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Phytoremediation potential of *Canavalia ensiformis* in copper- and zinc-contaminated soil

Rayane Reis Sousa[1], Niedja Bezerra Costa[2], Jorge Diniz de Oliveira[3], Ivaneide de Oliveira Nascimento[4], Fabrício de Oliveira Reis[5], Tiago Massi Ferraz[6]

ABSTRACT: Soil contamination by heavy metals is an agro–environmental and socioeconomic problem caused by anthropic action on the soil–plant system. More studies on the sustainable use of phytoremediators to remove these metals from the soil environment are needed. Thus, we evaluated the phytoremediation potential of Canavalia ensiformis L. for copper (Cu) and zinc (Zn) metals in the soil. The experiment was conducted in greenhouse, completely randomized design, and five Cu and Zn treatments at increasing doses of 0, 10, 20, 40 and 80 mg kg^{-1}, with four replicates. Growth data (height and number of leaves), dry mass, Cu and Zn concentration in the shoots and roots were assessed. Based on these results, the translocation factor (TF) calculations were performed. C. ensiformis has the potential for phytoextraction because the TF data were >1 for both metals in the shoot tissues of the plant. The highest metals concentrations were observed in the shoots, with a value of 14.13 and 9.36 mg kg^{-1} for Cu and Zn, respectively. The species had a tolerance index >70% for both metals. In conclusion, the C. ensiformis was efficient in phytoremediation of the evaluated heavy metals, with a progressive translocation process in a concentration–dependent manner.

Keywords: Bioaccumulation factor. Heavy metals. Jack–bean. Phytoextraction.

Autor correspondente: Rayane Reis Sousa
E-mail: rayanereis2324@gmail.com

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[1] Doutoranda no Programa de Pós-Graduação em Produção Vegetal (PG-PV) da Universidade Federal do Tocantins (UFT), Gurupi (TO), Brasil.

[2] Pós-doc (PNPD) no Programa de Pós-graduação em Agroecologia (PPGA) da Universidade Estadual do Maranhão (UEMA). Doutora em Agronomia pela Universidade Federal de Goiás (UFG). Brasil.

[3] Professor do Programa de Pós-Graduação em Agricultura e Ambiente (PPGAA/UEMA). Doutor em Química pela Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP). Brasil.

[4] Professora Adjunto II da Universidade Estadual da Região Tocantina do Maranhão (UEMASUL). Doutora em Agroecologia pela Universidade Estadual do Maranhão (UEMA). Brasil.

[5] Professor do Programa de Pós-Graduação em Agroecologia (PPGA/UEMA). Doutor em Produção Vegetal pela Universidade Estadual Norte Fluminense (UENF). Brasil.

[6] Professor dos Programas de Pós-Graduação em Agricultura e Ambiente (PPGAA), e Agroecologia (PPGA/UEMA). Doutor em Produção Vegetal pela Universidade Estadual Norte Fluminense (UENF). Brasil.

INTRODUCTION

Heavy metals cause environmental problems worldwide. These metals can degrade water quality and lead to health risks by accumulating in the food chain (Wongsasuluk *et al*., 2014). Among these, copper (Cu), nickel (Ni), lead (Pb), cadmium (Cd), chromium (Cr) and zinc (Zn) are frequently found as contaminants in various water resources and soil (Prasad; Freitas, 2005).

Soil contamination in agricultural systems is quite common and is derived primarily from the frequent use of fertilizers and pesticides to increase productivity and reduce the incidence of pests and diseases (Gonzaga *et al*., 2020). Metallic micronutrients, such as Cu and Zn, are essential for plant growth and development and are toxic when present in excess in the environment, affecting the interactions and dynamics of the soil–plant system (Menegaes *et al*., 2017).

In the soil, excess Cu can decrease the development potential of plants by acting on biochemical and physiological mechanisms, thus affecting cell organelles and drastically reducing photosynthesis (Menegaes *et al*., 2019); whereas high Zn concentrations can negatively affect plant growth and metabolism because of its involvement in enzyme activation, basal metabolism, protein degradation, and regulator biosynthesis (Negrão *et al*., 2021).

High Cu concentrations in food crops remarkably affect plant morphology and physiology at all growth stages (Li *et al*., 2018; Rehman *et al*., 2019; Zhou *et al*., 2019; Saleem *et al*., 2020). Therefore, the remediation of Cu contaminated agricultural soil is essential for avoiding its accumulation in the food chain and maintaining crop productivity (Gonzaga *et al*., 2020).

