

Article

Biomass and Nickel Tolerance: *Canavalia ensiformis* (L.) DC. as a Candidate Plant for Phytoremediation Applications

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Abstract

The use of high biomass production plants in studies of metal phytoremediation is an established practice. This strategy aims to identify plants that tolerate unusual amounts of metals such as nickel (Ni). When we compare the biomass production capacity of a Ni hyperaccumulator, for example *Alyssum bertolonii*, this rate is 4 to 5 kg/ha per crop cycle; on the other hand, species with a high biomass production capacity, for example *Canavalia ensiformis*, can produce 20 t ha⁻¹ to 25 t ha⁻¹ of green phytomass, 5 t ha⁻¹ to 8 t ha⁻¹ of dry phytomass and 1000 kg ha⁻¹ to 1800 kg ha⁻¹ of seeds. In this context, we planned an experiment to verify the tolerance and Ni accumulation capacity in *Canavalia ensiformis*. Our hypothesis was that increasing Ni concentration in the soil would not hinder the plant's biomass production. We conducted a completely randomized experiment with five concentrations of Ni added to the soil and five replicates in a greenhouse during the vegetative stage. We evaluated the plant's development, biomass production, and Ni accumulation in its organs. Our results demonstrated high tolerance to the metal, maintaining a biomass accumulation capacity of 68% of the dry mass in the soil with 277.8 mg kg⁻¹ of Ni at the highest concentration tested, compared to plants in the control soil. Considering that under these conditions the plants obtained a biomass of 10 g of leaves and 15 g of roots, and a nickel accumulation capacity of 75.05 mg kg⁻¹ in leaves and 102 mg kg⁻¹ in roots, the total Ni accumulation in the plants reached 2.37 mg Ni/plant in the soil with 277.8 mg kg⁻¹ of Ni. This soil Ni concentration would be lethal for most plants, and the metal concentration in the tissue exceeds the established limits for non-tolerant crops. With these results, this study aims to provide a foundation for improving the use of *Canavalia ensiformis* in phytoremediation.

Keywords: jack bean; tropical legume; cover crop; green manure; phytomanagement; heavy metal; contamination



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1. Introduction

Canavalia ensiformis (L.) DC., commonly known as jack bean, is a tropical legume species of significant scientific and agricultural importance within the Fabaceae family. This annual herbaceous plant displays an indeterminate growth pattern, producing either climbing or prostrate stems that may extend up to 1.2 m in length [1]. The species is morphologically characterized by trifoliolate compound leaves featuring elliptical-ovate leaflets

measuring 10–15 cm in length, along with racemose inflorescences bearing distinctive zygomorphic flowers with white to pinkish petals [2]. The fruit develops as a straight, dorsiventrally compressed legume pod ranging from 15 to 30 cm in length, containing 8–12 protein-rich seeds that demonstrate 25–30% protein content, including the notable storage protein canavalin which constitutes approximately 30% of total seed proteins [3].

From a scientific perspective, *Canavalia ensiformis* holds particular historical significance as the source material for the first crystallized enzyme (urease), isolated by James B. Sumner in 1926, an achievement that was later recognized with the Nobel Prize in Chemistry [4]. Contemporary research continues to investigate this species for its contributions to our understanding of nitrogen metabolism and enzyme kinetics [5,6]. The plant demonstrates considerable agronomic value through its efficient biological nitrogen fixation capability, typically achieving 50–190 kg N/ha/year via symbiotic relationships with rhizobial bacteria, combined with its notable adaptation to marginal soil conditions [2].

These characteristics, along with its utility as green manure, cover crop, and potential forage source (following appropriate processing to reduce antinutritional factors), establish *Canavalia ensiformis* as a valuable resource for sustainable agricultural systems in tropical regions [7]. The species' combination of unique biochemical properties and robust physiological adaptations positions it as both a subject of ongoing scientific inquiry and a practical solution for soil improvement and sustainable crop production [8]. Furthermore, the plant has a production capacity of 20 t ha⁻¹ to 25 t ha⁻¹ of green phytomass, 5 t ha⁻¹ to 8 t ha⁻¹ of dry phytomass, and 1000 kg ha⁻¹ to 1800 kg ha⁻¹ of seeds [9] while hyperaccumulators produce little biomass and exhibit slow growth; for example, the growth rate of *Alyssum bertolonii*, a Ni hyperaccumulator, is 4 to 5 kg/ha per crop cycle.

Phytoremediation is established as an advantageous bioremediation strategy due to its low cost and environmentally sound nature [10]. This technology utilizes plants and their physical, chemical, biological, and microbiological processes with the primary objective of minimizing the toxicity of pollutants in contaminated soils [11]. As a simple, economical, and non-disruptive method for the environment, it is considered a green technology with dual potential: to rehabilitate degraded soils and also generate useful by-products [12].

However, the implementation of this technique faces significant challenges. Among the main obstacles, phytotoxicity caused by dissolved metal ions, which directly inhibits plant growth, stands out. Added to this are intrinsic limitations of many hyperaccumulator species, such as their low growth rate and biomass production, coupled with limited root depth. [11,13]. For this reason, the use of species with high biomass production has been proposed, even if they do not exhibit hyperaccumulator characteristics, as their growth rate would enable greater removal of toxic elements per area even with a lower removal capacity per plant [11]. In this sense, we investigated the tolerance and metal accumulation characteristics of species already used in agriculture as cover crops, since their hardiness and growth profile could enable the gradual recovery of contaminated areas if these plants can absorb and translocate metals to their organs. Previous studies have already demonstrated the potential of jack bean (*Canavalia ensiformis*) for soil decontamination, both from organic compounds, such as the pesticides trifloxysulfuron-sodium [14], sulfentrazone [15] and from mineral elements, such as lead (Pb) [16,17] and manganese (Mn) [18]. However, regarding nickel (Ni), only one recent study has investigated the plant's tolerance, focusing on its germination and initial establishment [7].

Although the potential of species such as jack bean (*Canavalia ensiformis*) is evident, knowledge regarding the diversity of the Fabaceae family in general, especially under field conditions, remains limited. Therefore, prioritizing neglected crops (*orphan crops*) and conducting prolonged field studies are essential for a more comprehensive understanding of nickel's effects in agricultural settings [19]. This is particularly relevant given that Ni is classified as

an essential nutrient required in trace amounts for various enzymatic functions in biological systems [20]. However this element, has its nutritional limit critical and tolerance among plant species can have significant variation [21]. In addition, its toxic effect can occur when plants growing in an environment with a high availability of Ni are unable to compartmentalize and chelate high concentrations of the metal within cellular compartments [22,23]. The regulatory thresholds for Ni in soil are defined by two critical values: The prevention value (30 mg kg^{-1} dry weight) establishes the maximum concentration for maintaining the soil's primary ecological capacity; According to Brazilian legislation, exceeding the intervention value (190 mg kg^{-1} dry weight for agricultural use) indicates a potential risk to human health, triggering the need for remediation based on a generic exposure scenario [24]. The concentration of metals that plants with high biomass production can support is also useful information for phytoremediation and phytomanagement studies [13].

