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Research Article

Emergence and N metabolism of *Canavalia ensiformis* (L.) DC. seedlings in soil contaminated by nickel

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Abstract

Heavy metals such as nickel (Ni) can lead to bioaccumulation, affecting entire ecosystems and posing significant risks to various life forms, including plants. Although Ni is a micronutrient, it can be toxic by impacting enzyme activities and inhibiting seed germination and plant growth. In Brazil, official guidelines stipulate preventive and intervention values for Ni concentrations in soil to mitigate pollution and protect soil and groundwater quality. Soil samples classified as Typic Haplustox were artificially contaminated with $NiCl_2$ at concentrations of 120 (T120), 240 (T240), and 360 (T360) mg dm^{-3}, alongside a control treatment (T0). Seeds were cultivated under greenhouse conditions, and germination and growth parameters were analyzed after 15 days. Measurements included emergence speed index, germination percentage, root and shoot length, fresh and dry mass, and biochemical analyses of nitrogenous compounds and sugars. This study addresses the effects of toxic concentrations of $NiCl_2$ on plants, focusing on the germination and early growth stages. *Canavalia ensiformis* (L.) DC., a tropical legume with significant roles in green manure and phytoremediation, was chosen for its adaptability to various soils. The hypothesis is that *C. ensiformis* can withstand high soil Ni concentrations, maintaining growth despite environmental toxicity limits. The results indicated differential impacts of Ni, the emergence percentage decreases at 360 mg dm^{-3} soil with greater dry mass accumulation at 120 and 240 mg dm^{-3}, highlighting the importance of understanding plant responses to stress from potentially toxic elements for sustainable agricultural practices and environmental management.

Keywords: Fabaceae; germination; heavy metal; metabolism; micronutrient; potentially toxic element

Introduction

The presence of metals such as nickel, cobalt, cadmium, and copper in soil and water can cause bioaccumulation, affecting the entire ecosystem and negatively affecting the health of various life forms,

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including plants (Pokorska-Niewiada *et al.*, 2018). Nickel (Ni), despite being considered a micronutrient, is reported as toxic to most species at concentrations between 10–100 mg kg^{-1} in leaf tissue, varying between plant species and cultivars (Kabata-Pendias, 2010), besides affecting the activity of amylase, protease, and ribonuclease enzymes, thus delaying seed germination, reducing plant height, root length, fresh and dry weight, and increasing electrolyte leakage (Sethy and Ghosh, 2013).

For Ni, the prevention value (the ability of the soil to sustain its primary functions) is 30 mg kg^{-1} soil dry weight, and the intervention value (it is the concentration of a given substance in the soil above which there are potential direct or indirect risks to human health, considered a generic exposure scenario) for agriculture is 190 mg kg^{-1} soil dry weight (CETESB, 2021).

Legumes are critical to ecosystems, agriculture, agroforestry, industry, and low-nitrogen environments due to their symbiosis with rhizobia (Hasanuzzaman *et al.*, 2020). In this context, *Canavalia ensiformis* (L.) DC. is a legume with a wide tropical distribution and rustic characteristics. It has an annual or biannual cycle, with productivity ranging from 20 to 40 tons of green mass and 4 to 8 tons of dry mass per cycle. The species adapts to any type of soil, tolerates partial shade (Formentini, 2008), and is also used for green manure (Mangaravite *et al.*, 2023; Servín Niz *et al.*, 2023). Additionally, it has the potential for phytoremediation (Rangel *et al.*, 2023; Mendes *et al.*, 2024), and can be considered an orphan crop—also known as an indigenous or neglected crop—a category referring to plants that are not widely traded globally (Tadele, 2019; Dwyer *et al.*, 2022).

In this context, *C. ensiformis* was chosen for the study as a model of response to different concentrations of Ni in the soil for quantifying nitrogen compounds, total soluble sugars, seedling emergence speed, and growth measurements to understand the toxicity each availability may cause to the plants.

This study aims to address the knowledge gap regarding the effects of potentially toxic elements like nickel on plants, particularly from germination to the seedling stage, by analyzing changes in carbon and nitrogen metabolism and early growth. The hypothesis is that varying concentrations of Ni will impact plant growth and metabolic distribution.

