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Evaluation of Nickel (Ni) Induced Stress on Seed Germination, Growth, Photosynthetic Pigments, and Protein in *Lens culinaris* L.

Aradhana Pandey[1], Preeti Singh[2], Md. Akhlaqur Rahman[3]

[1]PhD Research Scholar, Department of Botany, S.S. Khanna Girls Degree College, Prayagraj, Uttar Pradesh, India
Email: *aradhanap587[at]gmail.com*

[2]Professor, Department of Botany, S.S.Khanna Girls' Degree College, Prayagraj, Uttar Pradesh, India

[3]Assistant Professor, Department of Zoology, S.S.Khanna Girls' Degree College, Prayagraj. Uttar Pradesh, India.
Email: *akhlaque.rh[at]gmail.com*
Email: *botany.ssk[at]gmail.com*

Abstract: ***Lens culinaris L. (commonly known as masoor) is among the oldest leguminous crops cultivated by mankind, holding a vital role in traditional agriculture, especially in semi-arid regions of the Indian subcontinent. Renowned for its drought resistance and nitrogen-fixation ability, this pulse crop is now increasingly exposed to environmental pollutants such as heavy metal contamination. Among these, nickel (Ni) has emerged as a significant concern due to its accumulation in agricultural soils through anthropogenic activities. On the other hand, Ni is an essential micronutrient involved in various enzymatic processes, its elevated concentrations adversely affect to plant physiology. In the present study, the phytotoxic effects of different concentrations of nickel (10, 30, and 50 mg/kg soil) were examined under in vivo conditions on Lens culinaris cultivar L4717. The results indicated a dual response to nickel (Ni) exposure. A lower Ni concentration (10 mg Ni/kg soil) stimulated plant growth parameters, specifically enhancing seed germination and seedling growth. This beneficial effect was accompanied by an increase in photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) and total protein content within the plant tissues. However, higher concentrations (30 and 50 mg Ni/kg soi) exhibited inhibitory effects, leading to visible toxicity symptoms, reduced seed germination, overall growth retardation, photosynthetic pigments, and protein degradation. These findings highlight the dose-dependent toxicity of nickel in lentils, suggesting a narrow threshold between its beneficial and toxic effects. The study emphasizes the importance of regulating heavy metal levels in agricultural soils to safeguard crop productivity and food quality.***

Keywords: *Lens culinaris*, chlorophyll, carotenoid, and protein

1. Introduction

Lens culinaris L., commonly known as lentil or *masoor*, is a crucial annual herbaceous legume (family Fabaceae) integral to global agriculture and particularly prominent in the semi-arid regions of the Indian subcontinent. As one of the earliest domesticated crops, its cultivation dates back approximately 8,000–9,000 years in the Fertile Crescent. *L. culinaris* exhibits a slender, erect growth habit, typically reaching 30 to 40 cm in height, and possesses a diploid chromosome number of 2n = 14. Renowned for its nutritional richness, drought resistance, and sustainability, lentil is a cornerstone of traditional farming systems. Its fibrous root system forms a critical symbiotic association with *Rhizobium* bacteria, facilitating atmospheric nitrogen fixation. This process significantly enhances soil fertility, reduces the need for chemical fertilizers, and supports sustainable yields, especially when rotated with cereals like wheat and barley. *L. culinaris* is cultivated as a rabi (winter) crop and displays considerable genetic variability, making it valuable for stress-tolerance breeding and crop improvement efforts.

Lens culinaris L. (lentil) serves as an effective model for phytotoxicity studies due to its sensitivity to heavy metals like lead (Pb) and nickel (Ni) [17]. Assessing changes in biochemical markers, such as chlorophyll, carotenoids, and protein content, provides valuable insights into environmental pollution levels.

Nickel, a prevalent transition metal, has become a widespread environmental contaminant due to both natural processes and increased human activities (WHO, 1991)[33,12]. Sources of accumulation in agricultural soils include industrial and municipal pollution, mining, smelting, fossil fuel combustion, and unsustainable agricultural practices, such as the excessive use of phosphate fertilizers and pesticide[2,3,4 ,5]. Elevated Ni concentrations in agricultural products pose risks to both plant growth and human health [14,33]

While nickel (Ni) is an essential micronutrient for plants, elevated concentrations prove phytotoxic[20,27]. Nickel toxicity is known to inhibit enzymatic activity, specifically affecting the Calvin cycle and chlorophyll biosynthesis, thereby decreasing photosynthetic efficiency [9]. Ni also interferes with nutrient uptake, causing deficiencies in iron (Fe) and zinc (Zn) and hindering the uptake of other heavy metals.[10,14]. Studies have shown that Ni stress reduces nitrogen concentrations in the roots and leaves of legume and leads to decreased protein and carbohydrate content in crops such as sunflower, soybean, and maize [10,11,12].

