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Scientific Article

Effects of iron and copper on emergence and physiology of *Canavalia ensiformis* (l.) DC

Efeito do ferro e cobre na emergência e fisiologia de *Canavalia ensiformis* (L.) DC

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ABSTRACT - Plants require iron (Fe) and copper (Cu) at low concentrations for metabolic functions; however, excess soil Fe and Cu can cause toxicity and affect plant development. The objective of this study was to assess the effects of soil Cu and Fe concentrations on seedling emergence, as well as on the metabolism of the main reserve compounds and the biomass in roots, stems, leaves, and cotyledons of *Canavalia ensiformis.* A greenhouse experiment was conducted in a completely randomized design; the Cu and Fe treatments in the soil were: (control, 50, 150, 250 and 350 mg dm^{-3}) over a period of 10 days. Seedling emergence was not affected by the evaluated treatments; however, the results showed an increase in the emergence speed index (EVI) for the Fe treatment at a dose of 150 mg dm^{-3} of soil compared to 350 mg dm^{-3} of soil. Treatment with Cu at a dose of 350 mg dm^{-3} of soil reduced root length. Cu treatments reduced root dry mass. Fe treatments affected the content of soluble amino acids in the stems, leaves and cotyledons, as well as the total soluble proteins in the cotyledons and the carbohydrate content in the leaves. Cu treatments increased protein and carbohydrate content in leaves and starch content in cotyledons. High concentrations of Cu and Fe modify the production of nitrogenous compounds in the plant and reduce root biomass.

Keywords: Legumes. Reserve compounds. Phytotoxicity. Trace elements.

RESUMO - As plantas necessitam de ferro (Fe) e cobre (Cu) em baixas concentrações para realizar funções metabólicas. Porém, o excesso de Fe e Cu no solo pode causar toxicidade e afetar o desenvolvimento das plantas. O objetivo desse estudo foi avaliar os efeitos das concentrações de Cu e Fe no solo na emergência das plântulas, bem como no metabolismo dos principais compostos de reserva e na biomassa de raízes, caules, folhas e cotilédones de *Canavalia ensiformis*. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado; os tratamentos de Cu e Fe no solo foram: (controle, 50, 150, 250 e 350 mg dm^{-3}) durante um período de 10 dias. A emergência das plântulas não foi afetada pelos tratamentos avaliados; entretanto, os resultados mostraram aumento do índice de velocidade de emergência (EVI) para o tratamento com Fe na dose de 150 mg dm^{-3} de solo em comparação com 350 mg dm^{-3} de solo. O tratamento com Cu na dose de 350 mg dm^{-3} de solo reduziu o comprimento das raízes. Os tratamentos com Cu reduziram a massa seca das raízes. Os tratamentos com Fe afetaram o conteúdo de aminoácidos solúveis nos caules, folhas e cotilédones, bem como as proteínas solúveis totais nos cotilédones e o conteúdo de carboidrato nas folhas. Os tratamentos com Cu aumentaram os teores de proteínas e carboidratos nas folhas e o teor de amido nos cotilédones. Altas concentrações de Cu e Fe modificam a produção de compostos nitrogenados na planta e diminuem a biomassa radicular.

Palavras-chave: Leguminosa. Compostos de reserva. Fitotoxicidade. Elementos-traço.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

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INTRODUCTION

The interaction of heavy metals with numerous non-metabolizable molecules is one of the main causes of their accumulation in living organisms. The accumulation of heavy metal in the environment causes extensive degradation of the contaminated area, affecting flora, fauna, and surface and groundwater, thus altering natural cycles and biochemical processes and posing health risks to humans (YADAV; GEORGE; DWIBEDI, 2023).

Copper (Cu) is an essential metal for carbohydrate, nitrogen, and lignin metabolism and chlorophyll synthesis in plants (CHIA et al., 2023). However, mining, industry, and agribusiness sectors contribute to waste and metal accumulations, mainly agribusinesses due to the extensive use of mineral fertilizers and pesticides (FASANI et al., 2022).

Iron (Fe) is essential for respiration, photosynthesis, biological nitrogen fixation, and electron transfer processes (GARCIA-MOLINA et al., 2020). However, excess Fe poses significant risks to human health and the environment; mining areas and their surroundings are examples of environments with Fe accumulation due to high amounts of mining waste with this metal (YANG et al., 2018).

Canavalia ensiformis (L.) DC (Fabaceae) is a rustic legume native to Central America (EMBRAPA, 1998; SHARASIA; GARG; BHANDERI, 2018).

This species is among cover crop legumes and stands out for its direct incorporation as green manure (ANDRADE et al., 2022). This species potential as green manure has been widely addressed in several studies (SOARES et al., 2022), especially in agricultural contexts (SANTOS et al., 2023).

Efficient emergence is a desired attribute in seedling formation, as the longer the seedling remains at initial developmental stages, the more vulnerable it is to adverse environmental conditions (MARTINS et al., 2012). Data on seedling emergence and partition and allocation of reserves in soils with excess heavy metals are scarce in the literature; this information is important for supporting essential processes of plant establishment and possible identification of species as potential phytoremediators.

Therefore, the hypothesis raised in this research was that increases in Fe and Cu concentrations in the soil lead to decreased emergence rate of *C. ensiformis* seedlings and affect nitrogen metabolism, reducing seedling growth and development. Thus, the objective of this study was to assess the effects of different soil Cu and Fe concentrations on seedling emergence, as well as on the metabolism of the main reserve compounds (starch, proteins, carbohydrates, and soluble amino acids) and the biomass in roots, stems, leaves, and cotyledons during the initial growth of *C. ensiformis*.