Materials and Methods

Soil classification and analysis

The soil used was Typic Haplustox (Oxisol) according to the USDA classification (Soil Survey Staff, 2022), it was collected from the experimental area of the "Fazenda de Ensino, Pesquisa e Extensão" (FEPE), in the vegetable production sector, in Selvíria, Mato Grosso do Sul — Brazil (20° 20' 24.9" S 51° 24' 19.7" W) and in the soil granulometric analysis (Teixeira *et al.*, 2017), the following proportions were verified: 130 g.kg^{-1} of clay, 868 g.$^{-1}$ of sand and 2 g.kg $^{-1}$ of silt, in the chemical attributes of the soil, the following values were obtained (Raij *et al.*, 2001): pH = 5.4 (determined with $CaCl_2$ 0.01M); Organic matter =13.0 g dm^{-3}; P = 6.0 mg dm^{-3} (resin); K = 1.0; Ca = 7 and Mg = 8.0 mmol c kg^{-1} (resin); B = 0.03 mg dm^{-3} (warm water); Cu = 0.3 mg dm^{-3}; Fe = 8 mg dm^{-3}; Mn = 2.8 mg dm^{-3} and Zn = 0.2 mg dm^{-3} (DTPA); potential acidity 12.0 mmol $_c$ dm^{-3} (SMP buffer); Al = 0.0 mmol$_c$ dm^{-3}; the sum of the bases 18 mmol$_c$ dm^{-3}; cation exchange capacity 30 mmol$_c$ dm^{-3} and base saturation 60%.

Ni contamination and quantification in soil

The soil was artificially contaminated with nickel (II) chloride hexahydrate ($NiCl_2 \bullet 6\ H_2O$), thus containing the control treatments and potentially toxic concentrations (120, 240, and 360 mg kg^{-1} dry weight of soil). The contamination was made from a stock solution and added to 3L of soil in each treatment. It was subsequently stabilized for approximately 30 days, and every 7 days, it was stirred to homogenize the contaminant.

To extract the total nickel content in the soil, the US-EPA method (US EPA, 2015a, 2015b), SW-846-3051A, was used, using soil samples (n=2) collected in each treatment repetition to evaluate the Ni availability. The reading was conducted using ICP-OES, optical emission spectrometry with inductively coupled plasma.

Growing conditions

The experiment was conducted over 15 days in a greenhouse located in the municipality of Ilha Solteira, São Paulo — Brazil (20° 25' 58 "S 51° 20' 33" W), with manual irrigation twice a day and under a completely randomized design, containing 4 treatments x 4 replications, totalling 16 experimental units. *C. ensiformis* seeds were obtained commercially and placed in seedbeds (Propylene Boxes for Seedlings), with 200 cells being separated into 50 cells per repetition.

Cultivation period and emergence analysis

During the initial development of the seedlings, the influence of the metal on the emergence speed was evaluated using the emergence speed index *(Maguire, 1962)* according to the equation below:

$$ESI = \frac{n1}{D1} + \frac{n2}{D2} + \cdots + \frac{nn}{Dn} \tag{1}$$

Where "n" represents the number of normal seedlings counted, and "D" is the number of days after sowing.

The final emergence count was calculated as a percentage of normal seedlings per treatment using the equation:

$$EP(\%) = \frac{(100*n)}{50} \tag{2}$$

Where "n" is the total number of seedlings that emerged from 50 seeds sown.

Experiment collection

After 15 days of cultivation, eight normal seedlings that emerged during the counting period were randomly selected in each replication, with four seedlings intended for measuring fresh mass (FM) and four seedlings for obtaining dry mass (DM) data.

Growth measurements were conducted according to Hunt and Benincasa (Benincasa, 1988; Hunt, 1990), obtaining the total length (TL) (root + shoot) with a millimeter ruler; subsequently, the seedlings were frozen in a -20 °C freezer for analysis of nitrogenous compounds and carbohydrates. The remaining plants were placed in a closed circulation oven at 60 °C for 72 hours (Raij *et al.*, 2001) to obtain the total dry mass (TDM).

Analysis of nitrogenous compounds

To extract total soluble compounds (Bieleski and Turner, 1966), 0.500 g of fresh mass was used for 5 mL of MCW (60% methanol, 25% chloroform, and 15% distilled water).

