Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

# Plant growth-promoting bacteria in sorghum development in copper-contaminated soil

## Bactérias promotoras do crescimento vegetal no desenvolvimento de sorgo em solo contaminado com cobre

*Juliano Cesar da Silva*[1], *Rodrigo Ferreira da Silva*[1], *Victorino Menegat dos Santos*[1], *Andréa da Rocha Giovenardi*[1], *Danni Maisa da Silva*[2], *Eduardo Canepelle*[1], *Ana Paula da Silva*[1]

## ABSTRACT

Copper (Cu) is a metal that can become toxic to sorghum when present in high concentrations in the soil. The use of plant growth-promoting rhizobacteria can be an alternative for the development of sorghum [*Sorghum bicolor* (L.) Moench] in soil contaminated with copper. The objective of this study was to determine the effect of *Azospirillum brasilense* inoculation on the development and copper levels of sorghum cultivated in soil contaminated with Cu, enabling the reuse of these areas. The experimental design was randomized blocks in a factorial arrangement (9 × 2), with nine doses of copper [(0: natural content of the soil), 30, 60, 90, 120, 150, 180, 240, and 300 mg of copper $kg^{-1}$ of soil] with and without *A. brasilense* inoculation, with six replicates. In the treatment without inoculation, a dose of 200 $kg^{-1}$ ha was applied. Plant height (PH), stem diameter, dry mass of the air part (DMAP), volume, length, and root dry mass (RDM), relative index of chlorophyll a and b, and copper contents in DMAP and RDM were evaluated. The inoculation with *A. brasilense* allows a higher index of chlorophyll a and b and the development of the aerial part of the sorghum, reducing copper content in the aerial part, enabling the reuse of soil containing 52.5% clay, contaminated with up to 300 mg of copper $kg^{-1}$ of soil. Inoculation with *A. brasilense* increases the specific surface area (SSA) of sorghum roots compared with non-inoculated plants grown only with nitrogen fertilization in soil contaminated with copper.

**Keywords:** rhizobacteria; *Azospirillum brasilense*; inoculation.

## RESUMO

O cobre é um metal que pode se tornar tóxico para o sorgo quando presente em altas concentrações no solo. A utilização de rizobactérias promotoras do crescimento de planta pode ser uma alternativa para o desenvolvimento do sorgo [*Sorghum bicolor* (L.) Moench] em solo contaminado com cobre. Objetivou-se determinar o efeito da inoculação de *Azospirillum brasilense* no desenvolvimento e teores de cobre do sorgo cultivado em solo contaminado com Cu, possibilitando o reaproveitamento dessas áreas. O delineamento experimental foi de blocos casualizados em arranjo fatorial (9 × 2), sendo avaliadas nove doses de cobre [(0: teor natural do solo), 30, 60, 90, 120, 150, 180, 240 e 300 mg de cobre $kg^{-1}$ de solo] com e sem inoculação de *Azospirillum brasilense*, com seis repetições. No tratamento sem inoculação foi realizada aplicação de nitrogênio na dose de 200 $kg^{-1}$ ha. Foram avaliados a altura de planta, o diâmetro do colo, a massa seca da parte aérea (MSPA), o volume, o comprimento e a massa seca radicular (MSR), o índice relativo de clorofila a e b e os teores de cobre na MSPA e MSR. A inoculação com *Azospirillum brasilense* possibilita maior índice de clorofila A e B e desenvolvimento da parte aérea do sorgo, reduzindo o teor de cobre na parte aérea, possibilitando o reaproveitamento de solo contendo 52,5% de argila, contaminado com até 300 mg de cobre $kg^{-1}$ de solo. A inoculação com *Azospirillum brasilense* aumenta a área superficial específica de raízes de sorgo em comparação a plantas não inoculadas cultivadas somente com adubação nitrogenada em solo contaminado com cobre.

**Palavras-chave:** rizobactérias; *Azospirillum brasilense*; inoculação.

[1]Universidade Federal de Santa Maria – Frederico Westphalen (RS), Brazil.

[2]Universidade Estadual do Rio Grande do Sul – Três Passos (RS), Brazil.

Correspondence address: Juliano Cesar da Silva – Universidade Federal de Santa Maria – Rua Sete de Setembro, s/n – Linha Sete de Setembro – CEP: 98400-000 – Frederico Westphalen (RS), Brazil. E-mail: julianocesaragro@hotmail.com

Conflicts of interest: the authors declare no conflicts of interest.

Funding: none

Received on: 06/27/2023. Accepted on: 12/20/2023.

https://doi.org/10.5327/Z2176-94781660

This is an open access article distributed under the terms of the Creative Commons license.

## Introduction

Copper, when present in high concentrations in the soil, can reduce plant development, inducing physiological, biochemical, and morphoanatomical disorders, which result in the inactivation of cytoplasmic enzymes, oxidative stress, and reduction of photosynthetic processes (Rodrigues et al., 2016; Marques et al., 2018). Furthermore, it interferes with the synthesis of pigments, causing a reduction in plant growth (Sánchez-Pardo et al., 2014), reduces the microbial activity in the soil, and affects soil fauna, compromising the biological quality of the soil (Karimi et al., 2021). Sources of soil contamination with copper are mining, smelting, fertilizers, fungicides, and biosolids used in agriculture, sludge from water treatment plants (Basso and Kiang, 2017), and repeated, uncontrolled application of animal waste, especially pig liquid in agricultural soils, which has a high concentration of this metal (Basso et al., 2012; Rhoden et al., 2017; Poggere et al., 2023). According to resolution nº 420, of December 28, 2009, the guiding value as a maximum limit of total copper in the soil, which determines the need for interventions in agricultural areas, is 200 mg kg$^{-1}$ of soil (CONAMA, 2009). Therefore, it is necessary to carry out scientific work that tests the ability of cultivated plants to survive this contaminant and that aims to reuse these areas environmentally degraded by the excess of this metal.

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal in the world, which is used for the production of bioenergy and animal and human food by millions of people (Ullmann et al., 2018; Maciel et al., 2019) and stands out as an alternative to the use of corn, due to similar nutritional characteristics, however, with higher protein content and lower lysine and methionine (Maciel et al., 2019). Furthermore, it has greater tolerance to water deficit compared with other plants (Menezes et al., 2021). Although copper toxicity in sorghum causes uniform bleaching and pale green color, which may cause the breakdown of the central vein with chlorosis, necrosis and induce iron deficiency (Lima Filho, 2016), this cereal is tolerant to copper, which may be a possibility for economic exploitation of areas contaminated with this metal (Silva et al., 2019). As a consequence, the reuse of these areas would lead to a reduction in the opening of new agricultural frontiers, minimizing the environmental impact caused by the expansion of agricultural activity, such as the 27% reduction in forest area and 31% in secondary vegetation evidenced in Amazonia in the State of Pará, Brazil, from 2000 to 2019 (Paula et al., 2022), as well as changes in the natural characteristics of water and soil (Hunke et al., 2015).