The previous study shows effects of Ni on plants are often dose-dependent, displaying both inhibitory and stimulatory responses. While higher concentrations generally lead to growth retardation, reduced water potential, and decreased yield [13,14], lower concentrations of $NiCl_2$ (e.g., <5mg/L) have been observed to stimulate seed germination, seedling growth, and biomass production in wheat and enhance the yield of some crops [15]. Notably, certain crops, such as barley, require Ni to complete their life cycle [16].

2. Objective

This study aims to investigate how increasing nickel (Ni) exposure affects the growth, leaf pigments, and soluble protein content of lentil (*Lens culinaris*), thereby quantifying the dose-response relationship and the toxicity threshold. By assessing these biomarkers, the study seeks to elucidate the extent of phytotoxicity caused by nickel exposure and contribute valuable insights into the tolerance mechanisms of *Lens culinaris* L. under heavy metal stress.

3. Methodology

Certified seeds of *Lens culinaris* cultivar (cv) L4717 were obtained from local seed store at Prayagraj, Uttar Pradesh, India. Seeds of uniform shape, size, and color were selected and surface-sterilized using 0.01% mercuric chloride ($HgCl_2$) for one minute, followed by thorough rinsing with distilled water. These sterilized seeds were then soaked in distilled water for the complete imbibition phase. Subsequently, the seeds were wrapped in muslin cloth and placed in a dark environment at room temperature for 24 hours under sterile conditions. For this investigation, soil was collected from the experimental plot of S.S. Khanna Girls' Degree College Prayagraj. The soil type was sandy clay loam, moderately rich in organic matter, and slightly acidic pH. To impose nickel stress, the soil was amended with nickel chloride ($NiCl_2$) to achieve final concentrations of 10 mg Ni/kg, 30 mg Ni/kg, and 50 mg Ni/kg of soil. Alongside, untreated soil was maintained as the control. The treated and control soils were filled in earthen pots, and the entire experiment was carried out under greenhouse conditions. Once sprouted, the seedlings were allowed to grow further in darkness until emergence.

Dose-Dependent Effects of Nickel on Seed Germination and growth in *Lens culinaris* L.

Seed germination percentage was recorded on the 5th and 10 days after sowing. Growth parameters such as root length and shoot length, were measured after 55 days after seedling emergence. Fresh weight and dry weight (after oven-drying at 70 °C until constant weight) were recorded to calculate biomass.

Physiological Response of Lentil to Nickel Toxicity: A Study on Photosynthetic Pigment Content: The 100 mg leaf from each sample was crushed and photosynthetic pigments were extracted in 80% acetone. The absorbance of the supernatant was recorded at 663,646 and 470 nm in UV-visible spectrophotometer and the amount of chlorophyll a, b and carotenoids were calculated by Arnon method [4]

Quantification of Total Soluble Proteins in Leaves of *Lens culinaris* L.

Protein content was quantified using the method described by Lowry et al., [18]. A 500 mg sample of the plant shoot was homogenized in 10 ml of a 20% trichloroacetic acid (TCA) solution. The mixture was then centrifuged at 3000 rpm for 10 minutes. The resulting supernatant was discarded, and the pellet was re-extracted with 5 ml of 0.1 N NaOH. A 1 ml aliquot of this final extract was transferred to a test tube, where it was incubated in the dark for 30 minutes. The absorbance of the prepared sample was then measured at 660 nm using a UV-visible spectrophotometer.

Statistical Analysis

Data were analyzed using mean ± standard error (SE). Significant differences among treatments were tested using one-way ANOVA, and results were compared against the control.

