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INFLUENCE OF MICROORGANISMS ON THE BIOREMEDIATION OF LEAD-CONTAMINATED SOIL CULTIVATED WITH CRAMBE ABYSSINICA UNDER PROTECTED CULTIVATION

INFLUÊNCIA DE MICRORGANISMOS NA BIORREMEDIAÇÃO DE SOLO CONTAMINADO COM CHUMBO CULTIVADO COM CRAMBE ABYSSINICA EM CULTIVO PROTEGIDO

INFLUENCIA DE LOS MICROORGANISMOS EN LA BIORREMEDIACIÓN DE SUELOS CONTAMINADOS CON PLOMO CULTIVADOS CON CRAMBE ABYSSINICA BAJO CULTIVO PROTEGIDO

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ABSTRACT

The study addresses soil contamination by Pb, a serious environmental and public health issue. Bioremediation using microorganisms and plants is a promising solution. This research evaluates the synergy between crambe (Crambe abyssinica) and growth-promoting microorganisms to remove Pb from soil. The experiment was conducted under protected cultivation between April and July 2023 using a randomized block design with four Pb concentration treatments and inoculation with two microbial species. Analyses included plant growth, macronutrient levels, and Pb content in plants and soil. Inoculation significantly increased crambe growth, including root and shoot length, and improved biomass. Microorganisms enhanced Pb solubilization and mobilization in soil, facilitating plant uptake, with stronger effects at higher Pb concentrations. The results confirm that combining crambe with growth-promoting bacteria is effective for bioremediating Pb-contaminated soils. These bacteria not only improved plant growth and health but also boosted the plants' capacity to accumulate and translocate Pb, underscoring the potential for large-scale application of this technique to recover areas contaminated by heavy metals.

Keywords: *Azospirillum brasilense*. Bioremediation. Lead. *Pseudomonas fluorescens*.

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RESUMO
O estudo aborda a contaminação do solo por chumbo (Pb), um grave problema ambiental e de saúde pública. A biorremediação utilizando microrganismos e plantas é uma solução promissora. Esta pesquisa avalia a sinergia entre a crambe (Crambe abyssinica) e microrganismos promotores de crescimento para a remoção de Pb do solo. O experimento foi conduzido em cultivo protegido entre abril e julho de 2023, utilizando um delineamento em blocos casualizados com quatro tratamentos de concentração de Pb e inoculação com duas espécies microbianas. As análises incluíram crescimento vegetal, níveis de macronutrientes e teor de Pb nas plantas e no solo. A inoculação aumentou significativamente o crescimento da crambe, incluindo o comprimento da raiz e da parte aérea, e melhorou a biomassa. Os microrganismos intensificaram a solubilização e a mobilização do Pb no solo, facilitando a absorção pelas plantas, com efeitos mais pronunciados em concentrações mais elevadas de Pb. Os resultados confirmam que a combinação da crambe com bactérias promotoras de crescimento é eficaz para a biorremediação de solos contaminados com Pb. Essas bactérias não apenas melhoraram o crescimento e a saúde das plantas, mas também aumentaram a capacidade das plantas de acumular e translocar Pb, ressaltando o potencial de aplicação em larga escala dessa técnica para recuperar áreas contaminadas por metais pesados.

Palavras-chave: *Azospirillum brasilense*. Biorremediação. Chumbo. *Pseudomonas fluorescens*.

RESUMEN
Este estudio aborda la contaminación del suelo por plomo (Pb), un grave problema ambiental y de salud pública. La biorremediación mediante microorganismos y plantas es una solución prometedora. Esta investigación evalúa la sinergia entre el crambe (Crambe abyssinica) y microorganismos promotores del crecimiento para eliminar el Pb del suelo. El experimento se llevó a cabo en cultivo protegido entre abril y julio de 2023, utilizando un diseño de bloques aleatorizados con cuatro tratamientos de concentración de Pb e inoculación con dos especies microbianas. Los análisis incluyeron el crecimiento de las plantas, los niveles de macronutrientes y el contenido de Pb en las plantas y el suelo. La inoculación incrementó significativamente el crecimiento del crambe, incluyendo la longitud de la raíz y el tallo, y mejoró la biomasa. Los microorganismos potenciaron la solubilización y movilización del Pb en el suelo, facilitando su absorción por las plantas, con efectos más pronunciados a mayores concentraciones de Pb. Los resultados confirman que la combinación de crambe con bacterias promotoras del crecimiento es eficaz para la biorremediación de suelos contaminados con Pb. Estas bacterias no solo mejoraron el crecimiento y la salud de las plantas, sino que también aumentaron su capacidad para acumular y translocar Pb, lo que subraya el potencial de aplicación a gran escala de esta técnica para recuperar áreas contaminadas por metales pesados.