The use of plant growth-promoting Rhizobacteria (PGPR) can be an alternative for growing sorghum in soil contaminated with copper. Among the species of microorganisms, the bacterium *Azospirillum brasilense* has been recommended by many researchers (Araújo et al., 2014; Hungria et al., 2016), as, in addition to its ability to fix $N_2$, it also promotes plant growth through the synthesis of phytohormones and compounds such as auxin, cytokinin, gibberellin, abscisic acid, ethylene, and salicylic acid (Pedraza, 2008), which increase the growth and absorption surface of plant roots and, consequently, the volume of soil explored (Milléo and Cristófoli, 2016). Furthermore, it helps mitigate abiotic stresses, such as salinity and drought, and mitigates the effects of excessive compounds and heavy metals (Fukami et al., 2018). The inoculation of sorghum seeds with *A. brasilense* results in an increase in the production of plant dry mass for silage (Nakao et al., 2018) and grain productivity (Mortate et al., 2020; Canepelle, 2023).

Concern about environmental contamination is crescent, especially those related to soil. Research in this area is justified because there is an urgent need to address this issue to ensure the maintenance of a healthy environment (Ferreira et al., 2016). The combination of PGPR, with the ability to fix nitrogen in grasses, is one of the most promising alternatives, as it also reduces the consumption of inorganic fertilizers, especially nitrogen fertilizers (Moreira et al., 2019). In this way, there would be less possibility of environmental contamination due to human activity related to the nitrification of surface and subsurface waters (Bartucca et al., 2016).

The nutritional and, in some situations, toxic effects caused by Cu on plants are well known. However, the question that still remains is whether the use of PGPR contributes to the development of sorghum in soil contaminated with this element (Cu). In this context, this study aimed to determine the effect of *A. brasilense* inoculation on the development and copper content of sorghum grown in soil contaminated with Cu, enabling the reuse of these areas.

## Materials and Methods

The experiment was installed and conducted in a protected environment, in the greenhouse belonging to the Forestry Engineering department of the UFSM Frederico Westphalen Campus. The soil used was collected from horizon A characterized as Red Oxisol (Santos et al., 2013), with 70% clay, which was mixed with 25% medium sand, enabling the creation of a substrate with a texture of 52.5% clay, to facilitate Cu reactivity and root analysis.

The substrate was dried in an oven with forced aeration at 65±1°C, ground and sieved through a 2-mm mesh, and analyzed in the soil chemistry laboratory whose physical and chemical characteristics are presented in Table 1, according to the methodology described by Mann and Ritchie (1993) for exchangeable copper (KCl extractor 0.005 mol L$^{-1}$) and Tedesco et al. (1995) for the other chemical elements.

**Table 1 – Physical and chemical characterization of the substrate used to grow sorghum crops inoculated with Plant Growth-Promoting Rhizobacteria and applied doses of copper.**

| Attribute | pH | OM | P | K | Mg | Al+H | Cu | Zn |
|---|---|---|---|---|---|---|---|---|
| Texture | 01:01 | % | mg/dm$^3$ | | | | mg/dm$^3$ | |
| 52.5% | 6.5 | 0.88 | 0.8 | 22.86 | 1.82 | 1.91 | 7.19 | 0.58 |

After chemical analysis, limestone was added to the substrate in sufficient quantity to reach a pH of 6.5 in water and then the substrate was incubated for 45 days, with humidity maintained at 80% of field capacity, to stabilize the physicochemical properties. Subsequently, the substrate was sterilized in an autoclave at a temperature of 121°C in 3 cycles of 30 min, and then contamination with copper was carried out, with the doses being applied 30 days before sowing, in the form of a copper sulfate solution ($CuSO_4$, $5H_2O$), which was homogenized by shaking in a plastic bag.

The sorghum cultivar used was TAMBO BMR, characterized by the presence of brownish pigments in the central vein of the leaves and in the stalk. To grow the plants in a greenhouse, plastic pots with a capacity of 3 L were filled with 2.7 kg of the substrate. In each pot, five seeds were sown, which were previously disinfected with 2% sodium hypochlorite for 15 min and washed in running water for 5 min. Thinning was carried out 8 days after germination, leaving three plants per pot until the end of the experiment. Irrigation was carried out daily with water, using an automatic drip irrigation system, maintaining humidity at 80% of field capacity.

The experimental design was randomized blocks in a factorial arrangement (9 × 2), with nine doses of copper [(0: natural soil content), 30, 60, 90, 120, 150, 180, 240, and 300 mg of copper $kg^{-1}$ of soil] with and without *A. brasilense* inoculation, with six replications. In the treatment without inoculation, the equivalent of 200 kg of N $ha^{-1}$ was applied, for an expected yield of 4 T of grains $ha^{-1}$, as recommended by CQFS-RS/SC (2016). After 30 days of applying the copper doses, a soil sample was taken from each pot to determine pseudo-total Cu according to the methodology described by Mann and Ritchie (1993).

The inoculant used was supplied by the company Total Biotecnologia Indústria e Comércio Ltda, based in the city of Curitiba – PR, and was developed by Total Biotecnologia and EMBRAPA. The liquid inoculant Azo Total Max® was used, composed of bacteria of the genus *A. brasilense* (AbV5 and AbV6) at a concentration of $7 \times 10^9$ colony-forming units (CFU) $ml^{-1}$, at a dose of 2 ml $kg^{-1}$ of seed, which resulted in a concentration of 2,000,000 CFU per seed, detected in the seeds at the time of sowing.

The determination of the relative chlorophyll index (RCI) of the leaves was carried out using a portable chlorophyllometer (ClorofiLOG®, Falker, Porto Alegre, model CFL 1030) (Falker Automação Agrícola Ltda, 2018), which provides results in dimensionless units called IRC values and works with readings at two wavelengths. IRC was measured in the fully expanded upper leaf of the sorghum crop and in the central leaf of the last trefoil, taking three readings per leaf, 60 days after sowing.

At the end of the experiment cycle, 100 days after sowing the sorghum, the following were quantified: plant height (PH), measured from the plant neck to the tip of the flag leaf; the neck diameter (ND), determined with a digital caliper (Black Jack®) in the hypocotyl region of the plant; root volume (RV), using a graduated cylinder; root length (RL); root dry mass (RDM), and aerial part dry mass (APDM), and for this purpose, the plants were sectioned in the collar region and both parts, root and aerial parts, were dried in an oven at 65±1°C until constant mass and weighed on an analytical balance.

