Influence of $CuSO_4$ application rates during the vegetative stage on agronomic characteristics of *Glycine max* (L.) Merrill

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Received: May 25, 2025 DOI: 10.14295/bjs.v4i7.758

Accepted: June 12, 2025 URL: https://doi.org/10.14295/bjs.v4i7.758

Abstract

Several micronutrients are essential for the development of agriculturally important crops, including copper (Cu). This study aimed to evaluate the effects of different doses of copper sulfate ($CuSO_4$), expressed as mg L^{-1} of elemental Cu, on early-maturing soybean during the vegetative phase. Plant parameters such as shoot and root length, fresh and dry biomass of shoots and roots, and Cu bioaccumulation (expressed in mg kg^{-1}) in roots and shoots were assessed. Eight Cu concentrations (0, 5, 15, 35, 85, 100, 125, and 600 mg L^{-1}) were prepared and applied directly into the planting furrow. A precocious soybean cultivar was used. Measurements were taken during the vegetative stage. Significant differences were observed at 100 and 125 mg L^{-1} doses for root length and root dry mass. The highest Cu bioaccumulation in roots and shoots occurred at 125 mg L^{-1}, while concentrations above this threshold showed toxicity to the early-maturing soybean cultivar. The Cu source applied at varying doses influenced only root development parameters—specifically root length and root dry mass—as well as Cu content accumulated in both roots and shoots during the vegetative growth stage.

Keywords: bioaccumulation, copper sulfate, plant development, toxic effect, oxidative effect.

Influência de doses de $CuSO_4$ durante a fase vegetativa sobre as características agronômicas de *Glycine max* (L.) Merrill

Resumo

Diversos micronutrientes são essenciais ao desenvolvimento dos vegetais de interesse agrícola, como o Cobre (Cu). Este estudo teve como objetivo avaliar diferentes doses de $CuSO_4$ expresso em mg L^{-1} de Cu na fase vegetativa de soja precoce e verificar os parâmetros de plantas como comprimento parte aérea e de raízes, massa fresca e seca da parte aérea e raízes e bioacumulação de Cu expresso em mg kg^{-1} nas raízes e parte aérea. Diferentes doses de Cu mg L^{-1} foram preparadas e aplicadas via suco nas concentrações (0, 5, 15, 35, 85, 100, 125, e 600 mg L^{-1} of Cu). Foi utilizado cultura de soja cultivar precoce. As análises foram realizadas na fase vegetativa para comprimento parte aérea e raízes, massa fresca e seca parte aérea e raízes e bioacumulação de Cu nos órgãos raízes e parte aérea. Foi verificado que doses 100 e 125 mg L^{-1} apresentaram diferenças significativas no comprimento de raízes e massa seca de raízes. A bioacumulação máxima foi observada na dose de 125 mg L^{-1}, doses superiores demonstraram toxicidade para a cultivar de soja precoce avaliada. A fonte de cobre (Cu) aplicada em diferentes doses influenciou apenas nos parâmetros de desenvolvimento, como comprimento e massa seca da raiz, bem como o teor de Cu bioacumulado nas raízes e na parte aérea de plantas de soja de maturação precoce durante o estágio vegetativo.

Palavras-chave: bioacumulação, sulfato de cobre, desenvolvimento vegetal, efeito tóxico, efeito oxidativo.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) holds a prominent position in Brazilian agriculture, with Brazil being one of the world's largest producers and exporters of this crop, which is essential for the production of animal feed, vegetable oils, pharmaceuticals, and food products (Lin et al., 2022; Mataveli et al., 2010). In addition to its economic importance, soybean is nutritionally valuable, being rich in proteins, both saturated and unsaturated fatty acids, and a wide range of minerals such as copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), and phosphorus (P), which are crucial for human and animal diets, as well as for maintaining soil mineral cycling (Sakai & Kogiso, 2016; Riaz et al., 2022).