Palabras clave: *Azospirillum brasilense*. Biorremediación. Plomo. *Pseudomonas fluorescens*.

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1 INTRODUCTION

Contamination by toxic metals, including Pb, is a global environmental and public health problem. Exposure to high Pb levels in soil can cause negative effects on human health, such as neurological damage and developmental disorders in children (Parvatiyar et al. 2005; Zhao et al. 2015). Therefore, remediating Pb-contaminated soils is crucial.

Environmental regulations set limits for Pb concentrations in soils to protect health and the environment. Bioremediation emerges as an efficient and sustainable option to meet these regulations, using microorganisms, plants, or their combinations to reduce pollutant concentrations, including Pb (Miller et al. 2009; Zhou et al. 2020). Among soil remediation technologies, bioremediation and phytoremediation stand out for their environmentally friendly ability to remove contaminants (Accioly and Siqueira 2000; Saha et al. 2021). Toxic metal removal can occur through physical, chemical, or biological methods. Physical and chemical methods include soil replacement, electrokinetic removal, thermal treatment, soil washing, vitrification, and chemical treatment with lime, phosphates, or organic compounds (Hou et al. 2020). These approaches are effective and fast, but costly and labor-intensive. They can also significantly alter soil quality indicators, compromising its agricultural suitability.

Bioremediation using microorganisms such as bacteria offers an economical and environmentally friendly alternative for contaminant degradation in soil. This technique uses metal-resistant bacteria that play a crucial role in solubilizing and mobilizing metals in soil, increasing their availability to plants (Ahmad 2015; Zhao et al. 2015). Such bacteria produce organic acids and siderophores that complex and solubilize toxic metals, aiding their uptake by plant roots. Bacteria including *Pseudomonas* spp. and *A. brasilense* spp. are notable for their tolerance to high Pb concentrations and abilities in solubilization, immobilization, and metal redox reactions (Miller et al. 2009; Zhou et al. 2020).

A study by Gonçalves Jr. et al. (2020) investigated the phytoremediation potential of *Crambe abyssinica* Hochst in artificially Pb-contaminated soil, where the plant showed high phytoremediation capacity despite reduced photosynthesis and increased respiration under contamination. This research reinforces the potential of metal-resistant bacteria to facilitate metal solubilization and mobilization in soil, making them more accessible to plants.

Given this context, this study aims to analyze the interaction between crambe, PGPB, and Pb removal from soils, evaluating the benefits of this association for bioremediation of contaminated soils.

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2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN AND PLANT ESTABLISHMENT

The study was conducted between April and July 2023, under protected cultivation on a rural property in the Espigão Azul district (24°50'26"S 53°27'26"W), in Southern Brazil. Laboratory analyses were carried out at SBS Laboratório – Análises Agronômicas e Veterinárias, in Cascavel, PR - Brazil.

The experimental design used was a randomized block design arranged in a factorial scheme with four replicates. Treatments consisted of inoculation with two species of microorganisms: *Azospirillum brasilense* (strains Ab-V5 and Ab-V6, both at $2x10^9$ CFU/mL) and *Pseudomonas fluorescens* (ATCC 13525, at $2x10^9$ CFU/mL), with each seed inoculated at 10^6 CFU/mL of viable cells. Cell viability was confirmed by viable cell counts 48 h before inoculation, applied directly into the furrow at planting. Regarding Pb contamination, four doses were established: control (no metal added), half of the maximum allowed dose for agricultural soils (90 mg kg^{-1}), according to CONAMA Resolution 420 (CONAMA 2009), the maximum allowed dose (180 mg kg^{-1}), and twice the maximum allowed dose (360 mg kg^{-1}).