Therefore, the hypothesis of this study is that jack bean, due to its growth and biomass accumulation characteristics, has potential for use in Ni phytoremediation or phytomanagement programs. It is believed that the species is capable of tolerating high levels of available Ni in the soil while maintaining its growth and translocating and accumulating quantities of Ni higher than those found in non-hyperaccumulator plants.

The objective of this study was to expose jack bean to increasing concentrations of available Ni in the soil to evaluate (1) the effects on growth and biomass accumulation; (2) the nodulation capacity in response to increasing Ni concentration; and (3) the Ni concentration levels in roots, stems, and leaves and the total accumulation per plant. It is expected that the results will provide a solid foundation for future studies on the effects of high Ni content in soils on legumes.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in a greenhouse at Ilha Solteira, São Paulo, Brazil ($20^{\circ}25'06.0''$ S, $51^{\circ}20'29.7''$ W) during middle summer. The greenhouse was covered with transparent, light-diffusing plastic film. The experiment was conducted under natural lighting, with an average ambient temperature of 27.5°C , ranging from a maximum of 31.8°C to a minimum of 22.1°C . The mean relative humidity was 79.8%, with extreme values of 98.3% and 53.4%. The daylight duration varied between 12 h and 31 min and 13 h and 1 min during the experimental period.

The soil consisted of a Typic Haplustox (Oxisol) [25] collected from the 0–0.40 m layer in an experimental area at UNESP's Teaching and Research Farm in Selvíria, Mato Grosso do Sul, Brazil ($20^{\circ}20'24.9''$ S, $51^{\circ}24'19.7''$ W), at cultivated lands.

2.2. Growing Conditions, Experimental Design and Treatments

The granulometric analysis of the soil [26] verified the following proportions: 169 (16.9%), 828 (82.8%) and 3 (0.3%) g kg^{-1} of clay, sand, and silt, respectively. The chemical attributes of the soil showed the following values [27]: pH = 5.0 (determined with CaCl_2 0.01 M); organic matter = 11.0 g kg^{-1} ; P = 2.0 mg kg^{-1} (resin); K = 0.8, Ca = 12; and Mg = $8.0 \text{ mmol}_c \text{ kg}^{-1}$ (resin); S = 6.0 mg kg^{-1} (Calcium phosphate); B = 0.06 mg kg^{-1} (warm water); Cu = 0.8, Fe = 16, Mn = 5.8 e Zn = 0.2 mg kg^{-1} (DTPA); Ni < 2 mg kg^{-1} (ICP-OES, HNO_3 EPA. 3051A); potential acidity $16.0 \text{ mmol}_c \text{ kg}^{-1}$ (SMP buffer); Al = $1.0 \text{ mmol}_c \text{ kg}^{-1}$; the sum of bases $18.8 \text{ mmol}_c \text{ kg}^{-1}$; cation exchange capacity $34.8 \text{ mmol}_c \text{ kg}^{-1}$ and base saturation 54%.

Thirty days before sowing, Ni was applied to the soil using nickel sulfate hexahydrate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) as the source. Individual solutions were prepared with the following Ni concentrations: 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg^{-1} (T5). A final volume of 500 mL of each solution was applied to the respective experimental units. The

soil was maintained in sealed transparent plastic bags for a 30-day incubation period, at room temperature, after which individual samples were collected to assess the effects of artificial contamination. The methodology for Ni extraction and quantification from the experimental units is detailed in the following section.

A completely randomized experimental design was employed, consisting of six treatments with five replications each, totaling 30 experimental units. Each experimental units had a capacity of 10 kg and was filled with 8 kg of soil sieved through a 2 mm mesh. The soil in each experimental units was sown with six commercially obtained jack bean seeds, placed approximately 4 cm below the surface.

2.3. Ni Concentration in Soil Samples and Plant Tissues

The analytical procedures for semi-total Ni quantification followed established EPA protocols: SW-846 [28] for general guidelines, 3051A [29] for soil analysis, and method 6010C [30] for plant tissue analysis. For soil samples, 500 mg aliquots were digested with concentrated nitro-perchloric using microwave-assisted digestion, followed by dilution to 50 mL with deionized water for ICP-OES (FAAS, Agilent, Model 55, Santa Clara, CA, USA) determination. Plant tissues were processed similarly, except for a final adjustment to 25 mL. The final mass and volume of the extracts are standardized; therefore, we used standard MDL and LOQ values (MDL, 0.4 mg kg^{-1} ; LOQ, 1.3 mg kg^{-1}). The SRM value adopted for plant tissue was 1570 a (Spinach leaves). For a sample without appreciable silicate content, EPA3051 can be considered the total content. The reference SRM value is 2.14. Our triplicate obtained a 95% recovery (average 2.0 mg kg^{-1}). For soil, the SRM value adopted was 2711 a Montana Soil. The reference values for EPA3051 adopted are in the range of range of 13 to 18 mg kg^{-1} . The average of the triplicates was within this range, and in this case, no recovery was reported.

Pre-sowing Ni bioavailability assessment was conducted on soil samples ($n = 4$ replicates per treatment) using Mehlich-1 extraction. The resulting available Ni concentrations showed a clear treatment gradient: control ($<2 \text{ mg kg}^{-1}$), 15 mg kg^{-1} treatment ($12.70 \pm 1.42 \text{ mg kg}^{-1}$), 30 mg kg^{-1} ($24.11 \pm 7.68 \text{ mg kg}^{-1}$), 60 mg kg^{-1} ($94.78 \pm 30.77 \text{ mg kg}^{-1}$), 120 mg kg^{-1} ($123.34 \pm 17.33 \text{ mg kg}^{-1}$), and 240 mg kg^{-1} ($277.83 \pm 92.55 \text{ mg kg}^{-1}$). Figure 1 demonstrates this dose-dependent increase in bioavailable Ni, confirming the successful establishment of concentration gradients for plant uptake studies. All ICP-OES measurements were performed following quality assurance protocols including calibration standards and blank samples.

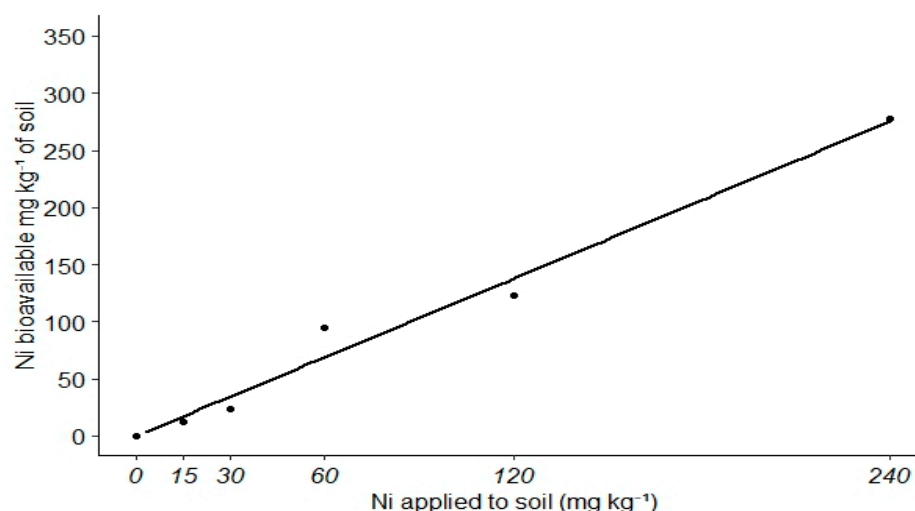


Figure 1. Validation of nickel (Ni) bioavailability following soil application at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg^{-1} (T5). The x-axis indicates treatment groups ($n = 4$), while the y-axis shows bioavailable Ni concentrations (mg kg^{-1}) after the incubation period.