Among essential micronutrients, copper (Cu) plays a key role as a cofactor in several proteins involved in fundamental physiological processes. These include cytochrome c oxidase, plastocyanin, ethylene receptors, and the enzyme Cu/Zn-superoxide dismutase (SOD), all of which are directly associated with mitochondrial respiration, photosynthesis, antioxidant defense, and carbon metabolism (Puig et al., 2007; Yuan et al., 2010). Approximately 70% of the Cu present in plant tissues is located within the chloroplasts, reflecting its importance in energy assimilation processes and oxidative stress regulation (Rai et al., 2018). Copper deficiency can lead to metabolic imbalances such as reduced photosynthetic activity, impaired root development, decreased fruit and seed production, and increased susceptibility to oxidative stress (Rai et al., 2018; Amorim et al., 2013).

On the other hand, excessive Cu application can result in severe phytotoxic effects. High concentrations of this micronutrient may cause chlorosis, necrosis, inhibition of root growth, and increased lignification of plant tissues, which impairs cell expansion and nutrient uptake. Furthermore, excess Cu can antagonize the absorption of other essential ions such as Fe, Zn, and Mn (Shaw & Hossain, 2013; Kulikova et al., 2011; Lequeux et al., 2010). Therefore, maintaining a balance between Cu deficiency and toxicity is critical for optimal physiological and productive performance in soybean cultivation.

In this context, studies investigating the effects of different Cu concentrations are essential for establishing safe and physiologically efficient ranges for use in soybean nutritional management. Recent research suggests that moderate Cu levels can enhance root growth, stimulate antioxidant enzymatic activity, and improve the uptake of other nutrients, provided they remain below toxicity thresholds (Cruz et al., 2022; Yusefi-Tanha et al., 2020).

This study aimed to evaluate the effects of different concentrations of copper sulfate ($CuSO_4$), expressed in mg L^{-1} of Cu, on the vegetative development of an early-maturing soybean cultivar, with a focus on biometric parameters and the bioaccumulation of Cu in both shoots and roots. Specifically, we assessed the impact of Cu applied in the planting furrow on agronomic traits and Cu bioaccumulation during the vegetative growth stage.

2. Materials and Methods

2.1 Experimental location

The experiment was conducted in the experimental area of the UniBRAS University Center in Rio Verde, Goiás, Brazil, from February to May 2025, at coordinates 17°48'16.6" S and 50°56'04.7" W, with an average altitude of 748 m.

2.2 Soil type

The soil used in the experiment was classified as a Dystroferric Red Latosol (LVdf), with a clayey texture, and the following chemical characteristics (Table 1).

Table 1. Chemical and physicochemical parameters of the planting soil.

Cmol$_c$ dm^{-3}					mg dm^{-3}			
Ca	Mg	Ca+Mg	Al	K	K	S	P	pH $CaCl_2$
4.67	1.19	5.86	0.00	0.19	74	151	9.0	4.9
Micronutrients mg dm^{-3}						g dm^{-3}	Cmol$_c$ dm^{-3}	
Na	Fe	Mn	Cu	Zn	B	O.M	CTC	SB
4.0	27.9	65.1	3.2	3.5	0.5	49.0	12.50	6.07
Texture			Relationships base			% bases CEC		
Clay	Silt	Sand	Ca/Mg	Ca/K	Mg/K	Ca/CEC	Mg/CEC	K/CEC
37.6	18.5	43.9	3.91	24.66	6.31	37.33	9.54	1.51

Note: CEC = Cation Exchange Capacity. Extractants P (Mel), K, Na, Cu, Fe, Mn, and Zn = Mehlich 1; Ca, Mg, and Al = 1N KCl; S = $Ca(H_2PO_4)_2$ in HOAc (acetic acid); Organic Matter (O.M.) = Colorimetric method; Total P = Sulfuric acid digestion, and B = $BaCl_2$. Source: Authors, 2025.

2.3 Climate

The region's climate is classified as Aw (Tropical Savanna) according to the Köppen-Geiger system. The average temperature ranges from 20 to 25 °C. The rainy season occurs from October to April, while the dry season extends from May to September. The average annual precipitation ranges from 1,567 to 1,611 mm.

2.4 Experimental design

The experimental design was completely randomized (CRD), with four replications. The treatments consisted of eight doses of $CuSO_4{\cdot}5H_2O$ P.A - ACS (Vatten Soluções Ambientais, Brazil) (0, 5, 15, 35, 85, 100, 125, and 600 mg/L^{-1} of Cu), applied in the planting furrow at the time of soybean sowing, totaling 32 sample plots. The Cu doses were randomly applied, ranging from low to high levels for soybean cultivation.