After collecting the plants, before being dried in an oven, the roots were washed with the aid of 1.0- and 0.5-mm mesh sieves, and a 0.1 g sample was taken for the evaluation of specific surface area (SSA), according to Tennant (1975). RDM and APDM were ground in a Wiley mill with a 10 mesh sieve to determine copper (Cu) content through nitric-perchloric digestion (3:1) and determination in atomic absorption spectrophotometry. To confirm the presence of *A. brasilense* in the endorhizosphere of sorghum plants, the roots were previously washed and superficially disinfected with 70% alcohol for 1 min, followed by 2% sodium hypochlorite for 5 min and washing with autoclaved distilled water. To prepare the concentrated solution, 1 g of the roots was added to 9 ml of saline solution and macerated with the aid of a crucible and pestle. From this solution, a serial dilution was carried out up to $10^{-6}$. Subsequently, 0.1 ml of dilutions $10^{-4}$–$10^{-6}$ were inoculated into three test tubes containing 5.0 ml of semi-solid NFB culture medium. Immediately after this, the tubes were closed with cotton and then incubated at 30°C in biochemical oxygen demand (BOD) (Döbereiner et al., 1995). The evaluation took place on the tenth day of incubation, with tubes that showed the formation of the film characteristic of the growth of diazotrophic bacteria being considered positive. From the positive tubes, the most probable number (MPN) of cells per gram of fresh root matter was estimated using the McCrady table for three replications (Döbereiner et al., 1995).

The results were subjected to analysis of variance using the "Statistical Analysis System" software (Sas, 2000). For the parameters with significant interaction between the variation factors (dose and inoculation), the copper doses within the inoculation were broken down, and in case of no significant interaction, the simple effects were broken down and polynomial regression analysis was applied for dose and Tukey for inoculation to 5%. In cases where no degree of the polynomial was significant, the Tukey test was applied at a 5% probability of error.

## Results and discussion

Analysis of the presence of *A. brasilense* in the endorhizosphere of sorghum plants using the MPN method showed the presence of these microorganisms in the root system of the studied crop (Table 2). The results showed that the treatment without inoculation did not present a population of PGPR, while, with inoculation, $10^7$ cells $g^{-1}$ were obtained, and the increase in copper doses did not change the population of *A. brasilense*. This result confirms the potential of PGPR inoculation in plants grown in areas contaminated with copper. The *Azospirillum* bacterium mitigates the effects of heavy metals in the soil on plants, especially copper, as it has mechanisms of resistance to high concentrations as well as the ability to transform these metals into less toxic forms, through mobilization reactions, which may be through autotrophic and heterotrophic leaching, chelation, and methylation, in addition to immobilization reactions.

**Table 2 – Population of Bacteria in the endorhizosphere of sorghum with and without inoculation with *Azospirillum brasilense*, cultivated with doses of copper in the soil.**

| Doses* | | 0 | 60 | 120 | 180 | 300 |
|---|---|---|---|---|---|---|
| With inoculation | Population ** | 4.67 a A | 4.16 a A | 3.63 a A | 3.73 a A | 3.56 a A |
| Without inoculation | Population ** | 0.00 a B | 0.00 a B | 0.00 a B | 0.00 a B | 0.00 a B |

Averages followed by the same lowercase letter in the row and capital letter in the column do not differ from each other at the 5% error probability level.
*mg of copper $kg^{-1}$ of soil; **bacteria population ($10^7$ cells $g^{-1}$).

Thus, the inoculation of this bacteria protects plants from the toxic effects of excess metals in the soil and also enables their development in contaminated areas (Gadd, 2004; Pérez, 2006; Bashan et al., 2010; Cassán et al., 2020; Barros, 2022).

Pseudo-total copper in the soil, under sorghum cultivation, linearly increases with the copper doses added in treatments with and without *A. brasilense* inoculation (Figure 1) resulting in values above 200 mg $kg^{-1}$ of soil, from an average dose of 168 mg of copper, which indicates the need for intervention in agricultural areas due to possible contamination (CONAMA, 2009). Values above those proposed by CONAMA are toxic to microorganisms, plants, and animals (Andreazza et al., 2013) and cause a reduction in plant growth due to morphological and physiological changes (Mateos-Naranjo et al., 2013; Guimarães et al., 2016). However, the copper content in the soil is not affected by inoculation with *A. brasilense* (Figure 1), and its concentration and availability in the soil result from the balance between the reactions of precipitation, dissolution, complexation, and adsorption, which are influenced by several factors, such as soil type, climate, culture, and chemical form of the elements (Medina et al., 2018; Souza et al., 2018).

Stem diameter (SD), PH, shoot dry mass (SDM), RDM, leaf area (LA), chlorophyll b (Cl b), and chlorophyll a (C1 a) showed no significant interaction between doses and inoculation, with only a single significant difference between inoculation for SD, PH, SDM, LA, Cl b, and Cl a. SD was significantly higher in sorghum plants inoculated with *A. brasilense* (Table 3). This can be attributed to mechanisms that promote growth and development, such as production of phytohormones, solubilization of phosphorus and potassium, and production of hydrolysis enzymes and siderophores (Araújo et al., 2012; Tan et al., 2014), as well as, to the nitrogen supply, coming from fixation by PGPR (Mortate et al., 2020). This result is important, as the reduction in diameter makes plants sensitive to lodging and breakage, negatively affecting crop harvest (May et al., 2012).

Sorghum PH was significantly bigger with *A. brasilense* inoculation (Table 3). This result corroborates other studies that also found an increase in the height of sorghum plants with *A. brasilense* inoculation (Nakao et al., 2014; Mortate et al., 2020; Barros, 2022). The bigger height of the inoculated sorghum plants is mainly attributed to the ability of this bacterium to biologically fix nitrogen and produce growth-stimulating phytohormones (auxins, cytokinins, and gibberellins) (Cassán et al., 2020; Canepelle, 2023). The bigger development of sorghum plants in height is a desired characteristic, as tall plants tend to produce higher amounts of dry matter (Oliveira et al., 2016).

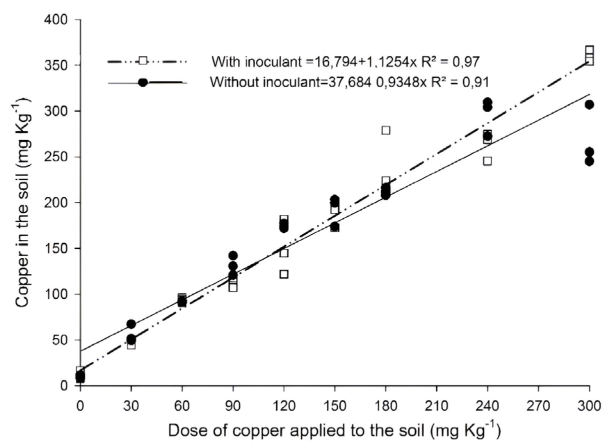


**Figure 1 – Pseudo-total copper content in the soil (Cu in soil) under sorghum cultivation in treatments without and with *Azospirillum brasilense* inoculation and under application of increasing doses of Cu in copper.**

**Table 3 – Means of stem diameter, plant height, aerial part dry mass, root dry mass, leaf area, chlorophyll a, and chlorophyll b of sorghum plants with or without inoculation with *Azospirillum brasilense*.**

| Inoculation* | SD (mm) | PH (cm) | LA ($cm^2$) | APDM (g) | Cl a (IRC) | Cl b (IRC) |
|---|---|---|---|---|---|---|
| With | 7.24 a | 70.25 a | 319.2 a | 4.08 a | 32.3 a | 7.3 a |
| Without | 5.01 b | 52.42 b | 193.26 b | 1.87 b | 30.4 b | 6.4 b |

*With or without inoculation of *Azospirillum brasilense*; SD: diâmetro do caule; PH: altura da planta; APDM: massa seca da parte aérea; RDM: massa seca da raiz; LA: área foliar; Cl a: clorofila a; Cl b: clorofila b. Means followed by the same letter do not differ significantly from each other using the Tukey test ($p<0.05$).

