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INFLUENCE OF BACTERIA ON THE BIOREMEDIATION OF SOIL CONTAMINATED WITH CADMIUM AND CULTIVATED WITH CRAMBE ABYSSINICA IN PROTECTED CULTIVATION

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ABSTRACT

The increasing concern about Cd contamination, a toxic metal that accumulates in the environment and enters the food chain, poses risks to human health and the environment. This research focuses on remediation methods such as phytoremediation using Crambe abyssinica and bioremediation with plant growth-promoting bacteria (PGPB), highlighting their potential to reduce Cd bioavailability. The experiments were carried out in a protected cultivation system in Paraná, Brazil, using a randomized block design. Seeds were inoculated with A. brasilense and P. fluorescens under varying Cd doses. Evaluated variables included plant growth, anatomical characteristics, and concentrations of macronutrients and Cd in plant tissue and soil. Results showed that bacterial inoculation significantly increased plant growth even under Cd contamination. Inoculated plants exhibited greater root and shoot length, as well as improved vascular structure in xylem and phloem. Nutrient analysis revealed better macronutrient uptake and greater Cd accumulation, especially in aerial plant parts. The results confirm that phytoremediation and bioremediation are effective strategies for mitigating Cd contamination in agricultural soils. Inoculation with PGPB not only improved plant health and growth but also enhanced Cd accumulation capacity, supporting their use in sustainable remediation strategies. The techniques presented offer sustainable approaches for managing environmental risks associated with toxic metals.

Keywords: Plant Growth-Promoting Bacteria. Phytoremediation. Toxic Metals. Microorganisms.

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INFLUÊNCIA DE BACTÉRIAS NA BIORREMEDIAÇÃO DE SOLO CONTAMINADO COM CÁDMIO E CULTIVADO COM CRAMBE ABYSSINICA EM CULTIVO PROTEGIDO

RESUMO

A crescente preocupação com a contaminação por Cd, um metal tóxico que se acumula no ambiente e entra na cadeia alimentar, representa riscos à saúde humana e ao meio ambiente. Esta pesquisa se concentra em métodos de remediação, como fitorremediação usando Crambe abyssinica e biorremediação com bactérias promotoras de crescimento de plantas (PGPB), destacando seu potencial para reduzir a biodisponibilidade de Cd. Os experimentos foram realizados em um sistema de cultivo protegido no Paraná, Brasil, usando um delineamento em blocos casualizados. As sementes foram inoculadas com A. brasilense e P. fluorescens sob doses variadas de Cd. As variáveis avaliadas incluíram crescimento da planta, características anatômicas e concentrações de macronutrientes e Cd no tecido da planta e no solo. Os resultados mostraram que a inoculação bacteriana aumentou significativamente o crescimento da planta, mesmo sob contaminação por Cd. As plantas inoculadas exibiram maior comprimento de raiz e parte aérea, bem como melhor estrutura vascular no xilema e floema. A análise de nutrientes revelou melhor absorção de macronutrientes e maior acúmulo de Cd, especialmente nas partes aéreas da planta. Os resultados confirmam que a fitorremediação e a biorremediação são estratégias eficazes para mitigar a contaminação por Cd em solos agrícolas. A inoculação com PGPB não apenas melhorou a saúde e o crescimento das plantas, mas também aumentou a capacidade de acumulação de Cd, apoiando seu uso em estratégias de remediação sustentáveis. As técnicas apresentadas oferecem abordagens sustentáveis para o gerenciamento de riscos ambientais associados a metais tóxicos.

Palavras-chave: Bactérias Promotoras do Crescimento de Plantas. Fitorremediação. Metais Tóxicos. Microrganismos.

INFLUENCIA DE LAS BACTERIAS EN LA BIORREMEDIACIÓN DE SUELOS CONTAMINADOS CON CADMIO Y CULTIVADOS CON CRAMBE ABYSSINICA EN CULTIVOS PROTEGIDOS

RESUMEN

La creciente preocupación por la contaminación con Cd, un metal tóxico que se acumula en el medio ambiente y entra en la cadena alimentaria, representa riesgos para la salud humana y el medio ambiente. Esta investigación se centra en métodos de remediación como la fitorremediación con Crambe abyssinica y la biorremediación con bacterias promotoras del crecimiento vegetal (PGPB), destacando su potencial para reducir la biodisponibilidad del Cd. Los experimentos se llevaron a cabo en un sistema de cultivo protegido en Paraná, Brasil, utilizando un diseño de bloques al azar. Se inocularon semillas con A. brasilense y P. fluorescens bajo diferentes dosis de Cd. Las variables evaluadas incluyeron el crecimiento de la planta, las características anatómicas y las concentraciones de macronutrientes y Cd en el tejido vegetal y el suelo. Los resultados mostraron que la inoculación bacteriana aumentó significativamente el crecimiento de la planta incluso bajo contaminación por Cd. Las plantas inoculadas exhibieron una mayor longitud de raíces y brotes, así como una mejor estructura vascular en xilema y floema. El análisis de nutrientes reveló una mejor absorción de macronutrientes y una mayor acumulación de Cd, especialmente en las partes aéreas de la planta. Los resultados confirman que la fitorremediación y la biorremediación son estrategias eficaces para mitigar la contaminación por Cd en suelos agrícolas. La inoculación con PGPB no solo mejoró la salud y el crecimiento de las plantas, sino que también incrementó la capacidad de acumulación de Cd, lo que respalda su uso en estrategias de remediación sostenibles. Las técnicas presentadas ofrecen enfoques sostenibles para la gestión de los riesgos ambientales asociados a los metales tóxicos.

Palabras clave: Bacterias Promotoras del Crecimiento Vegetal. Fitorremediación. Metales Tóxicos. Microorganismos.

1 INTRODUCTION

Pollution by toxic metals, especially Cd, has emerged as a global environmental and public health concern due to its persistence and capacity for bioaccumulation in soils, water, and the atmosphere, subsequently entering the food chain (Lima 2023). Cd stands out among toxic metals because of its widespread industrial release and the consequent health risk through food consumption (Chien et al. 2003).

Cadmium (Cd) exposure in humans is well-documented to cause severe health effects, including renal dysfunction, bone demineralization (Itai-itai disease), and an increased risk of cancer (Nordberg et al. 2018; Satarug et al. 2010). Chronic low-level exposure through contaminated food crops represents a significant route of human intake, especially in agricultural regions with Cd-enriched soils (Satarug et al. 2019). Therefore, reducing Cd bioavailability in agricultural soils is not only an environmental priority but also a critical public health objective to prevent its transfer into the food chain and mitigate human health risks (FAO/WHO 2010).

Soil contamination by Cd, exacerbated by the use of phosphate fertilizers and inappropriate agricultural practices, highlights the urgency of interventions to mitigate its adverse effects on the environment and human health (Rafiq et al. 2014).

The prevalence of Cd in agricultural soils, often resulting from the use of phosphate fertilizers, sewage sludge, and industrial waste, combined with the contribution of intensive agriculture, underscores the anthropogenic nature of this problem (Kumar et al. 2011; Zhong et al. 2020). This situation is further aggravated by insufficient regulation regarding the use of these inputs, resulting in high Cd concentrations in soil (Yang et al. 2004).

Given this scenario, it is essential to adopt effective remediation methods for Cd-contaminated soils. Phytoremediation and bioremediation stand out as sustainable solutions aimed at reducing Cd bioavailability and mitigating its impacts. Phytoremediation using plants such as Crambe (Crambe abyssinica) is a viable option, leveraging the high oil content of its seeds for applications such as biodiesel production (Ciciliano et al. 2023). Simultaneously, bioremediation with bacteria from the genera Azospirillum and Pseudomonas has demonstrated efficacy in reducing Cd concentrations, due to these microorganisms' tolerance to toxic metals (Miller et al. 2009; Cruz-Hernández et al. 2022).

Thus, this study aims to elucidate alternatives for Cd remediation, addressing both phytoremediation and bioremediation. It will discuss the mechanisms of plant uptake, bioaccumulation, and the deleterious effects of Cd on plants. However, it is crucial to recognize that both phytoremediation and bioremediation are influenced by environmental variables such as soil pH and organic matter, and that the effectiveness of these methods may vary depending on local conditions. Therefore, a detailed assessment of each situation is indispensable for selecting the most effective strategy.

By addressing the remediation of Cd-affected soils, this study contributes to the understanding of the relevance of phytoremediation and bioremediation techniques, emphasizing the role of plants such as Crambe and bacteria such as Azospirillum and Pseudomonas in the process.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN AND PLANT ESTABLISHMENT

The study was conducted from April to July 2023 in a protected cultivation system located on a rural property in the district of Espigão Azul (24°50'26"S 53°27'26"W), in Southern Brazil. Laboratory analyses were performed at SBS Laboratory – Agronomic and Veterinary Analyses, in the city of Cascavel, PR - Brazil.

The experimental setup followed a randomized block design, arranged in a factorial scheme with four replicates. Treatments consisted of inoculation with two bacterial species: *Azospirillum brasilense* (strains Ab-V5 and Ab-V6, both at 2×10^9 CFU/mL) and *Pseudomonas fluorescens* (ATCC 13525, at 2×10^9 CFU/mL), with each seed inoculated with 10^6 CFU/mL of viable cells. Cell viability was confirmed through viable cell counts performed 48 hours before inoculation, with the inoculum applied directly into the planting furrow at sowing.

Regarding Cd contamination, four doses were established: control (no metal addition), half the maximum permissible dose for agricultural soils (1.5 mg kg^{-1}) according to CONAMA Resolution 420 (CONAMA 2009), the maximum permissible dose (3.0 mg kg^{-1}), and twice the maximum permissible dose (6.0 mg kg^{-1}).

Soil contamination was performed by adding a standard cadmium chloride ($CdCl_2$) solution 24 hours before sowing and inoculation. Sowing consisted of ten seeds per pot, which was thinned to four plants per pot after ten days, as shown in Figure 1. Each experimental unit consisted of a pot with four plants, totaling 48 pots as outlined in Figure 2.

2.2 EXPERIMENTAL CONDITIONS AND SUBSTRATE PREPARATION

For cultivation, cylindrical plastic seedling bags were used, measuring 30 cm in height and 20 cm in diameter, with a 5 L capacity, filled with 5 kg of previously sieved red latosol (Figure 1.A). This substrate, initially presenting good agronomic characteristics for crambe cultivation, was adjusted with fertilization to achieve optimal growing conditions. Details on the fertility of the soil used are provided in Table 1. Treatments were irrigated every 48 hours to maintain soil moisture from sowing until the onset of flowering (Figure 1.F).

2.3 MORPHOMETRIC ANALYSES

On the 50th day after emergence (DAE), plants were removed from the pots for measurement of total length, shoot height, root length (using a graduated ruler), and stem diameter (with a digital caliper). Subsequently, plants were separated into roots and shoots, which were placed in paper bags and weighed to determine fresh mass. Samples were then dried in a forced-air circulation oven at 65 °C until constant mass was achieved, followed by determination of dry mass using a precision scale.

Anatomical analysis of the vascular bundles was performed through cross-sections at the collar region. Sample processing followed the protocol proposed by Gordon and McCandless (1973), with adaptations. Initially, fragments were treated with a 4% paraformaldehyde solution, adjusted to pH 7.20, and left immersed for 20 hours to ensure complete fixation. Samples then underwent a dehydration series with ethanol at increasing concentrations: 30%, 50%, 70%, 80%, and 90%, each step lasting 2 hours, followed by two stages in 100% ethanol for 2 hours each. After dehydration, samples were clarified in xylene I (a 50% mixture of 100% ethanol and xylene) for 1 hour, and then in pure xylene II for an additional hour.