4. Results

The Dual Nature of Nickel: A Study on Germination Response

Nickel (Ni), an essential micronutrient for many plants, exhibits a fascinating dual nature in its effect on seed germination: beneficial at low concentrations but detrimental at higher levels[26,28] In plants, nickel performs a vital function in a wide range of life processes and chemical reactions. It's a key component of the enzyme urease, which is vital for nitrogen metabolism by catalyzing the hydrolysis of urea into ammonia and carbon dioxide [19]. Additionally, nickel is involved in iron uptake, disease resistance, and seed viability. However, like many essential trace elements, the line between beneficial and toxic concentrations is narrow. Its toxicity to plants can manifest in several ways: High nickel levels can lead to the overproduction of reactive oxygen species (ROS), causing damage to cellular components like DNA, proteins, and lipids [6,20]. Nickel can bind to and inactivate various enzymes crucial for metabolic pathways, disrupting normal cellular function[21]

Nickel stress also influences protein metabolism. In the present study, total soluble protein content exhibited a biphasic pattern in response to Ni treatment. The control sample recorded 0.80 mg/mL of protein, while the 10 mg Ni/kg Ni treatment slightly increased protein content to 0.86 mg/mL. This initial increase may reflect a defense response involving stress-related protein synthesis. However, at higher concentrations—30 mg Ni/kg soil and 50 mg Ni/kg soil the protein levels declined to 0.68 mg/mL and 0.52 mg/mL, respectively. This reduction can be attributed to the inhibition of protein synthesis, enzymatic dysfunction, and potential degradation of existing proteins due to oxidative stress. Similar observations have been reported in *Hyptis suaveolens* and *Helianthus annuus* under Ni stress, where protein and carbohydrate content declined progressively with increasing metal concentration [22]

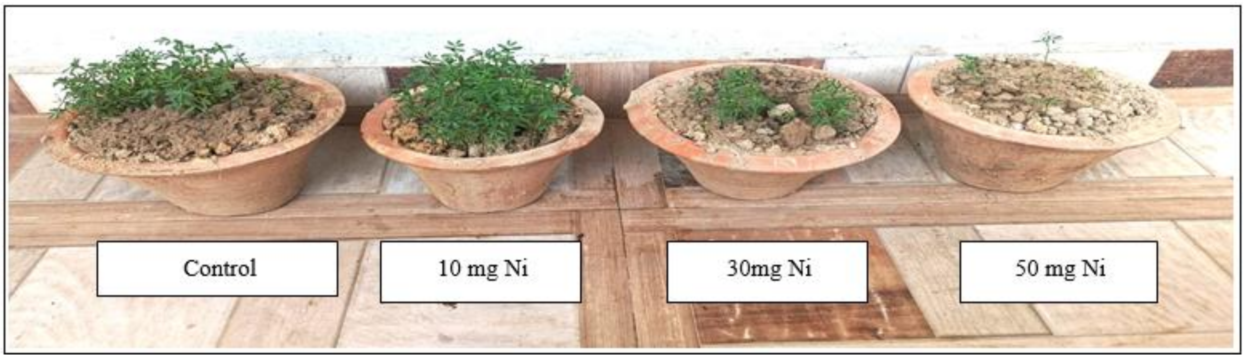
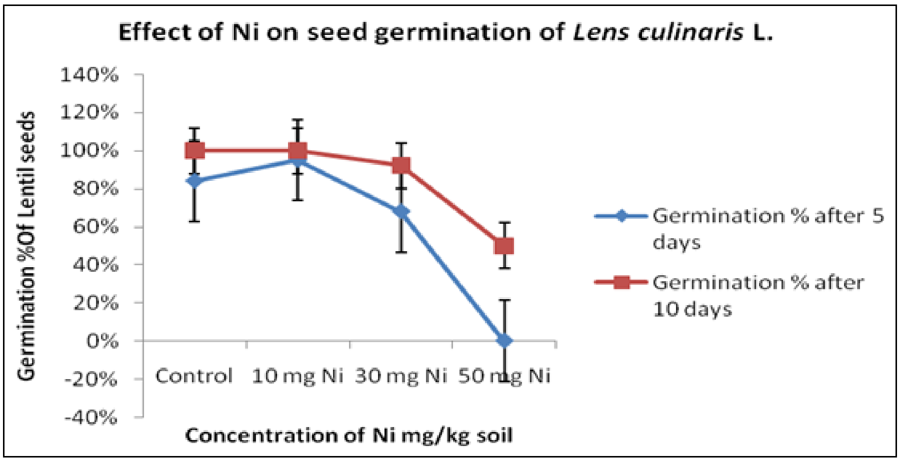


Figure 1: Dose-Dependent Effects of Nickel on the Germination and Early Growth of *Lens culinaris* L.

