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# Response of soybean crop to nitrogen fixation in copper-contaminated soil

# Resposta do cultivo de soja a fixação de nitrogênio em solo contaminado com cobre

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## Highlights

Heavy metals in the soil influence nitrogen-fixing bacteria.
Soybean development declines linearly with increasing soil Cu contamination levels.
Soil heavy metal contamination reduces the yield of soybean.
Soybean inoculated with nitrogen-fixing bacteria has a lower Cu translocation rate.
Soybean yield increases with *B. japonicum* inoculation in Cu-contaminated soil.

## Abstract

Copper (Cu) is an essential heavy metal for plants at adequate rates, but can be toxic to agricultural species at elevated levels. However, the use of nitrogen-fixing bacteria can be an alternative for growing soybean in Cu-contaminated areas. Tolerance to excess Cu by the roots occurs through immobilization of Cu in the cell wall, exclusion from or restricted uptake, and compartmentalization in the vacuole with soluble complexes. The objective of this study was to determine the influence of the use of fixing bacteria on the development,

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physiological response, and bioaccumulation of Cu in soybean cultivated in contaminated soil. The experiment was laid out in a completely randomized design, in a 7 × 2 factorial arrangement corresponding to seven rates of Cu (0, 80, 160, 240, 320, 400, and 480 mg kg-1 of soil) and two inoculation possibilities (with Bradyrhizobium japonicum and without inoculation [control]), in eight replicates. The following variables were evaluated: shoot height; stem diameter; number of grains per plant; shoot and root dry weights; leaf area; specific root surface area; Cu contents in the shoot, root, and grains; translocation factor; tolerance index; bioconcentration factor; bioaccumulation coefficient; chlorophyll parameters; and number and dry weight of nodules. Inoculation with B. japonicum increases the physiological traits of plant height, specific root surface area, and grain yield in soybean grown in Cu-contaminated soil. Soybean has low efficiency in translocating Cu to the shoot; however, the copper content in the grain makes it impossible to recommend the cultivation of this crop in soil contaminated with this metal.

**Key words:** *Bradyrhizobium japonicum. Glycine max* L. Heavy metal.

## Resumo

O cobre (Cu) é um metal pesado que nas doses adequadas é essencial para as plantas, mas em doses elevadas potencialmente pode ser tóxico às espécies agrícolas. Contudo, a utilização de bactérias fixadoras de nitrogênio pode ser uma alternativa para o cultivo de soja em área contaminado com cobre. A tolerância ao excesso de Cu pelas raízes se dá com a imobilização do Cu na parede celular, exclusão ou restrição da absorção, compartimentalização no vacúolo com complexos solúveis. Objetivou-se neste trabalho determinar a influência do uso de bactérias fixadoras no desenvolvimento, resposta fisiológica e na bioacumulação de cobre na soja cultivada em solo contaminado. O delineamento experimental foi inteiramente casualizado em arranjo fatorial 7 x 2, sendo, sete doses de cobre (0, 80, 160, 240, 320, 400 e 480 mg de cobre kg -1 de solo), duas inoculações (com Bradyrhizobium japonicum e sem inoculação - testemunha), com 8 repetições. Avaliou-se a altura da parte aérea, diâmetro do colo, número de grãos por planta, massa seca da parte aérea e sistema radicular, área foliar, área superficial específica de raízes, teores de cobre na parte aérea, radicular e grãos, índice de translocação e tolerância, fator de bioconcentração, coeficiente de bioacumulação, parâmetros da clorofila, número e massa seca de nódulos. A inoculação com B. japonicum proporciona incrementos nos caracteres fisiológicos, na altura de planta, área superficial específica de raízes e no rendimento de grãos da soja em solo contaminado com cobre. A soja apresenta baixa eficiência em translocar o cobre para parte aérea, porém seu teor no grão inviabiliza sua recomendação de cultivo em solo contaminado com este metal.

**Palavras-chave:** *Glycine max L.. Bradyrhizobium japonicum.* Metal pesado.

## Introduction

Copper (Cu) can occur naturally, through the weathering of rocks during pedogenesis, and exerts an influence on the soil heavy metal content (Hugen et al., 2013). However, the intense use of heavy metals by human activity has increased soil contamination with Cu, posing a risk to public health and the environment (Andreazza et al., 2013). Resolution no. 420 of the Conselho Nacional do Meio Ambiente [CONAMA] (2009) sets the guideline value of 200 mg kg$^{-1}$ of soil as the maximum limit for total copper in agricultural areas without intervention.

Excess Cu in plants causes changes in the electron transport system, affecting photosynthesis (Cambrollé et al., 2015), in addition to possibly damaging the root structure (Bochicchio et al., 2015). Research shows that Cu toxicity induces malformation of cell layers on the surface of the root meristem, thereby preventing the development of lateral roots, affecting their morphology (Marques, 2016), and reducing the uptake of water and mineral nutrients from the soil (Ambrosini et al., 2017). Therefore, the presence of high concentrations of Cu in the soil negatively affects plant growth and productivity (Oliveira, 2018). Thus, strategies must be adopted to mitigate the effect of Cu on plants.

With respect to the plant, soybean growing has expanded to several regions of Brazil, and its inoculation with nitrogen-fixing bacteria may be an alternative for its cultivation in Cu-contaminated soil. These bacteria synthesize growth-promoting substances that induce an increase in the rate of secondary-root appearance, hair density, and root surface (Mendes et al., 2010). Studies have demonstrated that in addition to promoting biological fixation of N from the air and making it available to the plant in an assimilable form (Tejo et al., 2019), bacteria of the genus *Bradyrhizobium* induce an increase in root volume and dry weight (Manteli et al., 2019). Additionally, in soil contaminated with heavy metals, bacteria of this genus exhibit greater survival and tolerance than those of the genus *Azorhizobium* (Matsuda et al., 2002). The symbiosis between leguminous plants and *Rhizobium* can reduce heavy metals in the soil while helping nitrogen fixation (Castro & Roll, 2020).