2.5 Soil Correction, planting, and soybean cultivar

Following soil analysis, correction was carried out considering the area as having high fertility, with over 20 years of cultivation history (soybean, corn, sorghum, and millet). Liming was performed to raise base saturation to 70%. A total of 300 kg of soil was collected from two depths (0–20 cm and 20–40 cm) and transported to the experimental area. Basal fertilization was conducted according to soil analysis results and interpretations based on Ribeiro et al. (1999), applying 80 kg ha^{-1} of P_2O_5 in the form of single superphosphate and 40 kg ha^{-1} of K_2O in the form of potassium chloride.

After correction, the soil was transferred to 15 L experimental units (pots), and five soybean seeds were sown per unit. After germination, thinning was carried out at the V1 stage, leaving three plants per experimental unit. The soybean cultivar used was *G. max* AS 3715 I2X Agroeste® (Bayer®). This cultivar belongs to the early-maturing group (relative maturity group 7.1), with high yield potential, good foliar and root health, and high soil fertility requirements. It exhibits a determinate growth habit and is resistant to anthracnose (*Colletotrichum truncatum*), frog-eye leaf spot (*Cercospora sojina*), and soybean cyst nematode (races 3 and 6).

2.6 Cultural practices

At the V3 stage, the commercial product FICAM® (Bendiocarb, Brazil) was used for pest control, as *Diabrotica speciosa* was observed. Irrigation was performed every six days or when low soil moisture was detected with an electronic moisture probe (BOM, model 6810, China).

2.7 Sampling and variable analysis

Sampling was carried out on soybean plants at the R1 phenological stage. The roots were washed with running water to remove soil particles. The plants were then transferred to the Soil and Foliar Laboratory at UniBRAS Rio Verde, where vegetative parameters were analyzed, including Plant Height (PH), Root Length (RL) expressed in centimeters (cm), Shoot Fresh Mass (SFM), Root Fresh Mass (RFM), Shoot Dry Mass (SDM), and Root Dry Mass (RDM), all expressed in grams (g). The Cu content in the root (Cu root) and the Cu content in the aerial part (Cu aerial) were analyzed.

2.8 Copper analysis

Copper (Cu) analysis was performed by atomic absorption spectrometry (AAS) following acid digestion (wet method), according to EMBRAPA (2011), using a Cu standard solution with concentrations ranging from 0.1 to 5.0 mg L^{-1}, based on a $CuSO_4$ standard salt. The wavelength (λ) used was 324.7 nm (main absorption line for Cu), with flame mode (F-AAS) and acetylene gas. The method used to digest the root and aerial part samples was the Nitric-perchloric mixture.

2.9 Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA), using the adopted experimental design. When significance was detected by the F-test ($p < 0.05$), regression analysis was performed, selecting the model with the highest significance and best fit (R^2). All analyses used the SISVAR statistical software (Ferreira, 2019).

3. Results

3.1 Statistical parameters obtained

The analysis of variance revealed significant differences among treatments for the variables root length (RL), root dry mass (RDM), shoot copper content (Cu aerial), and root copper content (Cu root) during the vegetative stage of soybean plants (Table 2). No significant differences were observed for plant height (PH), shoot fresh mass (SFM), root fresh mass (RFM), and shoot dry mass (SDM), according to the F-test.

Table 2. Statistical parameters of the vegetative phase (roots and shoots) and copper content in early-maturing soybean cultivars.

FV	Fc	Model	CV (%)
Plant height	0.68 ns	-	13.31
Root length	3.49*	Quadratic	21.29
Root fresh mass	1.96 ns	-	37.19
Shoot fresh mass	1.64 ns	-	25.22
Root dry mass	3.67*	Quadratic	39.46
Shoot dry mass	1.20 ns	-	25.92
Root Cu content	61.04*	Quadratic	9.50
Aerial part Cu content	0.00*	Quadratic	0.05

Note: FV = source of variation. Fc = ns = not significant. * statistic F. = significant. CV (%) = coefficient of variation. Source: Authors, 2025.