To determine amino acids (Yemm *et al.*, 1955), 10 µL of aliquot (extraction of total soluble compounds) was used in 990 µL of distilled water (H_2O) and subsequently added 500µL of 0.2M citrate buffer pH 5.0, 200 µL of ninhydrin in 5% methyl glycol, 1 mL of potassium cyanide (KCN) 0.0002M, and heated at 100 °C for 20 minutes, cooled for 10 minutes and added 1 mL of ethyl alcohol or 60% ethanol. The reading took place on a spectrophotometer at $\lambda = 570$ nm.

To determine proteins (Bradford, 1976), the MCW precipitate was used and 5 mL of 0.1M sodium hydroxide (NaOH) was added, and centrifuged at 8500 for 15 minutes. The supernatant was used to determine proteins, being the leaf and root organs, using 50 µL of aliquot and 50 µL of 0.1M NaOH for 5 mL of Bradford, in the cotyledon, 30 µL of aliquot and 70 µL of 0.1M NaOH for 5 mL from Bradford. The reading took place on a spectrophotometer at $\lambda = 595$ nm.

To determine total soluble sugars (Umbreit *et al.*, 1945), 200 µL of aliquot and 800 µL of distilled water were used in the cotyledons, while 500 µL of aliquot and 500 µL of distilled water were used in the leaf and root. After adding the sample, 1 mL of anthrone reagent was added to all tests, then stirred and heated in a water bath at 100 °C for 3 minutes, and then cooled to room temperature. The reading took place on a spectrophotometer at $\lambda = 660$ nm.

Statistical analysis

To analyze the independent variables, an analysis of variance (one-way ANOVA) was performed using the F test ($p < 0.05$). When the results were significant, the data were subjected to Tukey's *post-hoc* test ($p < 0.05$). The packages used for statistics and data organization were ExpDes.pt (Ferreira *et al.*, 2021), plyr (Wickham, 2008), dplyr (Wickham *et al.*, 2014) and the packages for building graphs were ggplot2 (Wickham *et al.*, 2023) and ggpubr (Kassambara, 2023). For Pearson correlation, the corrplot package (Wei and Simko, 2010) was used.

Statistical analysis was conducted using protocols developed in the software R version 4.4.1 (2024-06-14) - "Race for Your Life" (R Core Team, 2022) with the Integrated Development Environment (IDE) RStudio (Posit team, 2023).

Results

Ni availability in soil

Analysis of available nickel (Ni) concentrations in the soil, performed using the US-EPA method, was conducted before sowing (Table 1). The minimum value observed was less than 3.2 mg kg^{-1} at T0, while the maximum value was 74.15 mg kg^{-1} at T360. Ni values were presented without comparing means since the main objective is to demonstrate the presence of the element in the soil.

Table 1. Value of nickel available in the soil before the experiment

Source	Treatment	Ni concentration (mg kg^{-1})
$NiCl_2$	0 mg dm^{-3}(T0)	<3.2
	120 mg dm^{-3} (T120)	19.25
	240 mg dm^{-3}(T240)	33.05
	360 mg dm^{-3} (T360)	74.15

Seedling emergence and biometrics

EP (Figure 1A) did not differ significantly between treatments T0, T120, and T240, presenting 74%, 72%, and 70%, respectively. However, the T360 treatment exhibited the lowest emergence percentage, at 53%.

ESI (Figure 1A) was higher at T0, T120, and T240 (3.2) compared to T360 (1.7) (Figure 1E).

Concerning TL (Figure 1C), treatments T120 and T240 presented means of 22.6 cm and 20.8 cm, respectively, being significantly larger than T360 and T0, with a total length of 14.5 cm and 15.5 cm (Figure 1F).

The TDM (Figure 1D) of treatments T120 and T240, with 3.1 g and 3.2 g, respectively, was higher than treatments T0 and T360, which presented 2.2 g and 2.6 g.