In the above-described scenario, there remains doubt as to whether the use of nitrogen-fixing bacteria can help the development of soybean in Cu-contaminated soil. Therefore, the present study proposes to examine the development, physiological response, and bioaccumulation factors of soybean grown in Cu-contaminated soil in response to inoculation with *B. japonicum.*

## Material and Methods

The experiment was conducted over 120 days, between October 2019 and February 2020, in a non-air-conditioned greenhouse belonging to the Department of Agronomic and Environmental Sciences at the Federal University of Santa Maria (UFSM), located in Frederico Westphalen - RS, Brazil.

A completely randomized experimental design was adopted with a 7 × 2 factorial arrangement represented by seven rates of copper (0, 80, 160, 240, 320, 400, and 480 mg $kg^{-1}$ of soil) and two inoculation possibilities (with and without inoculation with nitrogen-fixing bacteria), in eight replicates. The experimental units consisted of 5-L polypropylene plastic pots filled with 4.5 kg of soil.

The soil used in the experiment was obtained from a 50% mixture (v v-1) of sieved sand (< 2.0 mm) and a 70% clay red Oxisol ('Latossolo') (Santos et al., 2018) collected from an agricultural area in the 0-20 cm layer, to allow the separation and analysis of the root system. The clay content was quantified by the method proposed by Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA] (2017), and chemical attributes as per F. C. Silva (2009) (Table 1).

**Table 1**
**Physicochemical characterization of the soil + sand mixture used to implement the experiment with the soybean crop.**

| Mixture | Clay* | pHwater | OM | P | K | Ca | Mg | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|
| | % | 1:1 | % | ---------------------- mg $dm^{-3}$ --------------------------- | | | | | |
| Soil + sand | 34 | 5.8 | 0.8 | 1.8 | 26.4 | 379.1 | 157.8 | 0.8 | 0.1 |

*Clay determined by the pipette method, EMBRAPA (2017).

Dolomitic limestone (relative TNV 80%: indicates % CaO and MgO) was applied to the mixed soil to achieve 65% base saturation, as recommended for the soybean crop (Sociedade Brasileira de Ciência do Solo [SBCS], 2016). After 30 days of limestone application (the time necessary for its reaction), the soil was sterilized in an autoclave at a temperature of 121 ºC in three 30-min cycles, and then the Cu rates were added in the form of a solution of copper sulfate ($CuSO_4.5H_2O$) applied 30 days before sowing, which was homogenized in the soil by agitation inside plastic bags. Immediately before sowing the soybean, soil samples were collected in each treatment and the pseudo-total levels of Cu were determined by method 3050b, described in United States Environmental Protection Agency [USEPA] (1996), followed by determination by atomic absorption spectrophotometry.

Inoculation was achieved using the liquid inoculant Simbiose®, a product composed of bacteria of the genus *Bradyrhizobium japonicum* (SEMIA 5079 and SEMIA 5080), with the concentration of 5.0 × 109 cfu $mL^{-1}$. The product was applied at a rate of 2 mL $kg^{-1}$ of seed, which were homogenized with the seeds at the time of sowing.

The soybean seeds used in the experiment were cultivar 5917 IPRO®, supplied by the Alfa Agro-industrial Cooperative, in Chapecó - SC. Five seeds were sown in each pot, which was previously disinfected with 2% sodium hypochlorite for 15 min and washed in running water for 5 min. Thinning was performed eight days after emergence, leaving one plant per pot until the end of the experiment. At the beginning of the experiment, soil moisture was standardized to field capacity and later maintained at 80% field capacity through drip irrigation. The pots were distributed inside the greenhouse at random.

At the end of the experiment, the following variables were measured: plant height (PH), using a graduated ruler, from the neck of the plants to the insertion of the last trefoil; stem diameter, using a Black Jack Tools® digital caliper; number of grains per plant; and 1000-grain weight per plant in the treatment. After separating the pods, the root system and the shoots were separated in the plant neck region and oven-dried at 60±1 ºC until reaching constant weight. Subsequently, they were weighed on an analytical scale to determine root dry weight (RDW) and shoot dry weight (SDW).

After being separated from the shoots, the roots were washed with running water over sieves with a mesh size of 0.5 mm and dried on blotting paper to determine root volume, following EMBRAPA (2017). To determine the specific surface area, root images were digitized using a scanner (HP D110) and processed using Safira 2.0® software for fiber and root analysis (Jorge & Silva, 2010). The root nodules were separated manually and the number of nodules per plant was quantified. Subsequently, nodule dry weight was determined by oven-drying at 60±1 °C until reaching constant weight and weighing on an analytical scale.

Leaf area was determined by the method that involves discs of known area, by randomly collecting five leaves at the R3/R4 stage (Adami et al., 2008).

The relative chlorophyll content of the leaves was determined at the R2 stage (full flowering) using a portable chlorophyll meter (ClorofiLOG®, Falker, model CFL 1030) (FALKER, 2008). Reading was performed at three wavelengths, with two emitters in the red range (near the peak of each chlorophyll, a and b) and one in the infrared range. The combination of these values generated the total relative chlorophyll content.