MATERIAL AND METHODS

Soil collection and experiment setup

The used for cultivating *Canavalia ensiformis* was obtained from the Teaching, Research, and Rural Extension Farm of the Faculty of Engineering of Ilha Solteira of the São Paulo State University (FEPE/FEIS/UNESP), in Selvíria, state of Mato Grosso do Sul, Brazil (20°22'S; 51°22'W) and characterized as a Typic Hapludox (Latossolo Vermelho distrófico típico; SANTOS et al., 2018). Soil samples were collected for chemical characterization (RAIJ et al., 2011), which showed the following results: pH 4.20; base saturation = 23.00; sum of bases = 9.50 mmolc dm^{-3}; Ca^{2+} = 4.00 mmolc dm^{-3}; K^{+} = 0.50 mmolc dm^{-3}; Mg^{2+} = 5.00 mmolc dm^{-3}; potential acidity (H^{+} + Al^{3+}) = 31.00 mmolc dm^{-3}; organic matter = 14.00 g.dm^{-3}; cation exchange capacity = 40.50 mmolc dm^{-3}; Al^{3+} = 10.00 mmolc dm^{-3}; P-resin = 1.00 mg.dm^{-3}; S-SO_4 = 5.00 mg.dm^{-3}; B = 0.21 mg.dm^{3}; Cu^{2+} = 0.90 mg.dm^{-3}; Fe^{2+} = 16.00 mg.dm^{-3}; Mn^{2+} = 6.90 mg.dm^{-3}; and Zn^{2+} = 0.10 mg.dm^{-3}.

Subsequently, the soil was artificially contaminated individually with Fe or Cu (treatments). Contaminating solutions of Cu and Fe were prepared using a solution of 800 mL of copper sulfate pentahydrate (31.433 g $CuSO_4*5H_2O$) and 800 mL of ferrous sulfate heptahydrate (39.825g of $FeSO_4*7H_2O$), respectively, both at a concentration of 10 g L^{-1}. The treatments consisted of Cu and Fe concentrations in the soil (0, 50, 150, 250, and 350 mg dm^{-3}). The contaminated soil was incubated in plastic bags for 15 days for stabilization and then transferred to plastic germination trays with individual cells.

Experiment installation

The emergence of *C. ensiformis* seedlings was analyzed by sowing seeds in 200-cell trays containing soil contaminated with the respective Cu and Fe concentrations; each cell was 2.3 cm diameter and 4 cm depth (volume of 13 mL). The experiment was conducted in a greenhouse with automatic irrigation three times a day, in Ilha Solteira, SP, Brazil, using a completely randomized design with 4 replications. The treatments consisted of different Cu and Fe concentrations in the soil (0, 50, 150, 250, and 350 mg dm^{-3}), comprising 10 fifty-seed treatments (Figure 1) for each contaminant, resulting in 2,000 cells.

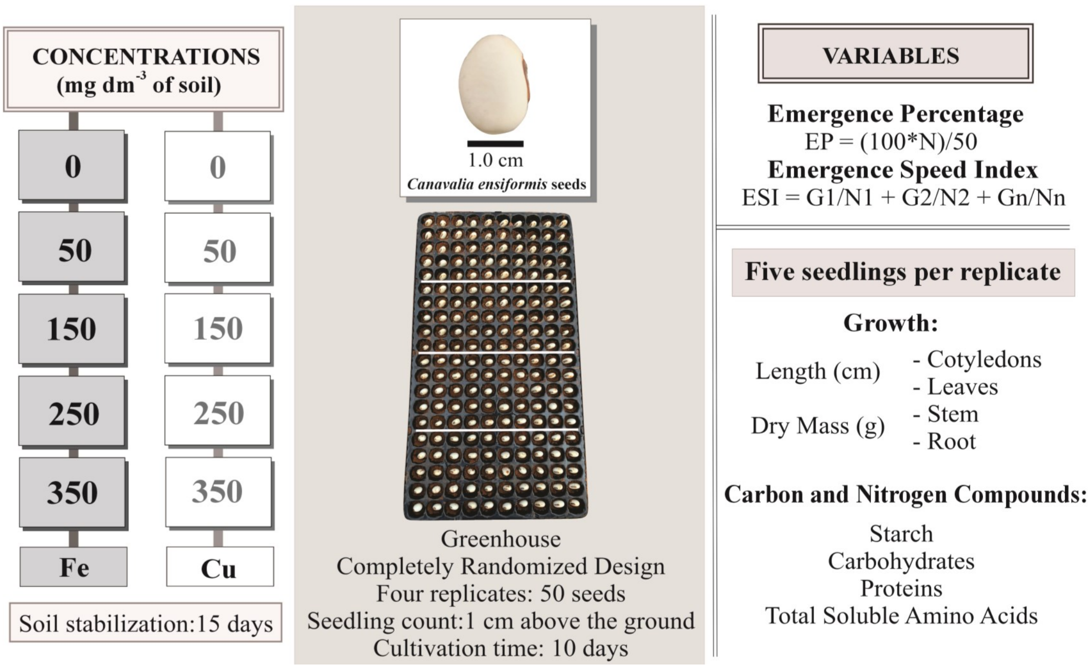


Figure 1. Fe and Cu concentrations, experimental design, and variables analyzed over 10 days.