Next, samples were embedded in paraffin through three stages of immersion, each lasting 6 hours. Paraffin blocks were prepared, and after 24 hours, once fully solidified, sections were cut using a manual microtome. Before staining, slides were heated at 50 °C in an oven for about 1 hour.

The staining process began with paraffin removal in pure xylene I and II for 10 minutes each, followed by a series of hydration steps in decreasing ethanol concentrations, and finally immersion in water. Staining was performed using 0.05% toluidine blue at pH 4.00, applied to the slides for 5 minutes at 55 °C. After staining, slides were rinsed under running water, dehydrated in ethanol, and cleared in xylene. Finally, slides were mounted with Entellan and coverslips.

Samples were observed under an optical microscope, enabling identification of xylem walls stained green or bluish-green, while phloem walls appeared reddish-purple. This differentiation is illustrated in Figures 3 and 4.

2.4 DETERMINATION OF TOTAL MACRONUTRIENT, MICRONUTRIENT, AND CADMIUM CONTENT IN SOILS AND PLANT TISSUE

To evaluate total macronutrient, micronutrient, and Cd contents in plant tissue (shoot and root), extraction was performed using a nitro-perchloric digestion method according to the Association of Official Analytical Collaboration (AOAC 2023). Determinations of Ca, Mg, Fe, Cu, Zn, Mn, and Cd were carried out by flame atomic absorption spectrometry (model GBC, SavantAA), while K was measured by flame photometry (Benfer, BFC 150). P was quantified by UV/Vis spectroscopy. N content was determined using the Kjeldahl method as modified by Vogel (Vogel 1981).

2.5 DETERMINATION OF AVAILABLE MACRONUTRIENT CONTENT IN SOIL

To determine available macronutrient, micronutrient, and Cd contents in soil, as well as pH, the methodology described by Embrapa (Silva 2009) was adopted. KCl extractor was used for Ca and Mg, and Mehlich 1 extractor for P and K. Determinations of Ca and Mg were performed by flame atomic absorption spectrometry (model GBC, SavantAA), while K was quantified by flame photometry (Benfer, BFC 150). P was quantified by UV/Vis spectroscopy.

2.6 LIMITS OF QUANTIFICATION

The limit of quantification (LOQ) is defined as the lowest analyte concentration that can be quantified in a sample with acceptable accuracy and precision. The LOQ is considered ten times the standard deviation of a series of blank measurements. The LOQ of the method using air-acetylene flame atomic absorption spectrophotometry is presented in Table 2.

2.7 ACCUMULATION AND TRANSLOCATION INDICES

The accumulation index was calculated as the ratio between the amount of metal accumulated in the plant and the amount accumulated in the substrate, following Zhang et al. (Zhang et al. 2007). Similarly, the translocation index was obtained by dividing the metal concentration in the shoot by the concentration in the root.

For statistical analysis, Analysis of Variance (ANOVA) was applied, and means were compared using Tukey’s test at a 5% probability level, with the statistical software Sisvar 5.6 (Ferreira 2019).

3 RESULTS AND DISCUSSION

3.1 MORPHOMETRIC ANALYSES

Inoculation resulted in a significant increase in the total length of crambe plants, as shown in Figure 5.A, regardless of metal concentration. Plants not exposed to the metal also exhibited increased total length when inoculated with *A. brasilense* and *P. fluorescens*. This effect on total plant length is attributed to marked shoot growth, as observed in Figure 5.B. In treatments without metal addition, increases of 32.70% with *A. brasilense* and 33.17% with *P. fluorescens* were recorded. For the highest metal concentration tested, the increase was even more pronounced, reaching 44.65% with *A. brasilense* and 58.13% with *P. fluorescens*. Root length also increased significantly in treatments without metal addition, with growth of 64.27% for *A. brasilense* and 79.21% for *P. fluorescens*. At the highest metal concentration, this increase was exceptionally high, reaching 166.66% with *A. brasilense* and 286.69% with *P. fluorescens*; these values can be found in Figure 5.C.

The use of plant growth-promoting bacteria (PGPB), such as *A. brasilense* and *Pseudomonas* sp., has proven effective in increasing root development and overall plant length, especially under environmental stress. These microorganisms support nutrient uptake and optimize plant physiology, essential characteristics for crops grown in Cd-contaminated soils.

Inoculation with *A. brasilense*, in particular, is advantageous because it promotes plant growth through multiple mechanisms, including phytohormone synthesis and improved N absorption. These interactions reduce stress and enable biological control of pathogens, boosting plant productivity (Bashan and De-Bashan 2010; Domenico 2019; Kargapolova et al. 2020).

Ferreira et al. (2020) highlight that *P. fluorescens* contributes to delaying plant senescence, mainly through the action of ACC deaminase, an important plant growth-promoting enzyme according to Glick (2014).

Additionally, bacteria such as *Azospirillum* and *Pseudomonas* can stimulate plant growth and reduce stress through the production of hormones such as auxin, gibberellin, and cytokinin (Khoshru et al. 2020; Lopes et al. 2021).

Studies conducted by Almeida (2020) and Silva (2022) on maize seeds inoculated with strains of *Azospirillum* sp., *Pseudomonas* sp., and *Bacillus aryabhattai* sp. revealed significant improvements in leaf growth and shoot green biomass. Both studies reported increases of over 10% compared to control groups, highlighting not only greater plant height and leaf area but also enhanced yield, demonstrating these microorganisms' effectiveness in promoting plant growth.

The results obtained using inoculation with *P. fluorescens* and *A. brasilense* are consistent with findings by Guimarães et al. (2021). That study specifically examined the co-inoculation of soybean seeds with *P. fluorescens* (strain ATCC 13525) and *Bradyrhizobium japonicum* along with phosphate fertilizer. *P. fluorescens* proved agronomically effective, promoting significant improvements in soybean growth and development. This finding reinforces the value of using such PGPB to enhance agricultural productivity.

A. brasilense plays a fundamental role in biological nitrogen fixation (BNF), partially meeting the N requirements of plants while synthesizing phytohormones such as auxins, gibberellins, and cytokinins, which are crucial for stimulating root growth (Hungria 2011). Hungria (2011) observed significant increases in root development and maize plant height after *Azospirillum* inoculation. Similarly, Cotrim et al. (2016) found that wheat seedlings treated with humic acid and *A. brasilense* exhibited improvements in root and shoot growth and in shoot dry mass.

Vasconcelos (2022) found that root length in rice plants inoculated with biological inputs, specifically *Azospirillum* and *Pseudomonas*, was 35.71% greater compared to treatments without inoculation. This result underscores the effectiveness of inoculants in promoting plant growth.

On the other hand, Gautam et al. (2016) emphasized that exposure to toxic metals such as Cd can adversely affect root growth. This reduction in growth is attributed to inhibition of cell division and the rate of cell elongation, often caused by irreversible blocking of the proton pump, a vital component for cellular metabolism.

Furthermore, all inoculated plants showed a significant increase in both fresh and dry mass, resulting in increased total biomass, as illustrated in Figure 6 for fresh and dry mass. Similar trends were observed in dry mass increment values, following the same patterns as fresh mass; these values are shown in Figure 6.

Studies conducted by Babu et al. (2015) showed that *Miscanthus sinensis*, in association with *P. koreensis* in soils contaminated by toxic metals from mining activities, exhibited high tolerance to heavy metals and significantly increased Cd solubilization. This association also resulted in a 54% increase in *M. sinensis* biomass, along with improved nutrient uptake and aboveground biomass production, benefiting soil microbiota (Hungria 2011).

Other studies, such as Chiarini et al. (1998), observed significant increases in both shoot and root fresh biomass in sorghum plants treated with *P. fluorescens*. Similarly, Gasoni et al. (2001) reported benefits limited to shoot fresh biomass in lettuce crops inoculated with *P. fluorescens*. Bulegon et al. (2016) also found promising results in soybean cultivation with *A. brasilense* inoculation.

The harmful effects of Cd on plants are well documented, including visible symptoms such as growth inhibition, chlorosis, necrosis, and wilting, as well as reductions in photosynthetic rate and cellular respiration (Navarro-León et al. 2019). Beyond these visible effects, Cd also causes less apparent impacts, such as reduced biomass, changes in mineral composition, and subcellular damage (Sanità di Toppi and Gabbrielli 1999). In extreme situations, the presence of Cd can drastically reduce biomass production and even lead to plant death (Dias et al. 2012).

Vasconcelos (2022) observed an increase in shoot fresh mass in rice plants treated with biological inputs compared to untreated controls. Additionally, Domingues et al. (2019) found that inoculation with *A. brasilense* significantly improved root growth in soybean plants, including increases in root length and radicle dry mass.

Evaluating biomass is essential for understanding phytoremediation strategies and plant development in contaminated environments. Greger (2003) highlights the distinction between hyperaccumulator plants, which have low biomass production but high metal accumulation capacity, and accumulator plants, which produce more biomass but accumulate less metal. This differentiation is crucial for optimizing metal removal per area, focusing not only on accumulation but also on the amount of biomass produced.

A. brasilense is well-known for its ability to improve nutrient absorption, leading to increased aboveground biomass production, which benefits soil microbiota, maintains moisture, and reduces nutrient leaching (Hungria 2011). This effectiveness was also observed in studies by Novinscak, Joly, and Filion (2019), where seed inoculation with *P. fluorescens* increased plant biomass and oil yield in crops such as soybean and canola.

Vasconcelos (2022) noted that the shoot dry mass of rice plants inoculated with *P. fluorescens* and *A. brasilense* increased significantly by 70.27%. In contrast, Marques et al. (2020) reported that shoot dry mass production in tree species was negatively impacted by soil contamination with Cd, compromising growth in some species.

Similarly, Košćak et al. (2023) demonstrated that inoculation with *A. brasilense* in hydroponically grown lettuce significantly increased fresh and dry mass, number of leaves, and chlorophyll production. This result suggests that these bacteria can be effective across various plant species and cultivation conditions.

Rafique et al. (2016) also supported these findings, demonstrating that inoculation with *A. brasilense* in wheat led to significant increases in plant fresh and dry weight, as well as greater root and leaf length. This beneficial effect is attributed to the bacteria's ability to produce phytohormones and improve plant nutrient efficiency.

The stem diameter of inoculated plants followed a similar pattern, being significantly larger than that of non-inoculated plants. In treatments without metal addition, increases of 61.03% with *A. brasilense* and 76.33% with *Pseudomonas* spp. were observed. At the highest metal concentration tested, the increase was even more pronounced, reaching 96.75% with *A. brasilense* and 92.82% with *P. fluorescens*, as shown in Figure 7.

Research on inoculation with PGPB such as *A. brasilense* and *P. fluorescens* has highlighted their effectiveness in producing significant changes in plant physiology. One of the observed effects is the increase in stem diameter, an indicator of more robust vegetative development and potentially greater resistance to environmental stresses, such as the presence of toxic metals including Cd.

Recent studies confirm that these bacteria not only increase stem diameter but also contribute to improved fruit quality and yield. For example, a study conducted on tomato plants inoculated with *P. fluorescens* and *A. brasilense* demonstrated that these bacteria substantially improved both stem diameter and overall plant performance, likely due to enhanced nutrition and hormonal modulation promoted by the bacteria (Pérez-Rodriguez et al. 2020).