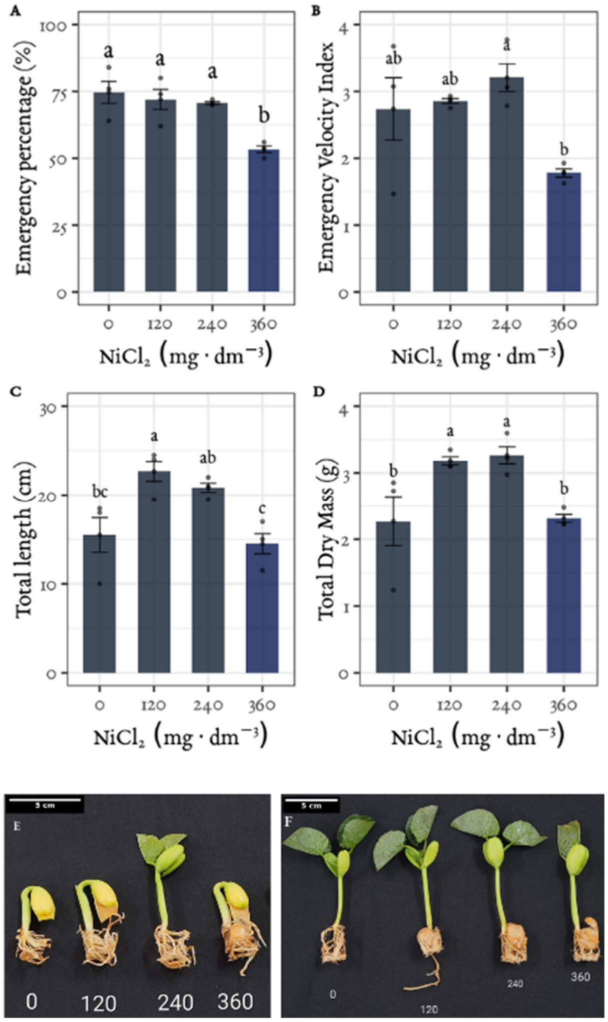


Figure 1. Initial growth of *C. ensiformis*, 15 days after planting in different concentrations of Ni in the form of $NiCl_2$. (A) Percentage of emergence and (B) emergence speed index from the 4th to the 8th days of *C. ensiformis* in different concentrations of $NiCl_2$. (C) Total length and (D) total dry mass. (E) *C. ensiformis* seedlings on the 8th day and (F) the 15th day after the start of the experiment

The seedlings were randomly selected for photography. Columns represent the mean value of the data, error bars represent standard error, and dots represent individual values. Significant differences ($p < 0.05$) were indicated with different letters between treatments (n=16).

Effect of Ni on carbohydrate and nitrogen metabolism

For total sugars and proteins (Figures 2A and 2B), there was no significant difference between treatments in cotyledons, roots, and leaves.

In the quantification of amino acids (Figure 2C), there was no significant difference between treatments in cotyledons and roots. However, the highest values were observed for leaves in treatments T0 and T360, while the lowest values were recorded in treatments T120 and T240.