3.2 Root length and root dry mass parameters

As shown in Figure 1, root length (RL) and root dry mass (RDM) exhibited statistically significant differences among the Cu treatments in early-maturing soybean plants, as determined by the F-test ($p < 0.05$). The highest mean RL was observed at the 100 mg L^{-1} Cu dose, reaching 71.0 cm, while the greatest RDM was recorded at the 125 mg L^{-1} dose, with a mean value of 0.98 g.

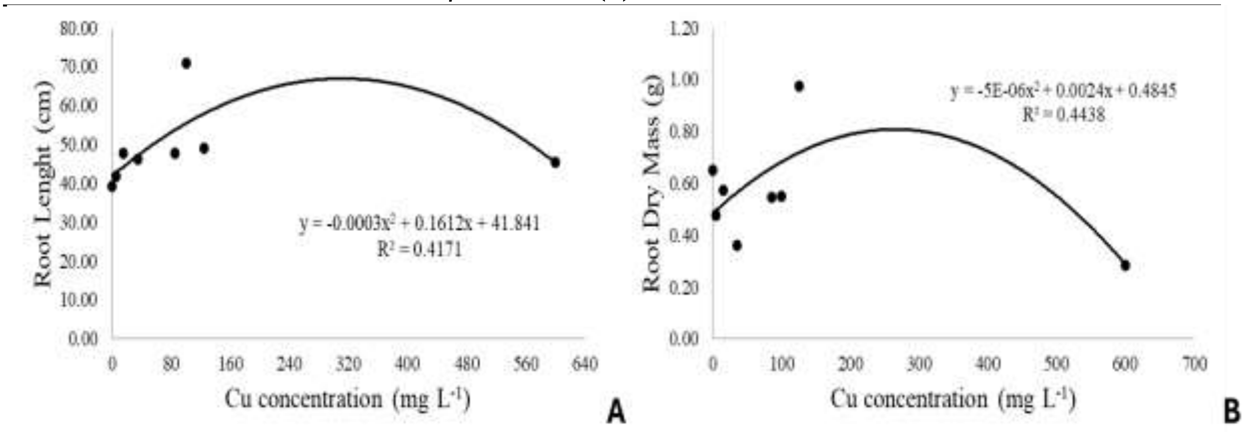

Figure 1. Root length (**A**) and root dry mass (**B**) of soybean plants at the vegetative stage under different copper (Cu mg L^{-1}) concentrations. Source: Authors, 2025.

3.3 Copper concentrations in shoots and roots

As shown in Figure 2, the highest copper contents in both shoots and roots were observed at the 125 mg L^{-1} Cu dose, with mean values of 128.54 mg kg^{-1} and 66.84 mg kg^{-1}, respectively. According to the F-test, these differences were statistically significant ($p < 0.05$).

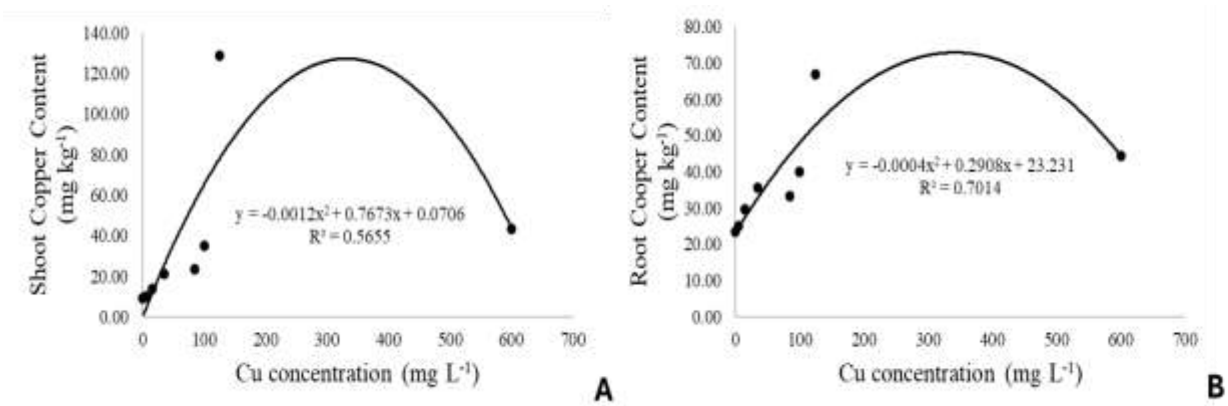

Figure 2. Copper content in the shoots (**A**) and roots (**B**) of early-maturing soybean plants at the vegetative stage under different copper concentrations, expressed as Cu (mg L^{-1}). Source: Authors, 2025.