Phytoremediation technology uses plants to degrade, extract, accumulate, volatilize, and stimulate the biodegradation of contaminants in the rhizosphere region (Hussain *et al*., 2018). It is a set of methods in which vegetation causes an environmental purification process through phytodegradation and phytostabilization, thus reducing the concentration of contaminants (Steliga; Kluk, 2020).

In recent years, the focus has primarily been placed on *in situ* methodologies that pose less risk to the environment and are economically viable. Bioremediation and phytoremediation are the best biotechnology options that meet these requirements (Oliveira *et al*., 2020; Verâne *et al*., 2020). Population growth and large–scale production indicators strongly demonstrate that the pressure on natural resources will further increase; thus, developing methods and plans for the recovery of degraded areas is essential.

According to Vasconcelos *et al*. (2020), *Canavalia ensiformis*, popularly known as "jack–bean" exhibits tolerance to high doses of imazapic–based herbicide; consequently, it is a plant phytoremediator for soils contaminated with imazapic. Madalão *et al*. (2017) recommended the use of *C. ensiformis* for the bioremediation of sulfentrazone contaminated soils, which can reduce the risk of environmental impacts. According to Ferraço *et al*. (2017), in addition to being tolerant to several herbicides, this species can release root exudates that activate the soil microbiota, which can, in turn, increase the decomposition of contaminating agents and promote biostimulation.

The primary challenge in phytoremediation application is obtaining information on plants that are tolerant to different metals, including Cu and Zn. Therefore, the objective of this study was to evaluate the potential of *C. ensiformis* as a phytoremediator of Cu and Zn in the soil. To the best of our knowledge, this is the first study to assess the impact of increasing doses of these metals on the growth, dry mass, metal concentration in shoots and roots, bioaccumulation factor (BF), translocation factor (TF), and tolerance index (TI) of this species.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL AND CULTIVATION CONDITIONS

The experiment was conducted in a greenhouse located at the Universidade Estadual da Região Tocantina do Maranhão (UEMASUL), Imperatriz *Campus*, MA, Brazil (5º31'32" S, 47º26'35" W; average altitude of 92 m). The climate is defined as Aw, with an average temperature and annual rainfall of 26.4°C and 1.476 mm, respectively.

The soil used in the experiment was obtained from preserved areas near a sanitary landfill in the municipality of Imperatriz, MA, Brazil (5º25'34.2 S, 47º 32' 26.4 W). Soil samples were collected for solid–waste sampling, according to ABNT/NBR 10007 standard (ABNT, 2004). Subsequently, the samples were transported and stored at the Campus Environmental Chemistry Laboratory, UEMASUL. The soil had the following characteristics: pH KCl: 4.68; pH H_2O: 5.68; cation exchange capacity (CEC): 11.18 $cmol_c\ dm^{-3}$; organic matter (OM): 4.81 $g\ kg^{-1}$.

The seeds of *C. ensiformis* were purchased from BRSEEDS SEMENTES LTDA, Araçatuba, SP, Brazil. The viability of the commercially purchased seeds was confirmed using sanity and germination tests (data not shown).

The experiment was based on the methodology proposed by Tavares *et al*. (2013) in which Cu and Zn contaminated soil was air–dried and its physicochemical properties and metal concentrations determined. Each experimental unit comprised a polyethylene pot with 2 kg of soil.

2.2 EXPERIMENTAL DESIGN

The experimental design was completely randomized, with treatments comprising five doses of each metal, and the values were calculated as follows using reference values determined by the Environmental Company of the State of São Paulo: 0 (control), 10, 20, 40, and 80 $mg\ kg^{-1}$ of zinc chloride ($ZnCl_2$) and copper sulfate ($CuSO_4$) were purchased from Sigma-Aldrich (St Louis, MO, USA), with four replicates each, totaling 40 experimental units.

After 130 days of *C. ensiformis* cultivation under different $ZnCl_2$ and $CuSO_4$ concentrations, all characteristics related to growth, dry mass, and Zn and Cu metal concentrations were assessed.

2.3 GROWTH PARAMETERS AND BIOMASS ACCUMULATION

At the end of the experiment, the shoot height (H) and dry matter of the plants were evaluated. Height was determined by measuring the height from the collar region of the seedling to the apical bud using a graduated ruler. To determine the dry matter of the shoots (SDM) and roots (RDM), both were dried in an oven at 60 ± 1°C until a constant mass was achieved.