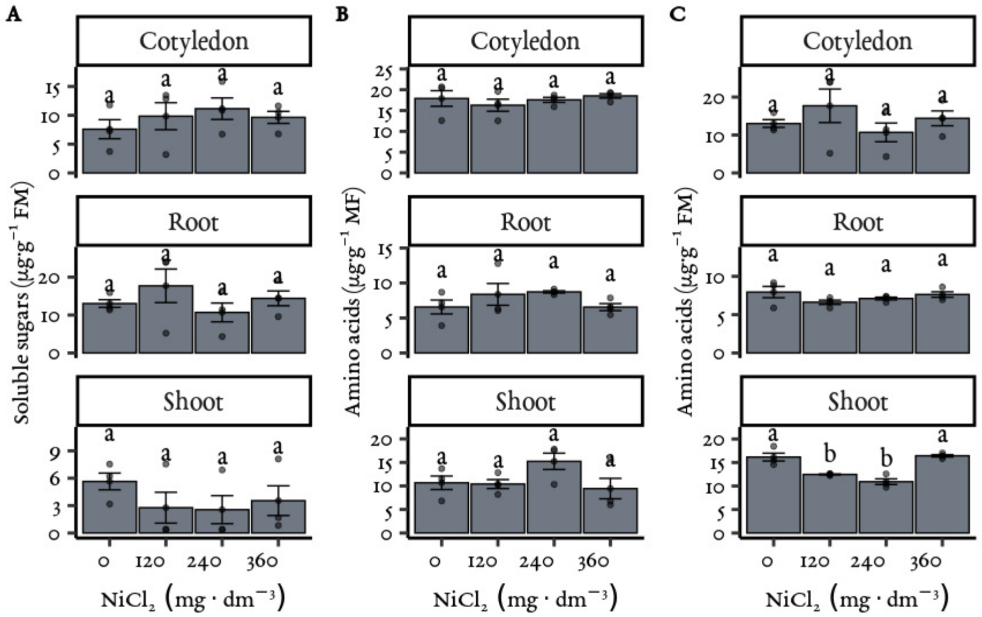


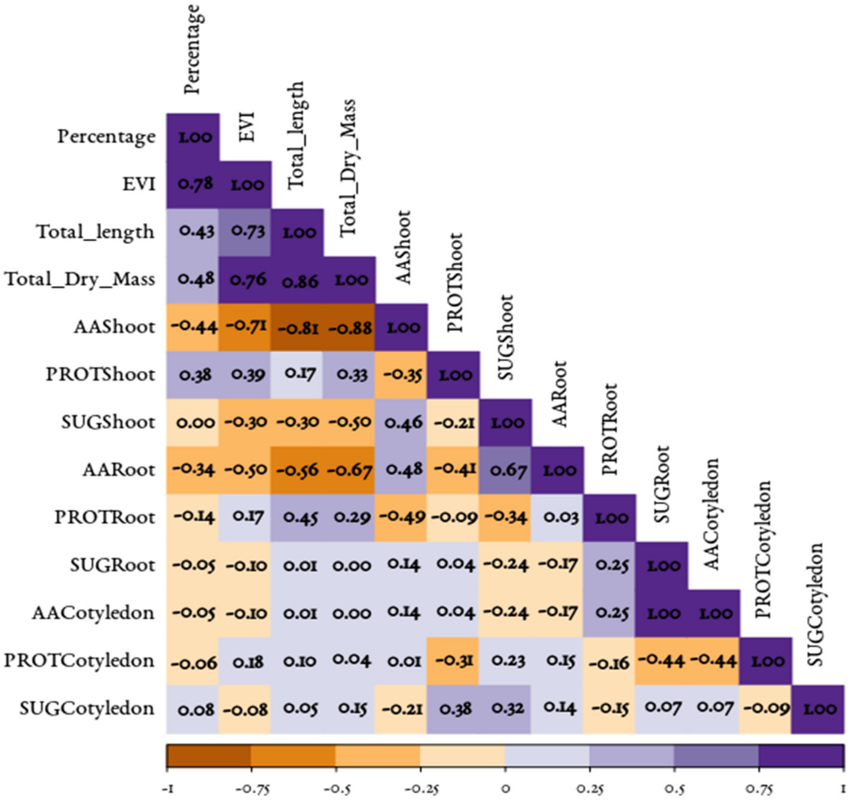
Figure 2. Quantification of compounds in leaves, roots, and cotyledons after 15 days of *C. ensiformis* in different concentrations of Ni in the form of $NiCl_2$. (A) Quantification of total soluble sugars, (B) total soluble proteins, and (C) total soluble amino acids

Columns represent the mean value of the data, error bars represent standard error, and dots represent individual values. Significant differences ($p < 0.05$) were indicated with different letters between treatments (n=16).

Correlation between emergence, biometrics, carbohydrate, and nitrogen metabolism

As shown in Figure 3, EP, ESI, TL, and TDM showed strong positive correlations with each other. These variables indicated that Ni in high concentrations, as a contaminant, negatively affects the initial stage of emergence, reducing the capacity to convert reserves, which is fundamental to accumulating plant biomass.

Furthermore, TDM and TL showed a strong negative correlation with the amount of amino acids in leaves, and a moderate correlation was observed in the roots (Figure 3). These correlations suggest that, relative to nickel, the lower amount of amino acids is associated with greater plant growth.