Dose-dependent effects of Nickel on the germination percentage of *Lens culinaris* L. The graph **visualizes the data for germination after 5 days and 10 days.**

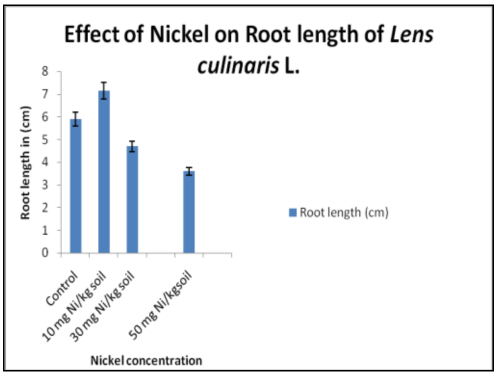


Graph 1: Effect of nickel on seed germination percentage of lentil (*Lens culinaris* L).

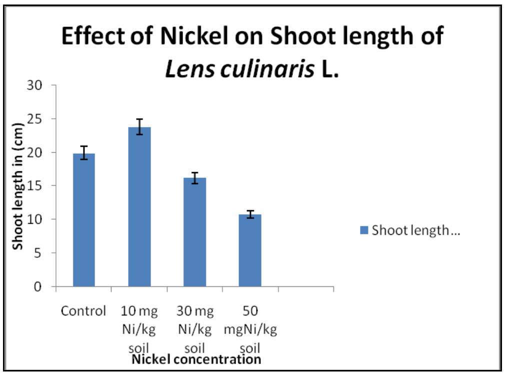
The graph 1. demonstrates that increasing concentrations of Nickel have a significant inhibitory effect on seed germination . At a low concentration of 10 mg Ni/kg soil Ni, the germination rate after 5 days (95%) is notably higher than the control (84%), suggesting a potential stimulatory effect at this level. Both the control and 10 mg Ni/kg soil treatments reached 100% germination after 10 days. Germination percentages sharply decrease as Ni concentration exceeds 10 mg Ni/kg soil. At 30 mg Ni/kg soil, germination dropped to 68% after 5 days and 92% after 10 days. The highest concentration, 50 mg Ni/kg soil, exhibits the most significant phytotoxicity, completely inhibiting germination after 5 days (0%). While some germination occurred by day 10 (50%), this remains a substantial inhibition compared to the control and lower Ni treatments (graph 1).

The present findings demonstrate a clear dose-dependent effect of Ni on seed germination in *Lens culinaris* L. A low concentration (10 mg Ni/kg soil) slightly enhanced germination compared to the control, indicating a possible stimulatory role of Ni at trace levels. However, as concentrations increased, a progressive inhibition was observed, with the highest dose (50 mg Ni/kg soil) causing severe suppression. Such biphasic responses, where low doses are beneficial but higher levels become toxic, are characteristic of heavy metal stress. The inhibitory effect at elevated Ni concentrations may be attributed to disturbances in essential metabolic processes such as water uptake, respiration, and enzyme activation, ultimately impairing germination efficiency.

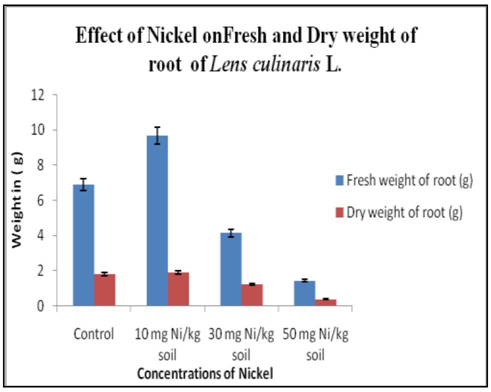
The effects of Ni on plants are known to be concentration-dependent, exhibiting a complex dual response where low doses may be beneficial, while elevated levels are detrimental. High concentrations of Ni can disrupt metabolic processes necessary for successful germination, including water uptake, respiration, and enzyme activation, leading to germination inhibition or delays.



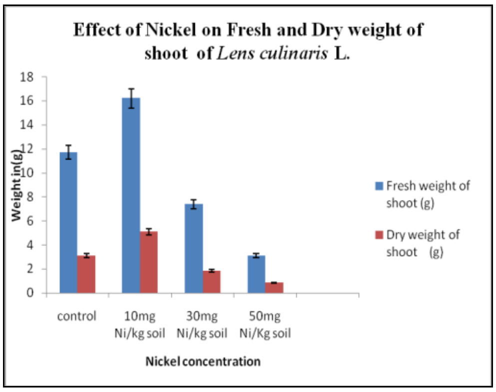
(a)



(b)



(c)



(d)

Graph 2: Effect of Nickel on Growth and Biomass of *Lens culinaris* L.