Although these studies did not focus specifically on Brassicaceae plants or Cd presence, they demonstrate the potential of bacterial inoculations to strengthen plants. This strengthening can be particularly advantageous in environments contaminated with heavy metals. The ability of these bacteria to modify plant physiology may provide improved protection against the toxic effects of metals like Cd, thereby increasing plant tolerance to such stresses.

3.2 ANATOMICAL ANALYSES

Regarding anatomical analyses, changes were observed in the collar anatomy of the plants in response both to Cd presence and to bacterial inoculation. In general, inoculation resulted in an increase in the number of cell layers in the xylem (Figure 8.B) and phloem (Figure 8.A), while Cd addition tended to reduce these layers. The differentiation and development of conducting vessels—xylem or phloem—are complex processes influenced by plant hormones. Gibberellins act in the early stages, while auxins and cytokinins regulate subsequent developmental steps, with ethylene contributing to the final formation of conducting vessels (Sorce et al. 2013).

On average, plants inoculated with *A. brasilense* showed an increase of 229.34% in phloem and 231.54% in xylem. Inoculation with *P. fluorescens* resulted in a 156.94% increase in phloem and 130.07% in xylem. For the pith cells observed (Figure 8.C), only *P. fluorescens* induced an 18.83% increase, while for the cortex observed in Figure 8.D, *A. brasilense* caused a 24.79% increase, as shown in Figure 9.

Inoculation with PGPB can effectively increase the size and number of cells in xylem and phloem vascular bundles. This capacity is attributed to several strategies employed by these microorganisms, including the production of phytohormones such as auxins, gibberellins, and cytokinins, which regulate plant growth and influence vascular tissue differentiation and improved plant nutrition.

Such processes enhance overall plant nutrition, which is fundamental for robust vascular system development. Additionally, root system modifications occur, where some microorganisms influence root architecture by promoting the development of lateral roots and root hairs. These changes can increase water and nutrient uptake, supporting a more developed and functional vascular system (Bush, Sethi, and Sablowski 2022; Yang and Wang 2016).

The formation of xylem, a crucial component of the plant vascular system, is regulated by a complex hormonal interplay involving decreased cytokinins and auxins and increased ethylene, which promotes the programmed cell death necessary for the formation of xylem vessels specialized in passive transport of water and nutrients (Sorce et al. 2013). The presence of *A. brasilense* has been shown to benefit xylem, especially under conditions of water stress or pathogen attack, by increasing the plant's hydraulic conductivity, which is essential for maintaining water balance (Pereyra et al. 2012; Romero, Vega, and Correa 2014). Studies such as those by Battistus (2019) and others (Boghdady and Ali 2013; El-Afry et al. 2012) also indicate improvements in xylem vascular structure in maize and wheat plants inoculated with *A. brasilense*.

Similarly, studies on phloem, another vital part of the vascular system, reveal that inoculation with *A. brasilense* can significantly enhance vascular structure, increasing the diameter of phloem vascular bundles. This suggests improved nutrient transport capacity within the plant, as well as a better response under stress conditions such as the presence of Cd (Battistus 2019; Boghdady and Ali 2013; El-Afry et al. 2012).

Regarding the pith, although there are no specific studies detailing the effects of inoculation on Brassicaceae plants exposed to Cd, as discussed, it is recognized that treatments with *A. brasilense* and *P. fluorescens* improve overall plant growth by influencing nutrient uptake and hormonal regulation. This can lead to beneficial modifications in the plant's internal structure, such as pith, which is crucial for nutrient storage and the conduction of water and solutes.

Finally, the cortex of plants also benefits from inoculation with these bacteria. Their ability to alter nutrient absorption and hormonal regulation can result in significant changes in cortex structure, which is essential for storing substances and conducting water and solutes within the stem. Although specific studies are limited, it is plausible that the improved nutritional and water-use efficiency promoted by these bacteria helps plants better cope with stress caused by heavy metals like Cd, strengthening the plant’s ability to maintain healthy anatomy, including the cortex.

3.3 TOTAL NUTRIENT CONTENT IN PLANT TISSUE – SHOOT

In the shoots, it was observed that inoculated plants exposed to Cd showed lower Ca levels compared to non-inoculated plants. Notably, at 1.5 mg kg^{-1} Cd, non-inoculated plants accumulated 79.10% more Ca than inoculated ones. At 3.0 mg kg^{-1} Cd, the difference increased to 84.34%, and at 6.0 mg kg^{-1} Cd, it was 50.84%. These values are presented in Table 3 below.

Mg showed a similar pattern, with its concentration decreasing as Cd concentration increased. However, as with Ca, inoculation reduced the accumulation of this element, with increases of 120.35%, 42.32%, and 78.57% respectively at Cd concentrations of 1.5 mg kg^{-1}, 3.0 mg kg^{-1}, and 6.0 mg kg^{-1}. N followed the same patterns observed for Ca and Mg, as shown in Table 3.

Regarding P and K, both showed a significant increase with inoculation. Overall, *A. brasilense* promoted a 53.25% increase in P concentration in the shoot and a 34.68% increase in K concentration, while inoculation with *Pseudomonas* resulted in a 235.87% increase for P and 94.12% for K.

In the root tissue, the macronutrients Ca, Mg, P, and K followed the same pattern observed for the shoot tissue, with lower Ca and Mg contents in plants inoculated with both bacteria. For P and K, the contents were higher in inoculated plants, especially with *Pseudomonas*, as shown in Table 3.

Also in Table 3, the values found for micronutrients in the shoot showed higher concentrations in treatments without inoculation, regardless of the microorganism used. The harmonic mean across all Cd concentrations clearly illustrated each treatment. Thus, in the shoot of non-inoculated plants, the contents of Cu, Zn, and Mn were respectively 5.01%, 18.18%, and 6.42% higher compared to inoculated plants. On the other hand, Fe showed a 24.71% increase in plants inoculated with *A. brasilense* compared to non-inoculated plants.

Inoculating plants with plant growth-promoting bacteria such as *A. brasilense* offers remarkable improvements in the absorption of essential nutrients, including N, P, K, Ca, Mg, S, B, Fe, Mn, and Zn. These bacteria not only increase the availability of these nutrients but also enhance crucial physiological processes such as photosynthesis and water-use efficiency. For example, a study on hydroponic lettuce inoculated with *A. brasilense* showed increased accumulation of these nutrients in the shoot, resulting in a significant rise in biomass and chlorophyll production (Zhang et al. 2014). This effect is particularly advantageous in cultivation systems like hydroponics, where nutrient absorption efficiency is critical (Hungria 2011).

P. fluorescens, in turn, is known for its multiple agricultural benefits, including pathogen control, phosphate solubilization, and the production of metabolites with antipathogenic properties (Kazi et al. 2016; Oliveira et al. 2015). This bacterium performs vital functions such as producing phytohormones and regulating ethylene levels, which facilitates better nutrient absorption (Hungria and Nogueira 2021).

Additionally, studies have shown that inoculation with *A. brasilense* can significantly increase nutrient contents in various crops. For example, research on brachiaria grass (*Urochloa brizantha*) showed a 13% increase in N content and a 10.4% increase in K after inoculation with *A. brasilense* (Hungria and Nogueira 2021). Similar results were observed in tamani grass, with notable increases in nutrients such as N, P, K, and Ca (Andrade et al. 2022).

The effectiveness of *Pseudomonas* in phosphate solubilization was demonstrated in bean plants, where inoculation with *Pseudomonas* sp. resulted in increased P content, improving nutrient uptake (Silva, Vitti, and Trevizam 2007). Additionally, inoculation with *P. fluorescens* in maize increased N availability, optimizing the efficiency of nitrogen fixation in the soil (De Siqueira et al. 2018). Inoculation with *P. aeruginosa* in tomato plants also promoted a significant increase in K content, enhancing plant development and productivity (Santos et al. 2015).

Finally, it is important to consider that the presence of heavy metals such as Cd can adversely affect the absorption, transport, and utilization of macronutrients and water, complicating plant nutritional management (Das, Samantaray, and Rout 1997; Jiang et al. 2005). Thus, inoculation with plant growth-promoting bacteria emerges as a potentially valuable strategy to mitigate these negative effects and improve plant health and productivity in contaminated environments.

3.4 TOTAL NUTRIENT CONTENT IN PLANT TISSUE – ROOT

In the root tissue, the results differed from those observed in the shoot. Without Cd presence, non-inoculated plants accumulated 7.75 times more Fe than inoculated plants. With 1.5 mg kg^{-1} Cd, *Pseudomonas* increased Fe accumulation by 2.49 times, and at concentrations of 3.0 mg kg^{-1} and 6.0 mg kg^{-1} Cd, *A. brasilense* was responsible for the highest increases, with 1.43 times and 2.52 times, respectively, compared to non-inoculated plants (Table 4).

For Cu, a significant increase was observed in plants not exposed to Cd and inoculated with *A. brasilense*, showing an 11.28-fold higher content compared to non-inoculated plants. At 1.5 mg kg^{-1} Cd, inoculation with *Pseudomonas* resulted in a 2.65-fold increase and with *A.*

brasilense a 2.09-fold increase in Cu content. For plants exposed to 6.0 mg kg^{-1} Cd, those inoculated with *A. brasilense* showed a 6.71-fold increase, while those inoculated with *Pseudomonas* had a 1.65-fold increase in Cu content (Table 4).

Regarding Zn in the root tissue, significant changes were observed only in plants exposed to 3.0 mg kg^{-1} Cd, where inoculation with *P. fluorescens* resulted in a 24.90% increase in Zn content. For plants not exposed to Cd or exposed to 1.5 mg kg^{-1}, Mn contents did not show significant differences between inoculated and non-inoculated plants. However, for exposures to 3.0 mg kg^{-1} and 6.0 mg kg^{-1} Cd, inoculation with *A. brasilense* was effective in increasing Mn content, with increments of 55.56% and 30.58%, respectively, as shown in Table 4.

Research by Guimarães et al. (2021) highlights the growing relevance of studies focused on phosphate-solubilizing microorganisms as a promising strategy for promoting plant growth and production. Phosphate is an essential nutrient, but its dynamics in the soil can be complex due to its tendency to become fixed in forms that are not readily available to plants. The ability of these microorganisms to solubilize phosphate makes it more accessible to plants, which can significantly improve crop yield and quality.

The use of these multifunctional microorganisms, which not only solubilize phosphate but also promote plant growth through various other functions such as phytohormone production and improved absorption of other nutrients, is fundamental for developing more sustainable agriculture. They offer an ecological solution to overcome soil nutritional limitations and maximize agricultural efficiency, reducing dependence on chemical fertilizers that can have adverse effects on both the environment and human health.

3.5 TOTAL CADMIUM CONTENT

The Cd concentration in the shoot was also influenced by inoculation. In the treatment with 1.5 mg kg^{-1} Cd, non-inoculated plants showed 0.10 mg kg^{-1} Cd in the shoot, while plants inoculated with *A. brasilense* showed a 50% increase, reaching 0.15 mg kg^{-1}. Plants inoculated with *P. fluorescens* presented 0.21 mg kg^{-1}, an increase of 110%. With the addition of 3.0 mg kg^{-1} Cd, non-inoculated plants had 0.14 mg kg^{-1} Cd in the shoot, while those inoculated with *A. brasilense* and *P. fluorescens* showed increases of 278.57% and 307.14%, with contents of 0.53 mg kg^{-1} and 0.57 mg kg^{-1}, respectively. For the treatment with 6.0 mg kg^{-1} Cd, non-inoculated plants had 0.59 mg kg^{-1} Cd in the shoot, while those inoculated with *A. brasilense* and *P. fluorescens* showed increases of 91.52% and 106.77%, with contents of 1.13 mg kg^{-1} and 1.22 mg kg^{-1}, respectively. These values are detailed in Table 5.

