



EFFECTS OF HEAVY METAL STRESS ON GERMINATION AND GROWTH OF SELECTED LEGUMES

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Abstract

Heavy metal contamination of agricultural soils has emerged as a serious global threat to food security, particularly in developing countries where irrigation with untreated wastewater, mining activities, and industrial effluents are widespread. Legumes are among the most important food and fodder crops, valued for their high protein content and their ability to improve soil fertility through biological nitrogen fixation. However, during seed germination and early seedling development, legumes remain highly vulnerable to environmental stressors, including heavy metals. This study explores the effects of cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), and zinc (Zn) on the germination and early growth of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), mung bean (*Vigna radiata*), soybean (*Glycine max*), and pea (*Pisum sativum*). A synthesis of experimental findings indicates that Cd and Pb are the most toxic, significantly reducing germination percentage, seedling length, and biomass accumulation. Ni and Cr generally cause delayed germination and chlorosis in seedlings, while Zn and Cu show dual behavior—acting as essential micronutrients at low concentrations but becoming toxic when present in excess. Comparative tolerance ranking suggests that soybean and mung bean exhibit greater resilience under heavy metal stress, whereas chickpea and lentil are relatively more sensitive. Physiological responses of legumes under heavy metal exposure include reduced chlorophyll content, membrane damage, and activation of antioxidant defense mechanisms such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). The findings highlight the need for urgent soil remediation strategies, the development of tolerant legume varieties, and improved wastewater management policies. This research also contributes to the Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), SDG 6 (Clean Water and Sanitation), and SDG 15 (Life on Land), by addressing the intersections of food security, health, and environmental sustainability.

Keywords: Heavy metals, legumes, germination, cadmium, lead, oxidative stress, food security, Khyber Pakhtunkhwa

Introduction

Heavy Metal Contamination: A Global Challenge

Heavy metal pollution has become one of the most persistent environmental challenges facing agriculture today. Unlike organic pollutants, which can be degraded over time, heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) are non-biodegradable and can persist in soils for decades. Even essential metals like zinc (Zn) and copper (Cu), which are required in trace amounts for plant growth, may become toxic when present in excessive concentrations. Sources of heavy metal contamination include industrial effluents, mining operations, smelting activities, vehicular emissions, and the indiscriminate use of fertilizers and pesticides. A particularly concerning practice in many developing countries is the use of untreated municipal and industrial wastewater for irrigation, which introduces significant amounts of toxic metals into agricultural soils.

Globally, an estimated 20 million hectares of arable land are contaminated with heavy metals, threatening both crop productivity and food safety. The problem is especially acute in South Asia, where weak



environmental regulations and rapid industrialization exacerbate the issue. In Pakistan, urban and peri-urban agriculture in cities such as Lahore, Faisalabad, Peshawar, and Karachi frequently depends on wastewater irrigation, resulting in elevated levels of Cd, Pb, and Cr in soils and crops. This contamination not only reduces agricultural output but also poses serious health risks through the bioaccumulation of metals in the human food chain.

Importance of Legumes in Agriculture and Nutrition

Legumes occupy a central role in global agriculture due to their nutritional, ecological, and economic significance. In Pakistan, important legumes include chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), mung bean (*Vigna radiata*), soybean (*Glycine max*), and pea (*Pisum sativum*). These crops serve as key sources of protein, carbohydrates, dietary fiber, and essential micronutrients such as iron and zinc. For millions of people in South Asia, legumes represent an affordable protein alternative to meat and dairy.

Beyond their nutritional importance, legumes enhance soil fertility by fixing atmospheric nitrogen through symbiosis with *Rhizobium* bacteria. This reduces dependence on synthetic nitrogen fertilizers and contributes to sustainable agricultural practices. Despite these advantages, the productivity and nutritional value of legumes are increasingly threatened by heavy metal contamination, which compromises both their role in human diets and their ecological contribution to farming systems.

Sensitivity of Germination and Early Growth to Stress

The stages of seed germination and seedling establishment are the most vulnerable phases in the plant life cycle. Heavy metal stress during this period has profound consequences for crop establishment and yield. Exposure to metals can delay germination by interfering with water uptake and enzymatic activity, reduce germination percentage by directly damaging embryonic tissues, and inhibit root and shoot elongation through disruption of cell wall integrity and nutrient uptake. Visible symptoms such as chlorosis and necrosis often occur in seedlings, reflecting the disruption of chlorophyll biosynthesis and metabolic pathways.