2.4 DETERMINATION OF CU AND ZN METALS IN SOIL AND *C. ENSIFORMIS* PLANT

Mehlich^{-1} method was used for the determination of heavy metals present in soils (Mehlich, 1953). Five grams of air-dried fine ground and dry mass of the aerial parts and roots of *C. ensiformis* were weighed in conical polyethylene flasks and 40 mL of Mehlich^{-1} extraction solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4). The mixture was then stirred for 5 min at 220 rpm in an orbital mechanical shaker and filtered through a quantitative filter paper (Whatman 44). During the extraction process, a blank control of the extracting solutions was performed for each sample set analyzed on the same day. The analyses were performed in quadruplicates.

The quantification of Cu and Zn metals in soil samples and *C. ensiformis* was performed using Varian AAS240FS model flame atomic absorption spectrometer (FAAS) equipped with a deuterium lamp for background correction and a Varian AA240 was used. Standard solutions used for instrument calibration were prepared based on aliquots of 1000 mg L^{-1} stock solution.

2.5 PHYTOREMEDIATION POTENTIAL OF *C. ENSIFORMIS*

Some factors (*e.g.*, BF, TF, and TI) that assess the response of metal in the plants were used (Patek–Mohd *et al.*, 2018) because some species may be tolerant to metals but are not always hyperaccumulators. The BF, TF, and TI were used to evaluate the potential of *C. ensiformis* to extract Cu and Zn from the soil and classify it as a hyperaccumulator species, according to Equations 1, 2, and 3, respectively (Arumugam *et al.*, 2018).

$$Bioaccumulation\ factor\ (BF) = \frac{Metal\ concentration\ in\ plant\ tissues}{Metal\ concentration\ in\ the\ soil} \tag{1}$$

$$Translocation\ factor\ (FT) = \frac{Metal\ concentration\ in\ the\ shoot}{Metal\ concentration\ in\ the\ roots} \tag{2}$$

$$Tolerance\ index\ (TI\%) = \frac{Dry\ mass\ of\ the\ plant\ at\ the\ dose\ of\ interest\ (g)}{Control\ dry\ mass\ (g)} x\ 100 \tag{3}$$

2.6 STATISTICAL ANALYSIS

Data were subjected to analysis of variance (ANOVA), and the means of growth analyses were compared using Tukey's test ($p < 0.05$). Phytoremediation assessments for each dose of metals were performed using regression analysis. The models were chosen based on the significance of the coefficients of determination (R^2) and regression. The statistical program used was R software (R core team, 2022), with *ExpDes.pt* (Ferreira *et al.*, 2021) and *Hmisc* (Harrel, 2022) packages.

3 RESULTS AND DISCUSSION

3.1 IMPACT OF COPPER AND ZINC ON *CANAVALIA ENSIFORMIS* BIOMASS

Treatments with different doses of copper (Cu) and zinc (Zn) metals were used because these are the extreme alterations that can cause a difference in the accumulation and form of metals and organic matter (OM) in the soil. Based on this, we evaluated whether *C. ensiformis* has tolerance and potential for phytoremediation in response to increasing doses of Cu and Zn. According to results the plant response to excess Cu and Zn in the soil was manifested by a significant reduction in shoot and root biomass at increasing levels of soil contamination.

In the soil contaminated with increasing doses of Cu, there was a significant effect ($p < 0.05$) on the height (H), shoot dry matter (SDM) and root dry matter (RDM) of the remedial species, *C. ensiformis* (Table 1). When compared to the control, the dose that most affected the height was 40 mg kg^{-1} of Cu, with a reduction of 8.28%. Doses of 10 and 20 mg kg^{-1} of Cu promoted an average increase of 6.59% in SDM, while doses of 40 and 80 mg kg^{-1} of Cu increased RDM by 73,3% and 90%, respectively, when compared to the control.

Table 1. Number of leaves, height (H), shoot dry matter (SDM) and root dry matter (RMS) of *Canavalia ensiformis* plants grown in soil contaminated with copper and zinc doses.

Treatment		**Number of leaves**	**H (cm)**	**SDM (g)**	**RDM (g)**
Cu (mg kg^{-1})	0	27	117.7 a	19.7 b	3.0 b
	10	24	117.7 a	21.0 a	3.2 b
	20	22	114.0 b	21.0 a	4.0 b
	40	19	108.7 c	19.0 b	5.2 a
	80	23	112.2 b	18.5 b	5.7 a
	p-value	0.2780 ^{ns}	1.22 e^{-06}***	0.0208*	0.0153*
	CV (%)	13.4	3.4	6.1	22.9
Zn (mg kg^{-1})	0	32 a	124.0 a	26.0 a	4.5 a
	10	31 a	122.0 a	27.2 a	3.5 b
	20	30 a	121.0 a	22.2 b	3.2 b
	40	28 a	119.7 b	19.7 b	3.0 b
	80	25 b	117.5 b	16.5 c	3.0 b
	p-value	0.0009***	3.42 e^{-07}***	8.20 e^{-10}***	8.30 e^{-09}***
	CV (%)	8.7	2.3	8.7	19.4

ns = not significant; * p<0.05; ** p<0.01; *** p<0.001. CV: Coefficient of variation. Means followed by the same letter in the column do not differ by Tukey's test at 5%.