Contamination was carried out by adding a standard lead chloride ($PbCl_2$) solution 24 h before planting and inoculation. Sowing involved ten seeds per pot, later thinned to four plants per pot after ten days, as shown in Figure 1. Each experimental unit comprised one pot with four plants, totaling 48 pots as schematized in Figure 2.

For cultivation, cylindrical seedling bags 30 cm in height and 20 cm in diameter with a capacity of 5 L were used, filled with 5 kg of pre-sieved red latosol (Figure 1.A). This substrate, initially with good agronomic characteristics for crambe cultivation, was amended with fertilizer to achieve ideal growing conditions. Details on the soil fertility are presented in Table 1.

Treatments were irrigated every 48 h to maintain soil moisture from planting until early flowering (Figure 1.F).

Morphometric analyses

At 50 days after emergence (DAE), plants were removed from the pots to measure total length, shoot height, root length (using a graduated ruler), and stem diameter (with a digital caliper). Plants were then separated into root and shoot, placed in paper bags, and weighed to determine fresh mass. Samples were then oven-dried in forced-air circulation at

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65 °C until constant weight was achieved, followed by dry mass determination with a precision balance.

The analysis of vascular bundle anatomy was performed through 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 h to ensure complete fixation. Samples then underwent a dehydration series in ethanol at 30%, 50%, 70%, 80%, and 90%, each step lasting 2 h, followed by two steps in 100% ethanol for 2 h each. After dehydration, samples were cleared in Xylol I (a 50% mixture of absolute ethanol and xylol) for 1 h and in pure Xylol II for another 1 h.

Next, samples were embedded in paraffin through three 6 h immersion steps. Paraffin blocks were prepared and, after 24 h of complete solidification, sections were cut using a manual microtome. Before staining, slides were heated at 50 °C in an oven for about 1 h.

The staining process began with paraffin removal in pure xylol I and II for 10 min each, followed by a series of hydration steps in decreasing ethanol concentrations and immersion in water. Staining was performed with 0.05% toluidine blue at pH 4.00 applied to the slides for 5 min at 55 °C. After staining, slides were washed in running water, dehydrated in ethanol, and fixed in xylol. Finally, slides were mounted with entellan and cover slips.

Samples were examined under an optical microscope, allowing identification of xylem walls stained green or bluish green, while phloem walls showed a reddish-purple color. This differentiation can be observed in Figures 3 and 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).

Determination of Available Macronutrient Content in Soil

To determine available macronutrient, micronutrient, and Pb contents in soil, as well as pH, the methodology described by Embrapa (Silva 2009) was adopted. KCl extractor was

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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.

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.

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 exposed to Pb, as shown in Figure 5.A, regardless of the applied metal concentration. Even in plants not exposed to the metal, there was an increase in total length when inoculated with *A. brasilense* and *P. fluorescens*. Specifically, in treatments without metal addition, the increase in total length was 1.32-fold for *A. brasilense* and 1.33-fold for *P. fluorescens*.

For the highest metal concentration tested, the increase was even more significant, especially for the shoot, reaching 1.44-fold for *A. brasilense* and 1.57-fold for *P. fluorescens*, as shown in Figure 5.B.

The observed increment also extended to root length. In the absence of metal, there was an increase of 1.64-fold for *A. brasilense* and 1.79-fold for *P. fluorescens*. For the highest Pb concentration tested, this increase was even more pronounced, being 2.57-fold for *A. brasilense* and 3.75-fold for *P. fluorescens*, as shown in Figure 5.C.

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Pb can cause significant adverse effects on plants, such as reduced germination and early growth rates, as well as increased levels of reactive oxygen species resulting in oxidative stress (Foyer et al. 1994; Mishra et al. 2017). However, the application of beneficial microorganisms, such as plant growth-promoting bacteria, has shown promise in reducing the impacts of Pb toxicity. These microorganisms can establish a symbiotic relationship with plant roots, improving nutrient uptake and resilience to stress conditions, including the presence of toxic metals (Ferrol et al. 2016; Gong and Tian 2019; Alotaibi et al. 2021).

Specifically, *A. brasilense* is known to enhance plant growth through various mechanisms, including the production of phytohormones, improved N uptake, stress reduction, and pathogen control, all contributing to overall productivity (Bashan and de-Bashan 2010; Domenico 2019; Kargapolova et al. 2020). Additionally, *A. brasilense* promotes biological nitrogen fixation and the synthesis of phytohormones such as auxins, gibberellins, and cytokinins, which favor root system development and drought stress resistance, resulting in increased biomass and benefits to soil microbiota (Hungria 2011).