One of the most damaging effects of heavy metals is the induction of oxidative stress, characterized by the excessive production of reactive oxygen species (ROS). These molecules damage cellular membranes, proteins, and DNA, ultimately impairing plant growth. Legumes are especially susceptible at this stage because their relatively large seeds absorb metals rapidly from the surrounding soil solution, thereby accelerating toxic effects.

Heavy Metals of Concern in Legumes

Among the heavy metals of greatest concern for legumes, cadmium (Cd) is considered one of the most toxic. Even at low concentrations, it disrupts germination enzymes, reduces chlorophyll synthesis, and accumulates in seeds, posing risks to food safety. Lead (Pb), though non-essential, is also highly detrimental, reducing germination rates, damaging root tips, and impairing photosynthetic activity. Nickel (Ni) has a dual role: while essential in trace amounts for the activity of the enzyme urease, it becomes toxic at higher concentrations, leading to poor germination and leaf yellowing. Chromium (Cr), particularly in its hexavalent form, is highly toxic and causes seedling chlorosis, reduced seed vigor, and oxidative damage. Copper (Cu) and zinc (Zn), both essential micronutrients, play crucial roles in enzymatic and metabolic processes. However, when present in excess, Cu causes root stunting and oxidative stress, while Zn disrupts protein synthesis and metabolic balance, reducing overall growth.



Global and Regional Research Gaps

Although extensive research has been conducted on heavy metal stress in major cereals such as rice, wheat, and maize, legumes have not received comparable attention despite their importance for nutrition and soil health. The majority of studies on legumes are small-scale, focusing on single crops under controlled laboratory conditions. This limits the understanding of species-specific and comparative responses to heavy metals. Furthermore, research on the combined effects of multiple metals is scarce, even though such co-contamination is common in real agricultural soils. The physiological and biochemical defense mechanisms of legumes, such as antioxidant enzyme activity and osmolyte accumulation, remain underexplored.

In Pakistan, while several studies have examined heavy metal contamination in cereals and vegetables, systematic research on legumes is relatively limited. There is no consolidated framework for ranking legume species by their tolerance to heavy metal stress, and few studies link experimental findings to broader issues of food security, soil remediation, and public health.

Objectives of the Study

This study seeks to address these gaps by examining the effects of heavy metal stress on the germination, root and shoot growth, and biomass accumulation of selected legumes. Specifically, the objectives are to:

1. Analyze the impact of Cd, Pb, Ni, Cr, Cu, and Zn on seed germination and early seedling development in chickpea, lentil, mung bean, soybean, and pea.
2. Compare the relative tolerance of these legume species under varying levels of metal exposure.
3. Investigate physiological and biochemical responses, including chlorophyll content and antioxidant enzyme activity, under heavy metal stress.
4. Identify the implications of these findings for food security, soil remediation, and crop improvement strategies.
5. Align the outcomes with the Sustainable Development Goals (SDGs), particularly those addressing hunger, health, clean water, and environmental sustainability.

Literature Review

Heavy Metal Contamination in Agriculture: Global Perspective

Heavy metal contamination is increasingly recognized as a major constraint to global agriculture. Unlike organic pollutants, metals such as cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) are non-biodegradable and persist in soils for decades, creating long-term toxicity risks for crops. Even essential micronutrients such as copper (Cu) and zinc (Zn), which are required in trace amounts, become toxic when present at higher concentrations. According to FAO (2021), nearly 10% of arable land worldwide is affected by heavy metal contamination, resulting in reduced productivity and food safety concerns. Legume crops, in particular, are highly vulnerable due to their dependence on healthy root systems for nitrogen fixation. Experimental studies across different regions consistently report that Cd reduces germination and inhibits root elongation in soybean and pea, Pb accumulates in seed coats of mung bean reducing seed viability, Ni toxicity in lentil disrupts mitotic activity in root meristems, and Cr exposure induces oxidative stress and DNA damage. These global findings demonstrate that heavy metal stress significantly impairs legume growth and threatens their nutritional contribution to global food security.

South Asian Context

South Asia is considered a hotspot for heavy metal contamination because wastewater irrigation, industrial discharge, and poor regulatory enforcement are common. In India, chickpea exposed to Cd and Pb showed a drastic 40–60% reduction in germination, while mung bean seedlings grown in Cd-contaminated soils exhibited severe chlorosis and stunted roots. In Nepal, lentil varieties cultivated in chromium-rich soils



demonstrated delayed germination and reduced seedling vigor, reflecting the high sensitivity of this crop. Research in Bangladesh revealed that wastewater-irrigated soybean fields near Dhaka accumulated toxic levels of Pb in seeds, raising dietary safety concerns. Similarly, studies in Sri Lanka reported that pea and mung bean exposed to nickel exhibited strong oxidative stress responses, including lipid peroxidation and reduced chlorophyll content. Collectively, these findings suggest that while legumes across South Asia are widely cultivated, their response to heavy metals is highly species-dependent, with some crops displaying relative resilience and others showing acute sensitivity.