2.4. Sample Collection, Measured and Calculated Parameters & Statistical Analysis

After sowing, the seedlings grew until the 10th day post-planting, at which point thinning was performed to leave two plants per experimental unit. These plants were cultivated until the 50th day after planting. The soil's field capacity was determined according to [31]. Throughout the experiment, evapotranspired water was manually replenished in each experimental unit to maintain soil moisture at 80% of the field capacity. No agricultural pesticides were used for pest or disease control, nor were any fertilizations or inoculations performed. No commercial inoculations or fertilizations were performed. The soil provided contained native bacterial strains capable of effective symbiotic association, as confirmed by the clear visual presence of nodules on the roots across all experimental units, which will be further discussed in subsequent sections.

On the 50th day of the experiment, the setup was dismantled for data collection. The pot containing the soil was placed inside a large tray and carefully overturned. While the soil was still moist, we gently extracted the roots from the soil clumps. Before proceeding with the root cleaning and washing steps, the soil firmly adhered to the root surface (operationally defined as rhizosphere soil) was carefully separated and collected for subsequent analysis of bioavailable Ni content. After removing this adhered soil, the remaining material was placed on a sieve and rinsed under running water in a dedicated soil-handling area.

At this stage, the shoots were separated from the roots using pruning shears. A second wash was carefully performed in a standard sink to remove any remaining impurities from the roots and shoots of the experimental plants. The collection order followed the sequence from T0 to T5 to prevent cross-contamination between samples. The washed plant parts were gently dried with paper towels and laid out on the bench for further separation.

The leaflets were separated from the plants and counted (Number of leaflets). Stem length was measured using a millimeter-graded ruler (Shoot length, cm), and stem diameter was measured with a caliper 5 cm above the root junction (Stem diameter, cm). Nodules were carefully separated from the roots, counted (Number of nodules), and immediately weighed (Fresh weight of nodules, g). The maximum vertical root length was measured using a millimeter-graded ruler, defined as the distance from the base of the shoot to the tip of the main primary root (Root length, cm).

All plant material except nodules was placed in labeled paper bags (marked with treatment codes) and dried in a forced-air oven at 60 °C until constant weight was achieved. The dried material was then weighed to determine dry biomass (Dry weight of root, stem, and leaf, g) and subsequently ground using a knife mill to prepare samples for elemental analysis. The elemental analysis methodology was described in the previous section.

Calculated variables were derived from mathematical relationships, including the tolerance index (1) and accumulation in organs and in plant (2).

Tolerance Index:

$$\text{Tolerance Index} = ((\text{Total dry biomass of treatment with Ni})) / ((\text{Total dry biomass of control plants})) \quad (1)$$

Accumulation:

$$\text{Metal Accumulation (mg)} = [\text{Metal}] \text{ (mg/kg)} \times \text{DW (g)} \times 0.001 \text{ (kg/g)} \quad (2)$$

The normality (Shapiro–Wilk) and homoscedasticity (Levene's test) of the data were tested ($p \leq 0.05$). For the comparison of differences in variables, analysis of variance was used one-way (ANOVA) through the F test ($p \leq 0.05$) and when significant, the variables were submitted to Tukey's post hoc test ($p \leq 0.05$). Statistical analysis and graphing were performed and documented using protocols developed in the R software version 4.2.1

(R Core Team 2022, Vienna, Austria) utilized the Integrated Development Environment (IDE) RStudio [32]. Among the presented data, only the post-incubation Ni concentration and tolerance index were not subjected to statistical analysis. Figure 2 summarizes the experimental methodology employed in this study.

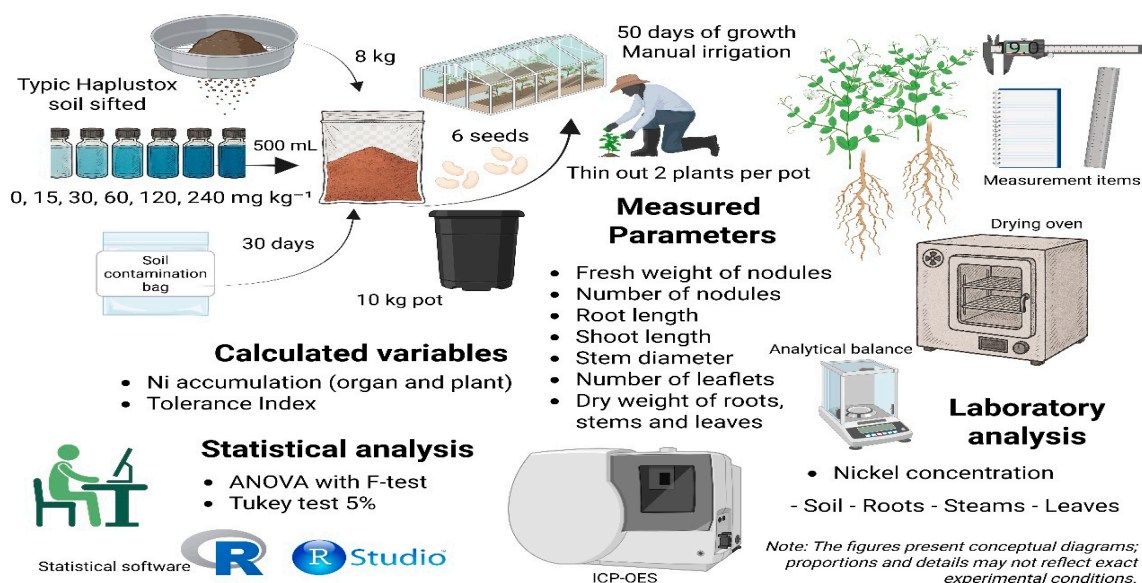


Figure 2. Schematic overview of the experimental methodology. The upper left quadrant depicts the soil contamination procedure and cultivation protocol. The upper right quadrant presents the measurement parameters and sampling strategy, including the number of plants collected per experimental unit. In the lower right quadrant, the analytical instrumentation and measured variables are shown, highlighting key equipment used for data collection. The lower left quadrant focuses on data processing. Created in BioRender. Aguilar, J. (2025) <https://BioRender.com/d7jqkjin> (accessed on 20 October 2025).