Studies show that the association of *P. fluorescens* with *A. brasilense* is beneficial for plant growth, corroborating research by Guimarães et al. (2021), which identified improvements in soybean crop development when co-inoculated with *P. fluorescens* and *Bradyrhizobium japonicum* in the presence of phosphate fertilizer. Ferreira (2020) also highlighted the efficiency of *P. fluorescens* in delaying plant senescence due to the enzyme ACC deaminase, an important plant growth promoter as described by Glick (2014). Inoculation with *A. brasilense* promoted advances in the development of *Brassica napus*, reinforcing that the symbiosis established between these microorganisms and plant roots not only improves nutrient uptake but also increases resilience to abiotic stress. Both microorganisms, through their ability to synthesize phytohormones, are crucial for plant growth, leading to significant biomass increases. These findings emphasize the vital role of microorganisms in plant development and mitigating the impacts of metal contamination, highlighting the promising use of biotechnological techniques in sustainable agriculture and soil recovery.

Hungria (2011) highlighted the benefits of using *A. brasilense* in promoting root development and increasing the height of maize plants after inoculation. Similarly, Cotrim et al. (2016) observed that treating wheat seeds with humic acid in combination with *A. brasilense* resulted in significant improvements in root growth, shoot development, and shoot dry mass. Furthermore, Vasconcellos (2022) reported that treating rice plants with biological

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inputs, including the association of *Azospirillum* and *Pseudomonas*, promoted a 35.71% increase in root length compared to treatments without inoculation, underscoring the effectiveness of microbial inoculation in stimulating plant growth. These evidences reinforce the value of plant growth-promoting microorganisms in agriculture, especially under abiotic stress conditions.

As a consequence of inoculation, there was a significant increase in both fresh and dry plant mass, resulting in a greater total biomass volume as illustrated in Figure 6 for fresh and dry mass. Similar patterns were observed for dry mass increments, as shown in Figure 6.

The study by Babu et al. (2015) on *Miscanthus sinensis* in combination with *Pseudomonas koreensis* in soils contaminated by toxic metals from mining activities demonstrated notable plant tolerance to toxic metals, with a substantial increase in Pb solubilization and a 54% increase in biomass. Greger (2003) highlights the importance of distinguishing between hyperaccumulator plants, which accumulate toxic metals but generate little biomass, and accumulator plants, which produce more biomass but with lower metal concentrations. This distinction is crucial for evaluating phytoextraction effectiveness, which depends not only on metal accumulation capacity but also on the volume of biomass generated.

Furthermore, the role of plant growth-promoting bacteria, such as *P. fluorescens*, is recognized as a promising approach to mitigate Pb toxicity in plants, improving plant resistance and biomass production, thus contributing to more sustainable and efficient practices in managing contaminated soils. Guimarães et al. (2023) and Duarte et al. (2020) show that both *A. brasilense* and *P. fluorescens*, through the synthesis of phytohormones such as indole-3-acetic acid, significantly boost plant growth and increase dry and fresh biomass production.

These investigations underline the effectiveness of these microorganisms in plant development and mitigating the negative impacts of metal contamination.

Studies such as those by Chiarini et al. (1998) and Gasoni et al. (2001) also illustrate the benefits of inoculation with *P. fluorescens* in crops like sorghum and lettuce, resulting in notable increases in shoot and root fresh mass, while Bulegon et al. (2016) report similar advances in soybean with *A. brasilense* inoculation. These results corroborate the capacity of these microorganisms to improve both root growth and biomass production in various

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agricultural crops, highlighting the potential of biotechnological strategies in sustainable agriculture and soil rehabilitation.

Biomass assessment is fundamental for understanding the effectiveness of phytoextraction techniques in metal-contaminated environments. As Greger (2003) notes, it is important to distinguish between hyperaccumulator plants, which have a high capacity to accumulate toxic metals but produce little biomass, and accumulator plants, which generate more biomass but with lower metal accumulation capacity. This distinction is crucial because the main goal of phytoextraction is not just to accumulate metals, but also to maximize the extraction of these contaminants per area through the biomass produced.