LA and SDM were significantly higher in plants with *A. brasilense* inoculation (Table 3). In the literature, other studies also showed an increase in sorghum LA and aerial dry mass with *A. brasilense* inoculation of plants (Barros, 2022; Canepelle, 2023). The increase in LA indicates better plant growth, as it is one of the morphological parameters that directly reflects the plant development of sorghum, as it is used to estimate the photosynthetic capacity of plants as well as to predict dry matter production and productivity (Lessa et al., 2018; Borrego et al., 2021). Therefore, inoculation with *A. brasilense* allows a significant increase in sorghum phenological parameters, surpassing nitrogen fertilization, as recommended in the literature (CQFS-RS/SC, 2016).

The inoculation with *A. brasilense* allowed higher averages of sorghum chlorophyll a and b (Table 3). Nitrogen plays a fundamental role in sorghum cultivation, as it is an essential constituent of proteins and directly interferes in the photosynthetic process, through its participation in the chlorophyll molecule (Frias et al., 2018), acting directly on the chemical composition of forage species (Simili et al., 2008). Sorghum plants inoculated with *A. brasilense* have a higher level of chlorophyll a and b than plants without inoculation, possibly because it has higher nitrogen levels, and their association with PGPR is characterized as alternatives to meet the plants' need for N without the use of chemical fertilizers, as these microorganisms increase the availability of nitrogen in the soil, through biological nitrogen fixation (BNF) (Breda et al., 2016), also resulting in greater production of APDM of plants (Table 3).

RL, RDM, SDM, and LA of sorghum (Table 4) showed a significant simple effect of copper doses. However, it was not possible to adjust for no degree of the polynomial for regressions; therefore, an analysis of the comparison of means was carried out (Table 4). Thus, from doses of 180 for CR and 240 mg of copper $kg^{-1}$ of soil for RDM, SDM, and LA, corresponding, respectively, to 145 and 198 mg of pseudo-total copper in the soil, the averages were significantly lower in relation to zero dose. Plants subjected to high doses of copper may show decreases in LA, SDM, and RL (Santos et al., 2009; Panziera et al., 2018), corroborating the research value for agricultural areas presented by COMANA 420 (2009).

There was a significant interaction between copper doses and inoculation for the copper content in the dry mass of the aerial and root parts of sorghum (Figure 2A). The copper content in the aerial part showed a quadratic increase with the copper doses, being lower with the inoculation of *A. brasilense* (Figure 2A). Diazotrophic bacteria has the ability to reduce the levels of copper and other metals in plants, due to reducing the transport of metals, through the complexation process and their accumulation inside cells, which consequently reduces their translocation to the interior of plants (Camargo et al., 2007; Andreazza et al., 2013; Barros, 2022).

**Table 4 – Root length, root dry mass, shoot dry mass , and leaf area of sorghum cultivated with copper doses in the soil.**

| Doses* | RL (cm) | RDM (g) | SDM (g) | LA ($cm^2$) |
|---|---|---|---|---|
| 0 | 52.0 a | 2.10 a | 4.6 a | 288.4 ab |
| 30 | 43.7 ab | 1.64 ab | 3.7 ab | 296.1 ab |
| 60 | 38.7 ab | 1.0 ab | 2.7 abc | 255.0 ab |
| 90 | 43.7 ab | 0.80 b | 2.4 bc | 212.0 ab |
| 120 | 45.5 ab | 1.4 ab | 2.9 abc | 305.5 a |
| 150 | 44.9 b | 1.1 ab | 3.3 abc | 286.7 ab |
| 180 | 43.7 ab | 1.0 ab | 2.2 bc | 241.9 ab |
| 240 | 35.6 b | 1.0 ab | 2.5 abc | 273.6 ab |
| 300 | 33.0 b | 0.60 b | 1.5 c | 128.6 b |

*mg of copper $kg^{-1}$ of soil; RL: root length; RDM: root dry mass; SDM: shoot dry mass; LA: leaf area.
Means followed by the same letter do not differ significantly from each other using the Tukey test ($p<0.05$).

In this sense, the research also reports that Rhizobacteria resistant to heavy metals can help in the acquisition of these contaminants from soil by helping in the growth of phytoextractive plants (Estrela et al., 2018); therefore, these organisms are an important strategy to recover areas contaminated with copper (Rajkumar and Freitas, 2008). It is worth noting that in this study, the copper content in the mass of the aerial part of sorghum, regardless of the dose of contaminant applied to the soil and inoculation, was below the maximum tolerable limit for food (dry matter), which is 30 mg $kg^{-1}$ proposed by ANVISA (1965).

The copper content in the root showed a linear increase with the copper doses in the soil in both inoculation treatments (Figure 2B), reaching an average of 445 mg $kg^{-1}$ SDM at the highest dose tested. This result corroborates those of Zancheta et al. (2011), who also showed that copper retention in the roots is the result of a tolerance mechanism to excess metal, and consequently, the reduction in the presence of free ions for transport to the shoot (Lasat, 2002). In this analysis, with an increase in the dose of copper in the soil, sorghum increases the copper content in the roots, regardless of whether or not the crop was inoculated with *A. brasilense*.

The volume and specific root surface area of sorghum showed a significant interaction between copper doses and inoculation (Figure 3A). The RV of sorghum without inoculation linearly reduced with the copper doses in the soil, resulting in 9727 $mm^3$ at a dose of 168 mg of copper, which corresponds to the limit of 200 mg of total copper in the soil, established by CONAMA (2009), while inoculation with *A. brasilense* resulted in an average of 19,857 $mm^3$ in all doses, 104% higher than the treatment without inoculation, which presented only 3975 $mm^3$ at the highest dose tested (Figure 3A). The literature reports that inoculation with diazotrophic bacteria increases the volume of grassroots (Quadros et al., 2014), the results of which can be attributed both to the effect of FBN and to mechanisms that promote morphological and physiological changes in the roots, with potential to maximize RV and the ability of plants to absorb and assimilate nutrients from the soil (Dobbelaere et al., 2001; Müller et al. 2016).

Increasing the dose of copper in the soil linearly reduced the SSA of sorghum roots without inoculation, resulting in 16,092 $mm^3$ at a dose of 168 mg of copper, while inoculation of *A. brasilense* induced a quadratic response with a maximum point at a dose of 386.272 mg of copper $kg^{-1}$ of soil, corresponding to an SSA of 36,584 $mm^2$, being significantly higher than the treatment without inoculation (Figure 3B). Inoculation with *A. brasilenses* makes it possible to modify the architecture of the plant's root, which causes an increase in root hairs and lateral roots and a curving of RL (Van Oosten et al., 2017), which consequently generates an increase in the root surface, enabling greater absorption of nutrients from the soil and root exudation (Spaepen and Vanderleyden, 2011). The increase in the SSA of roots allows for a greater capacity to absorb water and nutrients from the soil (Quadros et al., 2014).