	Percentage	EVI	Total_length	Total_Dry_Mass	AAShoot	PROTShoot	SUGShoot	AARoot	PROTRoot	SUGRoot	AACotyledon	PROTCotyledon	SUGCotyledon
Percentage	1.00												
EVI	0.78	1.00											
Total_length	0.43	0.73	1.00										
Total_Dry_Mass	0.48	0.76	0.86	1.00									
AAShoot	-0.44	-0.71	-0.81	-0.88	1.00								
PROTShoot	0.38	0.39	0.17	0.33	-0.35	1.00							
SUGShoot	0.00	-0.30	-0.30	-0.50	0.46	-0.21	1.00						
AARoot	-0.34	-0.50	-0.56	-0.67	0.48	-0.41	0.67	1.00					
PROTRoot	-0.14	0.17	0.45	0.29	-0.49	-0.09	-0.34	0.03	1.00				
SUGRoot	-0.05	-0.10	0.01	0.00	0.14	0.04	-0.24	-0.17	0.25	1.00			
AACotyledon	-0.05	-0.10	0.01	0.00	0.14	0.04	-0.24	-0.17	0.25	1.00	1.00		
PROTCotyledon	-0.06	0.18	0.10	0.04	0.01	-0.31	0.23	0.15	-0.16	-0.44	-0.44	1.00	
SUGCotyledon	0.08	-0.08	0.05	0.15	-0.21	0.38	0.32	0.14	-0.15	0.07	0.07	-0.09	1.00

Figure 3. Pearson correlation

The intensity and size of the circles relate to the degree of relationship between two variables (positive, purple, negative, brown). Classification of coefficients: r = 0.10 to 0.30 (weak); r = 0.40 to 0.6 (moderate); r = 0.70 to 1 (strong) (Dancey and Reidy, 2005). Abbreviations: ESI - emergency speed index; AA - amino acids; PROT - Protein; SUG - Soluble Sugar.

Discussion

The role of each trace element (TE) in plants can be characterized relative to several fundamental processes, such as absorption and transport within the plant, as well as their concentrations and forms of occurrence, in addition to ionic competition and interaction, and the concentrations of TEs are associated with the chemical composition of the growth medium. Therefore, these aspects should always be investigated for a given soil-plant system (Kabata-Pendias, 2010). In soils, nickel can be available in the form of soluble compounds (chlorides) or insoluble compounds (oxides), with soluble Ni compounds having greater mobility than insoluble Ni compounds in the soil, and this mobility of the element directly interferes with the amount of metal absorbed by plants (Nie *et al.,* 2015).

The data presented indicate that nickel, from a concentration of 74.1 mg kg^{-1} (T360), negatively influences the emergence of *C. ensiformis* seedlings. Initial growth can be influenced by physiological changes in plant metabolism since, in the cotyledon, reserve substances such as carbohydrates, proteins, and lipids are mobilized during the germination process so that during seedling development, they are degraded and used to obtain energy and produce metabolic precursors (Corte *et al.*, 2006).

The observed effects of Ni on the growth and metabolic processes of *C. ensiformis* seedlings highlight the complex interactions between Ni exposure and plant development. The seedling emergence and seedling emergence speed index were significantly reduced at higher concentrations of Ni, particularly in the T360 treatment, which had the highest concentration of available Ni. These results suggest that excessive Ni impairs seedling establishment by disrupting processes related to seed reserve mobilization and energy conversion, critical for early plant growth.

Nickel did not alter the amount of carbohydrates and proteins in *C. ensiformis* seedlings during the evaluated growth period, while the amount of amino acids in the leaves varied between treatments. This result can be explained for T360; with the delay of emergence, this amino acid pool was larger than T240 since arginine (Arg) is a nitrogen reservoir in seeds, and after the breakdown of storage proteins, Arg levels increase within the free amino acid pool, which provides nutrition for seed germination (Kawade *et al.*, 2023). In *Gossypium hirsutum* L., a similar result was observed at higher doses of Ni applied, where seedlings accumulated amino acids in the shoot, and the higher values of soluble amino acids may also be associated with lower values of fresh biomass, which seems to indicate difficulty in using reserve carbon to synthesize new tissues (Aguilar *et al.*, 2023).

In terms of nitrogen and carbohydrate metabolism, our findings indicate that Ni exposure did not significantly alter total sugars and proteins in the cotyledons, roots, and leaves of seedlings across different treatments. However, significant differences in amino acid concentrations were observed, particularly in the leaves, with T0 and T360 showing higher amino acid levels compared to T120 and T240. This variation could be linked to the plant's response to Ni toxicity, where excessive Ni may stimulate amino acid production in an attempt to detoxify the metal or protect against oxidative stress. Conversely, lower amino acid levels in the T120 and T240 treatments may indicate a less pronounced stress response at intermediate Ni concentrations, allowing for more efficient use of resources for growth.