3. Results

The increase in soil Ni concentration directly influenced Ni uptake by jack bean plants, with significant differences observed across all plant organs (Figure 3). Roots consistently exhibited the highest Ni concentrations across all treatments with general average 58.96 mg kg^{-1} , peaking at T5 ($102.57 \text{ mg kg}^{-1}$) and reaching the lowest levels in T0 (7.26 mg kg^{-1}). The positive linear trend where tissue Ni concentrations rose proportionally with soil availability confirms the expected dose–response pattern.

Figure 3 demonstrates that Ni concentrations in roots and leaves became remarkably similar at higher soil Ni treatments (120 and 240 mg kg^{-1}). At the 120 mg kg^{-1} treatment (T4), root Ni concentrations reached 75.05 mg kg^{-1} while leaves accumulated 75.24 mg kg^{-1} , showing virtually identical accumulation patterns. This trend persisted at the highest treatment level (T5), where roots contained $102.57 \text{ mg kg}^{-1}$ Ni compared to 95.65 mg kg^{-1} in leaves.

Stems showed the lowest overall Ni concentration (average: 14.79 mg kg^{-1}) among all plant organs, with minimal variation across treatments. Unlike the dose–response pattern observed in roots and leaves, stem Ni levels remained lowest in the control (T0: 2.64 mg kg^{-1}) and exhibited a stable linear trend, failing to increase proportionally with higher soil Ni concentrations (Figure 3).

Ni excess in soil significantly impaired nodulation in jack bean plants. Treatments T4 and T5 showed substantially lower root nodule production compared to T0, as evidenced in Figure 4B. Notably, plants from intermediate treatments (T1, T2, and T3) showed no statistical difference either from T0 or from the high-concentration treatments (T4 and

T5), suggesting a toxicity threshold for nodule formation between 60 and 120 mg kg⁻¹ of soil Ni.

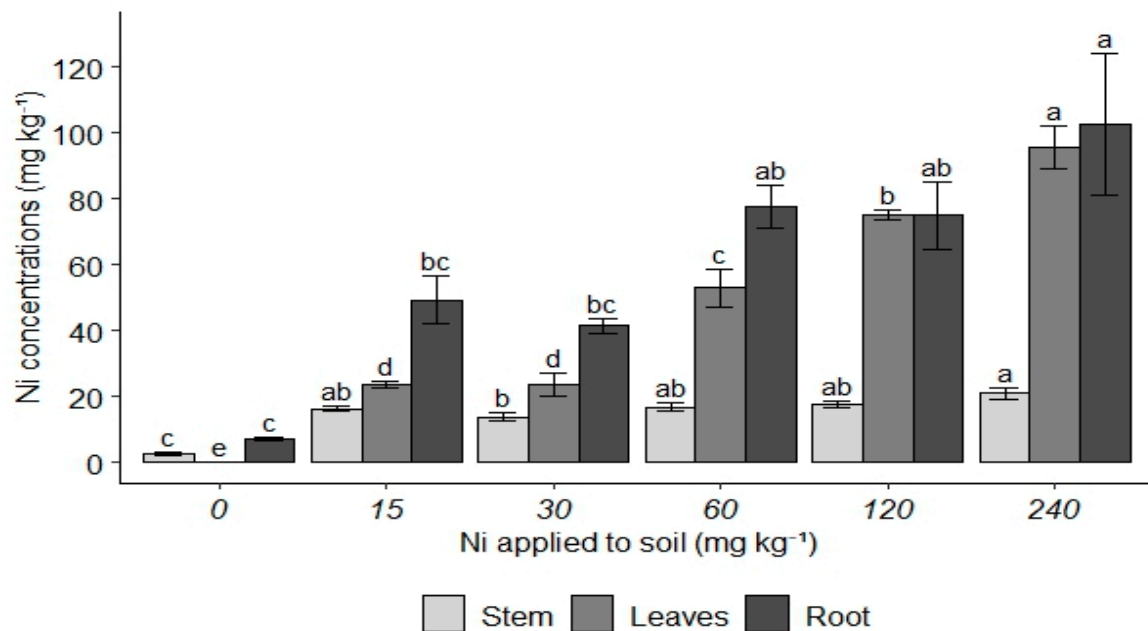


Figure 3. Ni concentration (mg kg⁻¹ of dry matter) in stems, leaves, and roots of jack bean plants at the vegetative stage grown in artificially Ni-contaminated soil at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg⁻¹ (T5). The x-axis represents the applied concentrations for each treatment, and the y-axis represents the analyzed variable. The data are plotted in three grouped columns per treatment, with the first column representing stems, the central column leaves, and the rightmost column roots. Different lowercase letters for the same plant organ indicate significant differences according to Tukey's test at 5% probability (n = 5).

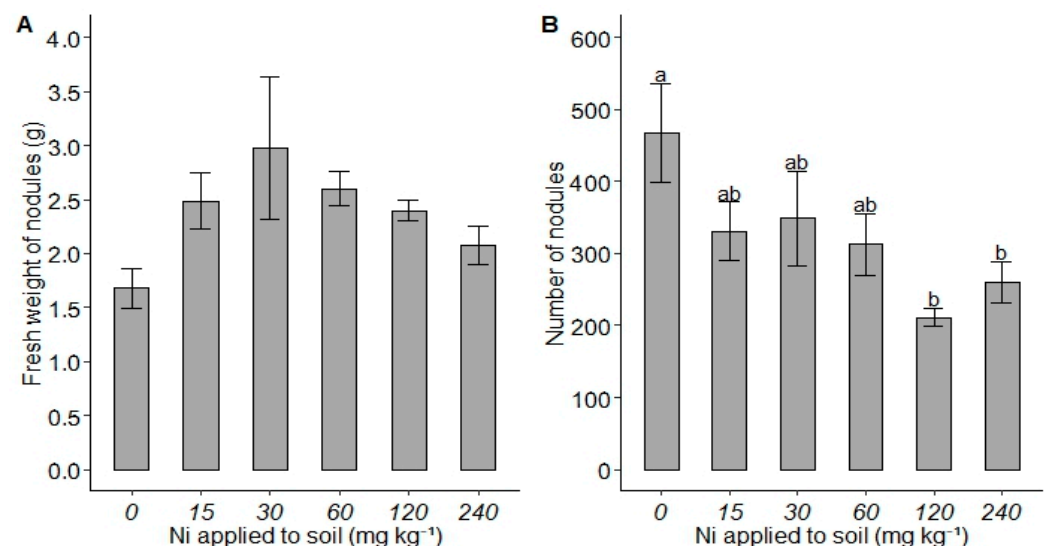


Figure 4. Nodule status: (A) Fresh weight of nodules (g) and (B) Number of nodules of jack bean plants at the vegetative stage grown in artificially Ni-contaminated soil at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg⁻¹ (T5). The x-axis represents the applied concentrations for each treatment, and the y-axis represents the analyzed variable. Different lowercase letters indicate significant differences according to Tukey's test at 5% probability (n = 5).