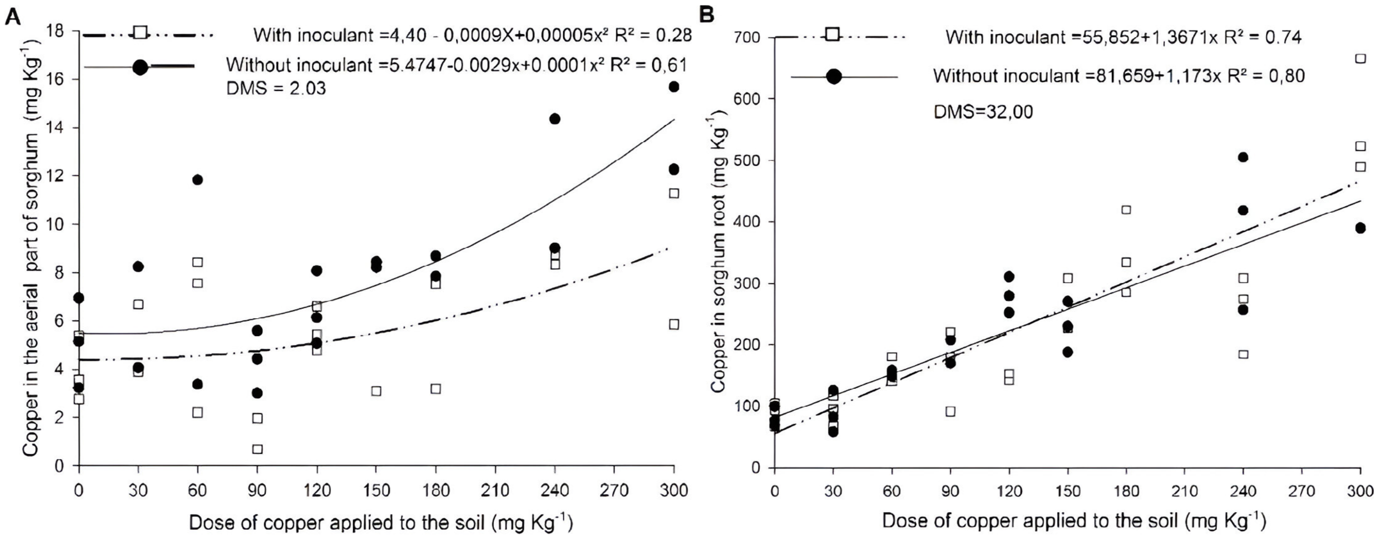


**Figure 2 – Copper content in the dry mass of the (A) aerial and (B) root parts of sorghum with and without inoculation with *Azospirillum brasilense*, cultivated with doses of copper in the soil.**

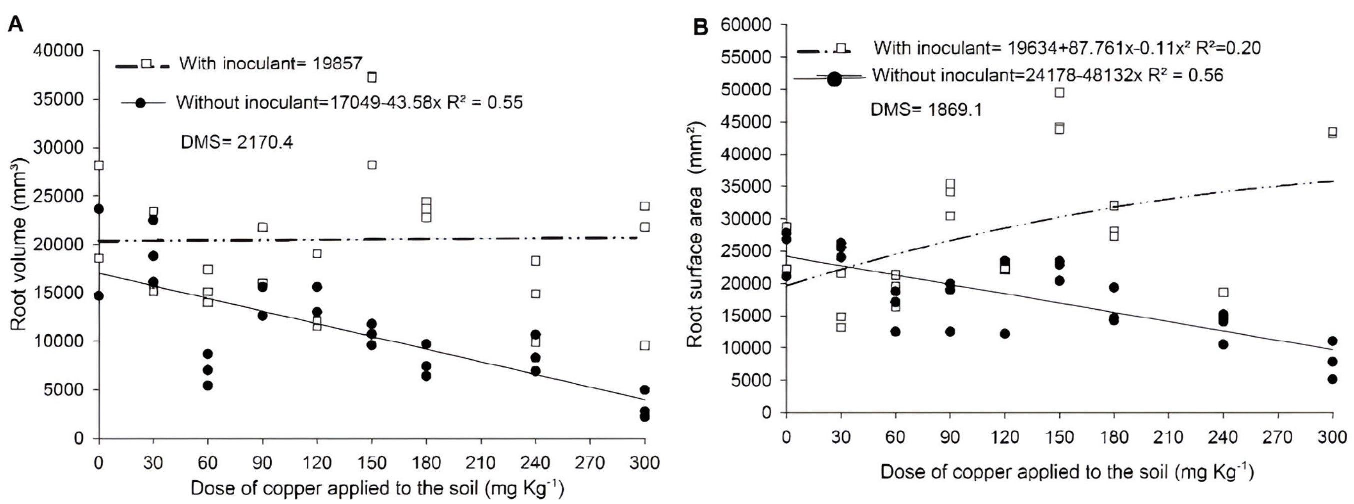


**Figure 3 – (A) Volume and (B) specific surface area of sorghum roots with *Azospirillum brasilense* inoculation, nitrogen application, and copper doses in the soil.**
VOL: volume; SSA: specific surface area.

Inoculation of sorghum by PGPR provides an increase in root surface area compared with non-inoculated plants.

## Conclusions

The inoculation with *A. brasilense* allows a higher level of chlorophyll a and b and the development of the aerial part of sorghum, reducing the copper content in the aerial part, enabling the reuse of soil with 52.5% clay, contaminated with up to 300 mg of copper $kg^{-1}$ ground.

Inoculation with *A. brasilense* increases the SSA of sorghum roots compared with non-inoculated plants grown only with nitrogen fertilization in soil contaminated with copper.

### Authors' contributions:

SILVA, J.C.: writing – review & editing. SILVA, R.F.: supervision. SANTOS, V.M.: writing – review & editing. SILVA, D.M.: writing – review & editing. GIOVENARDI, A.R.: writing – review & editing. CANEPELLE, E.: writing – review & editing. SILVA, A.P.: methodology.

## References

Agência Nacional de Vigilância Sanitário (ANVISA), 1965. Ministério da Saúde. Decreto nº 22.688, de 27 de agosto de 1965. Diário Oficial da União, Brasília.