Regarding shoot dry mass, studies such as that by Novinscak, Joly, and Filion (2019) demonstrated that seed inoculation with *P. fluorescens* can significantly increase plant biomass and oil production in crops such as soybean and canola. This capacity highlights the potential of plant growth-promoting bacteria not only to improve plant health and growth but also to increase efficiency in removing soil contaminants through phytoextraction.

Stem diameter also showed a significant increase in inoculated plants compared to non-inoculated ones. In treatments without metal addition, the increase was 61.03% for *A. brasilense* and 76.77% for *Pseudomonas*. For the highest metal concentration tested, the increase was even more pronounced, reaching 106.13% for *A. brasilense* and 98.11% for *P. fluorescens*, as detailed in Figure 7.

Research has shown that microorganisms such as *P. fluorescens* and *A. brasilense* not only increase stem diameter but also improve fruit quality and yield in plants. A specific study with tomato plants inoculated with these bacteria showed significant improvements in stem diameter and overall plant performance. This effect is attributed to improved nutrition and hormonal regulation promoted by these microorganisms, contributing to more robust and productive plant development (Pérez-Rodriguez et al. 2020).

3.2 ANATOMICAL ANALYSES

Significant anatomical changes were observed in the collar region of the plants, both under Pb exposure and after inoculation with the studied bacteria. In general, inoculation led to an increase in the number of cell layers in xylem components (Figure 8.B) and phloem (Figure 8.A), while the presence of the metal caused a gradual reduction in these layers. Based on the harmonic mean across all Pb concentration levels tested, plants inoculated with *A. brasilense* showed increases of 68.91% in phloem and 94.47% in xylem. Inoculation

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with *P. fluorescens* resulted in increases of 76.06% in phloem and 70.80% in xylem. The alteration and development of vascular tissues like xylem and phloem in plants are regulated by a cascade of plant hormones, playing critical roles at various stages. Initially, gibberellins act at the onset of vascular development. As the process advances, auxins and cytokinins regulate subsequent growth and differentiation of these tissues. Finally, ethylene contributes to the final stage, essential for the full maturation and functionality of the conductive vessels. This hormonal coordination ensures the proper structural and functional development of the plant vascular system, essential for efficient translocation of water and nutrients (Sorce et al. 2013).

Regarding the pith cells shown in Figure 8.C, only *P. fluorescens* provided an increase of 26%, while for the cortex shown in Figure 8.D, both microorganisms promoted an increase of about 27.61%, as seen in Figure 9. The introduction of plant growth-promoting bacteria has a significant impact on increasing the volume and number of cells in vascular tissues of both xylem and phloem. These microorganisms use multifunctional strategies, including the synthesis of plant hormones such as auxins, gibberellins, and cytokinins, which are essential for plant development and vascular tissue differentiation. Additionally, these bacteria improve the absorption of essential nutrients like N and P and produce siderophores that enhance nutrient availability for plants. These processes directly contribute to more robust vascular system development. Moreover, microorganism-induced changes in root architecture, such as promoting lateral roots and root hairs, enhance water and nutrient uptake, thereby supporting a more efficient and adapted vascular system (Yang and Wang 2016; Bush et al. 2022).

The differentiation and development of plant conductive vessels, both xylem and phloem, are intricate processes regulated by plant hormones. Initially, gibberellins play a fundamental role, while auxins and cytokinins drive the later stages, culminating in ethylene's involvement in final vessel formation. This process involves the induction of programmed cell death, essential for xylem formation, which is critical for the passive transport of water and nutrients (Sorce et al. 2013). The robustness of the vascular system, especially xylem, becomes crucial under stress conditions such as drought or pathogen attacks, directly influencing nutrient translocation efficiency. Interaction with beneficial microorganisms like *A. brasilense* can significantly improve the plant's hydraulic conductivity, essential for maintaining effective water balance (Pereyra et al. 2012; Romero et al. 2014). Furthermore, studies indicate that inoculation with *A. brasilense* can increase the diameter of vascular

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bundles in both xylem and phloem, promoting better nutrient translocation and reinforcing plant structure (El-Afry et al. 2012; Boghdady and Ali 2013; Battistus 2019). Therefore, the integration of plant hormones such as gibberellins, auxins, cytokinins, and ethylene, along with inoculation of plant growth-promoting bacteria, is fundamental for vigorous development of the plant vascular system. This dynamic not only contributes to plant resilience under adverse conditions but also highlights the potential of these interactions to improve agricultural productivity, offering promising pathways for sustainable farming (Pereyra et al. 2012; Sorce et al. 2013; Romero et al. 2014).

