



Effect of Heavy Metal Stress on Biochemical and Microbial Responses of *Cicer arietinum* L.

Gayatri Tiwari^{a*} and Neetu Singh^b

^{a,b}Department of Botany, T.D.P.G. College, Jaunpur (UP)

ARTICLE INFO	ABSTRACT
Article history: Received – 22 July 2026 Received in revised form – 05 Sept. 2026 Accepted – 21 Sept. 2026 Available online – 22 Sept. 2026	Heavy metal contamination of agricultural soil is an important environmental constraint that can adversely affect crop growth, metabolism and soil microbial communities. The present study was designed to evaluate the effects of increasing heavy metal stress on the biochemical characteristics of chickpea (<i>Cicer arietinum</i> L.) and the associated rhizospheric microbial population. Healthy seeds of <i>Cicer arietinum</i> L. were grown in soil amended with graded concentrations of a representative heavy metal. A control treatment without heavy metal addition was maintained for comparison. Biochemical parameters including chlorophyll, total soluble protein, total soluble carbohydrate, proline, total phenolic content and antioxidant enzyme activities were evaluated at the vegetative stage. Rhizospheric microbial population was assessed in terms of total viable bacterial and fungal counts. The representative results showed that increasing heavy metal concentration progressively reduced chlorophyll, protein and carbohydrate contents, whereas proline and phenolic compounds increased under moderate stress. Antioxidant enzymes such as superoxide dismutase, catalase and peroxidase showed increased activity at moderate stress but declined at the highest concentration, suggesting disruption of the antioxidant defense system under severe stress. Heavy metal exposure also reduced the cultivable bacterial and fungal populations of the rhizosphere. The findings indicate that heavy metal stress causes coordinated biochemical and microbial changes in chickpea and that the rhizosphere microbiome may play an important role in determining plant tolerance to metal toxicity.
Keywords: <i>Cicer arietinum</i>, chickpea, heavy metal, biochemical response, oxidative stress, antioxidant enzymes, rhizosphere, soil microorganisms, microbial population	

1. Introduction

Heavy metal contamination has become an important environmental problem because metals persist in soil and may accumulate in agricultural ecosystems. Industrial discharge, mining activities, sewage application, atmospheric deposition and excessive use of agricultural inputs can contribute to the accumulation of potentially toxic metals in soil. Unlike many organic pollutants, metals cannot be biologically degraded and may remain available in soil for long periods.

The presence of excessive metal concentrations can interfere with several physiological and biochemical processes in plants. Heavy metals may disturb nutrient uptake, photosynthesis, membrane stability and cellular metabolism. They can also promote the formation of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide. Excessive ROS production can result in lipid peroxidation, protein oxidation and damage to cellular structures. Plants consequently activate enzymatic and non-enzymatic antioxidant defense systems to maintain cellular redox balance (Sharma and Dietz, 2009; Gill and Tuteja, 2010).

The response of plants to heavy metal stress depends on the metal involved, its concentration, exposure period, soil characteristics and plant genotype. Essential metals such as Zn and Cu are required in small amounts but become toxic at excessive concentrations, whereas metals such as Cd and Pb have no known beneficial nutritional role at the concentrations generally considered toxic to plants.

Chickpea (*Cicer arietinum* L.) is an economically important grain legume and an important source of dietary protein, carbohydrates, minerals and other nutrients. Because chickpea is cultivated in soils with different physicochemical characteristics, understanding its response to metal contamination is relevant to agricultural productivity and food safety.

Heavy metal stress may also alter the soil microbial community. Soil microorganisms are particularly important in the rhizosphere because they participate in nutrient cycling, organic matter decomposition, nitrogen transformation and plant growth promotion. At excessive concentrations, heavy metals can inhibit microbial growth and enzyme activity. Conversely, some microorganisms possess mechanisms that allow them to tolerate metals through biosorption, bioaccumulation, precipitation, transformation and immobilization (Gadd, 2004; Tiwari and Lata, 2018).

The plant and rhizosphere microorganisms therefore constitute an interactive system under heavy metal stress. Root exudates can modify metal availability, while microorganisms can influence metal mobility and plant uptake. Plant-associated microorganisms may also improve plant tolerance by producing phytohormones, siderophores and other growth-promoting compounds (Tiwari and Lata, 2018; Mishra et al., 2017).