Although plants in T4 and T5 treatments developed fewer nodules, Figure 5A shows that fresh nodule mass remained statistically similar to T0 across all treatments. These find-

ings suggest that the remaining nodules in T4 and T5 may have undergone compensatory growth, potentially achieving larger individual sizes. Furthermore, peak fresh nodule mass occurred at T2, indicating that moderate Ni concentrations might have transiently stimulated nodule development.

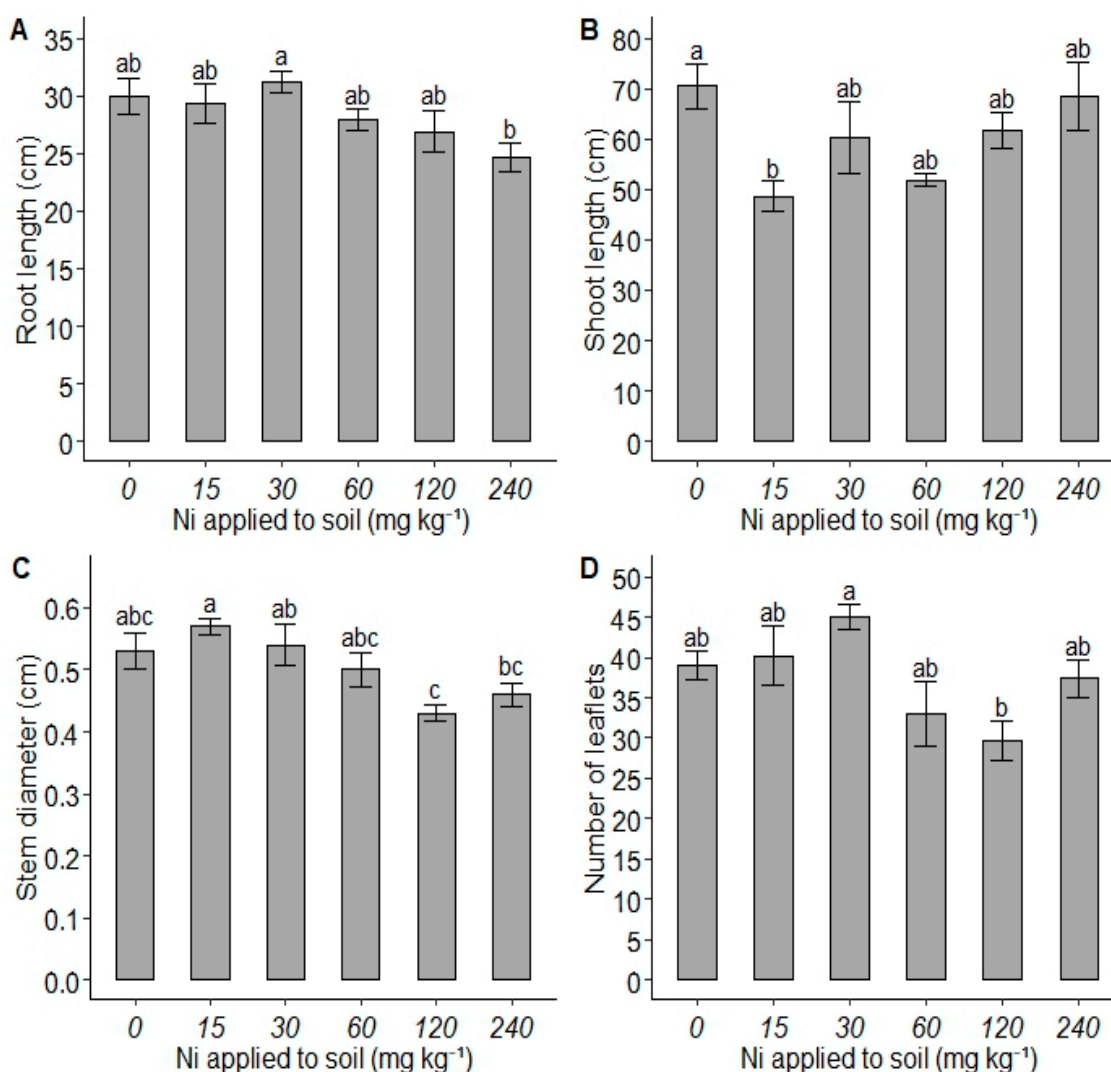


Figure 5. Growth variables: (A) Root length (cm), (B) Shoot length (cm), (C) Stem diameter (cm), and (D) Number of leaflets of jack bean plants at the vegetative stage grown in artificially Ni-contaminated soil at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg⁻¹ (T5). The x-axis represents the applied concentrations for each treatment, and the y-axis represents the analyzed variable. Different lowercase letters indicate significant differences according to Tukey's test at 5% probability ($n = 5$).

Shoot length was minimally affected by increasing Ni concentrations in soil and plant tissues, with an overall average of 60.3 cm. Both the control (T0; 70.5 cm) and highest concentration treatment (T5; 68.6 cm) showed statistically similar values, as did all other treatments when compared to either the control or the highest concentration treatment (Figure 5B). Root length was similarly unaffected by Ni presence in soil and tissues, exhibiting even less variation among treatments than shoot length (overall mean = 28.8 cm; Figure 5A). The shortest roots occurred in T5 (24.6 cm), while the smallest shoot length was observed in T1 (48.7 cm).

Stem diameter also showed minimal variation among Ni treatments, with the lowest value recorded at T4 and the highest at T1. Statistically, all other treatments were considered

similar to the control (Figure 5C). Plants in the T2 treatment produced more leaflets, whereas those in the T4 treatment produced fewer. Although variation between treatments was minimal, all averages remained statistically similar to the control (Figure 5D). Plant growth showed little response to increasing soil Ni concentrations. This was unexpected, as Ni is a potentially toxic metal, and higher tissue concentrations were anticipated to impair plant development.

Although increasing organ Ni concentrations did not significantly impair jack bean growth (Figure 4), dry mass analysis revealed moderate damage from excess Ni (Figure 6). Both root and leaf dry mass decreased linearly with higher Ni doses. Compared to T0, leaf dry mass was lower at T3, T4, and T5, while root dry mass declined at T4 and T5. In contrast, stem dry mass showed no differences across treatments. T5 reduced root and shoot dry mass by 36.8% and 25%, respectively, compared to T0.