After drying, the roots, shoots, and grains of soybean were ground in a Wiley mill with a 10-mm mesh sieve to determine the Cu contents by nitric-perchloric digestion (3:1), followed by atomic absorption spectrophotometry.

Total dry weight; the Cu contents (mg Kg$^{-1}$) of the root system (CuR) and shoot (CuS); and the accumulated amounts of Cu (µg plant$^{-1}$) in the root system (CuAR); shoot (ACuS), and in the total soybean plant (ACuT) at zero Cu (r0) and at the Cu rates from 80 to 480 mg kg$^{-1}$ (rn) were used to calculate the tolerance index (TI; Equation 1) and the translocation factor (TF; Equation 2).

$$\mathrm{TI} = \frac{\mathrm{TDWrn}}{\mathrm{TDWro}} * 100 \qquad \text{Equation 1}$$

$$\mathrm{TF} = \frac{\mathrm{ACuSrn}}{\mathrm{ACuTrn}} * 100 \qquad \text{Equation 2}$$

The bioconcentration factor was determined as the ratio between the metal concentration in the roots (mg kg$^{-1}$) and the pseudo-total concentration in the soil (mg kg$^{-1}$), whereas the bioaccumulation factor was calculated as the ratio between the metal concentration in the shoot (mg kg$^{-1}$) and the pseudo-total concentration in the soil (mg kg$^{-1}$), following Yoon et al. (2006).

Results were subjected to analysis of variance, and when they showed interaction, they were subjected to regression analysis of quantitative source of variation (rates) under each level of the qualitative factor (inoculation). For the parameters without interaction, the simple effects were decomposed and the means of the qualitative factor were compared by Tukey's test at 5% error probability and the means of the quantitative factor were subjected to polynomial regression analysis by SISVAR software (Ferreira, 2019).

## Results and Discussion

The Cu rates applied to the soil provided a linear increase in the pseudo-total concentrations of the metal (Figure 1), which reached values above the limit of 200 mg kg$^{-1}$ of soil established by the legislation (CONAMA, 2009) at the estimated Cu rate of 165.3 mg kg$^{-1}$ of soil.

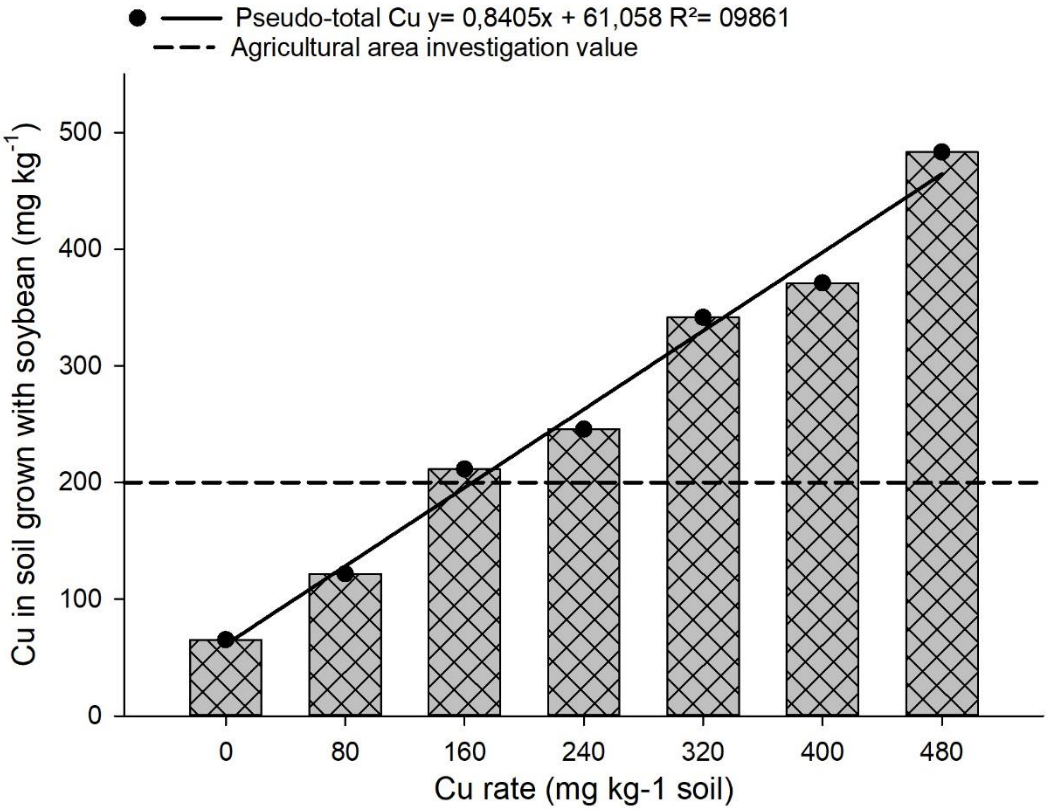

**Figure 1.** Pseudo-total copper contents as a function of rates of the metal (0, 80, 160, 240, 320, 400, and 480 mg kg$^{-1}$) added to the soil.

Results pertaining to the parameters of the soybean plants revealed an interaction effect between inoculation with *B. japonicum* and Cu rates applied to the soil on plant resistance, shoot dry weight (SDW), specific surface area, total chlorophyll content, number of grains per plant, and 1000-grain weight (Figure 2). In the uninoculated treatment, the Cu rates induced a linear reduction in plant height (PH) and SDW (Figure 2A and 2B). However, inoculation had a significantly higher impact, inducing a quadratic increase in these variables, which reached their maximum at the estimated Cu rates of 117 mg kg$^{-1}$ of soil (PH) and 175 mg kg$^{-1}$ of soil (SDW). Plant height is directly influenced by the amount of nitrogen and other nutrients absorbed through the root system, allowing greater photosynthetic capacity for the crop (Hungria, 2011). In this case, inoculation with *B. japonicum* directly influences the increase in the height of soybean plants (Bossolani et al., 2018). This result supports those described by Bizarro (2008), in which inoculant application increased the SDW of soybean plants.