Recent research on chickpea has demonstrated that heavy metal exposure can reduce growth and photosynthetic pigments while increasing oxidative stress markers and antioxidant enzyme activities. Microbial symbiosis has also been shown to influence

heavy-metal translocation, nodulation, nutrient uptake and biochemical responses in chickpea (Hasan et al., 2026).

The present investigation was therefore undertaken to study the biochemical and microbial responses of *Cicer arietinum* L. subjected to increasing heavy metal stress.

2. Materials and methods:

2.1 Plant material

Healthy and uniform seeds of *Cicer arietinum* L. were selected for the experiment. Seeds were surface sterilized using an appropriate sterilization procedure and washed several times with sterile distilled water before sowing.

2.2 Experimental soil

Topsoil was collected from an uncontaminated agricultural field. The soil was air-dried, homogenized and passed through a suitable sieve to remove stones and plant debris. The basic physicochemical characteristics of the soil were determined before experimentation.

Table 1
Initial physicochemical characteristics of experimental soil

Parameter	Initial value
pH	7.21 ± 0.08
Electrical conductivity (dS m ⁻¹)	0.31 ± 0.02
Organic carbon (%)	0.58 ± 0.04
Available nitrogen (mg kg ⁻¹)	238 ± 8.4
Available phosphorus (mg kg ⁻¹)	18.7 ± 1.2
Available potassium (mg kg ⁻¹)	184 ± 6.7
Soil texture	Loam
Moisture (%)	12.4 ± 0.6

Values are representative /illustrative and should be replaced with actual measurements.

2.3 Heavy metal treatment

For experimental evaluation, the soil was amended with graded concentrations of a representative heavy metal. A suitable experimental design may include the following treatments:

- T₀ = Control
- T₁ = 25 mg kg⁻¹
- T₂ = 50 mg kg⁻¹
- T₃ = 100 mg kg⁻¹
- T₄ = 150 mg kg⁻¹

The selected metal solution was thoroughly mixed with the soil to obtain uniform distribution. The treated soil was allowed to equilibrate before sowing.

Note: For an actual paper, the metal must be explicitly identified, for example Cd, Pb, Cr, Zn or Ni. The concentrations should be selected according to the specific metal, soil properties and applicable safety/laboratory protocols.

2.4 Experimental design

The experiment was conducted in pots containing a uniform quantity of soil. Seeds were sown at an equal depth and maintained under controlled environmental conditions. Each treatment was maintained in replicates.

Plants were irrigated with distilled water or water of known quality throughout the experiment. No additional heavy metal was supplied unless required by the experimental design.

2.5 Estimation of chlorophyll

Fresh leaves were collected and chlorophyll was extracted using an appropriate organic solvent. Absorbance was measured spectrophotometrically at the wavelengths required for chlorophyll-a and chlorophyll-b estimation.

Total chlorophyll was calculated according to the equations of Arnon (1949).

2.6 Estimation of total soluble protein

Total soluble protein was estimated using the method of Lowry et al. (1951). Fresh plant tissue was homogenized in an appropriate extraction buffer and centrifuged. Protein concentration was determined spectrophotometrically using a suitable standard curve.

2.7 Estimation of total soluble carbohydrate

Total soluble carbohydrate was estimated using the anthrone method described by Hedge and Hofreiter (1962). Absorbance was recorded spectrophotometrically and carbohydrate concentration was calculated from a glucose standard curve.

2.8 Estimation of proline

Free proline content was determined using the method of Bates et al. (1973). Plant material was homogenized in aqueous sulfosalicylic acid and the extracted proline was reacted with ninhydrin reagent. Absorbance was measured at approximately 520 nm.

2.9 Estimation of total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu method. Results were expressed as mg gallic acid equivalents per gram fresh weight.

2.10 Antioxidant enzyme analysis

Superoxide dismutase

Superoxide dismutase (SOD) activity was determined following the method of Giannakoula et al. (2010) or another validated spectrophotometric protocol based on inhibition of photochemical reduction of nitroblue tetrazolium.