Plants under doses of zinc showed a significant difference ($p < 0.05$) in the number of leaves and height of *C. ensiformis*. The effect of Zn addition in the soil was substantially reflected in the shoot and root mass variables (Table 1). The results exhibited that *C. ensiformis* had an average height of 120.84 cm and a low coefficient of variation (2.34%).

Different doses of Cu adjusted to the quadratic model showed a linear reduction in the height and number of leaves in *C. ensiformis* by 13.8% and 4.67%, respectively, concerning the maximum dose of Cu (80 mg kg^{-1}) (Figure 1A and 1B). Cu may have interfered in the photosystem I (PSI) electron transport chain, as mentioned by Taiz *et al.* (2017), thereby decreasing photoassimilate production and considerably attenuating apical growth. Under these conditions, the height of "jack–bean" plants were found to be greater in soils without Cu.

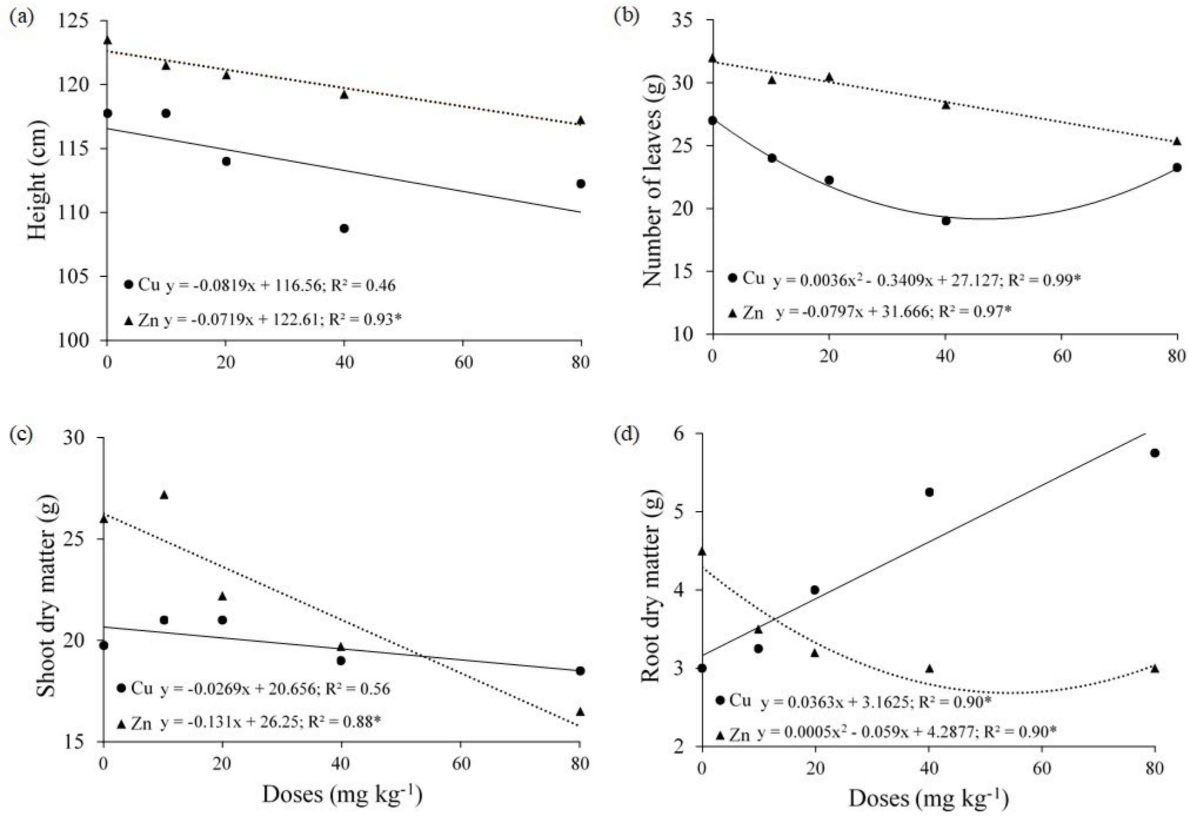

Figure 1. Morphometric evaluations of *C. ensiformis* grown in soils with increasing doses of Cu and Zn. (a) height, (b) number of leaves, (c) shoot dry matter and (d) root dry matter.