Seedling emergence evaluation and plant material collection

Emergence speed index and emergence percentage (%) were assessed throughout 10 days. Seedlings were then collected, evaluated for shoot and root lengths (cm) (BRASIL, 2009) and separated into roots, stems, leaves, and cotyledons. These plant parts were washed in water and dried on paper; a portion of the material was taken to a forced-air oven at 60 °C for 72 hours to obtain the shoot, cotyledon, and root dry weights (g) (BRASIL, 2009).

Emerged seedlings were defined as those with the leaf primordia braking through the soil surface, resulting in the emergence of cotyledon leaves. Normal emerged seedlings were those that had cotyledons 1 cm above the soil surface and showed no signs of toxicity (BRASIL, 2009). The emergence period of *C. ensiformis* seedlings typically ranges from 4 to 10 days after sowing (BRASIL, 2009).

Emergence speed index (ESI) was calculated using the equation: *ESI= (E1/N1 + E2/N2 + EN/EN)* (MAGUIRE, 1962), where *E* is the number of emerged plants and *N* is the number of days after sowing.

Extraction and quantification of nitrogen and carbon compounds

The extraction of carbohydrates and soluble amino acids followed the method described by Bieleski and Turner (1966), using 60% methanol + 25% chloroform + 15% distilled water (MCW) at a ratio of 1:10 (w v^{-1}). Proteins were extracted using 0.1 N sodium hydroxide, and starch was extracted using 30% perchloric acid. Fresh matter (FM) of roots, stems, cotyledons, and leaves of *C. ensiformis* seedlings were used for all analyses.

These extractions were performed as follows: 0.5 g FM + 5 mL MCW was homogenized and centrifuged at 8500 rpm for 15 min; 1 mL of chloroform and 1.5 mL of distilled water were added to each 4 mL of supernatant; samples were stored in a refrigerator for 24 hours to separate the water-soluble (upper) and fat-soluble (lower) phases; 5 ml of 0.1N sodium hydroxide was added to the precipitate resulting from the first extraction with MCW and then the samples were homogenized and centrifuged for 15 minutes.

Subsequently, the samples were subjected to acid hydrolysis using 5 mL of 30% perchloric acid added to the precipitate from the second extraction with 0.1 N sodium hydroxide; the samples were then homogenized and centrifuged. This extraction was carried out in two stages (2.5 mL + 2.5 mL) to obtain the maximum amount of hydrolysate, as described by (YEMM; WILLIS, 1954). The supernatant constituted the starch sample.

Quantification of soluble amino acids

The following reagents were used to quantify the content of amino acids in 1 mL of sample: 1 mL of 0.2 M citrate buffer (pH 5.0), 1 mL of 5% ninhydrin in methyl glycol, and 1 mL of 0.0002 M potassium cyanide. The samples were placed in a water bath at 100 °C for 20 minutes and subsequently cooled for 10 minutes at room temperature; then, 1 mL of 60% ethyl alcohol was added and the absorbance was measured at 570 nm using a spectrophotometer (YEMM; COCKING; RICKETTS, 1955).

Quantification of proteins

Protein content was quantified in 100 µL of sample using 5 mL of Bradford reagent. After three minutes, the samples were read at 595 nm on a spectrophotometer (BRADFORD, 1976).

Quantification of soluble carbohydrates

The content of soluble carbohydrates in 1 mL of sample was quantified using 2 mL of anthrone reagent (100 mg of anthrone in 2.5 mL of water + 50 mL of concentrated sulfuric acid). The tubes were shaken, sealed, heated in a water bath at 100 °C for 3 minutes, cooled to room temperature, and then read for absorbance at 660 nm on a spectrophotometer (UMBREIT et al., 1957).

Statistical analysis

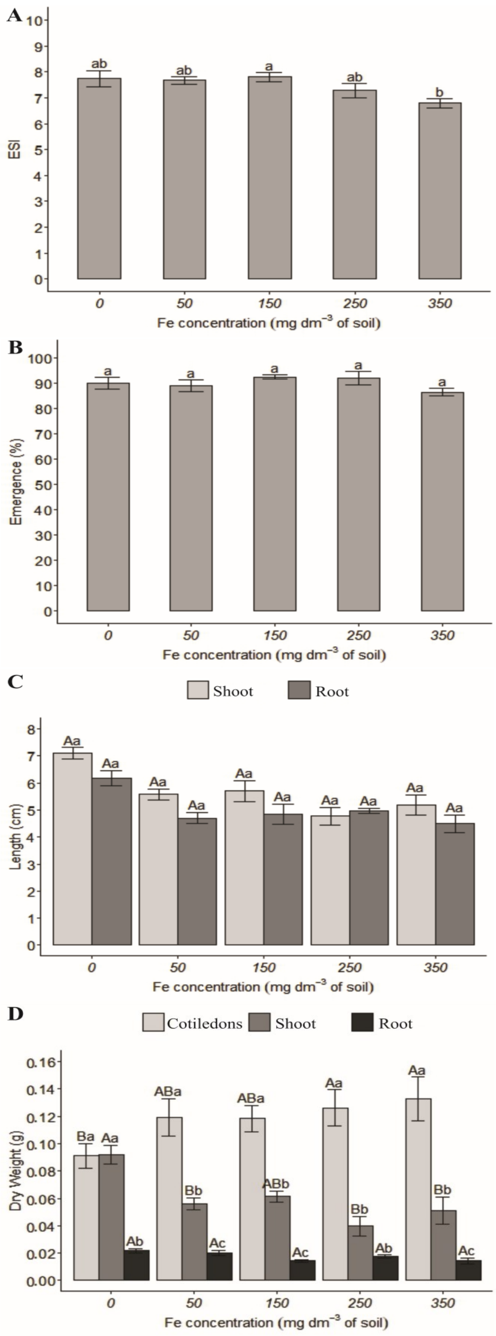
The obtained data were subjected to normality and homogeneity of variance analyses using Shapiro-Wilk and Bartlett tests, respectively. When significant differences between treatments were identified through ANOVA, means were compared using the Tukey's Test at a 5% significance level. The analyses were performed using the R packages ExpDes.pt and ggplot2.