Andreazza, R.; Camargo, F.A.O.; Antoniolli, Z.I.; Quadro, M.S.; Barcelos, A.A., 2013. Biorremediação de áreas contaminadas com cobre. Revista de Ciências Agrárias, v. 36, (2), 127-136. https://doi.org/10.19084/rca.16290

Araújo, F.F.; Guaberto, L.M.; Silva, I.F., 2012. Bioprospecção de rizobactérias promotoras de crescimento em *Brachiaria brizantha*. Revista Brasileira de Zootecnia, MG, v.41, (3), 52-527. https://doi.org/10.1590/S1516-35982012000300007

Araújo, R.M.; Araújo, A.S.F.; Nunes, L.P.L.; Figueiredo, M.V.B., 2014. Resposta do milho verde à inoculação com *Azospirillum brasilense* e níveis de nitrogênio. Ciência Rural, v. 44, (9), 1556-1560. https://doi.org/10.1590/0103-8478cr20130355

Barros, S., 2022. Arbuscular Mycorrhizal Fungi and Azospirillum brasilense in the development of sorghum and corn grown in soil contaminated with copper. Masters Dissertation, Universidade Federal de Santa Maria, campus Frederico Westphalen. Retrieved 23-06-2023, from http://repositorio.ufsm.br/handle/1/25791

Bartucca, M.L; Mimmo, T.; Cesco, S.; Buono, D.D., 2016. Nitrate removal frompolluted water by using a vegetated floating system. Science of the Total Environment, v. 542, 803-808. https://doi.org/10.1016/j.scitotenv.2015.10.156

Bashan, L.E.; Hernandez, J.; Nelson, K.N.; Bashan, Y.; Maier, R.M., 2010. Growth of quailbush in acidic, metalliferous desert mine tailings: effect of *Azospirillum brasilense* Sp6 on biomass production and rhizosphere community structure. Microbial Ecology, v. 60, 915-992. https://doi.org/10.1007/s00248-010-9713-7

Basso, C.J.; Ceretta, C.A.; Flores, E.M.M.; Girotto, E., 2012. Teores totais de metais pesados no solo após aplicação de dejeto líquido de suínos. Ciência Rural, v. 42, (4), 653-659. https://doi.org/10.1590/S0103-84782012000400012

Basso. J.B.; Kiang. C.H., 2017. Coeficiente de distribuição (kd) de cobre. potássio e cloreto em solos residuais do subgrupo itararé no estado de São Paulo. Águas Subterrâneas, v. 31, (1), 117-133.

Borrego, F.Z.; González, A.R.; Reyna, V.P.A.; Carrillo, M.G.; Moreno, V.M.R.; Rangel, P.P., 2021. Efecto de diferentes niveles de evapotranspiración sobre área foliar, temperatura superficial, potencial hídrico y rendimiento en sorgo forrajero. Terra Latinoamericana, v. 39, 1-14. https://doi.org/10.28940/terra.v39i0.954

Breda, F.D.F.; Alves, G.C.; Reis, V.M., 2016. Produtividade do milho na presença de doses de nitrogênio e inoculação com *Herbaspirillum seropedicae*. Pesquisa Agropecuária Brasileira, v. 51, (1), 45-52. https://doi.org/10.1590/S0100-204X2016000100006.

Camargo, F.A.O.; Bento, F.M.; Jacques, R.J.S.; Roesch, L.F.W.; Frankenberger, W.T., 2007. Uso de microrganismos para a remediação de metais. Tópicos Especiais em Ciência do Solo, v. 5, 467-496.

Canepelle, E., 2023. *Azospirillum brasilense* and arbuscular mycorrhizal fungi in the development of grain sorghum and its occurrence in soil contaminated with copper. Master Dissertation, Universidade Federal de Santa Maria, campus Frederico Westphalen. Retrieved 28-04-2023, from https://repositorio.ufsm.br/handle/1/29414

Cassán, F.; Coniglio, A.; López, G.; Molina, R.; Nievas, S.; Carlan, C.L.; Donadio, F.; Torres, D.; Rosas, S.; Pedrosa, F.O.; Souza, E.; Zorita, M.D.; Mora, V., 2020. Everything you must know about Azospirillum and its impact on agriculture and beyond. Biology and Fertility of Soils, v. 56, (4), p. 461-479. https://doi.org/10.1007/s00374-020-01463-y

Conselho Nacional Do Meio Ambiente (CONAMA), 2009. Resolução COMANA n. 420, de 28 de dezembro de 2009. Diário Oficial da União, Brasília.

Comissão de Química e Fertilidade do Solo (CQFS-RS/SC), 2016. Manual de calagem e adubação para os estados do Rio Grande do Sul e Santa Catarina. 11. ed. Sociedade Brasileira de Ciência do Solo, Porto Alegre, 376 p.

Dobbelaere, S.; Croonenborghs, A.; Thys, A.; Ptacek, D.; Vanderleyden, J.; Dutto, P.; Labandera-Gonzalez, C.; Caballero-Mellado, J.; Aguirre, J. F.; Kapulnik, Y.; Brener, S.; Burdman, S.; Kadouri, D.; Sarig, S.; Okon, Y., 2001. Responses of agronomically important crops to inoculation with Azospirillum. Functional Plant Biology, v. 28, (9), 871-879. https://doi.org/10.1071/pp01074

Döbereiner, J.; Baldani, V.L.D.; Baldani, J.I., 1995. Como isolar e identificar bactérias diazotróficas de plantas não leguminosas. Empresa Brasileira de Pesquisa Agropecuária – CNPAB, Brasília, 60 p.

Estrela, M.A.; Chaves. L.H.G.; Silva. L.N., 2018. Fitorremediação como solução para solos contaminados por metais pesados Phytoremediation. Revista Ceuma Perspectivas, v. 31, 160-172. https://doi.org/10.24863/rccp.v31i1.191

Falker Automação Agrícola Ltda. Manual do Medidor Eletrônico de Teor Clorofila (ClorofiLOG/CFL 1030), 2018 (Accessed September 06, 2022) at:. https://www.falker.com.br/br/suporte.

Ferreira, P.A. A.; Silva, Emanuela P.; Armas, R.D.; Lazzaretti, B.P.; Melo, G.W.B.; Zalamena, J.; Soares, C.R.F.S., 2016. Estratégias de amenização da fitotoxidez de metais pesados em solos de vinhedos. In: Melo, G.W.B.; Zalamena, J.; Brunetto, G.; Ceretta, C.A. Calagem. adubação e contaminação em solos cultivados com videiras. 7. ed. Embrapa Uva e Vinho, Bento Gonçalves, 111-138.

Lima Filho. O.F., 2016. Toxicidade de micronutrientes em sorgo- sacarino: diagnose visual. EMBRAPA, Dourados, 10 p.

Frias, D.B.; Coalho, M.R.; Costa, M.A.; Cizanska, I., 2018. Produtividade e qualidade do sorgo forrageiro na região norte do paraná submetido a diferentes níveis de adubação nitrogenada. Revista Terra & Cultura: Cadernos de Ensino e Pesquisa, v. 34, (Especial), 321-332.