4. Discussion

In our study, copper (Cu) doses significantly influenced only the parameters related to root development—specifically root length (RL) and root dry mass (RDM)—at intermediate concentrations of 100 and 125 mg L^{-1}, as well as Cu bioaccumulation in shoots and roots at the 125 mg L^{-1} level (expressed in mg kg^{-1}). Notably, plants exhibited enhanced root growth and higher root dry mass under these intermediate doses, which aligns with previous findings demonstrating that moderate Cu levels (~50 mg kg^{-1}) can stimulate early soybean development through antioxidant activation and improved nutrient use efficiency, despite the concurrent induction of oxidative stress (Gomes et al., 2021).

The expanded root system observed presents significant functional advantages, including increased water and nutrient uptake, improved soil aeration, and greater resilience under water deficit conditions—an idea widely supported by Gonçalves et al. (2017) and Beutler & Centurion (2004). Although this reference predates the past five-year window, its physiological insights remain highly relevant. More recent studies corroborate our results. Yusefi-Tanha et al. (2024) and Yusefi-Tanha et al. (2020) reported a clear pattern of Cu bioaccumulation in soybean tissues—root > leaf > stem > seed—which reinforces our findings of elevated Cu concentrations in shoots and roots at the 125 mg L^{-1} dose.

However, above the 125 mg L^{-1} threshold, Cu begins to exhibit toxic effects, in agreement with the observation by Yusefi-Tanha et al. (2020) that excessive Cu inhibits root growth and reduces biomass, even at relatively low concentrations, such as those evaluated in nanoparticle studies. Similarly, regression analysis conducted by Silva et al. (2021) in Cu-amended soils demonstrated that while Cu concentrations around 50 mg kg^{-1} supported early development, levels exceeding 133.5 mg kg^{-1} significantly suppressed root elongation, disrupted nutrient partitioning, and impaired photosynthetic performance (Gomes et al., 2021).

Additionally, the work of Fageria (2007) supports this toxic threshold concept, showing Cu-induced reduction in root growth in soybean cultivated in Oxisols—a response that varies depending on plant species and interactions with other micronutrients such as Zn and B. This highlights the importance of soil type and cultivar in determining Cu sensitivity.

Fageria (2001) also quantified crop-specific toxicity thresholds, identifying 15 mg kg^{-1} as the toxic Cu concentration for soybean, substantially lower than for other crops, demonstrating the narrow safety margin for this species. Although methodological approaches differ, our inferred toxic threshold (>125 mg L^{-1} or mg kg^{-1}) is qualitatively consistent with these earlier findings.

In summary, our results suggest an optimal “Cu window” between 100–125 mg L^{-1} that enhances root development and Cu uptake during the early vegetative phase of soybean. Exceeding this range induces phytotoxicity, particularly affecting the root system—a pattern strongly supported by recent literature. These insights are critical for guiding micronutrient management and avoiding Cu-induced root stress in soybean cultivation.

5. Conclusions

The copper (Cu) source applied at different doses influenced developmental parameters such as root length and root dry mass, as well as the bioaccumulated Cu content in the roots and shoots of early-maturing soybean plants during the vegetative stage. Furthermore, Cu doses above 125 mg L^{-1} exhibited toxic effects on the plants.

6. Authors’ Contributions

Richard Breno Sousa Castro: project writing, experimental setup and sample collection, data analysis, and manuscript writing. *Matheus Vinícius Abadia Ventura*: co-supervision, statistical analysis, post-review corrections. *Carlos Frederico de Souza Castro*: description and calculation of copper sulfate doses expressed as elemental Cu. *Antonio Carlos Pereira de Menezes Filho*: supervision, project writing, data collection, manuscript writing, post-review corrections, and final submission.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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