Inoculation resulted in an increase in Cd content in the root tissue for both bacteria, indicating that inoculation enhances the absorption of this metal. Under exposure to 1.5 mg kg^{-1} Cd, non-inoculated roots had a Cd content of 0.10 mg kg^{-1}, while those inoculated with *A. brasilense* and *P. fluorescens* showed contents of 0.22 mg kg^{-1} and 0.20 mg kg^{-1}, respectively. At 3.0 mg kg^{-1} Cd exposure, the concentration increased to 0.27 mg kg^{-1} in non-inoculated plants and to 0.30 mg kg^{-1} in plants inoculated with both bacteria. With 6.0 mg kg^{-1} Cd, non-inoculated plants showed a content of 0.30 mg kg^{-1} Cd, while those inoculated with *A. brasilense* and *P. fluorescens* exhibited contents of 1.27 mg kg^{-1} and 1.25 mg kg^{-1}, respectively, as shown in Table 5.

Plant roots are generally the primary points of contact with heavy metals in the soil, such as Cd, and tend to accumulate significant amounts of this metal. Studies like Oliveira et al. (2021) on *E. crassipes* and *Salvinia auriculata* show that roots accumulate more Cd, confirming earlier observations by Grant and Bailey (1998) suggesting that Cd can bind to negative charges on root cell walls and then be translocated to the shoot. However, Cd translocation through the xylem can occur independently of phytochelatin production, molecules that complex with heavy metals in the roots (Salt et al. 1995). This phenomenon suggests that other mechanisms may play roles in Cd mobility within plants.

Additionally, Dixit et al. (2001) point out that lower Cd accumulation in leaves may be a defensive plant strategy to protect photosynthetic functions from Cd-induced oxidative stress. This indicates a potential plant adaptation to mitigate the toxic effects of Cd by prioritizing the protection of vital processes like photosynthesis.

Comparative studies of Cd accumulation in different species, as reported by Wong et al. (1984) and Kayser et al. (1999) in *Brassica juncea*, highlight significant variations in Cd accumulation capacity even under similar contamination conditions. These results emphasize the variability between species and even between genotypes within the same species in response to Cd exposure.

Moreover, research by Kang et al. (2018) on the inoculation of *Leucaena leucocephala* with *Sinorhizobium saheli* in mine tailings soils illustrates a promising strategy. The inoculation not only improved plant growth and macronutrient absorption but also significantly reduced heavy metal uptake, achieving an 80% reduction in Cd absorption. This demonstrates the potential of bioaugmentation techniques with beneficial microorganisms for phytoremediation of contaminated soils, offering an effective and sustainable approach for managing heavy metal-contaminated soils.

3.6 TOTAL NUTRIENT CONTENT IN SOIL

In the soil, total Ca content was higher for non-inoculated plants. When assessing the harmonic mean across all Cd concentration treatments, which also reflects the individual means, a content of 19.75 g kg^{-1} of Ca was identified in non-inoculated soil, 19.14 g kg^{-1} in soil with *A. brasilense*, and 18.46 g kg^{-1} in soil inoculated with *Pseudomonas*. From these values, non-inoculated soil had an available Ca content of 7.47 cmolc/dm^3, while soil inoculated with *A. brasilense* showed 7.38 cmolc/dm^3 and soil with *P. fluorescens* had 7.30 cmolc/dm^3 of available Ca. The values for total nutrient contents can be found in Table 6, while the available nutrient contents are presented in Table 7.

The Mg content followed a similar pattern, with total contents of 5.41 g kg^{-1} in non-inoculated soils, 5.31 g kg^{-1} in soils inoculated with *A. brasilense*, and 5.20 g kg^{-1} in soils inoculated with *Pseudomonas*. The available Mg contents were 3.92 cmolc/dm^3 for non-inoculated soils, 3.78 cmolc/dm^3 for soils with *A. brasilense*, and 3.47 cmolc/dm^3 for soils with *Pseudomonas* (Tables 6 and 7).

There was a significant difference for total P in inoculated soils, where the total P content in non-inoculated soils was 52.25 mg kg^{-1} with an available level of 12.33 mg kg^{-1}. For soils inoculated with *A. brasilense*, the total P was 59.36 mg kg^{-1} and available P was 19.33 mg kg^{-1}. For soils with *Pseudomonas*, this difference was even more pronounced, with 65.23 mg kg^{-1} total P and 23.92 mg kg^{-1} available (Tables 6 and 7).

K showed a similar behavior to P, with non-inoculated soils presenting 289.34 mg kg^{-1} of total K and 0.48 cmolc/dm^3 available. Soils inoculated with *A. brasilense* showed a total content of 542.12 mg kg^{-1} and 0.66 cmolc/dm^3 available, while inoculation with *Pseudomonas* resulted in 535.28 mg kg^{-1} total K and 0.65 cmolc/dm^3 available (Tables 6 and 7).

Regarding micronutrients in the soil, inoculation with both bacteria induced an approximate increase of 8% in Fe levels and 6.5% for Mn. Inoculation with *A. brasilense* increased Cu levels by 7.6%, while inoculation with *Pseudomonas* increased Zn levels by 25% (Tables 6 and 7).

After the experiment, Cd dosage in the soil revealed impactful data. Soils without added metal, not previously contaminated, recorded 0 mg kg^{-1} of Cd. In soils with 1.5 mg kg^{-1} of Cd added, non-inoculated soils showed 1.26 mg kg^{-1} of Cd, while those inoculated with *A. brasilense* and *P. fluorescens* showed concentrations of 1.06 mg kg^{-1} and 0.99 mg kg^{-1}, respectively. With the addition of 3.0 mg kg^{-1} of Cd, non-inoculated soils exhibited 2.56 mg kg^{-1} of Cd, reducing to 2.11 mg kg^{-1} with *A. brasilense* and 2.04 mg kg^{-1} with *Pseudomonas*. For the highest Cd dose of 6.0 mg kg^{-1}, non-inoculated soils had 5.10 mg kg^{-1} of Cd, while inoculation with *A. brasilense* and *P. fluorescens* reduced concentrations to

3.22 mg kg^{-1} and 3.13 mg kg^{-1}, respectively, representing up to a 63% reduction in Cd levels in the soil.

Duarte (2021) also investigated the effects of combined inoculation of *Pseudomonas* sp. and *A. brasilense* on soil nutrient availability. The results indicated that this synergy between plant growth-promoting bacteria significantly increased the availability of essential nutrients such as N, P, and K, enhancing nutrient uptake by plants and consequently promoting more vigorous development.

However, in contexts of Cd contamination, inoculation can influence differently, in some cases reducing the bacteria's ability to solubilize nutrients. Recent studies emphasize the multifunctional characteristics of soil microorganisms and their beneficial impacts on plant development. For example, Terra et al. (2019), Rezende et al. (2021), and Oliveira et al. (2022) highlight that, in addition to improving nutrient availability, these microorganisms play crucial roles in the decomposition of organic residues. This process is essential for nutrient mineralization and the formation of chelators that complex and reduce the toxicity of pollutant compounds (Moreira and Siqueira 2006). Decomposition is facilitated by specific microbial groups that possess endo- or extracellular enzymes, effectively contributing to organic matter oxidation and soil quality improvement.

Regarding nutrient availability in soil, one of the most highlighted aspects in the literature is phosphate solubilization by plant growth-promoting bacteria. P, an essential macronutrient, plays a regulatory role in various metabolic pathways and biochemical reactions fundamental to plant growth (Taiz and Zeiger 2017). However, in many soils, especially in tropical regions such as the Cerrado with dystrophic Red Latosols, P is often found in forms that are insoluble and therefore inaccessible to plants. These forms are often bound to Fe and Al oxides present in the soil (Malavolta, Vitti, and Oliveira 1997).

The ability of microorganisms to solubilize phosphates is generally attributed to the exudation of a variety of organic acids. These include gluconic acid, 2-ketogluconic, lactic, isovaleric, isobutyric, acetic, glycolic, malonic, maleic, oxalic, propionic, tartaric, butyric, citric, fumaric, and succinic acids (Marciano Marra et al. 2012). These organic acids play a key role in breaking down insoluble phosphate complexes, making P available for plant uptake.

Thus, soil microorganisms not only directly contribute to plant health and growth through nutritional improvements but also play a vital role in the sustainability of agricultural systems, promoting a more efficient and environmentally friendly nutrient cycle.

The soil pH remained stable between 7.20 and 7.80 in all evaluated treatments, indicating that variations in nutrient and Cd contents were not accompanied by significant changes in soil pH, as shown in Figure 10.

The stability of soil pH throughout contamination and recovery studies is an important indicator, suggesting that the observed variations in heavy metal availability and absorption, such as Cd, are not directly linked to changes in pH. The constancy of pH indicates that the beneficial effects in modulating Cd absorption are attributable to factors other than simple changes in soil acidity or alkalinity.

This phenomenon can be explained by specific biochemical and physiological mechanisms induced by the activity of the inoculated microorganisms. Among these mechanisms are biosorption, bioprecipitation, and the chemical transformation of the metal, processes that can be mediated by enzymes or metabolites produced by the microbes. For example, certain microorganisms are capable of excreting chelating substances that bind to the metal, reducing its mobility and toxicity by forming stable complexes (Chavez Apare 2019; Domingos 1997; Florida Rofner et al. 2019).

Additionally, microorganisms can affect soil structure and organic matter, indirectly influencing the retention and mobility of toxic heavy metals. These changes can facilitate the immobilization of the metal in less accessible soil fractions, limiting its bioavailability.

Therefore, the absence of changes in soil pH reinforces the need to explore more deeply how interactions between plants, microorganisms, and toxic heavy metals can be used to develop more effective and environmentally sustainable bioremediation and phytoremediation strategies.

3.7 ACCUMULATION AND TRANSLOCATION INDEX

The accumulation index shown in Figure 11 revealed that inoculation significantly enhances the ability of *Crambe* plants to accumulate Cd, with a progressive increase as Cd levels in the soil rise. Among the studied bacteria, *A. brasilense* showed a lower accumulation index, increasing by 250%, 294.11%, and 1366.66% at soil Cd levels of 1.5 mg kg^{-1}, 3.0 mg kg^{-1}, and 6.0 mg kg^{-1}, respectively. *P. fluorescens*, on the other hand, promoted even higher indices, reaching 320%, 400%, and 1533.33% for the same Cd levels.

The translocation index shown in Figure 12 varied with inoculation only under conditions of 1.5 mg kg^{-1} Cd, where *P. fluorescens* reached an index of 1.08, while *A. brasilense* showed a lower value of 0.37, compared to 1.03 in non-inoculated plants. In treatments with 3.0 mg kg^{-1} Cd, the index for plants inoculated with *A. brasilense* was 2.03 and for *P. fluorescens* was 1.90, versus 0.49 for non-inoculated plants. With the addition of 6.0 mg kg^{-1} Cd, the index for non-inoculated plants was 1.97, while for those inoculated with *A. brasilense* it was 0.88 and for *P. fluorescens* it was 0.98.

Phytoextraction is a phytoremediation technique that involves using plants to remove contaminants from soil. This strategy relies on the ability of plants to absorb contaminants—especially metals—through their roots and accumulate them in aerial parts such as leaves and stems. This process is quantified using the accumulation factor and translocation factor, where an accumulation factor greater than one and a translocation factor greater than one indicate that the plant is effective at removing metals from the soil and translocating them to the shoot, making it an ideal candidate for phytoextraction (Vamerali, Bandiera, and Mosca 2010).