In general, a decrease in the number of leaves causes a reduction in the leaf area, which, in turn, reduces the photosynthetic rate and leads to lower production and translocation of photoassimilates to different parts of the plant (*e.g.*, root, stem and leaf), thereby negatively affecting growth. This may be associated with the end of the vegetative cycle, or the stress caused in the plants (Su *et al*., 2017; Taiz *et al*., 2017; Huang *et al*., 2018).

The dry biomass of shoots was reduced by different doses of Zn adjusted to the linear model, whereas Cu led to a reduction according to the quadratic model, with a significant variation ($p < 0.05$). This is because 100% soil phytoremediation by *C. ensiformis* did not occur, and soil exploration was limited because the experiment was carried out in a greenhouse with consequent stabilization of Zn interception/removal by the root system of *C. ensiformis*.

In Zn contaminated soil, the reduction of the dry mass demonstrated the intensity at which plant growth was affected, which may be associated with soil properties, such as low OM content and pH. The bond between OM and Cu can be toxic when the soil pH reaches values less than or equal to five (Meurer, 2006). On comparing the control with the highest applied dose (80 mg kg^{-1} of Cu and Zn each), we observed that the presence of Cu and Zn reduced the dry mass of the aerial parts by 6.32% and 36.53%, respectively (Figure 1C).

The RDM was reduced, demonstrating that *C. ensiformis* plants first emitted new and fine roots owing to stress induced by different Zn doses (Figure 1D); however, there was thickening and lower production of roots at higher doses, which was evident by 33.3% reduction in the RDM at the highest dose compared with that of the control.

The RDM of *C. ensiformis* demonstrated a positive linear response as the Cu concentration increased in the soil, with an increase of 91.7% compared with that of the control (Figure 1D), because Cu has an affinity with the root system (Marsola *et al*., 2005). This indicates that *C. ensiformis* is efficient in decontaminating the Cu contaminated soil, thus increasing the number of roots. In addition, a greater root proportion increases the release of root exudates, which activate the soil microbiota in the decomposition of organic compounds, promoting biostimulation.

3.2 COPPER AND ZINC CONTENT IN SOIL AND PLANT

Soil contamination indices quantify the concentrations of polluting elements and help in understanding the accumulation capacity and tolerance of various parts of plants, thus facilitating comparisons between different elements (Antoniadis *et al*., 2019).

The results in Table 2 clearly illustrate the variation in the metal levels of the *C. ensiformis* plant caused by increasing doses of Cu. According to the data obtained using flame atomic absorption, Cu and Zn were detected in all samples. $CuSO_4$ application resulted in a significant Cu accumulation ($p < 0.05$), with doses of 0, 10, 20, 40, and 80 mg kg^{-1} resulting in final Cu levels of 2.49, 5.69, 8.61, 14.4, and 28.5 mg kg^{-1}, respectively, in the soil (Table 2).

Table 2. Content of the Cu and Zn in soil, shoot and roots in *Canavalia ensiformis* plants grown in soil with increasing doses of copper and zinc.

Treatment		**Final content in the soil**	**Content (mg kg^{-1})**	
			Shoot	**Root**
Cu (mg kg^{-1})	0	2.49 e	1.22 b	1.17 c
	10	5.69 d	1.97 b	1.70 c
	20	8.61 c	2.87 b	3.49 b
	40	14.4 b	3.27 b	3.54 b
	80	28.5 a	14.13 a	7.07 a
	p-value	7.08 e^{-08}***	5.21 e^{-05}***	1.32 e^{-07}***
	CV (%)	20.1	25.2	22.4
Zn (mg kg^{-1})	0	0.91 e	0.51 c	0.24 c
	10	3.29 d	1.76 c	0.80 c
	20	11.19 c	7.04 b	2.89 b
	40	14.96 b	6.16 b	7.76 a
	80	19.20 a	9.36 a	8.94 a
	p-value	6.80 e^{-11}***	7.02 e^{-10}***	6.07 $^{e-09}$***
	CV (%)	8,12	19,0	24.5

ns = not significant; * p<0.05; ** p<0.01; *** p<0.001. CV: Coefficient of variation. Means followed by the same letter in the column do not differ by Tukey's test at 5%.

In the aerial part, the highest concentrations concerning the roots, with a concentration of 14.13 mg kg^{-1} of Cu, in which the roots exhibited 7.07 mg kg^{-1} of Cu in the highest treatment (80 mg kg^{-1}).