RESULTS AND DISCUSSION

Iron (Fe)

Fe application to the soil affected the emergence speed index of *Canavalia ensiformis* seedlings, which was higher in the treatment with 150 mg Fe dm^{-3} of soil, with a mean of 7.80±0.18, and lower in the treatment with 350 mg Fe dm^{-3} of soil (6.79±0.16) (Figure 2A).

Cotyledon dry weight increased in the treatments with 250 mg dm^{-3} of soil (0.12±0.01 g) and 350 mg dm^{-3} of soil (0.13±0.01 g) compared to the control treatment (0.09±0.008 g). Shoot dry weight was higher in the control (0.09±0.006 g) than in treatments with Fe at 50 (0.05±0.004 g), 250 (0.03±0.007 g), and 350 mg dm^{-3} of soil (0.05±0.009 g). Root dry weight did not significantly differ between treatments. Comparing the plant organs within the treatments, cotyledons yielded, overall, the greatest weight, followed by shoot and root weights, except for the control (Figure 2D).



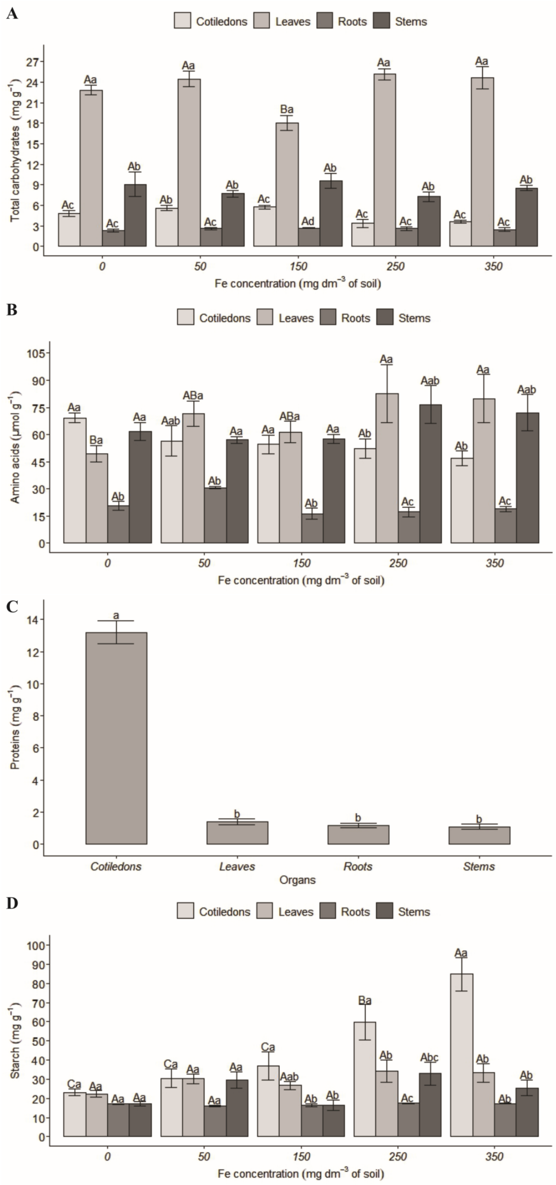
Bars with different uppercase letters comparing iron concentrations within plant organs, or lowercase letters comparing means of plant organs within iron concentrations, are significantly different from each other by the Tukey's test ($p<0.05$). Vertical lines represent the standard error

Figure 2. Emergence speed index (ESI) (A), emergence percentage (B), mean lengths (C), and mean dry weights (D) of *Canavalia ensiformis* seedlings grown under different iron concentrations in the soil.

Amino acid contents were higher in treatments with Fe at 250 and 350 mg dm^{-3} of soil (82.61±16.05 and 79.81±13.37 µmol g^{-1}, respectively) compared to the control. Comparing the organs within the Fe concentrations, amino acid contents differed significantly. Treatments with Fe at 0, 50, and 150 mg dm^{-3} of soil resulted in greater amino acid accumulation in stems, cotyledons, and leaves than in roots. Amino acid contents were higher in stems and leaves than in cotyledons and roots for treatments with Fe at 250 and 350 mg dm^{-3}of soil.

Protein contents differed significantly among plant organs, but were not statistically different among Fe concentrations (Figure 3C). Cotyledons had the highest protein content (13.20±0.72 mg g^{1}), and the accumulation of proteins in roots, stems, and leaves did not differ significantly.

Starch contents in stems, cotyledons, leaves, and roots differed significantly among Fe concentrations (Figure 3D). The highest starch contents in cotyledons (84.86±8.68 and 59.53±9.13 mg g^{1}) were found for the treatments with 350 and 250 and mg dm^{-3} of soil, respectively.



Bars with different uppercase letters comparing iron concentrations within plant organs, or lowercase letters comparing means of plant organs within iron concentrations, are significantly different from each other by the Tukey's test ($p<0.05$). Vertical lines represent the standard error.

Figure 3. Mean contents of carbohydrates (A), amino acids (B), proteins (C), and starch (D) in different organs of *Canavalia ensiformis* seedlings grown under different iron concentrations in the soil.