Studies such as those by Souza et al. (2013) and Boechat (2014) have shown that plants with a high translocation factor are classified as hyperaccumulators and are particularly useful in remediating contaminated soils, as they allow easy removal of metals through harvesting of the aerial biomass. The importance of this strategy lies in the possibility of recycling or reusing the extracted metals after processes such as drying and calcining the plant biomass (Kabata-Pendias and Pendias 2001; Zeittouni, Berton, and Abreu 2007).

However, the efficiency of phytoextraction can vary significantly among different plant species and even among varieties of the same species, as observed by Korentajer (1991). This variation is influenced by the specific capacity of each plant to absorb and distribute metals. For example, while some metals such as Zn, Cu, Pb, and Mn are generally poorly translocated to the shoot, Cd is notably mobile within plants, facilitating its extraction via phytoextraction (Hernández and Cooke 1997; McBride 1994).

Additionally, the capacity for metal uptake and accumulation can be influenced by soil conditions, including the presence of other elements, pH, and soil microbiota, which can alter the availability of metals to plant roots. For example, the addition of Cd to soil increases its availability and subsequent accumulation in all parts of the plants, as highlighted by Chaves and Souza (2014).

Therefore, the success of phytoextraction depends on the appropriate selection of plant species, proper management of soil conditions, and a deep understanding of the biochemical and physiological interactions that influence metal mobility in the environment.

4 CONCLUSION

Inoculation with *A. brasilense* and *P. fluorescens* significantly increased plant growth even under high Cd doses.

Inoculated plants exhibited improved vascular structure, with an increased number of cell layers in xylem and phloem.

Inoculated plants showed greater Cd accumulation capacity, especially in the shoot, facilitating phytoextraction.

Inoculation with *A. brasilense* and *P. fluorescens* significantly reduced Cd levels in the soil, with up to a 63% reduction in contaminated soils.

Plant growth-promoting bacteria proved effective in Cd bioremediation, making soils safer for agricultural use.

The application of *A. brasilense* and *P. fluorescens* represents a sustainable approach for remediating soils contaminated with toxic metals, contributing to food safety. These practices can be integrated into agricultural systems to improve productivity and environmental sustainability.

Inoculation with *A. brasilense* and *P. fluorescens* has proven to be an effective strategy for mitigating Cd contamination in agricultural soils. This study highlights the potential of these bacteria to promote plant growth and remediate contaminated soils, offering a sustainable solution to environmental and agricultural challenges associated with toxic heavy metal contamination.

These results also underscore the broader relevance of managing Cd contamination in agricultural soils, given the well-documented human health risks associated with cadmium exposure, including renal dysfunction, cardiovascular diseases, and carcinogenicity. By reducing soil Cd levels and improving plant Cd uptake for phytoextraction, these bacterial inoculations contribute to lowering the risk of cadmium entering the food chain, thereby supporting food safety and protecting public health.

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ATTACHMENTS

Table 1

Chemical characteristics for fertility and soil texture.

Attribute	Soil
pH in $CaCl_2$	5.21
Aluminum (Al) (cmolc/dm³)	0.00
Potential acidity (H+Al) (cmolc/dm³)	4.53
Sum of bases (S) (cmolc/dm³)	10.84
CEC at pH 7.0 (cmolc/dm³)	15.37
Effective CEC (cmolc/dm³)	10.84
Base saturation (V) (%)	70.53
Organic matter (OM) (g/kg)	39.63
Organic carbon (OC) (g/kg)	22.99
Calcium (Ca) (cmolc/dm³)	8.00
Magnesium (Mg) (cmolc/dm³)	2.16
Potassium (K) (cmolc/dm³)	0.68
Available phosphorus (P) (mg/dm³)	9.19
Copper (Cu) (mg/dm³)	11.13
Iron (Fe) (mg/dm³)	26.25
Manganese (Mn) (mg/dm³)	70.69
Zinc (Zn) (mg/dm³)	5.45
Boron (B) (mg/dm³)	0.55
Sulfur (S) (mg/dm³)	7.70
Sand (%)	16.30
Silt (%)	16.70
Clay (%)	67.00

Table 2

Limit of quantification for elements in air-acetylene flame atomic absorption spectrophotometry.

Element	Limit of quantification (mg kg^{-1})
Calcium	0.009
Magnesium	0.005
Iron	0.01
Copper	0.01
Zinc	0.01
Manganese	0.01
Cadmium	0.005

Table 3

Nutrient levels of macro and micronutrients in the shoots of crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.

Total nutrient content in shoot									
		Zero (0,0 mg kg^{-1} Cd)		Half (1,5 mg kg^{-1} Cd)		Dose (3,0 mg kg^{-1} Cd)		Double (6,0 mg kg^{-1} Cd)	
Ca (g kg^{-1})	*P. fluorescens*	15,45	(a)	13,77	(b)	12,76	(c)	10,60	(d)
	A. brasilense	11,61	(a)	11,58	(a)	7,92	(c)	9,42	(b)
	Control	12,66	(c)	20,74	(a)	14,6	(b)	14,21	(b)
Mg (g kg^{-1})	*P. fluorescens*	2,29	(a)	1,89	(b)	1,74	(b)	1,76	(b)
	A. brasilense	1,76	(a)	1,91	(a)	1,13	(b)	1,40	(b)
	Control	2,39	(b)	2,69	(a)	2,49	(ab)	2,50	(ab)
	P. fluorescens	52,71	(a)	51,34	(b)	42,38	(d)	45,08	(c)

N (g kg⁻¹)	*A. brasilense*	48,68	(b)	55,58	(a)	45,43	(d)	47,32	(c)
	Control	50,78	(a)	51,10	(a)	49,56	(b)	50,89	(a)
Fe (mg kg⁻¹)	*P. fluorescens*	999,60	(c)	1795,39	(a)	1748,87	(a)	1153,38	(b)
	A. brasilense	1223,00	(b)	467,54	(c)	441,44	(c)	7829,91	(a)
	Control	573,50	(d)	3330,44	(a)	1676,75	(c)	2406,93	(b)
Cu (mg kg⁻¹)	*P. fluorescens*	14,25	(d)	18,61	(b)	28,81	(a)	17,55	(c)
	A. brasilense	14,05	(d)	23,70	(c)	25,20	(b)	41,49	(a)
	Control	21,50	(c)	19,00	(d)	36,44	(a)	32,76	(b)
Zn (mg kg⁻¹)	*P. fluorescens*	0,41	(c)	0,58	(a)	0,48	(b)	0,51	(b)
	A. brasilense	0,43	(b)	0,44	(b)	0,28	(c)	0,62	(a)
	Control	0,48	(c)	0,51	(bc)	0,56	(a)	0,55	(ab)
Mn (mg kg⁻¹)	*P. fluorescens*	78,22	(a)	72,66	(b)	78,73	(a)	65,08	(c)
	A. brasilense	67,43	(d)	68,43	(c)	74,22	(b)	78,77	(a)
	Control	81,02	(b)	73,01	(c)	68,11	(d)	85,26	(a)

Notes: Limits of quantification (LQ): Ca = 0.009; Mg = 0.005; Fe = 0.01; Cu = 0.01; Zn = 0.01, Mg = 0.01. Means followed by the same letters do not differ statistically by Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Table 4

Nutritional levels of macro- and micronutrients in the root tissue of Crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.

Total nutrient contents in roots									
		Zero (0,0 mg kg⁻¹ Cd)		Half (1,5 mg kg⁻¹ Cd)		Dose (3,0 mg kg⁻¹ Cd)		Double (6,0 mg kg⁻¹ Cd)	
Ca (g kg⁻¹)	*P. fluorescens*	111,90	(b)	62,13	(d)	127,18	(a)	80,77	(c)
	A. brasilense	82,04	(c)	104,33	(b)	97,75	(b)	116,47	(a)
	Control	110,20	(b)	101,16	(bc)	89,97	(d)	147,92	(a)
Mg (g kg⁻¹)	*P. fluorescens*	14,96	(a)	7,02	(b)	15,83	(a)	13,76	(a)
	A. brasilense	12,71	(b)	7,31	(c)	15,70	(a)	6,50	(c)
	Control	24,00	(a)	9,66	(c)	13,76	(b)	12,33	(bc)
Fe (mg kg⁻¹)	*P. fluorescens*	192,50	(bc)	273,98	(b)	438,15	(a)	165,73	(c)
	A. brasilense	388,30	(b)	146,16	(d)	535,58	(a)	242,67	(b)
	Control	915,10	(a)	109,67	(c)	374,16	(b)	96,17	(c)
Cu (mg kg⁻¹)	*P. fluorescens*	15,51	(c)	46,69	(b)	14,47	(c)	91,76	(a)
	A. brasilense	175,00	(b)	36,93	(c)	15,56	(d)	373,14	(a)
	Control	33,86	(b)	17,60	(c)	33,16	(b)	55,60	(a)
Zn (mg kg⁻¹)	*P. fluorescens*	39,65	(c)	32,85	(c)	59,44	(a)	48,25	(b)
	A. brasilense	45,90	(b)	35,87	(c)	48,48	(b)	65,04	(a)
	Control	62,33	(a)	32,99	(c)	47,90	(b)	57,91	(a)
Mn (mg kg⁻¹)	*P. fluorescens*	128,20	(a)	52,91	(c)	107,86	(b)	56,27	(c)
	A. brasilense	63,20	(c)	58,15	(c)	132,06	(a)	77,54	(b)
	Control	129,20	(a)	58,59	(c)	84,89	(b)	59,38	(c)

Notes: Limits of quantification (LQ): Ca = 0.009; Mg = 0.005; Fe = 0.01; Cu = 0.01; Zn = 0.01. Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Table 5

Cd contents found in soil, roots, and shoots of Crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.

Total Cadmium Content (mg kg⁻¹)									
		Zero (0,0 mg kg⁻¹ Cd)		Half (1,5 mg kg⁻¹ Cd)		Dose (3,0 mg kg⁻¹ Cd)		Double (6,0 mg kg⁻¹ Cd)	
Soil	*P. fluorescens*	<LQ	(d)	0,99	(c)	2,04	(b)	3,13	(a)
	A. brasilense	<LQ	(d)	1,06	(c)	2,11	(b)	3,22	(a)
	Control	<LQ	(d)	1,26	(c)	2,56	(b)	5,10	(a)
	P. fluorescens	<LQ	(d)	0,20	(c)	0,30	(b)	1,25	(a)

Roots	*A. brasilense*	<LQ	(c)	0,22	(b)	0,27	(b)	1,27	(a)
	Control	<LQ	(c)	0,10	(b)	0,30	(a)	0,30	(a)
Shoot	*P. fluorescens*	<LQ	(d)	0,21	(c)	0,57	(b)	1,22	(a)
	A. brasilense	<LQ	(d)	0,15	(c)	0,53	(b)	1,13	(a)
	Control	<LQ	(d)	0,10	(c)	0,14	(b)	0,59	(a)

Notes: Limits of quantification (LQ): Cd = 0.005. Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability (n=4), within the same group. The group always corresponds to means found in the same row.

Table 6

Total macro and micronutrient contents in soil after cultivation of Crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.