The graph 2. graphs (a–d) illustrate that the experimental findings indicated that nickel (Ni) exposure markedly affected the growth performance of *Lens culinaris* L. At a lower concentration (10 mg Ni/kg soil), root length increased by 12.5%**,** while higher concentrations (30 and 50 mg Ni/kg soil) caused reductions of 15.8% and 36.8%**,** respectively, compared with the control. Shoot length also increased by 10.8% at 10 mg Ni/kg soil but declined by 13.3% and 30.9% at 30 and 50 mg Ni/kg soil, respectively. The fresh weight of roots showed a significant increase of 15.5% at the lower concentration, whereas reductions of 18.1% and 40.5% were recorded at 30 and 50 mg Ni/kg soil, respectively. Similarly, shoot fresh weight was enhanced by 13.4% at 10 mg Ni/kg soil but decreased by 17.7% and 39.4% under higher concentrations (30 and 50 mg Ni/kg soil, respectively). These observations were recorded 55 days after seedling emergence.(graph 2)

The experimental data (Graphs a–d) revealed that nickel (Ni) exposure exerted a significant influence on the growth performance of *Lens culinaris* L. seedlings. At a lower concentration (10 mg Ni/kg soil), growth parameters exhibited a stimulatory response, as evidenced by increases in root length (12.5%), shoot length (10.8%), root fresh weight (15.5%), and shoot fresh weight (13.4%) compared with the control. However, with increasing Ni concentrations (30 and 50 mg Ni/kg soil), all measured traits showed a progressive decline. Root length was reduced by 15.8% and 36.8%, while shoot length decreased by 13.3% and 30.9% at 30 and 50 mg Ni/kg soil, respectively. Similarly, root fresh weight declined by 18.1% and 40.5%, and shoot fresh weight by 17.7% and 39.4% under the same treatments. These contrasting trends indicate that Ni at low levels may stimulate early growth, but elevated concentrations exert pronounced phytotoxic effects, leading to significant suppression of biomass accumulation.

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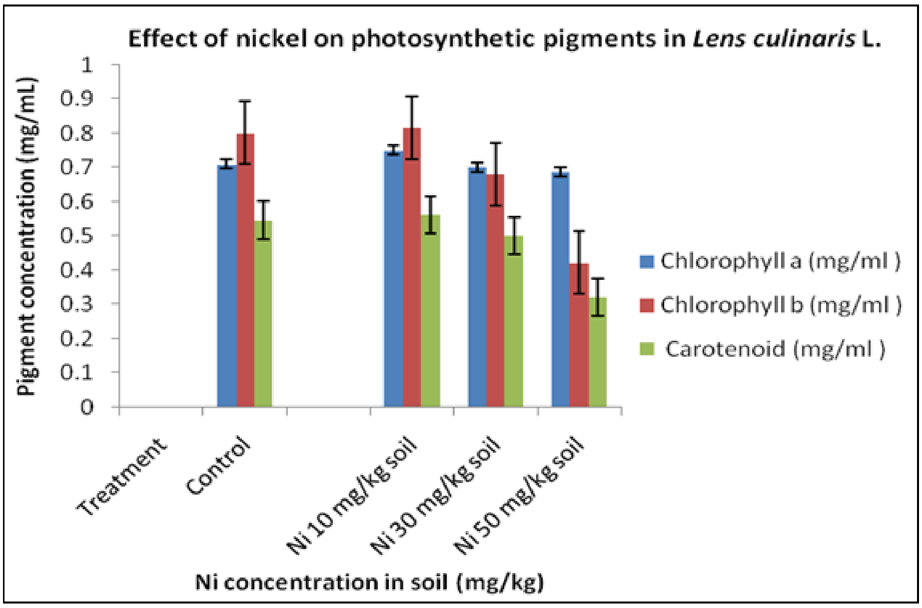


Graph 3: Effect of Nickel on Photosynthetic pigments in *Lens culinaris* L.

