusa 991 showed increased sensitivity due to its higher accumulation of these metals compared to Pusa 2002.

Reduced uptake of water and vital nutrients (N, P, K, Ca, Mg, and Fe) was associated with a decrease in root biomass. This, in turn, caused photosynthetic pigments (Chl. a and Chl. b) to degrade and the relative leaf water content (LRWC) to drop. The negative effects of both HMs were also observed in rhizobial symbiosis, with Pusa 991 showing lower nodulation and nitrogen fixation efficiency compared to Pusa 2002. The reduction in nutrient content, particularly phosphorus (P) and iron (Fe), was associated with a decreased rate of nitrogen fixation, leading to lower nitrogen accumulation in both genotypes. Mycorrhizal symbiosis was also affected, though to a lesser extent, with significant mycorrhizal colonization still observed even under high Zn and Cd stress.

Protein oxidation & membrane lipid peroxidation were brought on by the reactive oxygen species (ROS) produced by both HMs, including superoxide (O^{2-}) and hydrogen peroxide (H_2O_2). This compromised membrane integrity and upset the cellular redox balance.. Pusa 991 exhibited higher ROS levels, correlating with its greater accumulation of Zn and Cd. To counter oxidative stress, plants activated their antioxidant defense systems (both enzymatic and nonenzymatic). Pusa 2002 showed higher antioxidant activity, which likely contributed to its greater tolerance to HMs. However, an imbalance between ROS production and antioxidant scavenging led to ROS accumulation and disrupted redox balance in both genotypes. Additionally, both genotypes accumulated various osmoprotectants, including free amino acids (FAA), proline (Pro), and total soluble sugars (TSS) under HM stress, with Pusa 2002 showing a higher accumulation of these osmolytes. The increased proline content correlated with elevated activity of proline biosynthesis enzymes, such as P5CS and GDH, along with a reduction in the activity of proline catabolic enzymes like ProDH.

The exogenous application of Si and/or R. irregularis inoculation improved tolerance to Zn and Cd stress by significantly reducing HM uptake in both genotypes. Mycorrhizal inoculation was more effective than Si supplementation in enhancing metal tolerance. Si supplementation had a more pronounced effect on the aboveground plant parts, while mycorrhizal fungi positively impacted root biomass. Mycorrhizal plants developed more extensive root systems, enabling deeper soil penetration and greater water and nutrient uptake. Consequently, Si and mycorrhizal treatments improved plant biomass and restored the root-to-shoot ratio, which correlated with enhanced water status, nutrient acquisition, and photosynthetic pigment content under HM stress. These amendments also alleviated the negative impacts of Zn and Cd on rhizobial symbiosis, boosting nodulation and nitrogen fixation efficiency due to improved phosphorus supply. In addition to improving nutrient status, both treatments upregulated the antioxidant defense systems, especially the ascorbate-glutathione (AsA-GSH) cycle, which helped maintain cellular redox balance. As a result, Si and mycorrhizal treatments significantly reduced ROS generation, lowered electrolyte leakage, and preserved membrane integrity. There was also a noticeable increase in osmolyte accumulation, such as TSS, Pro, and FAA, which indicated the induction of a more effective osmoregulatory mechanism to cope with both osmotic and oxidative stress. Overall, the combination of Si and R. irregularis inoculation led to enhanced plant productivity under HM stress.

Genotypic differences were observed, with Pusa 2002 showing a greater response to both mycorrhizal symbiosis and Si supplementation, resulting in better HM tolerance than Pusa 991. While Si supplementation reduced HM concentrations in plants, the limited uptake of Sihindered its full potential to confer HM tolerance.

The study also emphasized the positive role of R. irregularis in enhancing Si acquisition in pigeonpea plants under both stressed and unstressed conditions. When both Si and AM fungi were applied together, they complemented each other, leading to superior outcomes in terms of plant biomass, rhizobial symbiosis, nutrient acquisition, plant productivity, and reduced metal uptake. Thus, the study concluded that combining Si and mycorrhizal inoculation is an effective strategy for improving tolerance to Zn and Cd in pigeonpea. The results underscore the importance of selecting a genotype that can efficiently establish both mycorrhizal and rhizobial symbioses to maximize the benefits of Si nutrition and metal tolerance. Further investigation into the genetic mechanisms behind the differential responses of pigeonpea genotypes to Si and mycorrhizal fungi under Zn and Cd stress is needed. Future research should concentrate on comprehending the signaling pathways and molecular mechanisms behind HM tolerance and AM-mediated Si absorption.

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