Total Nutrient Contents in Soil									
		Zero (0,0 mg kg^{-1} Cd)		Half (1,5 mg kg^{-1} Cd)		Dose (3,0 mg kg^{-1} Cd)		Double (6,0 mg kg^{-1} Cd)	
Ca (g kg^{-1})	*P. fluorescens*	22,30	(a)	17,67	(b)	17,07	(bc)	16,82	(c)
	A. brasilense	17,97	(b)	23,83	(a)	17,42	(b)	17,36	(b)
	Controle	22,25	(a)	21,24	(b)	17,24	(d)	18,28	(c)
Mg (g kg^{-1})	*P. fluorescens*	5,32	(b)	6,39	(a)	4,78	(c)	4,33	(d)
	A. brasilense	5,34	(b)	6,03	(a)	5,33	(b)	4,56	(c)
	Controle	6,25	(a)	5,31	(b)	4,65	(c)	5,42	(b)
Fe (mg kg^{-1})	*P. fluorescens*	1012,00	(c)	971,72	(d)	1030,96	(b)	1080,56	(a)
	A. brasilense	1047,00	(c)	916,94	(d)	1085,71	(a)	1067,05	(b)
	Controle	1061,00	(a)	767,79	(d)	1004,09	(b)	977,51	(c)
Cu (mg kg^{-1})	*P. fluorescens*	82,27	(b)	88,60	(a)	75,61	(c)	77,64	(c)
	A. brasilense	82,89	(c)	102,64	(b)	72,89	(d)	112,25	(a)
	Controle	64,37	(d)	72,82	(c)	81,81	(b)	125,33	(a)
Zn (mg kg^{-1})	*P. fluorescens*	1,77	(a)	1,09	(b)	0,82	(c)	0,72	(d)
	A. brasilense	0,57	(b)	0,46	(d)	0,53	(c)	0,74	(a)
	Controle	0,94	(b)	1,16	(a)	0,75	(c)	0,68	(d)
Mn (mg kg^{-1})	*P. fluorescens*	13,26	(a)	12,73	(b)	11,82	(c)	9,50	(d)
	A. brasilense	11,79	(a)	11,89	(a)	12,28	(a)	10,62	(b)
	Controle	9,57	(c)	9,34	(c)	13,63	(a)	11,80	(b)

Notes: Limits of quantification (LQ): Ca = 0.009; Mg = 0.005; Fe = 0.01; Cu = 0.01; Zn = 0.01, Mg = 0.01. Means followed by the same letters do not differ statistically according to Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Table 7

Available/exchangeable macronutrient contents for soil fertility in soil after cultivation of crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.

Available Macronutrient Contents in Soil									
		Zero (0,0 mg kg^{-1} Cd)		Half (1,5 mg kg^{-1} Cd)		Dose (3,0 mg kg^{-1} Cd)		Double (6,0 mg kg^{-1} Cd)	
Ca (cmol$_c$/dm^3)	*P. fluorescens*	7,36	(a)	6,79	(b)	7,51	(a)	7,54	(a)
	A. brasilense	7,74	(b)	6,61	(c)	6,85	(c)	8,34	(a)
	Controle	7,49	(b)	7,98	(a)	6,78	(c)	7,63	(b)
Mg (cmol$_c$/dm^3)	*P. fluorescens*	2,89	(c)	3,44	(b)	4,19	(a)	3,34	(b)
	A. brasilense	2,80	(c)	4,42	(a)	3,72	(b)	4,18	(a)
	Controle	3,34	(d)	4,16	(b)	3,85	(c)	4,31	(a)
K (cmol$_c$/dm^3)	*P. fluorescens*	0,63	(c)	0,63	(c)	0,69	(a)	0,66	(b)
	A. brasilense	0,63	(c)	0,66	(b)	0,72	(a)	0,64	(bc)
	Controle	0,52	(a)	0,50	(b)	0,47	(c)	0,45	(c)
	P. fluorescens	24,46	(a)	24,22	(ab)	23,85	(b)	23,14	(c)

P (mg/dm³)	*A. brasilense*	20,43	(a)	19,93	(b)	19,13	(c)	17,85	(d)
	Controle	14,94	(a)	13,92	(b)	11,85	(c)	8,62	(d)

Notes: Limits of quantification (LQ): Ca = 0.009; Mg = 0.005; Fe = 0.01; Cu = 0.01; Zn = 0.01, Mg = 0.01. Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Figure 1

Phenological stages of crambe at each experimental stage. (A) implementation; (B) start of germination – 4 days after sowing; (C) development and thinning – 15 days after sowing; (D) development after thinning – 20 days; (E) appearance of the first flowers; (F) uniform flower development; (G) plant prior to harvest.

Figure 2

Randomized block design arranged in a factorial scheme with four replicates, four metal doses, and two microorganisms. (A - Control = 0 mg kg⁻¹ Cd; B - Half = 1.5 mg kg⁻¹ Cd; C - Dose = 3 mg kg⁻¹ Cd; D - Double = 6 mg kg⁻¹ Cd, 1 – No inoculation, 2 – Inoculation with A. brasiliense, 3 – Inoculation with P. fluorescens).

Figure 3

Differentiation between xylem vessel staining in blue and phloem in purple.

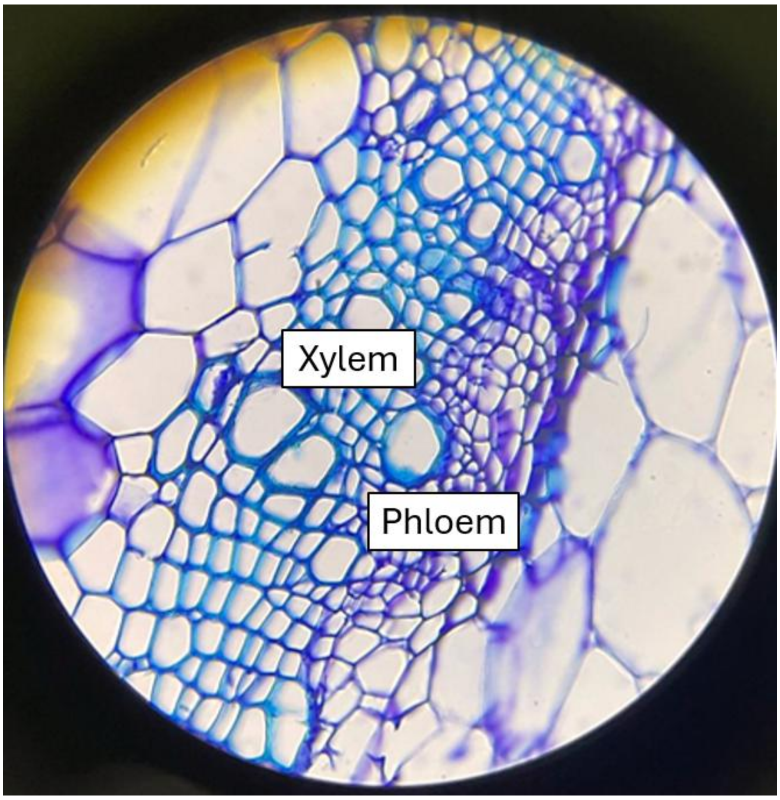


Figure 4

Histological section and morphological differentiation of the crambe collar at the onset of flowering, stained with toluidine blue.

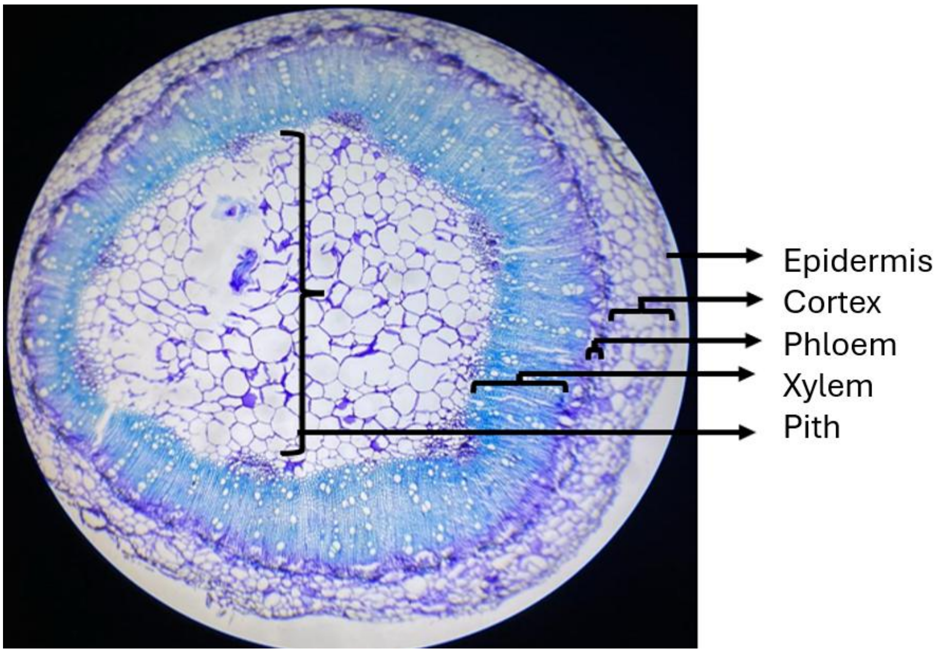
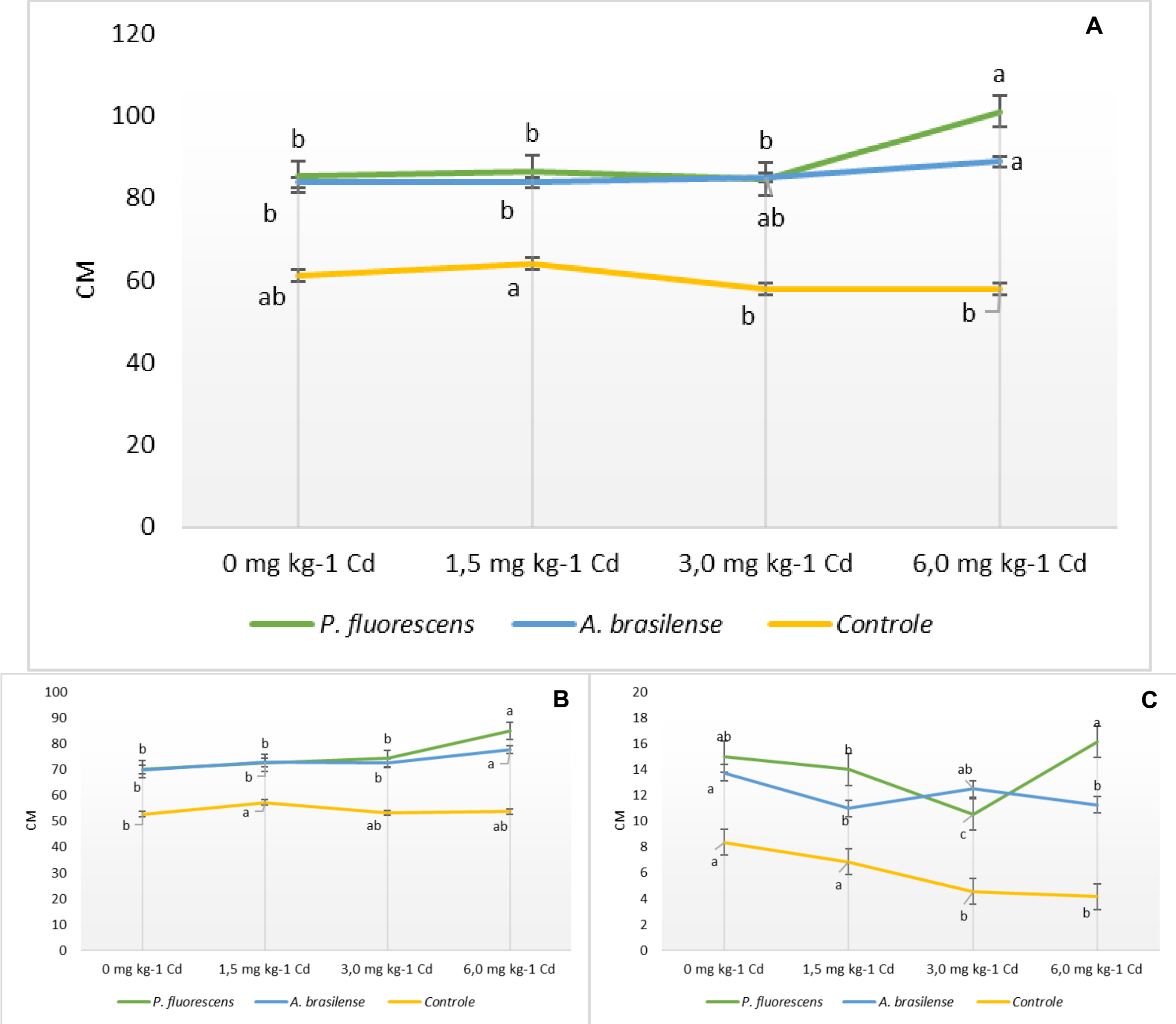