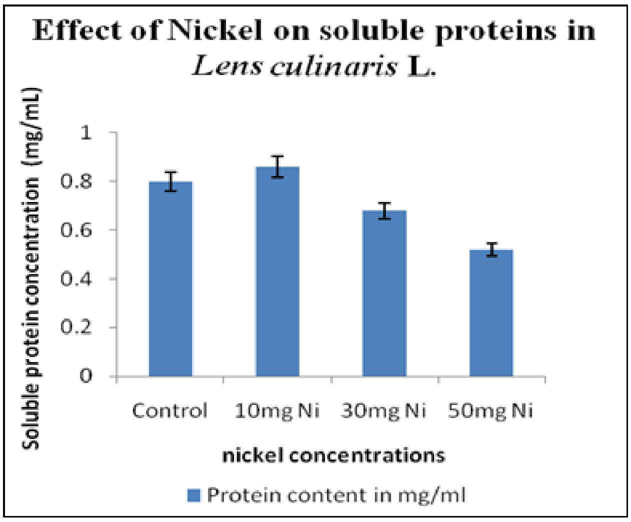
The graph 3. illustrates the dose-dependent effects of increasing Nickel (Ni) concentrations in soil on the content of photosynthetic pigments (Chlorophyll a, Chlorophyll b, and Carotenoid), using Arnon's method in the plant tissues.

The graph demonstrates that low concentrations of Nickel (10 mg Ni/kg soil) have a negligible or slightly beneficial effect on pigment content. However, increasing Ni concentration above this level results in a significant reduction in Chlorophyll a, Chlorophyll b, and Carotenoid concentrations, indicating a clear inhibitory effect on the plant's photosynthetic capacity.

Stimulatory Effect at Low Concentration (10 mg Ni/kg soil): Compared to the Control group, the treatment with 10 mg/kg soil of Ni shows a slight increase across all measured pigments. Chlorophyll a content increases slightly from 0.710 mg/g to 0.750 mg/g, Chlorophyll b from 0.801 mg/g to 0.816 mg/g, and Carotenoid from 0.546 mg/g to 0.562 mg/g.

Inhibitory Effects at Higher Concentrations (30 and 50 mg Ni/kg soil): As the concentration of Ni increases to 30 mg Ni/kg soil and 50 mg Ni/kg soil, a notable decline in all pigment concentrations is observed, indicating phytotoxicity.

Dose-Dependent Decline: The most pronounced reduction is seen at the highest concentration (50 mg Ni/kg soil). At this level, Chlorophyll b shows the sharpest decline, dropping significantly to 0.422 mg/g from 0.801 mg/g (Control). Carotenoid content also decreases substantially to 0.321 mg/g from 0.546 mg/g.



Graph 4: Effect of Varying Nickel Concentrations on Soluble Protein in *Lens culinaris* L.

The graph 4. illustrates the dose-dependent effect of Nickel (Ni) concentration on the soluble protein content of *Lens culinaris* L. plants.

The above data reveals a dual response to Nickel treatment:

Stimulation at Low Concentration: At 10 mg N/kg soil, the protein content shows a slight increase compared to the control group (0.86 mg/ml versus 0.80 mg/ml).

Inhibition at Higher Concentrations: As Ni concentration increases to 30 mg Ni/kg soil and 50 mg Ni/kg soil, there is a clear inhibitory effect on protein synthesis. Protein content significantly decreases, reaching its lowest level at 50 mg Ni (0.52 mg/ml).

5. Discussion

The present investigation aimed to evaluate the phytotoxic effects of nickel (Ni) on *Lens culinaris* L. under in vivo conditions, with a specific focus on plant growth parameters, protein content, chlorophyll pigments, and carotenoid levels. The results clearly indicate that exposure to elevated concentrations of nickel leads to significant physiological and biochemical disruptions in lentil plants, depending upon the concentration and duration of exposure [23].

Nickel, although essential in trace amounts for plant metabolism particularly in urease activity and nitrogen assimilation becomes toxic when accumulated beyond permissible levels [24,30]. Heavy metals can alter the balance of plant hormones (gibberellins and abscisic acid) that regulate germination. A shift towards dormancy-promoting hormones or away from germination-promoting ones can lead to delayed or reduced germination [5,25,26]. Interestingly, some studies suggest that abiotic stress tolerance mechanisms such as drought resistance may confer partial resilience to heavy metal stress, possibly by regulating water balance and enhancing antioxidant defense[26,27] Exogenous application of abscisic acid (ABA) has been reported to alter the translocation pattern of cadmium and nickel by enhancing their retention in roots, thereby reducing their movement to aerial parts. This mechanism may be beneficial in limiting the physiological damage caused by heavy metals in sensitive crop species.