Copper (Cu)

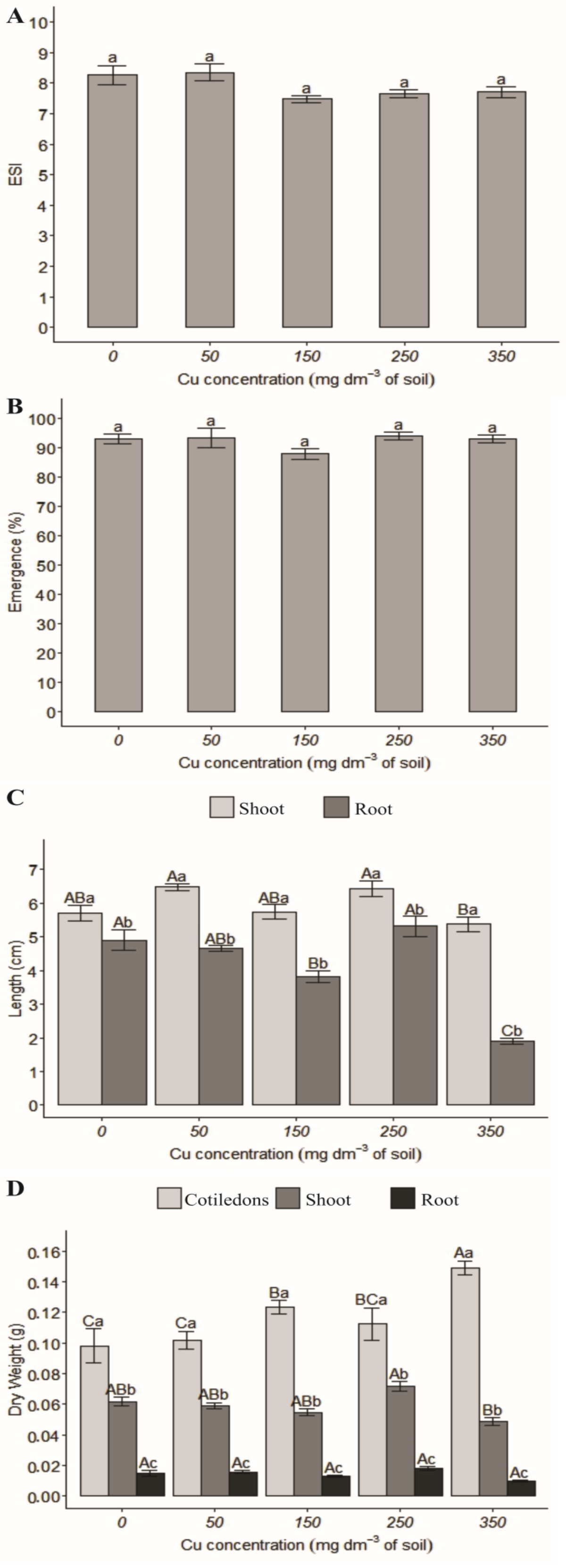
Treatments with Cu at 50 and 250 mg dm^{-3} of soil resulted in higher shoot lengths (6.48±0.10 and 6.42±0.23 cm, respectively) compared to that with 350 mg dm^{-3} of soil (5.37±0.21 cm). Roots were longer in the treatment with 250 mg dm^{-3} of soil (5.31±0.30 cm) and in the control (4.90±0.30 cm), and lower in the treatment with 350 mg dm^{-3} of soil (1.89±0.09 cm) (Figures 4C and 6A).

The cotyledon dry weights ranged from 0.10±0.01 to 0.15±0.004 g; the highest mean was found for the treatment with 350 mg dm^{-3} of soil, which differed significantly from the other treatments. Shoot dry weights ranged from 0.05±0.002 (Cu at 350 mg dm^{-3} of soil) to 0.07±0.003 g (Cu at 250 mg dm^{-3} of soil).

Carbohydrate contents showed no significant difference among Cu concentrations, but differed significantly among plant organs (Figure 5A). The highest carbohydrate content (26.94±0.87 mg g^1) was found in leaves, whereas the lowest (2.92±0.10 mg g^1) was found in roots.