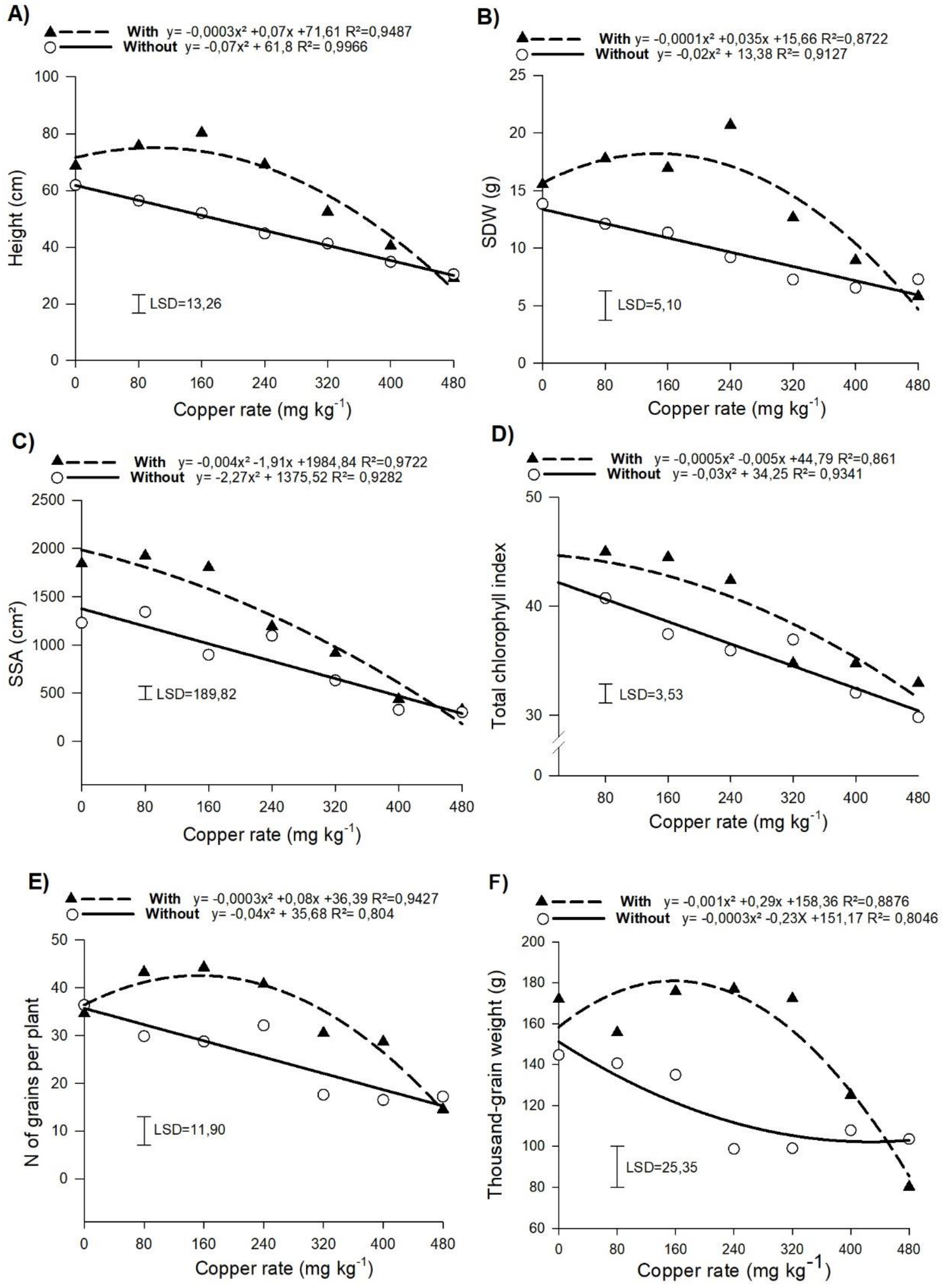

**Figure 2.** Plant height (A), shoot dry weight (SDW; B), specific surface area (SSA; C), total chlorophyll content (TCI; D), number of grains per plant (NGP; E), and 1000-grain weight (TGW; F) of soybean plants subjected to rates of copper in the soil with and without inoculation with *Bradyrhizobium japonicum.*

Specific root surface area decreased linearly with increasing rates of Cu applied to the soil in the treatments without and with inoculation. Inoculation provided a 69% larger specific surface area, on average, compared with the treatment without inoculation at zero Cu rate (Figure 2C). This result corroborates those reported by Schwalbert (2018), in which excessive Cu applied to the soil also affected the roots of soybean; and J. C. Silva (2019), in which the root surface area of the soybean crop decreased with the application of Cu to the soil. Common symptoms of heavy metal toxicity include reduced growth, particularly of the root system (Kukkola, Rautio, & Huttunen, 2000). As for inoculation, in addition to biological nitrogen fixation, most of these bacteria also have the capacity to produce plant growth hormones. The production of these growth-promoting substances can stimulate an increase in root hair density, secondary-root appearance rate, and root surface area (Mendes et al., 2010).