Additionally, treatments T120 and T240, with available nickel concentrations of 19.2 mg kg^{-1} and 33.0 mg kg^{-1}, respectively, showed positive effects on seedling growth, indicating that nickel, an essential micronutrient, can help in these variables. Furthermore, research on Ni fertilization also needs to be considered, for example, the application of Micro-Ni $(OH)_2$ and Macro-$NiSO_4$ may represent a viable alternative to the treatment of *Glycine max* (L.) seeds. Merr., with Ni sources with a positive impact on the initial development of seedlings, increasing the germination rate, root length, and Ni distribution in the tissues (Oliveira *et al.,* 2022).

The main role of urease in seeds is to process stored N, and arginine is often the most abundant form of stored N in seeds, being degraded to urea by arginase, which is abundant in the cotyledons of some plants, especially legumes (Tsadilas *et al.*, 2019). In general, the presence of TE reduces plant yield and production, impacting health levels and the economy, making it imperative to manage their use, release, and distribution in the environment, developing environmental agencies and government bodies so that necessary measures are taken to ensure that pollution is minimized (Majhi and Sikdar, 2023).

The strong correlations between the percentage of emergence, emergence speed index, total length, and total dry mass emphasize that high concentrations of nickel, especially at T360, negatively impact early plant growth by disrupting essential metabolic processes. Furthermore, the negative correlation between total dry mass, total length, and amino acid content in leaves suggests that nickel-induced inhibition of amino acid metabolism may be associated with reduced growth, as amino acids are fundamental for protein synthesis and overall plant development. These findings underscore the complex mechanisms by which nickel interferes with plant growth, possibly through direct toxicity or alterations in metabolic pathways essential for normal development.

Although Ni application is an emerging practice in modern agriculture, determining the ideal method is crucial to ensure adequate absorption and utilization of this micronutrient, resulting in a viable and sustainable management technology, and research is essential to establish official guidelines for Ni application in agricultural practices (Rodak *et al.*, 2024). In this sense, understanding plant responses in association with Ni from the emergence process to the harvest phase has fundamental applications for reducing pollution and the negative effects that the metal can cause when excessively used (Adriano, 2001; Kabata-Pendias and Szteke, 2015; Tsadilas *et al.,* 2019; Prado, 2021; Aguilar *et al*., 2023). This research reinforces the environmental importance of studies that demonstrate the responses of different species from germination to understand how Ni can affect plant growth.

Understanding the mechanistic pathways of nickel metabolism in leguminous plants requires further investigation into the roles of key enzymes such as nickel transporters, metallothioneins, and phytochelatins, which are critical for metal homeostasis and detoxification. Future studies exploring these molecular mechanisms in *C. ensiformis* could offer valuable insights into its responses to nickel stress and contribute to the development of effective strategies for mitigating nickel toxicity in leguminous crops.

Conclusions

The emergence percentage of *Canavalia ensiformis* (L.) DC. decreases at 360 mg Ni dm^{-3} soil in the form of $NiCl_2$, and the maximum capacity to maintain seedling growth is at 120 mg Ni dm^{-3}. The greatest accumulation of dry mass in seedlings occurs at 120 and 240 mg Ni dm^{-3}, in contrast, it negatively affects the amino acid pool in the leaves at these concentrations.

The species sustains initial growth in Ni concentrations considered harmful to the environment by Brazilian legislation.

Authors' Contributions

Conceptualization, L.S.C. and T.C.F.; methodology, G.S.R., T.C.F., F.A.A.M., A.R.C.; formal analysis, T.C.F., F.A.A.M., A.R.C.; investigation, G.S.R., T.C.F., M.L.G.O.; data curation, G.S.R., T.C.F., M.L.G.O.; writing—original draft preparation, G.S.R., T.C.F., M.L.G.O.; writing—review and editing, M.L.G.O., F.A.A.M., L.S.C.; supervision, L.S.C.; project administration, L.S.C.; funding acquisition, L.S.C

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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