$ZnCl_2$ application in the soil at doses of 0, 10, 20, 40, and 80 mg kg^{-1} resulted in final Zn levels of 0.91, 3.29, 11.19, 14.96, and 19.20 mg kg^{-1}, respectively. The accumulation of Zn was smaller compared with that of Cu in the soil, suggesting that Cu and Zn are adsorbed on different types and sizes of particles in the soil solution, thus having different mobilities.

The highest Zn concentration was found in the aerial part at 9.36 mg kg^{-1} on comparing the average Zn concentration (Table 2). These results were more remarkable for the control treatment (without the addition of metals) samples because they represented all the metals present in the soil. Soil phytoremediation occurred in all treatments. Thus, an average soil decontamination rate of 85% was obtained, with a positive linear increase of the content of both metals for shoots and roots (Figure 2).

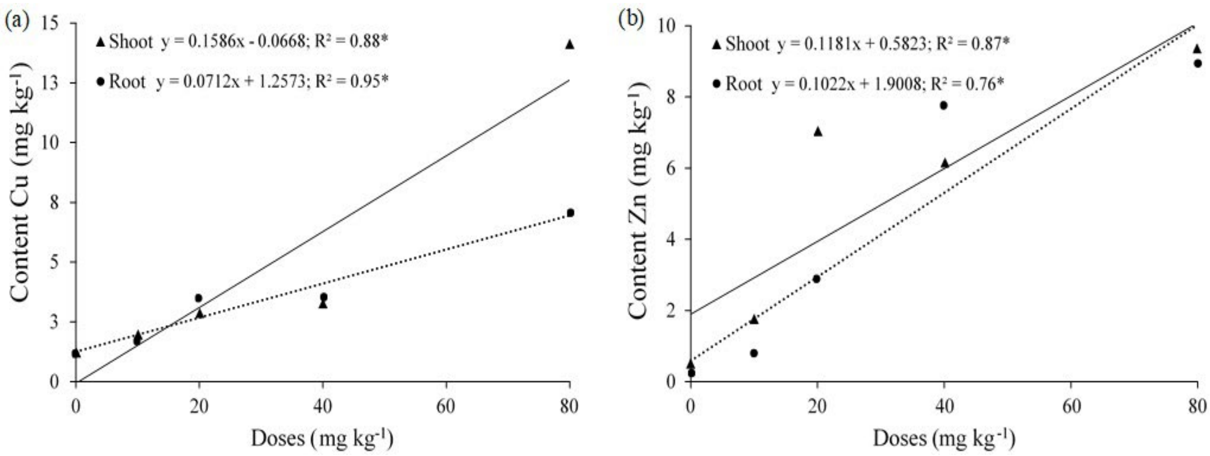

Figure 2. Regression equations of the Cu (a) and Zn (b) content in shoot and root in *Canavalia ensiformis* plants cultivated in soil maintained with increasing doses of copper and zinc.

The Cu concentration in the shoot of *C. ensiformis* was approximately 1047% higher than that of the control plants. In this study, *C. ensiformis* was considered an efficient phytoextractor in treatments with concentrations lower than 80 mg kg^{-1}, with an increase of 504% in the root system (Figure 2A).

Roots are physical barriers at soil–plant interfaces that restrict the absorption of different elements. Zn concentration in the shoots and roots increased by 1735% and 3625%, respectively, at the highest dose, compared with that of the control treatment (Figure 2B). Arumugam *et al.* (2018) reported that plants in uncontaminated soils can exhibit lower Cu levels than plants in contaminated soils, suggesting that the potential of a plant to act as an accumulator depends on the concentration of the element in the soil.

3.3 BIOACCUMULATION FACTOR, TRANSLOCATION FACTOR AND TOLERANCE INDEX

The bioaccumulation factor (BF), tolerance index (TI), translocation factor (TF), and final Cu concentration in the soil were significant ($p < 0.05$) (Table 2 and 3).

Table 3. Bioaccumulation factor (BF), tolerance index (TI), translocation factor (TF) in *Canavalia ensiformis* plants grown in soil with increasing doses of copper and zinc.

(Continua)

Treatment		BF	TF	TI (%)
Cu (mg kg^{-1})	0	1.02 a	0.96 c	114 ab
	10	0.64 c	1.43 bc	121 a
	20	0.77 b	0.87 bc	125 a
	40	0.49 c	0.92 b	121 b
	80	0.74 b	2.08 a	103 b
	p-value	6.12 e^{-07}***	5.18 e^{-05}***	0.0028**
	CV (%)	21.5	43.4	6.2

(Continua)

Treatment		BF	TF	TI (%)
Zn (mg kg^{-1})	0	1.11	0.49 b	117 a
	10	0.80	0.38 b	118 a
	20	0.80	0.21 c	98 b
	40	0.72	1.12 a	87 bc
	80	0.83	1.26 a	75 c
	p-value	0.1206 ^{ns}	5.54 e^{-07}***	2.81 e^{-06}***
	CV (%)	21.6	17.0	8.4

ns = not significant; * p<0.05; ** p<0.01; *** p<0.001. CV: Coefficient of variation. Means followed by the same letter in the column do not differ by Tukey's test at 5%.