The total chlorophyll content decreased linearly in the uninoculated treatment and was significantly higher with inoculation, with a maximum achieved at the Cu rate of 5 mg kg$^{-1}$ (Figure 2D). The leaf chlorophyll content is used to predict the nutritional level of N in plants (Pereira et al., 2016). Nitrogen is a fundamental nutrient in plant metabolism that acts in the processes of biosynthesis of amino acids, chlorophyll, nucleic acids, and nitrogenous bases (Long et al., 2015). In the process of photosynthesis, excess Cu can cause inhibition of electron flow, changes in the composition of chloroplast membranes, and inhibition of synthesis and/or changes in the structure of photosynthetic pigments such as chlorophylls and carotenoids (Mendoza et al., 2013). Soybean cultivars studied by Schwalbert (2018) showed a reduction in the concentration of photosynthetic pigments in the presence of 40 μM of Cu kg$^{-1}$ of soil. The results indicate that inoculation with *Bradyrhizobium* leads to a higher chlorophyll content, even in Cu-contaminated soil.

The number of grains per plant also declined linearly with the rates of Cu applied to the soil in the uninoculated treatment (Figure 2E) and was significantly higher in the inoculated treatment, reaching a maximum of 42 grains per plant at the Cu rate of 133 mg kg$^{-1}$ (Figure 2E). Seed inoculation with *B. japonicum* increases grain yield in soybean, due to the greater biological nitrogen fixation provided by the action of these microorganisms (Braccini et al., 2016). Tolerance to heavy metals by nitrogen-fixing bacteria (NFB) seems to be related to the dense polysaccharide capsule around the cells, especially in strains with *Bradyrhizobium,* which retain the metals, preventing their absorption (Angle et al., 1993).

Thousand-grain weight decreased quadratically in the uninoculated treatment, up to 107.1 g per plant at the estimated Cu rate of 383 mg kg$^{-1}$ of soil. With inoculation, the result was significantly higher, with a quadratic increase that reached 179.4 g per plant up to the Cu rate of 145 mg kg$^{-1}$ of soil (Figure 2F). Inoculation with *B. japonicum* provides a positive effect on 1000-grain weight when compared with the lack of this treatment, since NFB can invigorate soybean plants, enhancing root system growth and, consequently, increasing grain yield (Braccini et al., 2016). Manteli et al. (2019) also showed an increase in 1000-grain weight and yield of soybean with the use of *B. japonicum.*

There was no interaction effect between Cu rate and inoculation on the Cu contents in the shoot, root system, or grain, which were only affected by the Cu rate (Figure 3). The shoot Cu content increased linearly with the rates of Cu applied to the soil (Figure 3A), similar to the findings reported by Schwalbert (2018). However, high concentrations of the element cause a toxic effect, reducing plant growth (Dechen & Nachtigall, 2006).

The root Cu content showed a linear increase with the rates applied to the soil, reaching 316.56 mg $kg^{-1}$ at the maximum rate tested (Figure 3B). The immobilization of Cu by extracellular carbohydrates in the root cell wall causes fewer ions to remain free in the cytoplasm to be transported to the shoot, resulting in higher concentrations of this metal in the roots (Lasat, 2002). The restriction of Cu translocation to the shoot is a plant survival strategy aimed at maintaining a lower concentration of the metal in the most photosynthetically sensitive organs, with the excess being accumulated in the less sensitive organs such as the roots (Yang et al., 2011). Nonetheless, high concentrations of Cu in the soil can stimulate the translocation of this element to the shoot and, thus, interfere negatively with several physiological processes (Cambrollé et al., 2013).

The Cu content in the grains showed a quadratic response with a maximum point at the applied Cu rate of 325 mg $kg^{-1}$ of soil, which resulted in a Cu content of 24 mg $kg^{-1}$ in the grains (Figure 3C). Magalhães et al. (2015) reported the level of 7.6 mg $kg^{-1}$ in soybean grown in soils without Cu application. A considerable increase of this metal in the grains was observed when soybean was grown under high rates of Cu in the soil, exceeding the maximum limit of 10 mg $kg^{-1}$ allowed for this element in the grains of this legume as established by the legislation (Agência Nacional de Vigilância Sanitária [ANVISA], 1998).

There was an interaction effect between the factors (Cu rates and inoculation) on the tolerance index (TI). This variable showed a quadratic increase in the inoculated treatment with maximum point at the Cu rate of 120 mg $kg^{-1}$. This treatment was superior to its uninoculated counterpart, in which TI decreased linearly with the applied Cu rate (Figure 4A). Plants with a TI greater than 60% are highly tolerant to contaminants (Lux et al., 2004). The treatment with *B. japonicum* inoculation allowed a TI above 60% up to the Cu rate of 370 mg $kg^{-1}$ in the soil and an increase of 57.9% in soybean tolerance at the rate of 120 mg $kg^{-1}$.