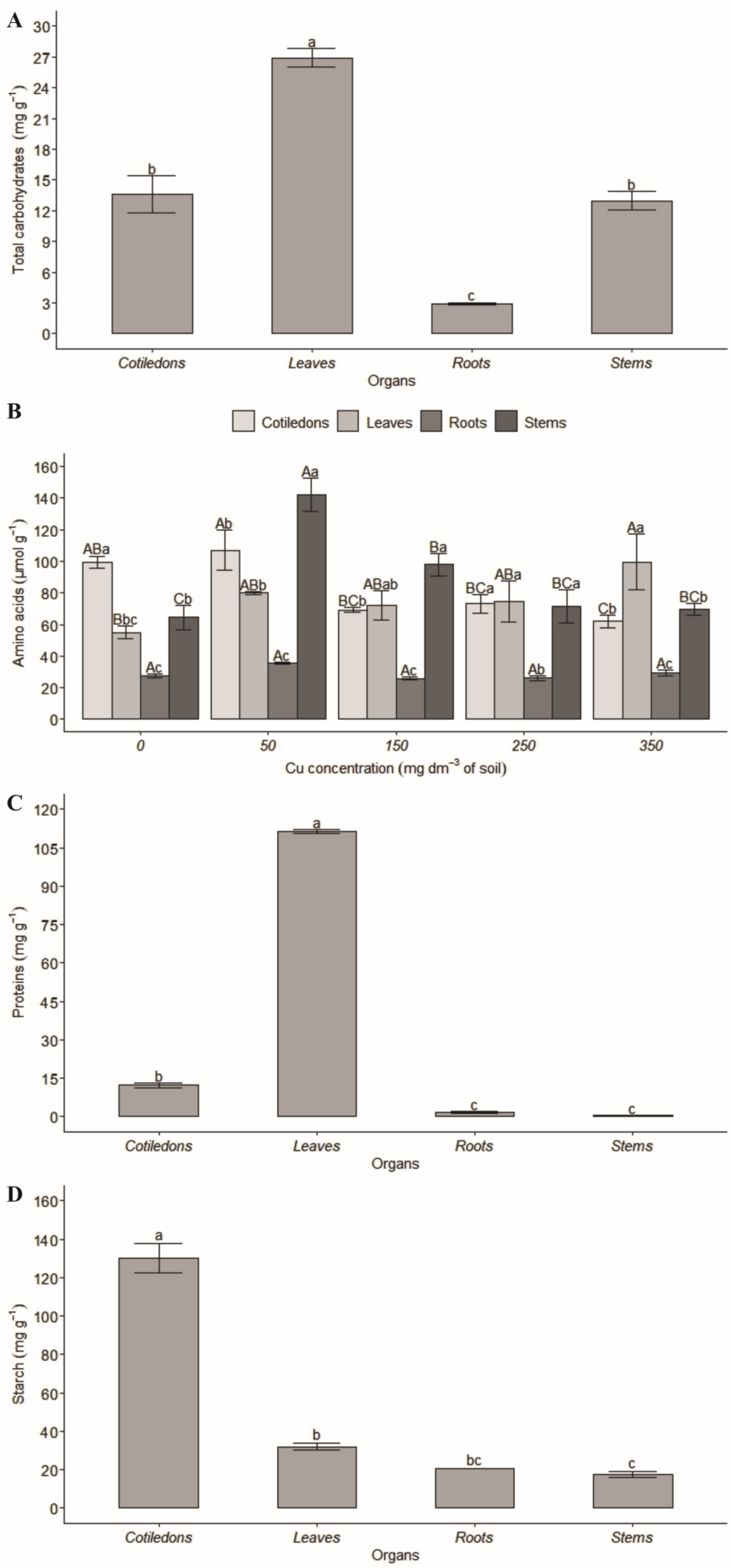
Amino acid contents differed significantly among Cu concentrations, except for roots (Figure 5B). The highest amino acid content in stems (142.17±10.53 µmol g^{-1}) was found for the treatment with 50 mg dm^{-3} of soil. Considering the treatment with 350 mg dm^{-3} of soil, leaves showed the highest amino acid content (99.57±17.58 mg g^1).

The highest protein content was found in leaves (111.48±0.74 mg g^1), differing significantly from those found in roots, stems, and cotyledons (1.64±0.24, 0. 47±0.04, and 12.24±0.89 mg g^1, respectively) (Figure 5C).



Bars with different uppercase letters comparing copper concentrations within plant organs, or lowercase letters comparing means of plant organs within copper concentrations, are significantly different from each other by the Tukey's test ($p<0.05$). Vertical lines represent the standard error

Figure 4. Emergence speed index (ESI) (A), emergence percentage (B), mean lengths (C), and mean dry weights (D) in different plant organs of *Canavalia ensiformis* seedlings grown under different copper concentrations in the soil.



Bars with different uppercase letters comparing copper concentrations within plant organs, or lowercase letters comparing means of plant organs within copper concentrations, are significantly different from each other by the Tukey's test ($p<0.05$). Vertical lines represent the standard error

Figure 5. Mean contents of carbohydrates (A) amino acids (B) proteins (C), and starch (D) in different plant organs of *Canavalia ensiformis* seedlings grown under different copper concentrations in the soil.

Toxicity effects of Fe and Cu on *C. ensiformis* seedlings

High Fe and Cu concentrations can lead to the absorption and accumulation of these elements in plant tissues; this excess accumulation causes toxicity symptoms in plants (TAIZ et al., 2017). Under these conditions, Fe and Cu can induce physiological responses, causing severe reductions in plant growth and yield (YANG et al., 2018).

Seedling emergence percentage was not significantly affected by Fe and Cu concentrations in the soil. Emergence speed index (Fe) differed statistically, showing a reduction in the treatment with Fe at 350 mg dm^{-3} of soil compared to 150 mg dm^{-3} of soil. Excess metals can interfere with the plant development (GHOSH; SETHY, 2013). Consequently, high metal concentrations can reduce germination and emergence rates, as found by Sun et al. (2020) for *Vigna radiata* (L.) R. Wilczek (Fabaceae) subjected to treatment with Fe at 1000 mg L.

Although both Fe and Cu are categorized as micronutrients, they are heavy metals that can bioaccumulate throughout the food chain. However, there are no studies evaluating the effects of high concentrations of soil Cu and Fe in the soil on germination of *C. ensiformis* plants.

Shoot length was longer than root length in all Cu treatments, and the shortest roots were found for the treatment with Cu at 350 mg dm^{-3} of soil (Figures 4C and 6A). This was also found for *Medicago sativa* (DUAN et al., 2019) and *C. ensiformis* plants with and without treatment with arbuscular mycorrhizal fungi (ANDRADE et al., 2010) grown under high Cu concentrations. This can be explained by disturbances in plant growth and development caused by excess Cu and its effect on important physiological processes (TAIZ et al., 2017), as it reduces root cell division and elongation, directly affecting root length (AMIN et al., 2021). Contrastingly, Fe concentrations did not result in differences in lengths of all evaluated plant organs (Figure 6B).