The TI had averages values above 100%, with low variability (CV = 6.2%), in all treatments, indicating that *C. ensiformis* is highly tolerant to Cu. The average Cu levels in shoots exhibited high variability (CV = 25.2%) and a significant difference between the treatments ($p < 0.05$). The Cu concentration in the roots was also significant ($p < 0.001$) (Table 2). The Cu and Zn levels in the study area were lower than the reference values, indicating that there is no risk of pollution by these metals, even though it is an area with anthropogenic influence.

The significant difference ($p < 0.05$) was observed between the Zn concentration of the shoots and roots (Table 2); the BF was not significant ($p > 0.05$), whereas the TF was significant ($p < 0.05$) for the metal levels in plant tissues (Table 3). Significant differences ($p < 0.05$) were observed between the final Zn concentration in the soil, shoots, and roots and the TF and TI values (Table 2 and 3).

In this study, Cu did not have an accumulating characteristic in the roots, as the results showed a BF <1 (Table 3). The OM at 4.81 g kg^{-1} may have limited the phytoremediation; thus, it is necessary to verify whether the plants present better results with the addition of softeners. Chandrasekhar (2019) concluded that BF is highly dependent on the Cu concentration in the soil and there is a different response for each plant species, even though it is present with the same physicochemical properties in the soil.

The TF was not significant ($p < 0.05$), and the BF was less than one; however, it had high levels both in the aerial part and root, indicating that the plant translocated Cu to different parts for some reason, which implies that the studied plant is not an accumulator of Cu.

The TI showed R^2 equal to or greater than 0.96 (Figure 3B) and linearly decreased with an increasing Cu dose. When this index >60%, plants are highly tolerant of contaminants (Lux *et al.* 2004). Thus, as *C. ensiformis* presented values >70% at all tested Cu doses, it may be a promising plant for phytoremediation in clayey and acidic soils contaminated with up to 80 mg kg^{-1} of Cu.