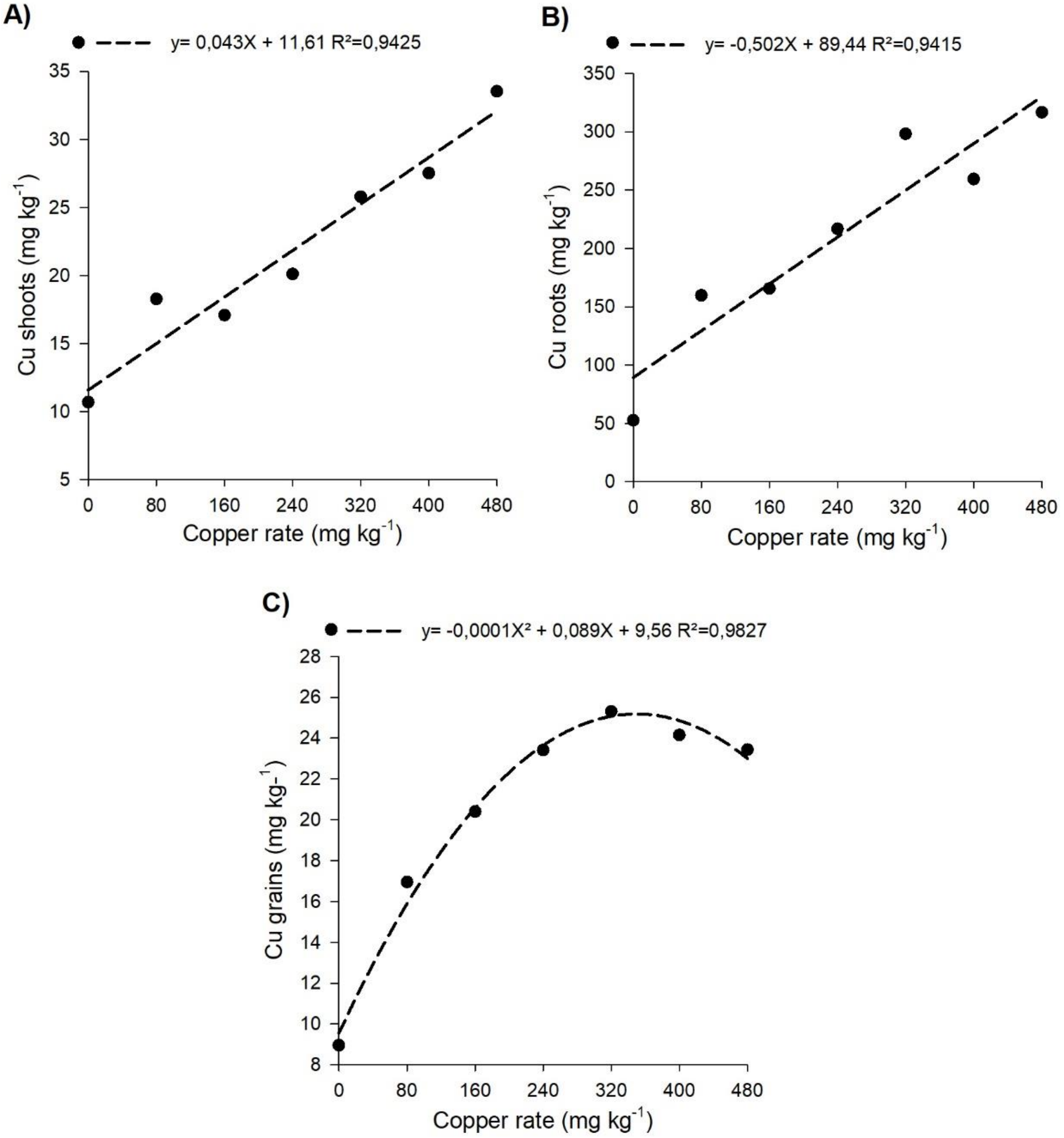

**Figure 3.** Copper contents in the shoot (CuS; A), root system (CuR; B), and grain (CuG; C) of soybean plants subjected to rates of copper in the soil.