Fukami, J.; Cerezini, P.; Hungria, M., 2018. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express, v. 8, (1), 73. https://doi.org/10.1186/s13568-018-0608-1

Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma, v. 122, (2-4), 109-119. https://doi.org/10.1016/j.geoderma.2004.01.002

Guimarães, P.R.; Ambrosini, V.G.; Miotto, A.; Ceretta, C.A.; Simão, D.G.; Brunetto, G., 2016. Black Oat (*Avena strigose* Schreb.) Growth and Root Anatomical Changes in Sandy Soil with Different Copper and Phosphorus Concentrations. Water, Air, & Soil Pollution, v. 227, (6), 192-201. https://doi.org/10.1007/s11270-016-2900-5

Hunke, P.; Mueller, E.N.; Schroder, B.; Zeilhofer, P., 2015. The Brazilian Cerrado: assess-ment of water and soil degradation in catchments under intensive agriculturaluse. Ecohydrology, v. 8, (6), 1154-1180. https://doi.org/10.1002/eco.1573

Hungria, M.; Nogueira, M.A.; Araujo. R.S., 2016. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics. Agriculture. Ecosystems and Environment, v. 221, 125-131. https://doi.org/10.1016/j.agee.2016.01.024

Karimi, B.; Maron, P.; Ranjard, L., 2021. Ecotoxicity of copper input and accumulation for soil biodiversity in vineyards. Environmental Chemistry Letters, v. 19, 2013-2030. https://doi.org/10.1007/s10311-021-01260-5

Lasat, M.M., 2002. Phytoextraction of Toxic Metals: A Review of Biological Mechanisms. Journal of Environmental Quality, v. 31, 109-120. PMID: 11837415

Lessa, B.F.T.; Silva, T.M.; Dutra, A.S.; Oliveira, A.B., 2018. Modelos matemáticos para a estimativa da área foliar total em sorgo sacarino. Energia na Agricultura, v. 33, (2), p. 175-182. https://doi.org/10.17224/EnergAgric.2018v33n2p175-182

Maciel, M.P.; Moura, V.H.S.; Aiura, F.S.; Arouca, C.L.C.; Souza, L.F.M.; Silva, D.B.; Said, J.L.S., 2019. Níveis de proteína em rações com milho ou sorgo para codornas japonesas. Archivos de Zootecnia, v. 68, (261), 110-118. https://doi.org/10.21071/az.v68i261.3946

Mann, S.S.; Ritchie, G.S.P., 1993. Changes in the Forms of Cadmium with Time in Some Western Australian Soils. Australian Journal of Soil Research, v. 32, (2), 241-250. https://doi.org/10.1071/SR9940241

Mateos-Naranjo, E.; Andrades-Moreno, L.; Cambrollé, J.; Perez-Martin, A., 2013. Assessing the effect of copper on growth, copper accumulation and physiological responses of grazing species *Atriplex halimus*: ecotoxicological implications. Ecotoxicology and Environmental Safety, v. 90, 136-142. https://doi.org/10.1016/j.ecoenv.2012.12.020

Marques, D.M.; Silva, A.B.; Mantovani, J.R.; Pereira, D.S.; Souza, T.C., 2018. Growth and physiological responses of tree species (*Hymenaea courbaril* L.; *Peltophorum dubium* (Spreng.) Taub. and *Myroxylon peruiferum* LF) exposed to different copper concentrations in the soil. Revista Árvore, v. 42, (2), e420202. https://doi.org/10.1590/1806-90882018000200002

May, A.; Campanha, M.M.; Silva, A.R.; Coelho, M.A.O.; Parrella, R.A.A.; Schaffert, R.E.; Filho, I.A.P., 2012. Variedades de sorgo sacarino em diferentes espaçamentos e população de plantas. Revista Brasileira de Milho e Sorgo, v. 11, (3), 278-290. https://doi.org/10.18512/1980-6477/rbms.v11n3p278-290

Medina, E.F.; Pires, J.M.M.; Oliveira, F.S.D.; Silva, B.E.C., 2018. Seleção de solos da região de viçosa para utilização como barreira química para metais pesados. The Journal of Engineering and Exact Sciences, v. 4, (3), 0349-0352. https://doi.org/10.18540/jcecvl4iss3pp0349-0352

Menezes, C.B.; Fernandes, E.A.; Parrella, R.A.C.; Schaffert, R.E.; Rodrigues, J.A.S., 2021. Importância do sorgo para o abastecimento de grãos, forragem e bioenergia no Brasil. In: Menezes, C.B. Melhoramento genético de sorgo. Embrapa, Brasília, 546 p.

Miyazawa, M.; Pavan, M.A.; Muraoka, T.; Carmo, C.A.F.S.; Melo, W.J., 2009. Análise química de tecido vegetal. In: Silva, F. C. Manual de análises químicas de solos, plantas e fertilizantes. 2. ed. Embrapa Informação Tecnológica, Brasília, pp. 59-85.

Milléo, M.V.R.; Cristófoli, I., 2016. Avaliação da eficiência agronômica da inoculação de *Azospirillum* sp. na cultura do milho. Scientia Agraria, v. 17, (3), 14-23.

Moreira, R.C.; Valadão, F.C.A.; Valadão Júnior, D.D., 2019. Desempenho agronômico do milho em função da inoculação com *Azospirillum brasilense* e adubação nitrogenada. Amazonian Journal of Agricultural and Environmental Sciences, v. 62. https://doi.org/10.22491/rca.2019.2865

Mortate, R.K.; Nunes, B.M.; Costa, E.M.; Rocha, E.M.F.; Ventura, M.V.A.; Pereira, L.S., 2020. Resposta de sorgo inoculado com *Azospirillum brasilense* a doses de nitrogênio em cobertura. Ciência Agrícola, v. 18, (1), 65-72.

Müller, T.M.; Sandini, I.E.; Rodrigues, J.D.; Novakowiski, J.H.; Basi, S.; Kaminski, T.H., 2016. Combination of inoculation methods of *Azospirillum brasilense* with broadcasting ofnitrogen fertilizer increases corn yield. Ciência Rural, v. 46, (2), 210-215. https://doi.org/10.1590/0103-8478cr20131283

Nakao, A.; Souza, M.F.P.; Dickman, L.; Centeno, D.C.; Rodrigues, R.A.F., 2014. Resposta do sorgo granífero à aplicação de diferentes doses e épocas de inoculante (*Azospirillum brasilense*) via foliar. Enciclopédia Biosfera, v. 10, (18), 2702-2714.