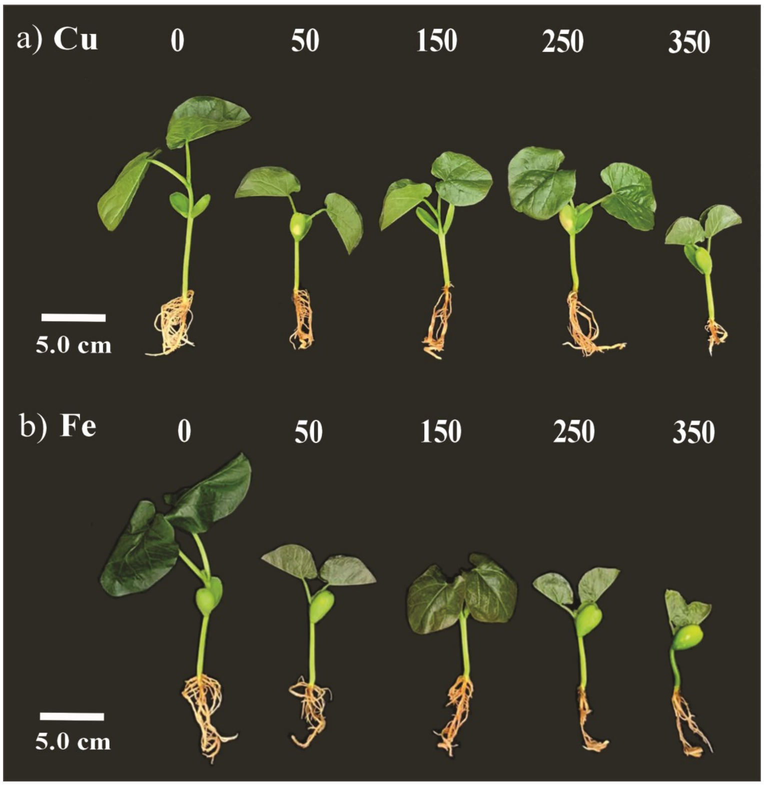


Figure 6. Randomly chosen *Canavalia ensiformis* seedlings grown in a greenhouse in soils with copper (a) and iron (b) concentrations of 0 (control), 50, 150, 250, and 350 mg dm^3 of soil, 10 days after sowing.

The species used in the study has large-sized cotyledons (Figure 1), which affects the dry weight. Thus, the lower the seedling development, the higher the cotyledon weight, as cotyledon reserves were used to a lesser extent by the seedling. The results showed that the lower the shoot and root dry weights within the Cu concentrations, the greater the cotyledon dry weight (Figures 4D and 6A). This correlation between dry weights was observed in all treatments with Fe (Figures 2D and 6B).

Carbohydrate contents were higher in leaves and lower in roots of seedlings grown with excess Fe or excess Cu. Carbohydrate metabolism is affected by heavy metals, as reported by Wiszniewska et al. (2019), who found increased soluble sugar contents in *Aster tripolium* under Cd and Pb stress. This higher carbohydrate content in leaves may be attributed to a lower allocation of reserves in leaves due to the initial growth phase, or to the onset of leaf photosynthetic activity, with production and export of carbon to the other plant parts (ALAGOZ; LAJAYER; GHORBANPOUR, 2023).

Seedlings grown in soils with excess Fe accumulate more amino acids in stems and leaves. This can be explained by the function of amino acids in regulating osmotic control and maintaining turgor pressure under adverse conditions to sustain plant growth (SUPRASANNA; NIKALJE; RAI, 2016).

Higher cotyledon protein contents in soils with excess Fe can be explained by nitrogen compounds, which are retained in some species as storage proteins in cotyledons; this is important at the initial seed development phase, as they are responsible for seedling nutrition when physiological demands are not yet fully met by the root system (CAMARGOS et al., 2013).

Cotyledon starch contents increased as the Fe concentration was increased. The Starch contents were higher in cotyledons than in other organs of seedlings grown with excess Cu. Cotyledons are reserve organs, thus, they accumulate soluble sugars and starch to relocate them to the epicotyl region and ensure the supply of carbon and nitrogen to enable shoot development according to environmental biotic and abiotic conditions (ALAGOZ; LAJAYER; GHORBANPOUR, 2023).

Excess Fe and Cu in the soil affected the distribution and allocation of reserves and biomass of *C. ensiformis* seedlings, leading to a strategic allocation of reserve compounds to different organs (starch in cotyledons; and amino acids, carbohydrates, and proteins in leaves). This adaptation assists in mitigating phytotoxicity, mainly for roots, which have high biomass and are directly exposed to these metals.

CONCLUSION

The high toxicity of excess iron and copper in the soil resulted in reductions in biomass of *Canavalia ensiformis* roots, affecting the production of nitrogen compounds, concentrating them in shoots (mainly leaves and cotyledons), whereas roots had the lowest concentrations. However, these metals did not affect seedling emergence. Further studies should evaluate metal ion transport proteins and anatomical changes in vegetative organs of *Canavalia ensiformis* plants, evaluating their complete cycle and monitoring their responses throughout plant development.

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REFERENCES

ALAGOZ, S. M.; LAJAYER, B. A.; GHORBANPOUR, M. Biossíntese de prolina e carboidratos solúveis e seus papéis em plantas sob estresses abióticos. In: GHORBANPOUR, M.; SHAHID, M. A. (Eds.). **Mitigadores de estresse de plantas: Tipos, Técnicas e Funções**. 1. ed. Cham, Suíça: Springer, 2023. cap. 10, p. 169-185.