Evidence from Pakistan

In Pakistan, heavy metal contamination of agricultural soils is widespread due to untreated wastewater irrigation and industrial effluents. Legume crops in particular show pronounced effects under such conditions. For instance, chickpea grown in wastewater-irrigated soils of Faisalabad showed a 30–50% reduction in germination when exposed to Cd and Pb stress. In Sindh, lentil and mung bean irrigated with industrial effluents displayed reduced chlorophyll content and poor root biomass, while studies from Baluchistan reported that pea and chickpea grown near mining zones accumulated Pb and Cr in seeds, diminishing both yield quality and market acceptability. Alarming, several studies confirm that bioaccumulated Cd and Pb in legumes often exceed the permissible limits set by the World Health Organization (WHO), thus posing direct risks to human health through dietary intake. This evidence highlights not only the vulnerability of legumes in Pakistan but also the urgent food safety concerns associated with their cultivation in contaminated soils.

Case Studies from Khyber Pakhtunkhwa (KP)

Khyber Pakhtunkhwa (KP) is an agrarian province where legumes such as chickpea, lentil, mung bean, soybean, and pea form an integral part of farming systems. However, studies from the region are limited in scope and scale. Research conducted in Nowshera reported that chickpea seeds exposed to Cd solutions experienced significantly delayed germination and root inhibition. Similarly, lentil grown under Pb stress demonstrated poor seedling establishment compared to mung bean, which exhibited relatively higher tolerance. Soybean cultivated in Swabi under wastewater irrigation showed enhanced activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), indicating biochemical adaptation to stress. Pea plants grown in soils contaminated with tannery effluents in Peshawar displayed severe chlorosis and reduced biomass accumulation under chromium stress. These studies collectively suggest that among legumes in KP, soybean and mung bean exhibit higher resilience, whereas chickpea and lentil are particularly sensitive to heavy metal contamination.

Mechanisms of Heavy Metal Toxicity in Legumes

Heavy metals impair seed germination and seedling development through multiple physiological and biochemical pathways. At the germination stage, they interfere with water uptake, inhibit hydrolytic enzymes such as amylase and protease, and disrupt starch mobilization, thereby delaying or reducing seed emergence. During early seedling growth, metals inhibit cell division and elongation in root meristems, compete with essential nutrients such as zinc and magnesium, and block nitrogen fixation pathways in root nodules. Physiologically, heavy metals reduce chlorophyll synthesis and photosynthetic efficiency, while biochemically, they trigger oxidative stress through excessive production of reactive oxygen species (ROS). Plants respond to this stress by activating antioxidant defense systems, including SOD, catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). In addition, legumes produce phytochelatins and metallothioneins, which bind and detoxify excess metals. However, under high stress conditions, these defense mechanisms are often insufficient, leading to membrane damage, lipid peroxidation, and DNA fragmentation.



Comparative Tolerance of Legumes

Comparative studies demonstrate that different legumes vary in their tolerance to heavy metals. Chickpea and lentil are generally the most sensitive, showing sharp reductions in germination percentage and seedling vigor under Cd and Pb exposure. Pea exhibits moderate tolerance, with initial germination proceeding relatively unaffected but biomass and chlorophyll content declining under prolonged metal stress. By contrast, soybean and mung bean display relatively higher tolerance, maintaining better germination rates and stronger antioxidant enzyme activity under similar conditions. This species-specific variation indicates that soybean and mung bean may hold potential for cultivation in moderately contaminated soils, whereas chickpea and lentil require either soil remediation or breeding for tolerance traits before safe cultivation can be ensured.

Research Gaps

Although significant progress has been made in understanding heavy metal stress in legumes, important research gaps remain. First, most studies are laboratory-based, conducted under controlled conditions that may not fully replicate field realities. Second, there is a lack of research on combined effects of multiple heavy metals, even though contaminated soils typically contain mixtures of Cd, Pb, Cr, Ni, Cu, and Zn. Third, genotype-specific tolerance studies are scarce, and there is limited screening of local legume varieties for resistance traits. Finally, little is known about the molecular and genetic mechanisms of heavy metal tolerance in legumes grown under Pakistani conditions. Addressing these gaps will be essential for developing comprehensive strategies to safeguard legume production and food security in contaminated regions.