Figure 5

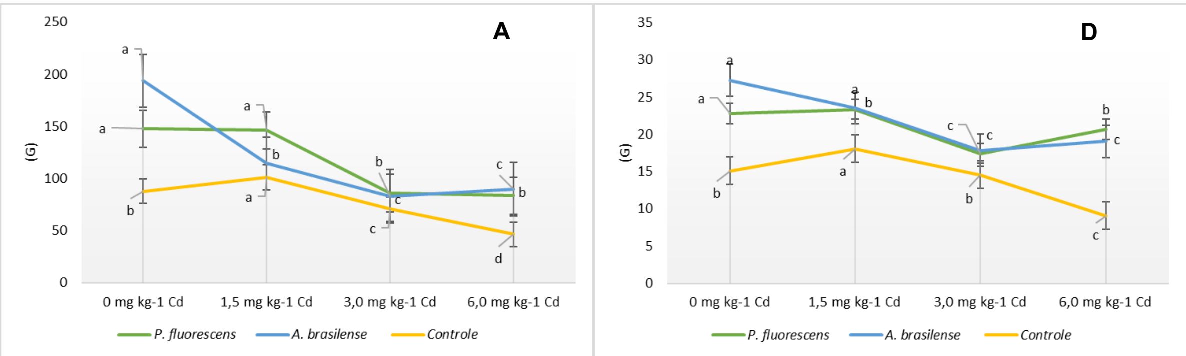
(A) Total length, (B) shoot length, and (C) root length of Crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.

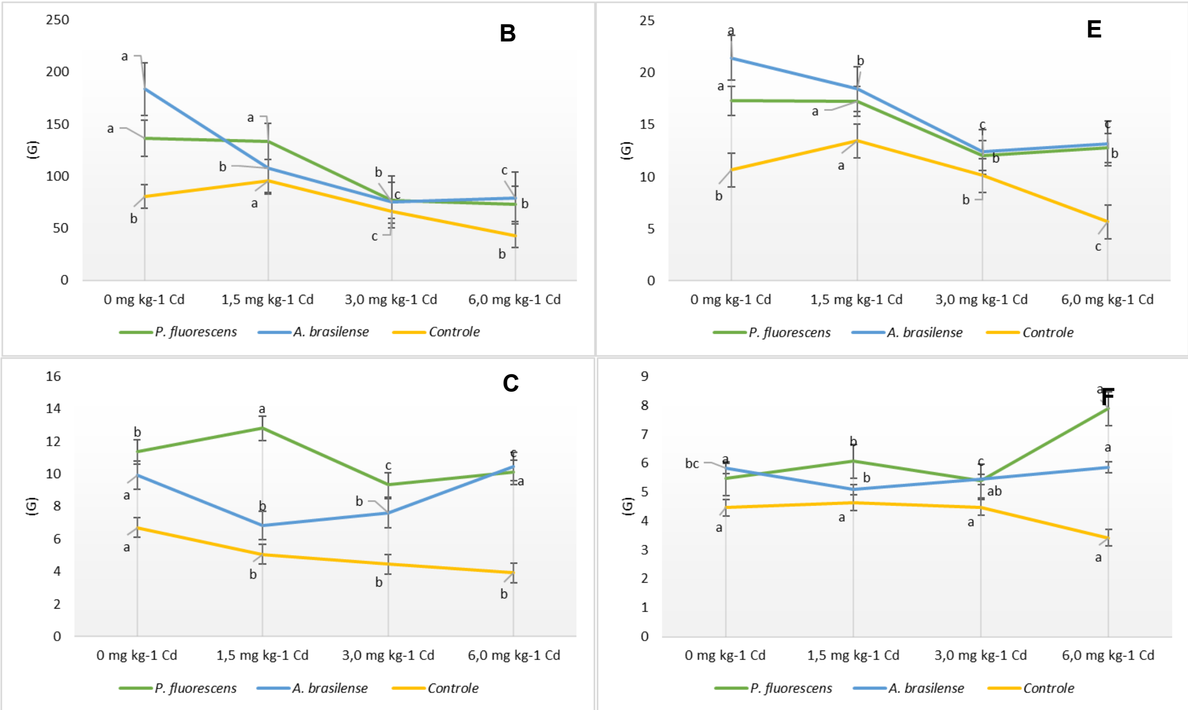


Means followed by the same letter do not differ statistically according to Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Figure 6

Relationship of (A) total fresh mass, (B) shoot fresh mass, (C) root fresh mass, (D) total dry mass, (E) shoot dry mass, and (F) root dry mass of crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.

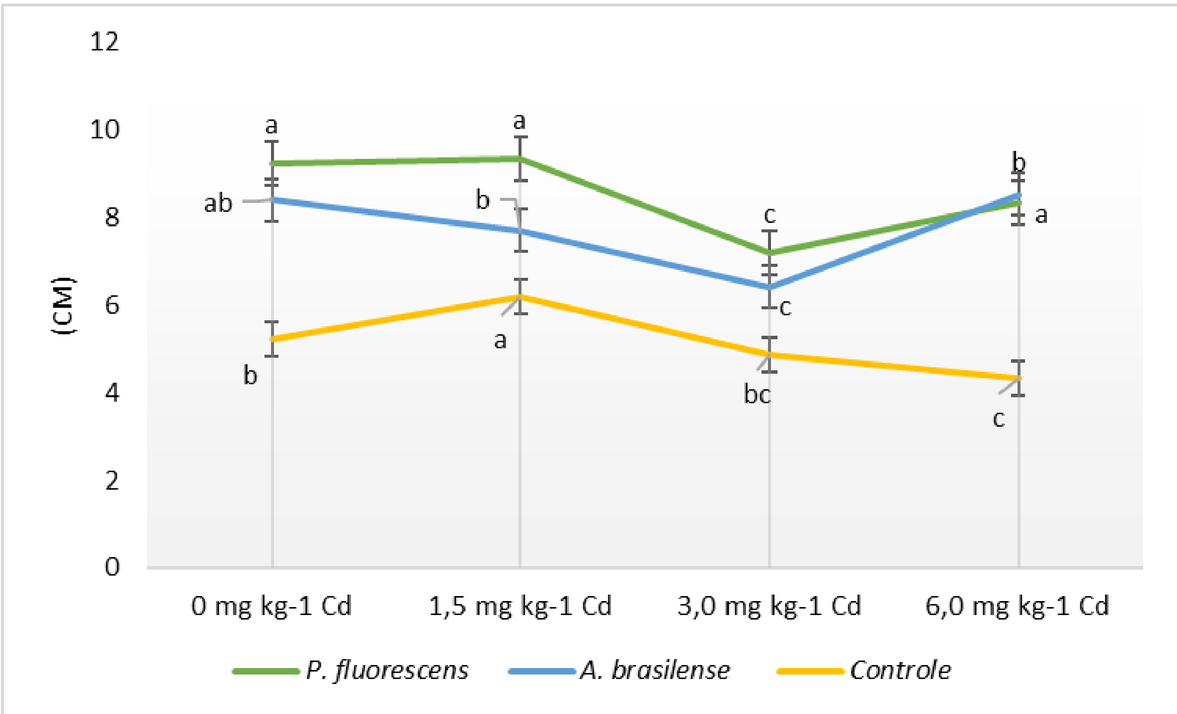




Means followed by the same letters do not differ statistically by Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Figure 7

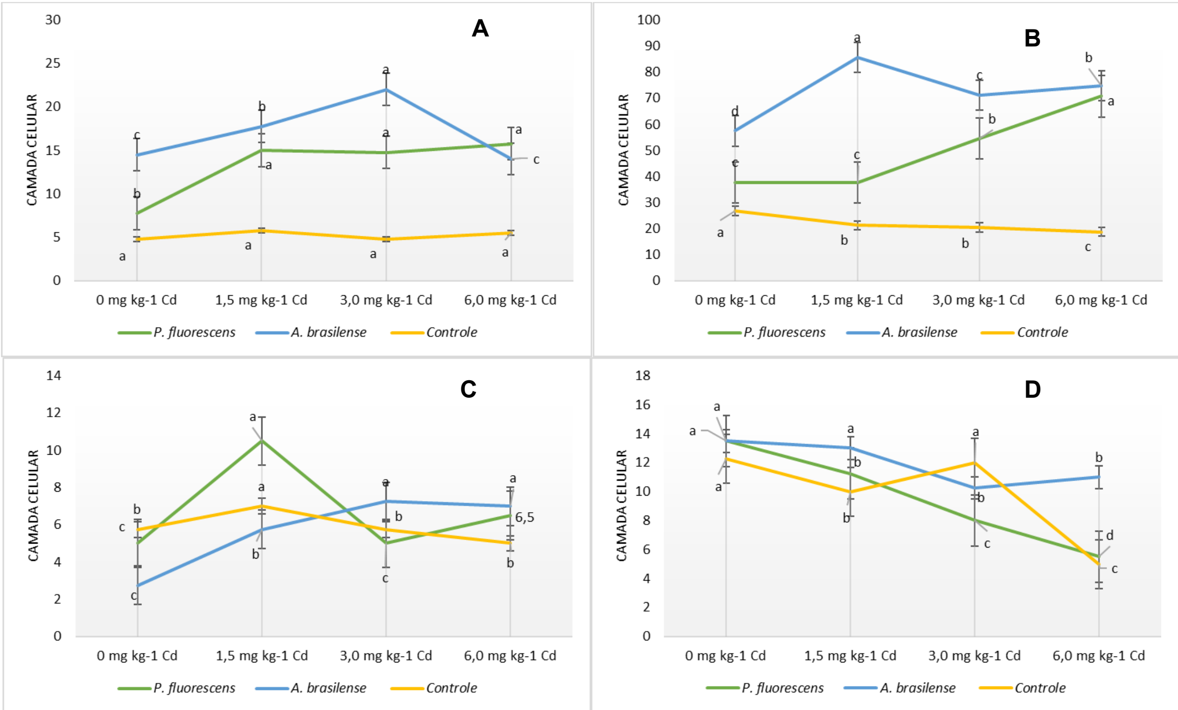
Stem diameter of crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.



Means followed by the same letters do not differ statistically by Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Figure 8

Histomorphological analysis of (A) phloem, (B) xylem, (C) pith, and (D) cortex in the collar of crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses. SOURCE: Author, 2024.