AMIN, H. et al. Copper (Cu) tolerance and accumulation potential in four native plant species: a comparative study for effective phytoextraction technique. **Geology, Ecology, and Landscapes**, 5: 53-64, 2021.

ANDRADE, R. A. et al. Cover crop rates and decomposition of green manure in Southwestern Amazon. **Revista em Agronegócio e Meio Ambiente**, 15: 10, 2022.

ANDRADE, S. A. L. et al. Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. **Environmental and Experimental Botany**, 68: 198-207, 2010.

BIELESKI, R. L.; TURNER, N. A. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. **Analytical Biochemistry**, 17: 278-293, 1966.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, 72: 248-254, 1976.

BRASIL - Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. 1. ed. Brasília, DF: Secretaria de Defesa Agropecuária, 2009. 399 p.

CAMARGOS, L. S. et al. Alocação de compostos nitrogenados de reserva durante a germinação de sementes de *Canavalia brasiliensis*. **Biotemas**, 26: 4, 2013.

CHIA, J. C. et al. Loss of OPT3 function decreases phloem copper levels and impairs crosstalk between copper and iron homeostasis and shoot-to-root signaling in Arabidopsis thaliana. **The Plant Cell**, 35: 2157-2185, 2023.

DUAN, C. et al. Deciphering the rhizobium inoculation effect on spatial distribution of phosphatase activity in the rhizosphere of alfalfa under copper stress. **Soil Biology and Biochemistry**, 137: 107574, 2019.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. **Feijão de porco, leguminosa para adubação verde e cobertura do solo**. Belém, PA: EMBRAPA-CPATU, 1998. 1 p.

FASANI, E. et al. Metal detoxification in land plants: from bryophytes to vascular plants. STATE of the Art and Opportunities. **Plants**, 11: 237, 2022.

GARCIA-MOLINA, A. et al. Systems biology of responses to simultaneous copper and iron deficiency in Arabidopsis. **The Plant Journal**, 103: 2119-2138, 2020.

GHOSH, S.; SETHY, S. Effect of heavy metals on germination of seeds. **Journal of Natural Science, Biology and Medicine**, 4: 272, 2013.

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor 1. **Crop Science**, 2: 176-177, 1962.

MARTINS, C. C. et al. Posição da semente na semeadura e tipo de substrato sobre a emergência e crescimento de plântulas de *Schizolobium parahyba* (Vell.) S. F. Blake. **Ciência Florestal**, 22: 845-852, 2012.

RAIJ, B. V. et al. **Análise química para avaliação da fertilidade de solos tropicais.** Campinas, SP: Instituto Agronômico. 2011. 285 p.

SANTOS, H. G. et al. **Sistema brasileiro de classificação de solos**. 5. ed. Brasília, DF: Embrapa, 2018. 356 p.

SANTOS, L. F. C. et al. Growth and mineral composition of legume cover crops for sustainable agriculture in southern Mexico. **Tropical Agriculture**, 100: 182-190, 2023.

SHARASIA, P. L.; GARG, M. R.; BHANDERI, B. M. Beans. In: SHARASIA, P. L.; GARG, M. R.; BHANDERI, B. M. (Eds.). **Pulses and their by-products as animal feed**. Roma, Itália: Food and Agriculture Organizations of the United Nations, 2018. cap. 2, p. 8-9.

SOARES, D. O. et al. Chemical properties of soil and cassava yield as a function of weed management by cover crops in the Amazon ecosystem. **Sustainability**, 14: 1886, 2022.

SUN, Y. et al. Phytotoxicity of iron-based materials in mung bean: Seed germination tests. **Chemosphere**, 251: 126432, 2020.

SUPRASANNA, P.; NIKALJE, G. C.; RAI, A. N. Osmolyte accumulation and implications in plant abiotic stress tolerance. In: NOUSHINA, I.; RAHAT, N.; NAFEES A. K. (Eds.). **Osmolytes and plants acclimation to changing environment: emerging omics technologies**. New Delhi: Springer India, 2016. cap. 1, p. 1-12.

TAIZ, L. et al. **Plant physiology and development**. 6. ed. Porto Alegre, RS: Artmed. 2017. 88 p.

UMBREIT, W. W. et al. A colorimetric method for transaminase in serum or plasma. **Journal of Laboratory and Clinical Medicine**, 49: 454-459, 1957.

WISZNIEWSKA, A. et al. Insight sobre mecanismos de tolerância a múltiplos estresses em um halófito Aster tripolium submetido a estresse de salinidade e metais pesados. **Ecotoxicology Environmental Safety**, 180: 12-22, 2019.

YADAV, M.; GEORGE, N.; DWIBEDI, V. Emergence of toxic trace elements in plant environment: Insights into potential of silica nanoparticles for mitigation of metal toxicity in plants. **Environmental Pollution**, 333: 122112, 2023.

YANG, Q. et al. A review of soil heavy metal pollution from industrial and agricultural regions in China: Pollution and risk assessment. **Science of the Total Environment**, 642: 690-700, 2018.

YEMM, E.; WILLIS, A. J. The estimation of carbohydrate in plant extracts by anthrone. **Biochemical Journal**, 57: 508-514, 1954.

YEMM, E.W.; COCKING, E. C.; RICKETTS, R. E. The determination of amino-acids with ninhydrin. **Analyst**, 80: 209-213, 1955.