Nickel-treated plants showed a marked reduction in chlorophyll *a*, chlorophyll *b*, and total carotenoid contents compared to the control. This decline suggests that nickel interferes with chlorophyll biosynthesis, possibly by replacing magnesium ions in the chlorophyll molecule or by affecting enzymes such as δ-aminolevulinic acid dehydratase [29]. Additionally, Ni-induced oxidative stress may accelerate chlorophyll degradation through lipid peroxidation and reactive oxygen species (ROS) production [6,30]. The reduction in carotenoids, which play a vital role in photoprotection and stabilization of light-harvesting complexes, further exacerbates photosynthetic efficiency loss. These findings strongly corroborate previous research, demonstrating significant pigment degradation in legumes subjected to heavy metal exposure [33]

Protein content, a vital indicator of nitrogen metabolism and overall plant vigor, also declined significantly with increasing nickel concentration [11,15,18,31]. This reduction may result from inhibited nitrate reductase activity, protein denaturation due to ROS accumulation, or impaired amino acid synthesis. Nickel toxicity has been known to affect protein folding and enzyme structure, leading to metabolic imbalances. Previous studies [16,24,32] have similarly highlighted decreased protein content in metal-stressed plants, indicating a common mode of physiological disturbance under heavy metal stress.

Interestingly, in the lower concentration of Ni (10 mg Ni/kg soil), a slight stimulatory effect was occasionally noted, particularly in pigment content. This may be due to a hormetic response, where trace amounts of Ni temporarily enhance metabolic activity before reaching a threshold of toxicity. Such biphasic responses are not uncommon in metal stress studies and warrant further investigation. Given that staple foods like lentils are a significant part of people's diets, it's crucial for public health research to focus on measuring the amounts of both essential trace metals and harmful heavy metals present in these foods. This helps ensure food safety and prevent potential health issue [33].

Overall, the findings of the current study emphasize that *Lens culinaris* L. is sensitive to elevated nickel levels, which adversely affect key physiological processes. The observed alterations in protein metabolism, photosynthetic pigment synthesis, and growth performance confirm that nickel poses a serious threat to legume productivity when present in excess in agricultural soils. These results are crucial in the context of increasing industrial pollution and the potential accumulation of heavy metals in cultivable land.

6. Conclusion

In the present study, a progressive decline in shoot and root length, along with overall biomass, was observed as the concentration of nickel in the soil increased [26, 28]. This suppression of growth may be attributed to nickel-induced impairment in cell division, disruption of enzymatic processes, and oxidative damage to cellular structures. Similar findings have been reported by Gajewska and Sklodowska (2009), who noted stunted growth in *Phaseolus vulgaris* under nickel stress

The present study highlights the deleterious effects of nickel (Ni) toxicity on the physiological and biochemical parameters of *Lens culinaris* (L.) under in vivo conditions. While low concentrations of nickel appeared to temporarily stimulate certain pigments and protein levels likely due to an initial stress response higher concentrations (30 and 50 mg Ni/kg soil) led to significant reductions is seed germination, photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids), and soluble protein content.

The above graph demonstrates that low concentrations of Nickel (10 mg Ni/kg soil) have a negligible or slightly beneficial effect on pigment content. However, increasing Ni concentration above this level results in a significant reduction in Chlorophyll a, Chlorophyll b, and Carotenoid concentrations, indicating a clear inhibitory effect on the plant's photosynthetic capacity.

These findings confirm that nickel, though essential in trace amounts, becomes phytotoxic when accumulated beyond threshold levels. The observed decrease in photosynthetic pigments and protein content under elevated nickel exposure suggests impairment of key metabolic and enzymatic processes, ultimately leading to reduced photosynthetic efficiency and seed germination in *Lens culinaris* L. Visible symptoms such as chlorosis and necrosis further support the toxic impact of nickel stress.

Given the increasing environmental burden of heavy metals due to industrialization and improper waste management, the sensitivity of *Lens culinaris* to nickel toxicity poses a serious concern for sustainable agriculture. The study emphasizes the need for regular monitoring of soil metal content, adoption of safe irrigation practices, and exploration of metal-tolerant cultivars or soil amendments that can mitigate heavy metal uptake.

Overall, the results contribute valuable insights into the impact of heavy metal stress on pulse crops and underscore the importance of developing strategies to protect crop health and productivity in contaminated soils.

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