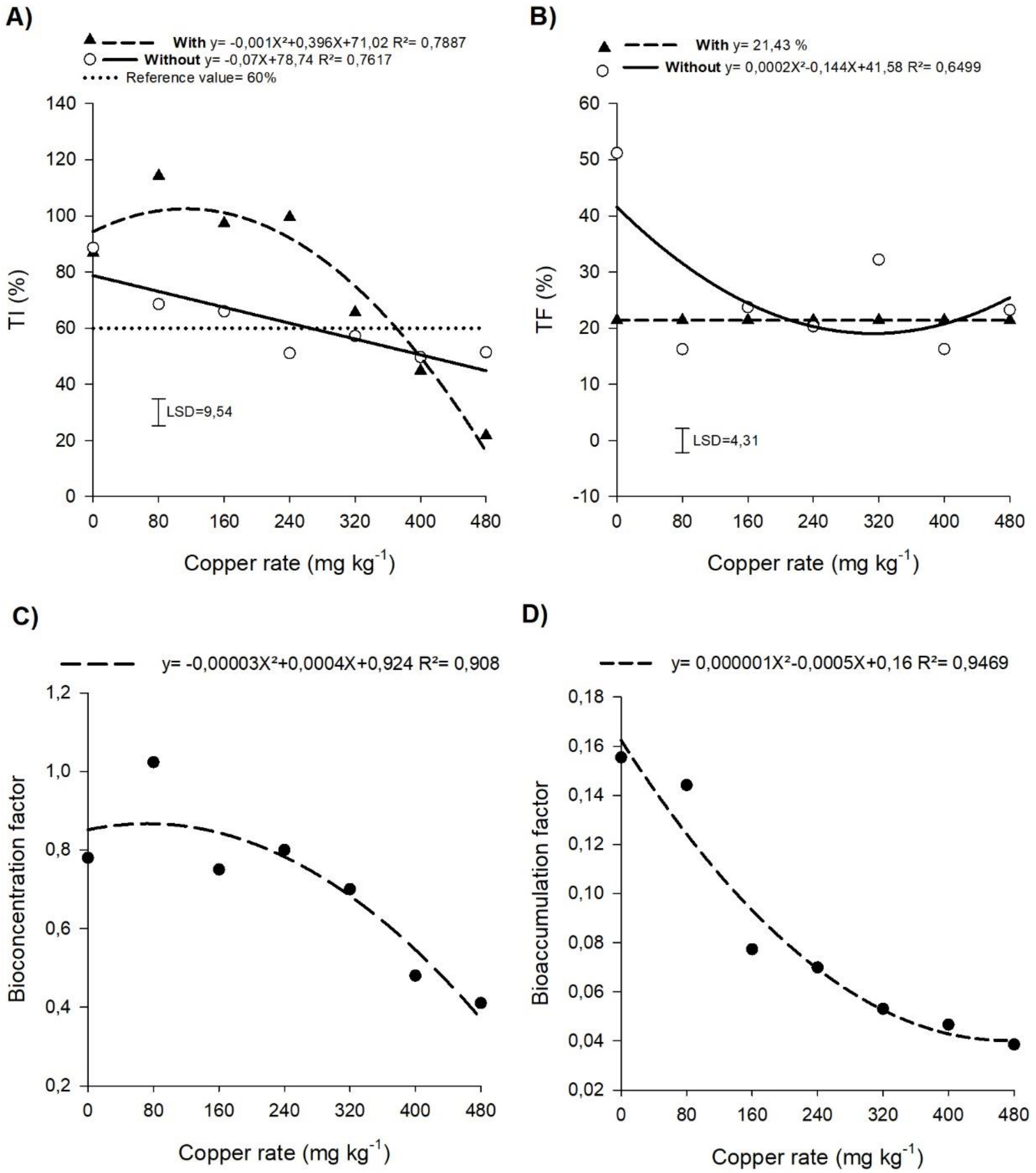

**Figure 4.** Interaction effect between inoculation and copper rates on the tolerance index (TI; A) and translocation factor (TF; B) and simple effect of copper rates on the bioconcentration factor (C) and bioaccumulation coefficient (D) in soybean plants.

The translocation factor (TF) decreased quadratically to 19.37% at the Cu rate of 313.39 mg kg$^{-1}$ soil in the uninoculated treatment, and showed no fit in the inoculated treatment, averaging 21% (Figure 4B). The uptake of metals by the root system and their low translocation to the shoot are considered mechanisms that the plant uses to increase tolerance to heavy metals (Pulford & Watson, 2003). The lower or closer to zero TF is, the greater the probability of survival and growth of the species in a contaminated environment (Scheid et al., 2018).

There was no interaction effect between Cu rates and inoculation on the bioconcentration factor or the bioaccumulation coefficient, which were only affected by the effect of Cu rates in isolation (Figure 4C and 4D). Copper rates reduced the bioconcentration factor (Figure 4C). Plants with a bioconcentration factor greater than 1.0 can be recommended for phytoextraction of soil contaminants (Mcgrath & Zhao, 2003). This result may be related to the lower dry weight shown by plants subjected to Cu rates applied to the soil (Figure 2B). Plants subjected to high Cu rates have their shoot growth and dry weight reduced (Yruela, 2013).

The bioaccumulation coefficient decreased quadratically with the rates of Cu applied to the soil, showing values below 1.0 at all rates applied (Figure 4D). This indicates low efficiency in translocating Cu to the shoot (Fuksová et al., 2009). Negrini (2017) evaluated the species *E. grandis, E. saligna, E. dunnii,* and *C. citriodora* and observed similar results in soil contaminated with Cu. Coinaski (2019) also described similar findings in *Ilex paraguariensis,* which showed values below the classification value (1.0). The results demonstrate that soybean is not a Cu-extracting plant.

There was no interaction effect between Cu rates and inoculation for leaf area, root dry weight, root volume, stem diameter, number of nodules, or nodule dry weight (Figure 5). Leaf area decreased quadratically with the Cu rates (Figure 5A). This result was possibly due to the reduction in chlorophyll content, which induces less photosynthetic capacity and, consequently, less accumulation of dry matter (Ambede et al., 2012). The reduction in photosynthesis due to the excess of Cu is related to the decrease in the number and volume of chloroplasts, whose organelles are responsible for photosynthetic activity in plants (Panou-Filotheou et al., 2001).

Root dry weight, root volume, and stem diameter decreased linearly with increasing rates of Cu applied to the soil (Figure 5B, 5C, and 5D). Research has shown that 50 μM of $CuSO_4$ in the soil is sufficient to stop root growth in soybean (Kulikova et al., 2011) and that stem diameter in this crop reduces with increasing rates of Cu in the soil (Silva, 2019). Excess Cu results in poor formation of cell layers on the surface of the root meristem and prevents the development of lateral roots, affecting their morphology (Marques, 2016).

The number of nodules and nodule dry weight showed a quadratic response with increasing Cu rates, reaching maximum points of 113 nodules root$^{-1}$ at 172 mg kg$^{-1}$ of soil and 1.81 g root at 150 mg kg$^{-1}$ of soil, respectively (Figure 5E and 5F). A study by Gitti (2015) showed that the inoculation of soybean seeds positively influenced the increases in the number of nodules per plant, which averaged 88.4. Copper is a micronutrient used by plants in small amounts and is important for nitrogen fixation that occurs inside the root nodules (Cancian, 2018).

By analyzing the simple effect, we observe that inoculation increased leaf area, root dry weight, the number of legumes, and root volume (Table 2). Studies report that bacteria of the genus *Bradyrhizobium* sp. induce an increase in root volume and root dry weight (Manteli et al., 2019). This fact may be related to the supply of N provided by the symbiosis, which is required in large amounts for the maintenance and formation of pods in the soybean crop (Bulegon et al., 2016).