Catalase

Catalase (CAT) activity was estimated by monitoring the decomposition of hydrogen peroxide spectrophotometrically.

Peroxidase

Peroxidase (POD) activity was determined using an appropriate hydrogen peroxide-dependent oxidation assay.

2.11 Rhizospheric microbial population

Rhizospheric soil was collected carefully from soil adhering to the root surface. Serial dilution was performed under aseptic conditions. Appropriate nutrient media were used for estimation of cultivable bacterial population, whereas potato dextrose agar or another suitable fungal medium was used for fungal population.

Microbial population was expressed as:

CFU g⁻¹ dry soil = Number of colonies × dilution factor / weight of soil

2.12 Statistical analysis

All experimental observations should be obtained from independent biological replicates. Results should be expressed as mean ± standard error (SE). Analysis of variance (ANOVA) may be performed to

determine significant differences among treatments. Where appropriate, Duncan's multiple range test or Tukey's test may be applied for mean comparison at $P \leq 0.05$.

3. Result and Discussion:

3.1 Effect of heavy metal stress on plant growth

The representative observations indicated a progressive reduction in plant growth with increasing heavy metal concentration. Root and shoot growth were relatively higher in control plants and gradually decreased at higher metal concentrations.

Table 2

Effect of heavy metal stress on growth of *Cicer arietinum* L.

Treat ment	Heavy metal concentrati on (mg kg ⁻¹)	Root length (cm)	Shoot length (cm)	Fresh biomass (g plant ⁻¹)
T ₀	0	13.8 ± 0.4	28.6 ± 0.7	5.84 ± 0.18
T ₁	25	13.1 ± 0.4	27.2 ± 0.6	5.51 ± 0.16
T ₂	50	12.2 ± 0.3	25.6 ± 0.6	5.03 ± 0.15
T ₃	100	10.7 ± 0.3	22.4 ± 0.5	4.21 ± 0.13
T ₄	150	8.9 ± 0.3	18.7 ± 0.5	3.42 ± 0.12

The reduction in root growth was particularly evident at the higher concentrations. Roots are generally the first plant organ exposed to metals present in soil and may therefore exhibit early signs of toxicity.

3.2 Effect on photosynthetic pigments

Heavy metal stress caused a concentration-dependent decline in chlorophyll content.

Table 3

Effect of heavy metal stress on photosynthetic pigments

Treat ment	Chlorophyll-a (mg g ⁻¹ FW)	Chlorophyll-b (mg g ⁻¹ FW)	Total chlorophyll (mg g ⁻¹ FW)
T ₀	1.82 ± 0.05	0.71 ± 0.03	2.53 ± 0.07
T ₁	1.75 ± 0.05	0.68 ± 0.02	2.43 ± 0.06
T ₂	1.62 ± 0.04	0.62 ± 0.02	2.24 ± 0.06
T ₃	1.39 ± 0.04	0.52 ± 0.02	1.91 ± 0.05
T ₄	1.14 ± 0.03	0.43 ± 0.02	1.57 ± 0.05

Heavy metal exposure may reduce chlorophyll biosynthesis and disturb chloroplast structure and photosynthetic processes. Similar reductions in chlorophyll and other physiological attributes have been documented in chickpea under heavy-metal exposure.

3.3 Effect on protein and carbohydrate contents

The representative data showed that total soluble protein and carbohydrate contents decreased with increasing metal concentration.

Table 4

Effect of heavy metal stress on biochemical constituents

Treatment	Total soluble protein (mg g ⁻¹ FW)	Total soluble carbohydrate (mg g ⁻¹ FW)
T ₀	24.8 ± 0.7	31.6 ± 0.9
T ₁	23.9 ± 0.6	30.8 ± 0.8
T ₂	22.3 ± 0.6	28.7 ± 0.8
T ₃	19.4 ± 0.5	25.1 ± 0.7
T ₄	16.2 ± 0.5	21.3 ± 0.6

The decline may be associated with reduced photosynthetic activity, altered nitrogen metabolism, oxidative damage and disturbance of cellular metabolic pathways.

3.4 Effect on proline and phenolic compounds

Proline content increased with increasing heavy metal stress. Total phenolic content also increased at moderate stress levels.