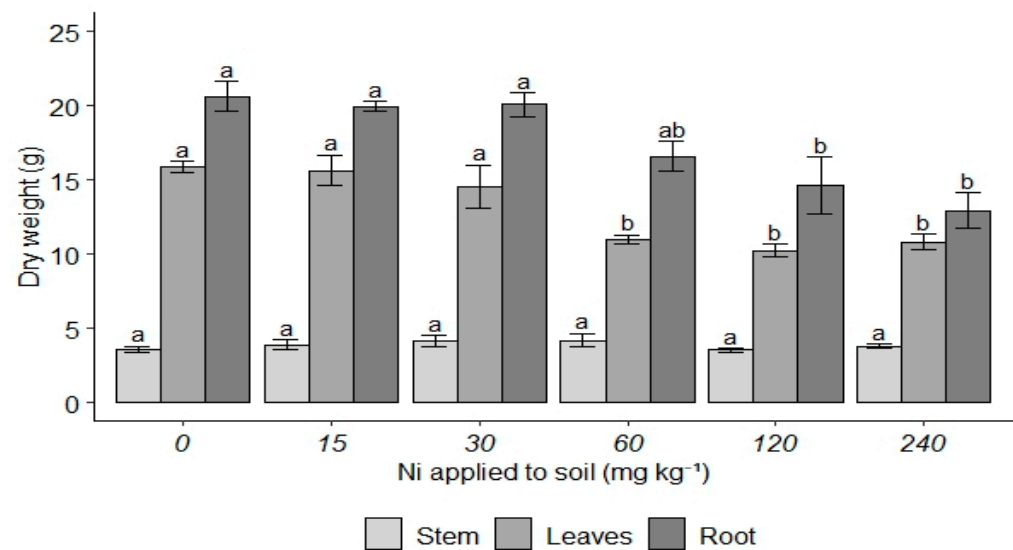


Figure 6. Dry weight of stems, leaves, and roots of jack bean plants at the vegetative stage grown in artificially Ni-contaminated soil at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg⁻¹ (T5). The *x*-axis represents the applied concentrations for each treatment, and the *y*-axis represents the analyzed variable. The data are plotted in three grouped columns per treatment, with the first column representing stems, the central column leaves, and the rightmost column roots. Different lowercase letters for the same plant organ indicate significant differences according to Tukey's test at 5% probability ($n = 5$).

Ni accumulation was lower in stems and higher in roots (Figure 7). In stems, accumulation remained low (general average 0.06 mg) due to both low Ni concentration (Figure 3) and reduced dry mass (Figure 6), though it was still significantly higher in Ni-treated plants than in controls. Root accumulation patterns indicate that this plant contains a Ni reservoir in its roots, consistently absorbing large amounts of Ni regardless of external concentration, with a general average of 1.09 mg in Ni treatments. This is supported by the statistical equivalence of Ni accumulation across all treated groups, which exhibited high root Ni concentrations unlike control plants, which showed minimal Ni absorption and accumulation.

The highest variation in Ni accumulation per organ occurred in leaves (Figure 7), with treatments forming four distinct statistical groups. T5 showed the highest isolated accumulation value (1.03 mg). While T3 and T4 also exhibited high accumulation (general average 0.68 mg), their values remained below T5. Among Ni-treated plants, T1 and T2 displayed the lowest accumulation levels (general average 0.35 mg). As expected, the control treatment (T0) showed the lowest isolated value with no Ni accumulation.

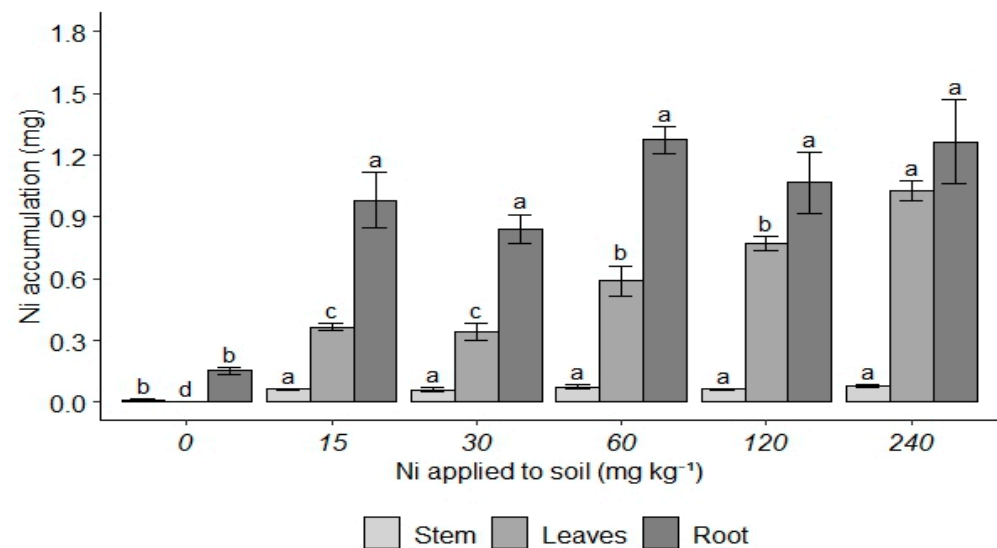


Figure 7. Ni accumulation (mg organ^{-1}) in jack bean plants at the vegetative stage grown in artificially Ni-contaminated soil at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg^{-1} (T5). The x-axis represents the applied concentrations for each treatment, and the y-axis represents the analyzed variable. Different lowercase letters for the same plant organ indicate significant differences according to Tukey's test at 5% probability ($n = 5$).

Total Ni accumulation per plant followed the same pattern observed in leaves (Figure 8A). The results show that plant Ni accumulation increased with soil concentrations, though non-linearly, suggesting potential detoxification mechanisms at intermediate doses.

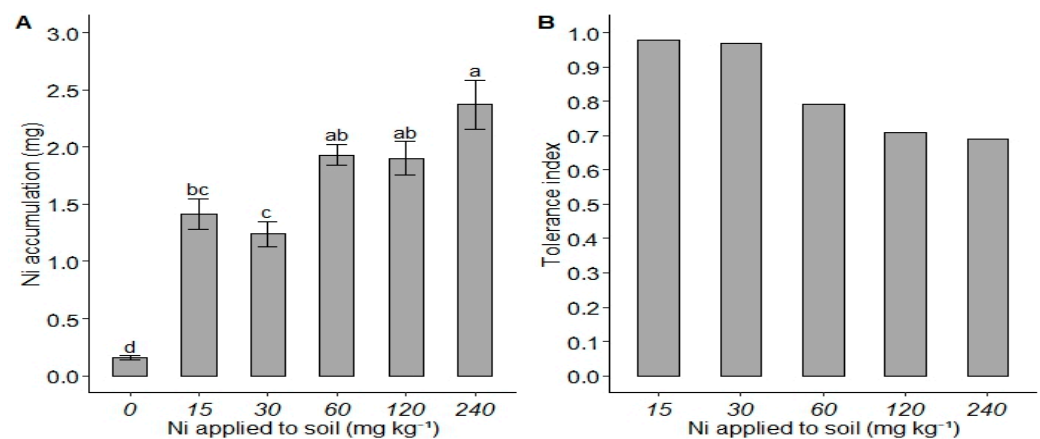


Figure 8. (A) Ni accumulation (mg plant^{-1}), and (B) Tolerance index of jack bean plants at the vegetative stage grown in artificially Ni-contaminated soil at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg^{-1} (T5). The x-axis represents the applied concentrations for each treatment, and the y-axis represents the analyzed variable. Different lowercase letters indicate significant differences according to Tukey's test at 5% probability ($n = 5$).

The tolerance index decreased under high Ni concentrations, demonstrating the metal's toxic effects on growth (Figure 8B). As the tolerance index is biomass-dependent, this reduction indicates approximately 30% lower biomass production at the highest concentration a significant yet optimal result for this study, considering most plants would exhibit severe toxicity symptoms at such elevated levels.