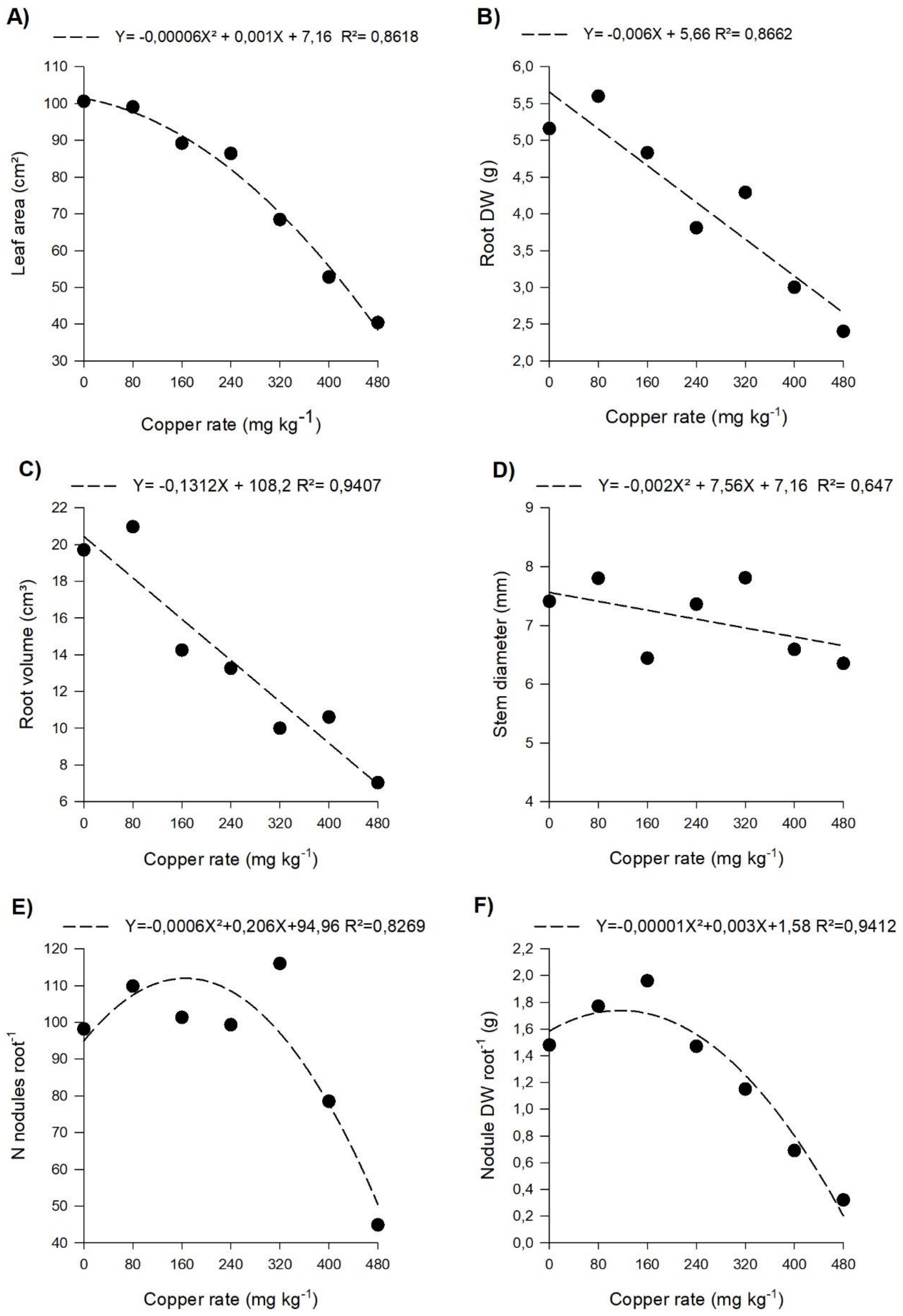

**Figure 5.** Leaf area (LA; A), stem diameter (SD; B), root dry weight (RDW; C), root volume (RV; D), number of nodules (NN; E) and nodule dry weight (NDM; F) of soybean plants subjected to rates of copper in the soil.

The results described in this study show that inoculation with NFB contributed to improving plant morphology, physiology, and, consequently, the number of grains in copper-contaminated soil. This study indicated that it is possible to grow soybean in 34% clay soil contaminated with up to 370 mg Cu kg$^{-1}$, since, when inoculated with *B. japonicum*, this plant tolerates Cu in the soil. However, mechanisms to avoid an increase in the Cu content in the soybean grain must be investigated.

**Table 2**
**Effect of inoculation on leaf area, stem diameter, root dry weight (RDW), number of legumes, and root volume of soybean plants with and without inoculation with the bacterium *B. japonicum.***

| Inoculation | Leaf area (cm²) | Diameter (mm) | RDW (g) | N of legumes | Root volume (cm³) |
|---|---|---|---|---|---|
| Without | 65.37 B* | 6.99 A | 3.56 B | 13.54 B | 5.67 B |
| With | 93.88 A | 7.24 A | 5.13 A | 17.92 A | 6.66 A |

* Means followed by the same letter in the column do not differ by the t test (LSD) at 5%. NFB: *B. japonicum.*

## Conclusions

Inoculation with *Bradyrhizobium japonicum* increases physiological traits such as plant height, specific root surface area, number of grains per plant, and 1000-grain weight in soybean grown in copper-contaminated soil. Therefore, copper values below 370 mg kg$^{-1}$ in the soil do not harm the crop.

Soybean has low efficiency in translocating copper to the shoot; however, the copper content in the grain makes it impossible to recommend the cultivation of this crop in soil contaminated with this metal.

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