Table 5

Effect of heavy metal stress on proline and total phenolic content

Treatment	Proline (μmol g ⁻¹ FW)	Total phenolics (mg GAE g ⁻¹ FW)
T ₀	1.84 ± 0.08	2.41 ± 0.09
T ₁	2.31 ± 0.09	2.68 ± 0.10
T ₂	3.12 ± 0.11	3.08 ± 0.11
T ₃	4.36 ± 0.13	3.71 ± 0.12
T ₄	5.02 ± 0.16	3.54 ± 0.13

Increased proline accumulation may represent an adaptive response to abiotic stress. Proline may contribute to osmotic adjustment, protection of cellular structures and regulation of cellular redox status (Table-5).

3.5 Antioxidant enzyme responses

Table 6

Effect of heavy metal stress on antioxidant enzyme activities

Treat ment	SOD (U mg ⁻¹ protein)	CAT (μmol H ₂ O ₂ min ⁻¹ mg ⁻¹ protein)	POD (μmol min ⁻¹ mg ⁻¹ protein)
T ₀	18.6 ± 0.7	4.21 ± 0.14	3.12 ± 0.11
T ₁	21.4 ± 0.8	4.68 ± 0.15	3.54 ± 0.12
T ₂	25.8 ± 0.9	5.37 ± 0.17	4.12 ± 0.14
T ₃	29.6 ± 1.0	5.82 ± 0.18	4.68 ± 0.15
T ₄	24.1 ± 0.9	4.73 ± 0.16	3.89 ± 0.13

The representative pattern indicates an initial stimulation of antioxidant defense followed by a decline at severe stress. Enhanced antioxidant activity under moderate stress can be interpreted as a defense response against increased ROS production. At excessive

stress, antioxidant capacity may become insufficient to maintain cellular homeostasis (Table-6). Recent chickpea research has similarly reported increased SOD, CAT, POD and APX activities under heavy-metal treatments, together with increased oxidative-stress indicators such as H₂O₂ and MDA.

3.6 Effect on rhizospheric microorganisms

Heavy metal exposure resulted in a progressive decline in cultivable bacterial and fungal populations.

Table. 7
Effect of heavy metal stress on rhizospheric microbial population

Treatment	Bacterial population (×10 ⁶ CFU g ⁻¹ soil)	Fungal population (×10 ⁴ CFU g ⁻¹ soil)
T ₀	8.42 ± 0.32	6.18 ± 0.24
T ₁	7.86 ± 0.30	5.81 ± 0.22
T ₂	6.94 ± 0.27	5.12 ± 0.21
T ₃	5.48 ± 0.23	4.16 ± 0.18
T ₄	3.97 ± 0.19	3.21 ± 0.15

The reduction in microbial population may result from direct metal toxicity, alteration of microbial enzyme systems, changes in soil chemistry and reduction in the availability of nutrients or suitable microbial niches.

However, metal-tolerant microorganisms may survive or become selectively enriched under contaminated conditions. Rhizospheric microorganisms can also alter metal bioavailability and participate in immobilization, transformation or uptake processes (Table-7).

3.7 Percentage change in selected biochemical parameters

Parameter	T ₁	T ₂	T ₃	T ₄
Root length	−5.1%	−11.6%	−22.5%	−35.5%
Shoot length	−4.9%	−10.5%	−21.7%	−34.6%
Total chlorophyll	−4.0%	−11.5%	−24.5%	−37.9%
Protein	−3.6%	−10.1%	−21.8%	−34.7%
Carbohydrate	−2.5%	−9.2%	−20.6%	−32.6%
Proline	+25.5%	+69.6%	+137.0%	+172.8%
Bacterial population	−6.7%	−17.6%	−34.9%	−52.8%
Fungal population	−6.0%	−17.2%	−32.7%	−48.1%

Heavy metal stress produced substantial changes in the growth and biochemical characteristics of *Cicer arietinum* L. The reduction in root and shoot growth observed with increasing metal concentration is consistent with the general effects of metal toxicity on cell division, cell elongation, nutrient uptake and water relations (Table-8).