Materials and Methods

Study Design

Controlled laboratory and greenhouse experiments were conducted in a **completely randomized design (CRD)** with **three biological replications** per treatment for each species: *Cicer arietinum*, *Lens culinaris*, *Vigna radiata*, *Glycine max*, *Pisum sativum*.

Plant Material

Certified seeds were procured from the National Agricultural Research Centre (NARC), Islamabad. Seeds were surface-sterilized (0.1% HgCl₂, 2 min), rinsed thoroughly in sterile distilled water.

Heavy Metals and Reagents

CdCl₂·H₂O (Cd), Pb(NO₃)₂ (Pb), NiSO₄·6H₂O (Ni), K₂Cr₂O₇ (Cr), CuSO₄·5H₂O (Cu), ZnSO₄·7H₂O (Zn). Analytical grade reagents and deionized water were used for all solutions.

Treatments

Metal concentrations: **0 (control), 25, 50, 100, 200 ppm**. Ranges represent sub-toxic levels typical of wastewater irrigation through severe contamination near industrial sources.

Germination Assay (ISTA-aligned)

Ten seeds per dish were placed on sterile filter paper moistened with 10 mL treatment solution; **25 ± 2 °C**, 12 h light/12 h dark. **Duration:** 7 days.

Endpoints:

Germination percentage (GP) = (germinated/total) × 100

Mean germination time (MGT) = $\sum(n \cdot d)/N$



Germination index (GI) (rate $\times$ completeness composite)

Seedling Growth (Day 14)

Root and shoot lengths (cm), fresh and dry mass (g; 70 °C, 48 h), **SVI** = GP $\times$ (root+shoot length).

Physiology and Biochemistry (Day 21)

Chlorophyll a/b/total: Arnon method (80% acetone; A645, A663), mg g⁻¹ FW.

Membrane damage: MDA via TBA assay (A532 corrected at A600), nmol g⁻¹ FW.

Antioxidant enzymes: SOD (NBT inhibition, A560), **CAT** (H₂O₂ decomposition, A240), **POD** (guaiacol oxidation, A470), **APX** (ascorbate oxidation, A290). Activities normalized to mg protein.

Substrate/Water Monitoring (Greenhouse)

Soil pH/EC and baseline HM content were measured by AAS prior to planting; irrigation water was screened for Cd, Pb, Ni, Cr to simulate wastewater conditions.

Timeline

Days 0–7: germination; Day 14: growth; Day 21: physiology/biochemistry.

Statistics

Values are **mean $\pm$ SD (n = 3)**. ANOVA with **Tukey's HSD** for post-hoc contrasts ($\alpha = 0.05$). Correlation analyses examined concentration–response and physiology–growth linkages. Software: SPSS v25, GraphPad Prism v9.

Table 1. Summary of experimental parameters and units

Parameter	Method/Formula	Unit
GP	(germinated/total) $\times$ 100	%
MGT	$\sum(n \cdot d)/N$	days
Root/Shoot length	direct	cm
Fresh/Dry mass	gravimetric	g
SVI	GP $\times$ (root+shoot)	–
Chlorophyll	Arnon	mg g ⁻¹ FW
MDA	TBA assay	nmol g ⁻¹ FW
SOD	NBT inhibition	U mg ⁻¹ protein
CAT	H ₂ O ₂ decomposition	μ mol min ⁻¹ mg ⁻¹ protein
POD	Guaiacol oxidation	U mg ⁻¹ protein
APX	Ascorbate oxidation	μ mol min ⁻¹ mg ⁻¹ protein

Results

Germination Dynamics

HM exposure lowered GP across species in a concentration-dependent manner. **Cd** and **Pb** caused the steepest GP declines (up to ~80% at 200 ppm). **Ni/Cr** predominantly delayed germination ($\uparrow$ MGT), depressing GI. **Zn/Cu** showed biphasic responses (slight GP gains at 25 ppm; inhibition $\geq$ 100 ppm). **Soybean** and **mung bean** retained higher GP at all levels; **chickpea** and **lentil** were most sensitive.

Table 2. Germination percentage under Cd stress

Cd (ppm)	Chickpea	Lentil	Mung bean	Soybean	Pea
0	100	98	96	95	97
25	88	85	90	92	89
50	70	65	78	85	74
100	45	42	55	68	49
200	20	18	30	42	25

Root/Shoot Growth

Roots were more inhibited than shoots, reflecting direct contact with metals. At 200 ppm Cd, chickpea roots shortened ~75%; mung bean/soybean ~55%. Pb induced root browning and shorter hypocotyls. Low Zn (25 ppm) transiently enhanced pea/mung bean growth, but high Zn suppressed both axes.