Nakao, A.H.; Andreotti, M.; Soares, D.A.; Modesto, V.C.; Dickman, L., 2018. Intercropping *Urochloa brizantha* and sorghum inoculated with *Azospirillum brasilense* for silage. Revista Ciência Agronômica, v. 49, 501-511. https://doi.org/10.5935/1806-6690.20180057

Oliveira, R.M.D.E.; Aspiazú, I.; Silva, K.M.J.; Portugal, A.F.; Silva, A.F.; Carvalho, A.J., 2016. Interferência de plantas daninhas em sorgo sacarino em diferentes espaçamentos no semiárido mineiro rena. Revista Brasileira de Milho e Sorgo, v. 15, (3), 481-489. https://doi.org/10.18512/1980-6477/rbms.v15n3p481-489

Panziera, A.G.; Swarowsky, A.; Estefanel, V.; Gomes, B.C.V., 2018. Potencial de fitoextração de cobre por aveia preta em área de vinhedos no Sul do Brasil. Revista Engenharia na Agricultura, v. 26, (6), 565-573. https://doi.org/10.13083/reveng.v26i6.780

Paula, D.S.; Escada, M.I.S.; Ortiz, J.O., 2022. Análise multitemporal do uso e cobertura da terra na Amazônia: A expansão da Agricultura de Larga Escala na Bacia do Rio Curuá-Una. Revista Brasileira de Cartografia, v. 74, (2), p. 379-398. https://doi.org/10.14393/rbcv74n2-63206

Pedraza, R.O., 2008. Recent advances in nitrogen-fixing acetic acid bacteria. International Journal of Food Microbiology, v. 125, (1), 25-35. https://doi.org/10.1016/j.ijfoodmicro.2007.11.079

Pérez, O.C., 2006. Natural attenuation of soils contaminated with toxic waste of mining origin. Isolation and microbial characterization. Internal report of activities of the Faculty of Biology, University of Havana, Havana, Cuba, from: http://www.ucm.es/info/biohidro/Coto_Orquidea.pdf.

Poggere, G.; Gasparin A.; Barbosa, J.Z.; Melo, G.W.; Corrêa, R.S.; Motta, A.C.V., 2023. Soil contamination by copper: Sources, ecological risks, and mitigation strategies in Brazil. Journal of Trace Elements and Minerals, v. 4, 100059. https://doi.org/10.1016/j.jtemin.2023.100059

Quadros, P.D.; Roesch, L.F.W.; Silva, P.R.F.; Vieira, V.M.; Roehrs, D.D.; Camargo, F.A.O., 2014. Desempenho agronômico a campo de híbridos de milho inoculados com *Azospirillum*. Revista Ceres, v. 61, (2), 209-218. https://doi.org/10.1590/S0034-737X2014000200008

Rajkumar. M.; Freitas. H., 2008. Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard. Bioresource Technology, v. 99, (9), 3491-3498. https://doi.org/10.1016/j.biortech.2007.07.046

Rhoden, A.C.; Klein, V.; Feldmann, N.A.; Muhl, F.R.; Balbinot, M.; Ritter, A.F. S., 2017. Parâmetros químicos do solo influenciados por aplicação de dejetos de suínos em áreas agrícolas do oeste catarinense. Revista de Ciências Agroveterinárias e Alimentos, v. 2, 1-12.

Rodrigues, A.C.D.; Santos, A.M.; Santos, F.S.; Pereira, A.C.C.; Sobrinho, N.M.B.A., 2016. Mecanismos de respostas das plantas à poluição por metais pesados: possibilidade de uso de macrófitas para remediação de ambientes aquáticos contaminados. Revista Virtual de Química, v. 8, (1), 262-276. https://doi.org/10.5935/1984-6835.20160017

Sánchez-Pardo, B.; Fernández-Pascual, M.; Zornoza, P., 2014. Copper microlocalisation and changes in leaf morphology, chloroplast ultrastructure and antioxidative response in white lupin and soybean grown in copper excess. Journal of Plant Research, v. 127, 119-129. https://doi.org/10.1007/s10265-013-0583-1

Santos, H.C.; Fraga, V.S.; Raposo, R.W.C.; Perreira, W.E., 2009. Cu e Zn na cultura do sorgo cultivado em três classes de solos: I . Crescimento vegetativo e produção. Revista Brasileira de Engenharia Agrícola e Ambiental, v. 13, (2), 125-130. https://doi.org/10.1590/S1415-43662009000200003

Santos, H.G., 2013. Sistema Brasileiro de Classificação de Solos. Embrapa, Brasília, 342 p.

Sas, L.E., 2000. Getting started with the SAS Learning Edition, Cary.

Silva, T.J.; Hansted, F.; Tonello, P.S.; Goveia, D., 2019. Fitorremediação de Solos Contaminados com Metais : Panorama Atual e Perspectivas de uso de Espécies Florestais. Revista Virtual de Quimica, v. 11, (1), 1-17. https://doi.org/10.21577/1984-6835.20190003

Simili, F.F.; Reis, R.A.; Furlan, B.N.; Paz, C.C.P.; Lima, M.L.P.; Bellingieri, P A., 2008. Do Híbrido De Sorgo-Sudão E Potássica : Composição Química E Digestibilidade in Vitro. Ciência Agrotécnica, v. 32, (2), 474-480. https://doi.org/10.21577/1984-6835.20190003

Souza, E.L.; Damasceno, F.; Schirmer, G.K.; Ramires, M.F.; Bisognin, R.P.; Bohrer, R.E.G.; Vasconcelos, M.C.; Cezimbra, J.C.G., 2018. Resíduos contaminantes no solo: possibilidades e consequências. Revista Gestão & Sustentabilidade Ambiental, v. 7, (2), 484-509. https://doi.org/10.19177/rgsa.v7e22018484-509

Spaepen, S.; Vanderleyden, J., 2011. Auxin and plant-microbe interactions auxin and plant-microbe interactions. Cold Spring Harbor Perspectives in Biology, v. 7, (3), 1-13. https://doi.org/10.1101/cshperspect.a001438

Tan, K.Z.; Radziah, O.; Halimi, M.S.; Khairuddin, A.R.; Habib, S.H.; Shamsuddin. Z.H., 2014. Isolation and characterization of rhizobia and plant growth-promoting rhizobacteria and their effects on growth of rice seedlings. American Journal of Agricultural and Biological Science, v. 9, (3), 342-360. https://doi.org/10.3844/ajabssp.2014.342.360

Tedesco, M.J.; Gianello, C.; Bissani, C.A.; Bohnen, H.; Volkweiss, S.J., 1995. Análise de solo, plantas e outros materiais. Departamento de Solos da UFRGS, Porto Alegre, 170 p.

Tennant, D., 1975. A test of a modified line intersect method of estimating root length. Journal of Ecology, v. 63, (3), 995-1001. https://doi.org/10.2307/2258617

Ullmann, R.; Resende, O.; Rodrigues, G.B.; Chaves, T.H.; Oliveira, D.E., 2018. Qualidade fisiológica das sementes de sorgo sacarino submetidas à secagem e ao armazenamento. Revista Engenharia na Agricultura, v. 26, (4), 313-321. https://doi.org/10.13083/reveng.v26i4.960

Van Oosten, M.J.; Pepe, O.; Pescale, S.; Silletti, S.; Maggio, A., 2017. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chemical and Biological Technologies in Agriculture, v. 4, 1-12. https://doi.org/10.1186/s40538-017-0089-5

Zancheta, A.C.F.; Abreu, C.A.; Zambrosi, F.C.B.; Erismann, N.M.; Lagôa, A.M.M.A., 2011. Fitoextração de cobre por espécies de plantas cultivadas em solução nutritiva. Bragantia, v. 70, (4), 737-744. https://doi.org/10.1590/S0006-87052011000400002