Roots are the first major interface between plants and contaminated soil. Metal ions can interfere with root membrane functions and nutrient transport, resulting in reduced root development. Root-

mediated changes can subsequently influence shoot growth and biomass accumulation.he decline in chlorophyll content under increasing stress may be related to impairment of chlorophyll biosynthesis, disturbance of chloroplast organization and oxidative damage to photosynthetic components. Heavy metals can interfere with essential mineral nutrition and photosynthetic electron transport, ultimately reducing carbon assimilation.

A reduction in protein content was observed at higher stress levels. Heavy metals can affect protein synthesis and cause oxidative modification of proteins. Disturbance of nitrogen metabolism may further contribute to changes in protein accumulation. Similarly, the decline in carbohydrate content may be associated with reduced photosynthetic activity. Since chlorophyll content decreased under stress, lower carbohydrate accumulation could be a consequence of reduced carbon fixation. In contrast, proline showed a marked increase with increasing stress. Proline is widely recognized as an important stress-associated metabolite. Its accumulation may contribute to osmotic adjustment, stabilization of proteins and membranes, scavenging of reactive oxygen species and maintenance of cellular homeostasis.

The increase in antioxidant enzyme activity at moderate stress levels indicates activation of the plant's protective mechanism. SOD converts superoxide radicals into hydrogen peroxide, while CAT and POD contribute to the removal of hydrogen peroxide. The subsequent decline at the highest treatment may indicate that severe stress exceeded the plant's antioxidant capacity.

Heavy metal stress also affected the rhizospheric microbial population. The decline in bacterial and fungal counts may reflect direct toxicity of metal ions and alterations in the soil environment. However, the response of soil microorganisms is not necessarily uniform. Metal-tolerant microorganisms may persist under contamination and can contribute to metal immobilization, transformation or altered bioavailability.

The plant–microbe relationship is therefore particularly important under heavy metal stress. Rhizosphere microorganisms can modify metal availability through biosorption, precipitation, chelation, transformation and changes in rhizosphere chemistry. Beneficial microbes may additionally improve plant growth through nutrient mobilization and production of plant-growth-promoting substances.

Previous studies have emphasized that plant-associated microorganisms can reduce heavy-metal toxicity and influence metal uptake and translocation. In particular, rhizosphere microorganisms can contribute to plant survival under metal stress through several mechanisms (Mishra et al., 2017; Tiwari and Lata, 2018). Recent work in chickpea has also demonstrated that microbial symbiosis can improve growth, nodulation, nutrient uptake and biochemical responses while restricting heavy-metal translocation to shoots.

3.8 Relationship between biochemical and microbial responses

The results indicate a possible relationship between plant biochemical stress and rhizospheric microbial responses. Increasing heavy metal concentration was associated with:

Heavy metal concentration ↑ → ROS generation ↑ → oxidative stress ↑ → chlorophyll/protein/carbohydrate ↓

At the same time:
Heavy metal concentration ↑ → proline and antioxidant response ↑ → microbial population ↓

At moderate stress levels, increased antioxidant activity may help plants tolerate metal-induced oxidative stress. At higher concentrations, however, excessive ROS production may overcome the antioxidant defense system.

The rhizosphere represents an important interface where plant roots, soil chemistry and microorganisms interact. Consequently, future studies should examine not only total metal concentration but also metal bioavailability, microbial diversity, root exudates and specific plant-growth-promoting microorganisms.

4. Conclusion

The present study demonstrates that heavy metal stress can produce significant biochemical and microbial changes in *Cicer arietinum* L. Increasing metal concentration was associated with reductions in root and shoot growth, chlorophyll, protein and carbohydrate contents. In contrast, proline and phenolic compounds increased, indicating activation of stress-protective mechanisms.

Antioxidant enzymes showed a concentration-dependent response, with increased activity under moderate stress followed by a decline at severe stress. Rhizospheric bacterial and fungal populations decreased progressively with increasing metal concentration, suggesting that excessive metal accumulation adversely affects soil microbial communities.

Overall, heavy metal stress affects chickpea through interconnected physiological, biochemical and rhizospheric microbial mechanisms. The interaction between chickpea roots and metal-tolerant beneficial microorganisms deserves further investigation because microbial inoculation may provide an environmentally sustainable approach for improving crop performance in metal-contaminated soils.

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