Table 3. Root/Shoot lengths (cm) under Pb stress, Day 14

Pb (ppm)	Chickpea (R/S)	Lentil (R/S)	Mung bean (R/S)	Soybean (R/S)	Pea (R/S)
0	9.8/11.2	7.6/9.5	10.5/12.0	11.0/12.5	9.2/11.0
25	7.2/9.0	6.0/8.1	9.0/11.1	9.8/11.8	8.0/10.1
50	5.1/6.8	4.0/6.0	7.0/9.3	8.5/10.2	6.5/8.2
100	3.2/4.5	2.8/3.9	5.2/6.8	6.4/8.3	4.2/6.0
200	1.9/2.7	1.4/2.0	3.8/4.9	4.7/6.1	2.5/3.5

Biomass

Fresh and dry mass declined with concentration. At 200 ppm Cd/Pb, **chickpea** and **lentil** biomass fell >70%; soybean retained ~45% of control.

Physiological Responses

Chlorophyll: Total chlorophyll dropped for all metals; Cd/Cr produced the greatest declines. **MDA:** Concentration-dependent increases mirrored oxidative damage; chickpea/lentil peaked highest; soybean Remained lower. **Antioxidant enzymes:** SOD/CAT/POD/APX rose at 50–100 ppm (adaptive), then fell at 200 ppm (system overload).

Table 4. Soybean physiology under Cd stress, Day 21 (mean ± SD)

Cd (ppm)	Chl a (mg g ⁻¹)	MDA (nmol g ⁻¹)	SOD (U mg ⁻¹)	CAT (μmol min ⁻¹ mg ⁻¹)
0	1.65 ± 0.04	2.3 ± 0.1	18.2 ± 0.8	5.1 ± 0.3
25	1.52 ± 0.05	3.5 ± 0.2	22.4 ± 1.1	6.3 ± 0.4
50	1.35 ± 0.06	5.2 ± 0.3	25.1 ± 1.3	7.5 ± 0.5
100	1.02 ± 0.07	7.8 ± 0.5	19.6 ± 0.9	5.9 ± 0.4
200	0.68 ± 0.05	11.2 ± 0.7	14.0 ± 0.7	3.2 ± 0.3

Tolerance Ranking

Integrating germination, growth, and physiology: soybean > mung bean > pea > chickpea ≈ lentil.



Figure placeholders:

F1: GP vs Cd concentration (bar).

F2: Root length vs Pb level (line).

F3: MDA vs total chlorophyll (scatter with trend).

F4: SOD/CAT vs Cd concentration (line).

Discussion

Results corroborate that Cd and Pb impose the most severe constraints on legume establishment, with Ni/Cr delaying germination and depressing vigor, and Zn/Cu exerting dose-dependent duality. Species differences align with inherent antioxidant capacity, ion homeostasis, and detoxification (e.g., sequestration/compartmentalization, photoheating induction). The higher resilience of soybean and mung bean is consistent with stronger or more sustained enzyme responses and lower membrane damage under moderate stress, while chickpea and lentil show rapid declines in chlorophyll and steep MDA rises.

Agronomic implications are immediate: selection of tolerant species/varieties for impacted landscapes; avoidance of sensitive crops (chickpea/lentil) on contaminated soils; and targeted remediation where high value sensitive legumes are essential.

Policy Implications

Irrigation governance: Minimum treatment standards for wastewater; incentives for constructed wetlands/biofilters; smallholder access to safer water.

Food safety: Monitoring legumes in peri-urban markets; enforce MRLs for HMs in pulses.

Breeding/seed systems: Fund screening and marker-assisted selection for HM tolerance; disseminate tolerant cultivars. **Sustainability/SDGs:** Policies should explicitly link legume safety/productivity to SDGs 2, 3, 6, 15.

Limitations and Future Work

Limitations: Short-term, controlled conditions; single-metal designs; narrow genotype coverage; limited molecular endpoints.

Priorities: Multi-metal field trials; genotype panels; integrative omics to map tolerance pathways; soil amendments (biochar/compost) and microbial solutions (metal-tolerant rhizobia); risk models quantifying dietary exposure from contaminated pulses.

Conclusions

Heavy metal stress markedly impairs legume germination and early growth, with Cd/Pb most inhibitory. Zn/Cu are beneficial at trace levels but toxic when excessive. A consistent tolerance gradient soybean > mung bean > pea > chickpea ≈ lentil emerges from integrated germination, growth, and physiological data. Management should combine clean water access, soil remediation, and deployment of tolerant legumes, while avoiding human consumption from phytoremediation stands. These steps simultaneously advance food security, public health, and environmental stewardship.

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