Figure 9 presents representative plants from each treatment, documenting phenotypic characteristics at harvest. The control plants (T0) exhibited the longest stems and had already developed visible flowers. While plants in other treatments appeared morphologi-

cally similar to each other, none showed visible signs of leaf toxicity. Notably, unlike the controls, none of the treated plants had initiated flowering by harvest (Figure 9).

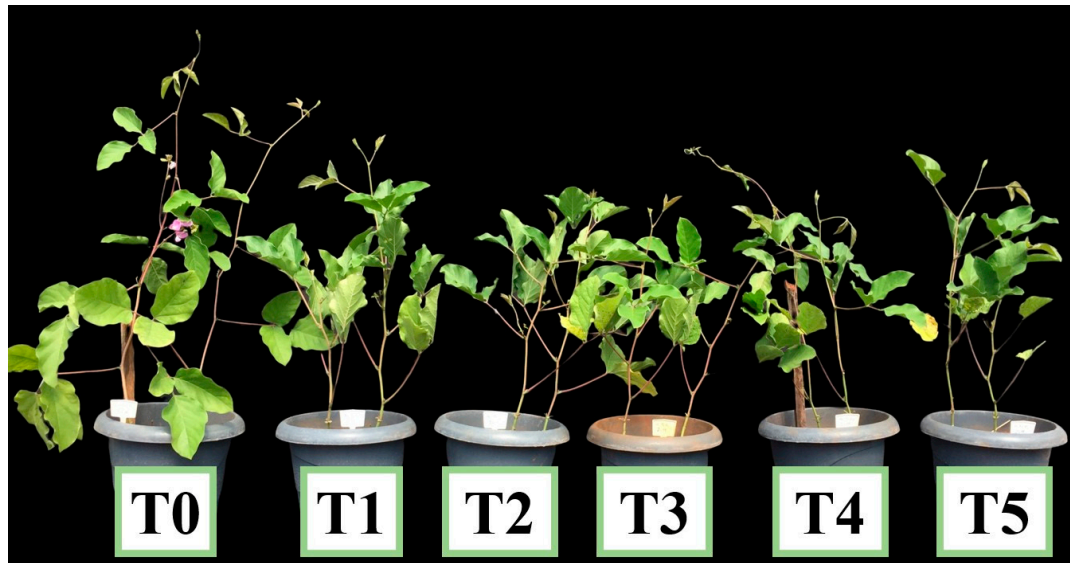


Figure 9. Representative plants from each treatment. One randomly selected plant per replicate ($n = 5$) for each treatment (T0 to T5) was arranged left to right and photographed against a black background on the final harvest day. Images depict the visual appearance of jack bean plants at the vegetative stage grown in artificially Ni-contaminated soil at concentrations of 0 (T0), 15 (T1), 30 (T2), 60 (T3), 120 (T4), and 240 mg kg^{-1} (T5).

4. Discussion

Jack bean is a highly versatile plant. Its use as a green manure has been widely encouraged due to its rapid growth rate and ability to perform BNF even in hostile environments such as exposed soils with high weed pressure, low organic matter, and low fertility [1,9,33]. In our study, the key finding lies in the high tolerance of this species to Ni under the cultivation conditions. The remarkable acclimation plasticity of this plant in response to increasing Ni availability in the soil is noteworthy. In our experiment, the plant managed to produce 70% of the control biomass (Figure 6) while containing 102.57 and 95.65 mg kg^{-1} of Ni in root and leaf tissues, respectively (Figure 3).

In most conventional crops and non-adapted plant species, similar Ni concentrations would be sufficient to cause severe metabolic collapse [23,34,35]. In cotton plants, on the treatment with 15 mg kg^{-1} , the average root content was 26.99 mg kg^{-1} while with 90 mg kg^{-1} the root Ni content was 93.41 mg kg^{-1} , under these conditions, the plant exhibited impaired photosynthetic capacity and reduced dry matter accumulation [21]. The tolerance response of jack bean to high Ni levels in its tissues is justified by the plant's efficient ability to interact with stressful abiotic agents, mitigating their deleterious effects [7,14,15,17,36,37]. The mitigation of deleterious effects could be manifested through the inhibition or delay in flowering. This phenomenon represents a strategic resource-allocation under stress, where the plant favors sustained vegetative growth and defense over reproduction. From a phytoremediation standpoint, this response offers two significant practical advantages: it extends the harvest window for maximum biomass and Ni accumulation, and it mitigates the risk of contaminant dispersion via seeds, which helps prevent the plant from becoming a weedy contaminant in the remediated area.

The growth pattern observed under Ni excess differed significantly from that seen in sensitive species, as the biometric variables did not follow a typical dose–response relationship. For instance, in soybean crops, a dose of 9.0 mg kg^{-1} was sufficient to

induce toxicity, as verified by alterations in the plant's ionome, these changes resulted in suboptimal plant development due to a significant accumulation of Ni in tissues [38]. In this context, soybean exemplifies a Ni-sensitive organism, demonstrating that this metal can be highly detrimental to plant growth even at relatively low concentrations.

This absence of a linear typical dose–response does not indicate that development proceeded normally in the presence of the metal. On the contrary, the reduction in dry mass accumulation observed in Ni-treated plants demonstrates that, despite metabolic tolerance, the imposed stress affected plant growth a fact directly reflected in the plant's tolerance index. The mere 30% reduction in the tolerance index indicates that the plant mobilized efficient metabolic acclimation mechanisms, enabling the maintenance of homeostatic balance and sustaining growth even under high Ni concentrations in tissues.

In a previous study with jack bean, the presence of Ni during seed germination enhanced the process when seeds were germinated in soil containing 19 to 33 mg kg^{−1} of bioavailable Ni compared to the control. However, seedling emergence was impaired at a concentration of 74.15 mg kg^{−1}, resulting in a 21% reduction in emergence rate relative to the Ni-free treatment [7]. Although this study provides a valuable comparative baseline, it should be noted that the bioavailable Ni concentrations reported were substantially lower than those observed in our investigation. Furthermore, due to the short evaluation period in the earlier study, more comprehensive comparisons remain limited. The enhanced nitrogen metabolism observed in the presence of low nickel concentrations during germination suggests that this plant may utilize Ni to mobilize seed reserves, potentially through increased demand for urease activity during this critical physiological process [39,40].

The organ-specific accumulation pattern reveals distinctive biometric characteristics indicative of specialized biochemical strategies for metal management. Evidence suggests this species employs unique mechanisms for metal absorption, chelation, and transport, potentially involving metabolic pathways such as phytochelatin synthesis, vacuolar compartmentalization, and differential expression of metal transporters [41]. These metabolic adaptations not only confer tolerance to metal stress but also enhance extraction efficiency for soil contaminants, positioning this plant as a promising model for phytoremediation applications.