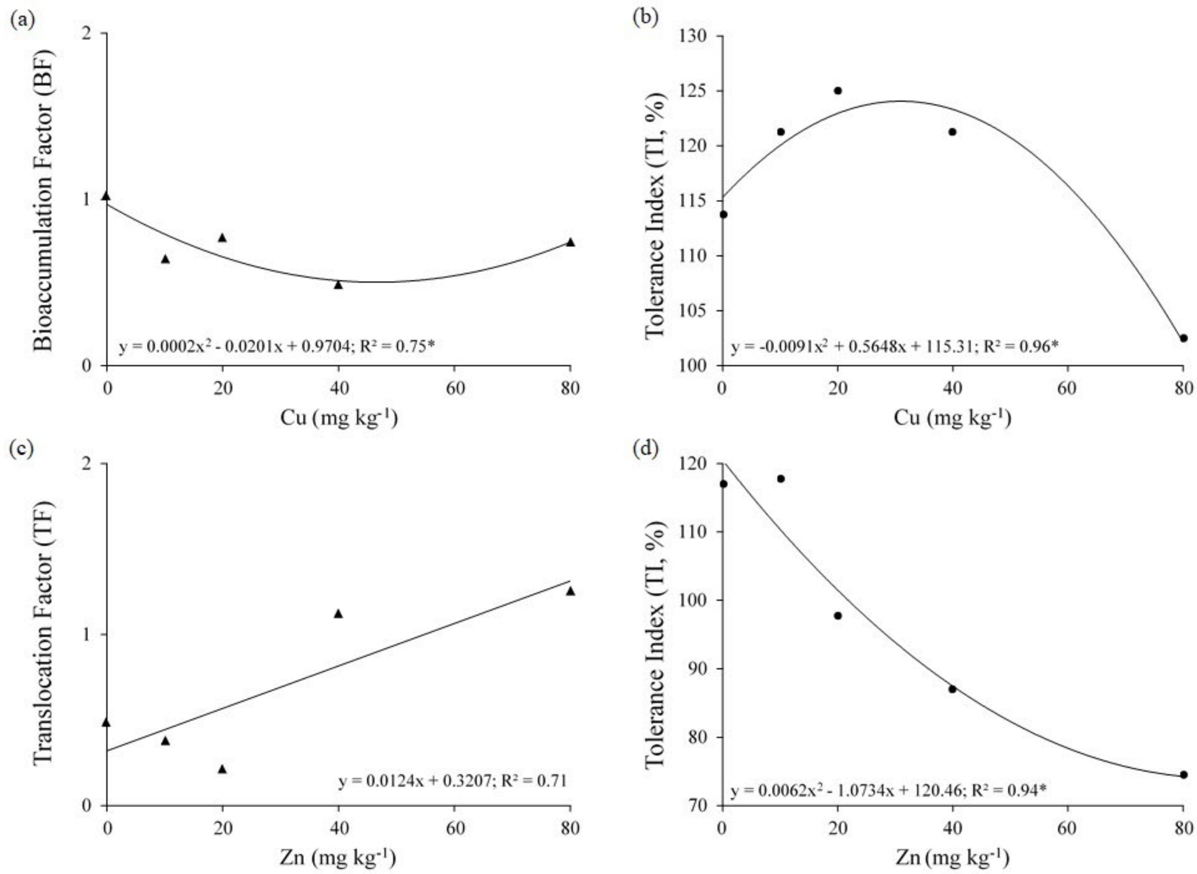

Figure 3. Regression equations for (a) bioaccumulation factor and (b) tolerance index of Cu; and (c) translocation factor and (d) tolerance index of Zn in *Canavalia ensiformis* plants cultivated in soil maintained with increasing doses of copper and zinc.

The TF is a common indicator for assessing the capacity of plant leaves to absorb metals. In the linear model, the increasing Zn doses positively influenced the TF in the soil, which was 52% higher in the Zn dose of 80 mg kg^{-1} when compared with that of the control treatment. Thus, TF values indicate high translocation of Zn in *C. ensiformis* (Figure 3C).

For the Zn concentration in *C. ensiformis*, the linear regression data of the TF are illustrated in Figure 3C, which exhibits that the TF was higher at 40 mg kg^{-1} dose, with a value of 1.29, thus indicating its phytoextractor potential. This implies that the plant was not affected and translocation to the aerial part was not impaired owing to metal contamination. *C. ensiformis* plants absorb and translocate Zn to the shoots, in addition to accumulating large amounts of this element in their roots.

The Zn TI of *C. ensiformis* plants showed a negative linear response. This species exhibited the highest TI (117%) at 10 mg kg^{-1} of Zn in the soil (Figure 3D). We observed that these *C. ensiformis* plants present values >70% at all evaluated doses, indicating that it is a promising species for phytoremediation in soils contaminated with up to 80 mg kg^{-1} of Zn. However, these concentrations were not harmful to the vitality of the species, indicating its tolerance to the presence of Zn in the soil. In addition, few species can survive in Zn contaminated soils (Ashraf *et al*., 2019).

In summary, most treatments were statistically similar, and the TF was less than 1, which indicates that *C. ensiformis* did not translocate the metal from its root to leaves in most treatments. Plant pods should be analyzed to check whether there is phytotoxicity and whether the metal is translocated to that organ.

The residue of *C. ensiformis* after phytoremediation can be a source of lignocellulosic material for dry mass processing. In addition, its use in the synthesis of biofuels and other materials can lead to more sustainable management of resources as it would have otherwise been completely neglected in terms of its commercial potential (Santos, 2019).

Most plant species can accumulate metals; however, they demonstrate a reduction in their performance when exposed to high concentration levels (Bai *et al*., 2018). This has limited the use of phytoremediation to sites contaminated with high concentrations of metals. Although *C. ensiformis* reduced agronomic characteristics, it tolerated and was able to phytoremediation an essential concentration of heavy metals, which can serve as a basis for future studies.

The survival of *C. ensiformis* to metal exposure is due to its tolerance, which is marked by its ability to absorb, translocate, and concentrate metals in different tissues and organs. To the best of our knowledge, this is the first study to analyze the impact of increasing doses of Zn and Cu metals on *C. ensiformis.* The results obtained in this study provide insights for establishing an agronomically and environmentally focused Cu and Zn phytoremediation program with a legume.

4 FINAL CONSIDERATIONS

Canavalia ensiformis has a high tolerance to Zn and Cu and may be used in the phytoremediation of soils contaminated with up to 80 mg kg^{-1} of Cu and/or Zn. Therefore, this species should be included in phytoremediation programs for soils contaminated with these metals, as it can provide mitigation to the damaged environment and help in a sustainable recovery.

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