Means followed by the same letters do not differ statistically by Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Figure 9

Histological section of the collar of crambe plants, (a) plants without inoculation and exposed to double the maximum Cd dose (9 mg kg ⁻¹), (b) plants inoculated with P. fluorescens and exposed to double the maximum Cd dose (9 mg kg ⁻¹); both observed at 40× magnification.

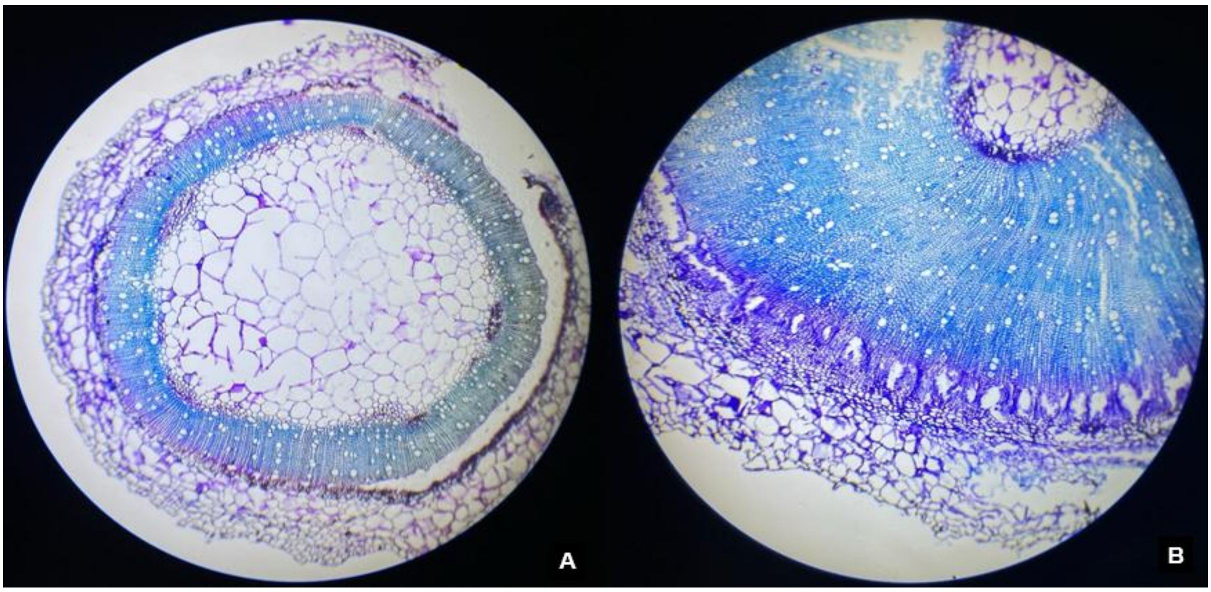
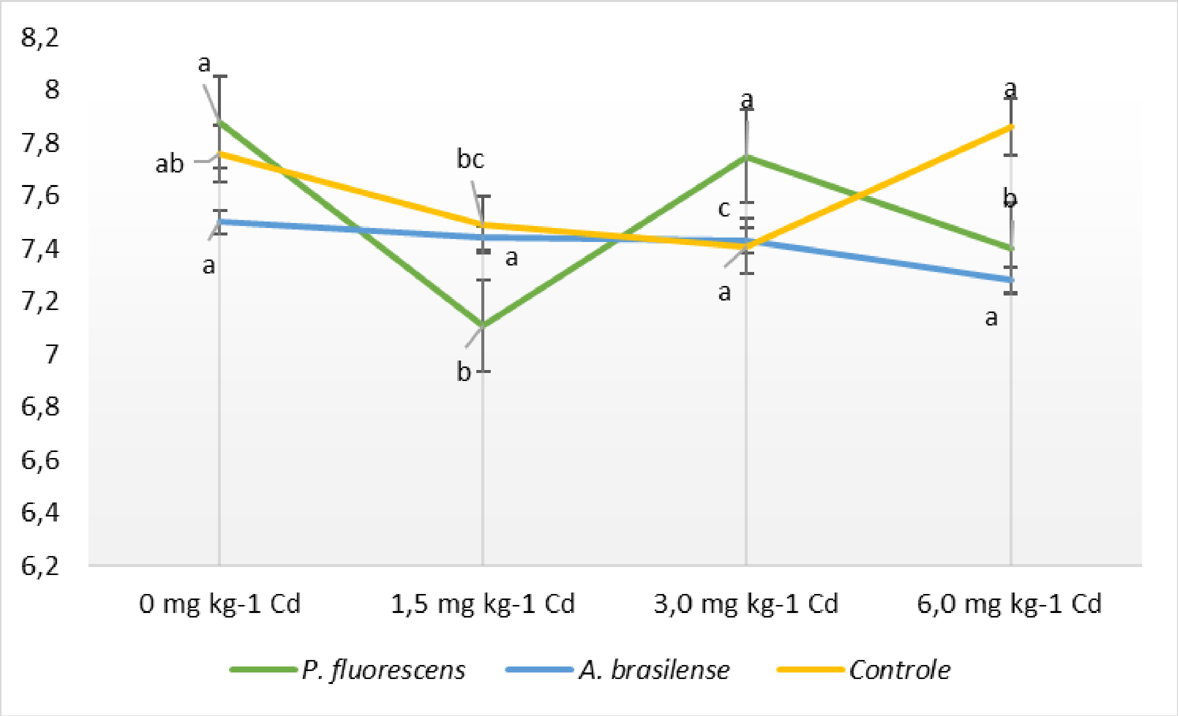


Figure 10

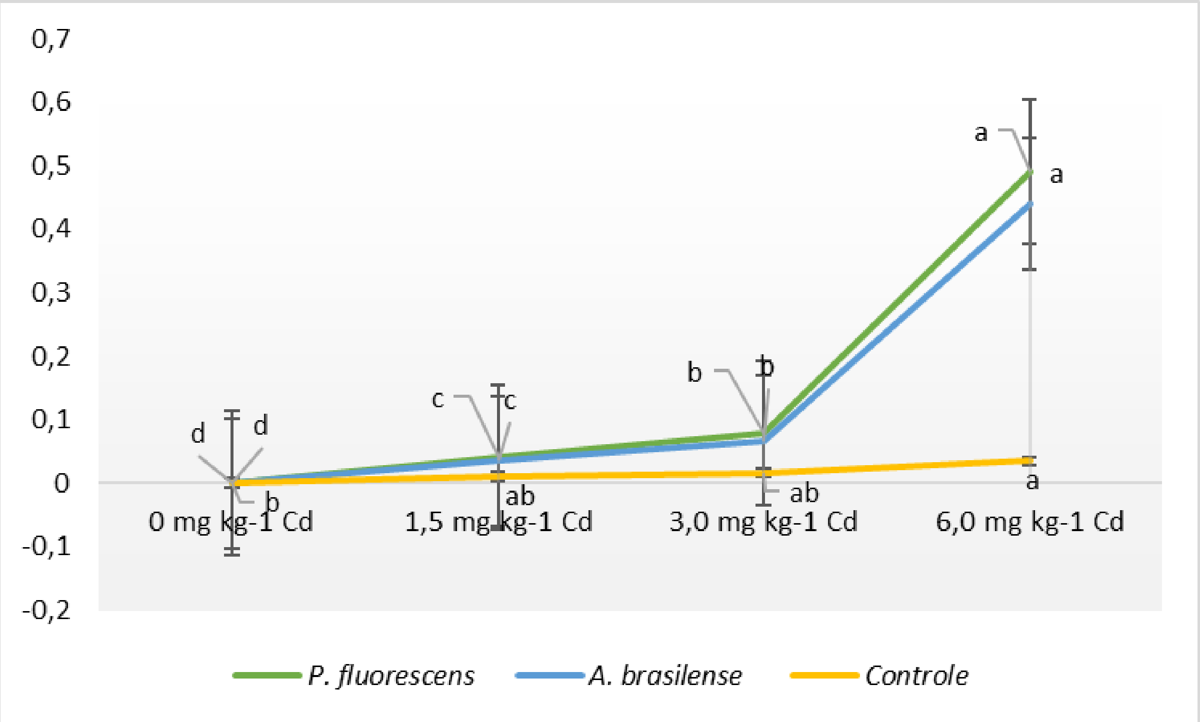
Influence of soil pH in treatments with Crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.



Means followed by the same letter do not differ statistically by Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Figure 11

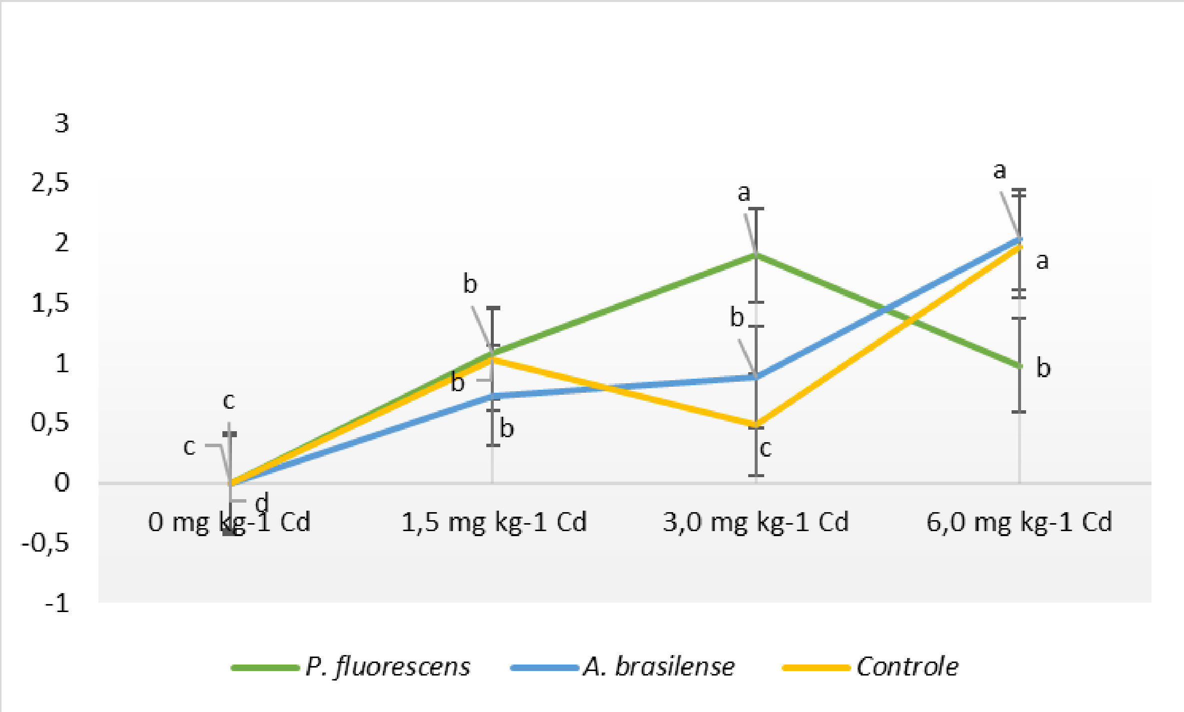
Accumulation index for Crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.



Means followed by the same letters do not differ statistically according to Tukey's test at 5% probability (n=4) within the same group. The group always corresponds to means found in the same row.

Figure 12

Translocation index for crambe plants inoculated with A. brasilense and P. fluorescens in response to different Cd doses.



Means followed by the same letters do not differ statistically according to Tukey's test at 5% probability (n=4), within the same group. The group always corresponds to means found in the same row.