Studies suggest that legumes detoxify heavy metals primarily through two mechanisms: chelation by organic acids (such as citrate and malate) and sequestration, which are common strategies across plant families [42,43]. Furthermore, based on the unique metabolic profile of *Canavalia ensiformis*, we propose a potential involvement of canavanine—a key non-protein amino acid—in the overall Ni detoxification or tolerance process, possibly acting as a secondary chelating agent or defense metabolite [44]. However, detailed investigation and validation of organic acid chelation or specific protein expression (e.g., canavalin) were beyond the scope of this study, which focused on biomass production and accumulation capacity under in a greenhouse setting.

We hypothesize that tropical legumes adapted to hostile environments possess intrinsic physiological traits that enhance their capacity to tolerate and acclimate to abiotic stresses [19,45–48]. In our study, nodulation was significantly affected by high Ni availability. However, this effect cannot be categorically classified as negative or positive: while plants exposed to elevated Ni concentrations developed fewer nodules, these structures exhibited greater individual mass. This morphophysiological compensation suggests an adaptive response to metal stress, wherein the plant prioritizes nodule quality over quantity, potentially to maintain nitrogen fixation efficiency under adverse conditions [49].

The observed reduction in dry biomass leads us to hypothesize that if nodule quantity is indeed linked to nitrogen acquisition efficiency, the decrease in biomass may be a secondary response to impaired functioning of the nitrogen-supplying structures. Since

reduced growth is a common plant response to nitrogen limitation, further analysis is required to determine whether the compensatory nodulation response observed here ultimately constrains nitrogen flux to plant growth tissues.

It is well-established that nickel hyperaccumulator plant species are prime candidates for bioremediation research and applications [10,50]. While metal accumulation mechanisms may share similarities between hyperaccumulators and non-hyperaccumulators, the magnitude of tissue concentration remains undeniably higher in the former [51]. Here, we demonstrate that a non-hyperaccumulator organism can tolerate high tissue Ni levels even without additives that enhance such traits. This finding opens diverse approach possibilities for enabling this plant's use in phytoremediation applications, as illustrated in Figure 10.

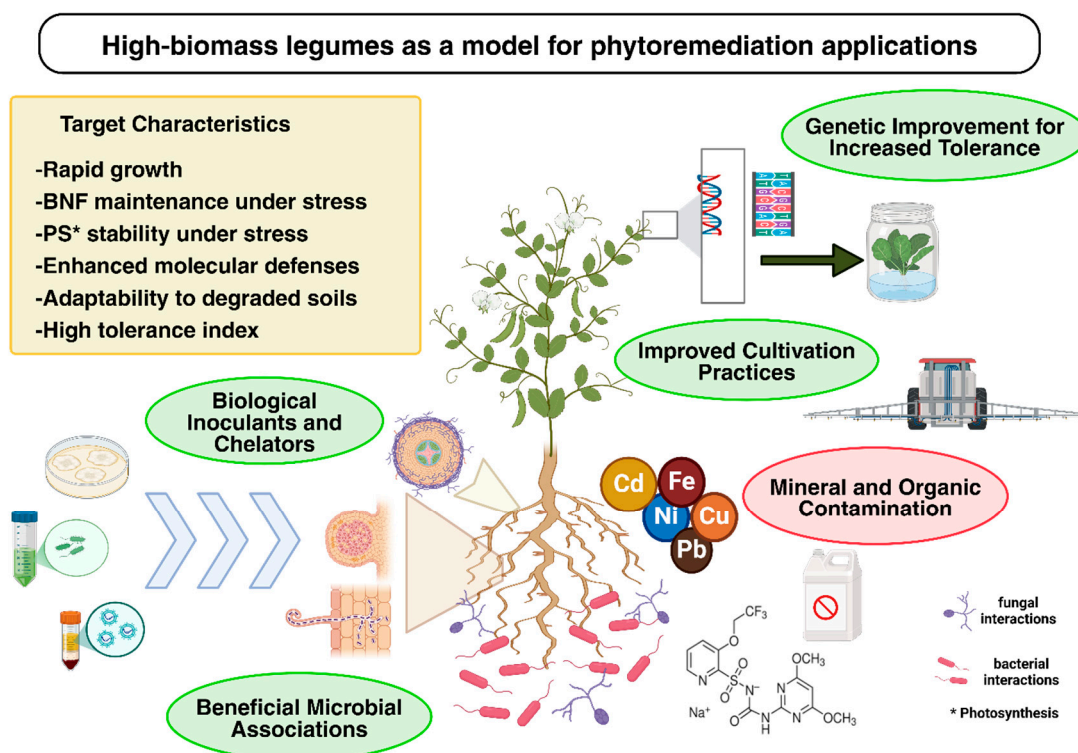


Figure 10. Jack bean as a Model Legume for Phytoremediation: Potential and Perspectives: Similarly to other high-biomass legumes reported in the literature, jack bean shows significant potential for remediating soils contaminated with both organic and inorganic pollutants. Key strategies to enhance its phytoremediation and phytomanagement capacity include: optimizing root-microbial interactions, applying chelating agents and biological inoculants, and employing genetic and phytotechnical improvements. * PS = Photosynthesis. Created in BioRender. Aguilar, J. (2025) <https://BioRender.com/ie13s2s> (accessed on 20 October 2025).

Hyperaccumulator plants often exhibit slow growth and low biomass production. In contrast, high-biomass plants capable of tolerating elevated levels of metals or organic contaminants grow rapidly and accumulate significant biomass per life cycle [11,52]. Contemporary research focuses on enhancing phytoremediation systems through bioinputs such as microbial inoculants and chelating agents to improve plant tolerance and contaminant uptake efficiency [22,53–55]. This approach is grounded in the understanding that rhizosphere biology plays a fundamental role in mitigating metal-induced abiotic stress [56,57].

Additionally, strategies involving polycultures of hyperaccumulator and non-hyperaccumulator species are being developed to operationalize phytoremediation [58]. These agroecosystems aim to optimize seed production, management practices, and field-scale applicability. While the current findings establish *C. ensiformis* as a highly tolerant

and effective nickel (Ni) accumulator, further research is warranted on its long-term viability and the precise cellular and molecular mechanisms of Ni uptake to fully optimize its use in these large-scale remediation programs. Genetic engineering further complements these efforts by enabling the development of plants with enhanced metal tolerance and accumulation traits.

5. Conclusions

The biometric growth measures exhibited limited variation, with only a 30% reduction in dry mass production observed even at the highest Ni concentration. This result demonstrates a notably high level of metal tolerance, sustained even under elevated Ni exposure.

BNF was maintained across all treatments. In response to increasing Ni concentration, a compensatory nodulation response was observed: while the nodule number decreased, individual nodule mass increased. This adaptive mechanism indicates a high functional tolerance of the symbiotic system under metal-induced stress conditions.

Canavalia ensiformis (L.) DC. demonstrated a pronounced capacity to withstand high levels of bioavailable soil Ni, effectively absorbing and accumulating the metal in all evaluated organs. A clear concentration–response pattern was evident in roots and leaves. Tissue Ni concentrations at the highest treatment level significantly exceeded the toxicity thresholds reported for non-accumulator species, with accumulation reaching 2.37 mg Ni per plant in soil containing 277.8 mg kg^{−1} of Ni.

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