3.3 TOTAL NUTRIENT CONTENT IN PLANT TISSUE – SHOOT

In Pb-exposed and inoculated plants, a reduction in Ca concentration in the shoot was observed compared to non-inoculated plants, especially at Pb concentrations of 180 mg kg^{-1} and 360 mg kg^{-1}, where the non-inoculated treatment showed values 14.64% and 21.23% higher, respectively. In contrast, at 90 mg kg^{-1} Pb, inoculation with *A. brasilense* resulted in a 19.08% increase in Ca concentration, while *P. fluorescens* promoted a 32.42% increase. In the absence of Pb, only *P. fluorescens* induced a 33.07% increase in Ca concentration compared to non-inoculated plants, as shown in Table 3.

For Mg and N, no significant differences were observed in any of the treatments. However, for P and K, both nutrients showed a significant increase with inoculation. Overall, *A. brasilense* increased P concentration in the shoot by 42.12% and K by 21.30%, while *P. fluorescens* increased P by 293.45% and K by 102.34%. The values for total nutrient content in plant tissue are presented in Table 3.

P. fluorescens is recognized for its significant contribution in agriculture, particularly in combating phytopathogens by producing metabolites with antipathogenic properties. This species can also convert unavailable phosphates into forms accessible to plants, a process demonstrated in studies such as those by Kazi et al. (2016) and Oliveira et al. (2015). Additionally, Hungria and Nogueira (2021) highlight the multifunctionality of *Pseudomonas*, from phosphate solubilization and phytohormone synthesis to ethylene regulation via ACC deaminase enzyme and siderophore production, all essential for optimizing nutrient uptake by plants.

In contrast, research by Felix et al. (1999) showed that *Brassica juncea* extracted low amounts of Pb in highly contaminated soils, reaching only 0.6 mg kg^{-1}. Meanwhile, *A. brasilense* plays a crucial role in N assimilation and in influencing plant hormone secretion,

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as discussed by Okon and Itzigsohn (1995), underscoring its importance in plant growth. Hungria et al. (2011) and subsequent studies confirmed these benefits in various plants, including *Urochloa brizantha* and tamani grass, with significant increases in N, P, K, and Ca after inoculation with *A. brasilense* (Hungria and Nogueira 2021; Andrade et al. 2022).

Additionally, phosphate solubilization by *Pseudomonas* is fundamental, with studies such as those by Silva et al. (2007) and Siqueira et al. (2018) demonstrating significant increases in P and N content in bean and maize, respectively. In the context of Pb contamination, the situation becomes more complex, as shown by Li et al. (2016), who pointed out the adverse effects of Pb on microbial activity and macronutrient availability. More recent research, such as that by Shaari et al. (2024) and Collin et al. (2022), suggests that Pb can reduce macronutrient availability by forming insoluble complexes and inducing oxidative stress.

Other investigations highlight the ability of certain bacteria to transform Pb into less toxic forms, potentially reducing its bioavailability to plants (Violante et al. 2010). Symbiosis with microorganisms, including fungi and bacteria such as *Azospirillum* and *Pseudomonas*, is crucial for optimizing plant mineral nutrition, especially in Pb-contaminated soils (Ma et al. 2013; Upadhayay et al. 2020). Finally, Flores-Aguilar et al. (2020) confirm the effectiveness of inoculation with *A. brasilense* in significantly increasing K, N, and P levels in *Brassica oleracea* var. Royal Vantage, highlighting the potential of microbial inoculation strategies to mitigate soil contaminant effects and promote plant health and nutrition, outlining a promising path for more sustainable agricultural practices.

3.4 TOTAL NUTRIENT CONTENT IN PLANT TISSUE – ROOTS

In root tissue, there was no increase in Ca accumulation in inoculated plants, with values remaining similar or lower than those of the non-inoculated treatment, except for *A. brasilense* at a concentration of 180 mg kg^{-1} Pb, where there was a 2.63% increase compared to the non-inoculated treatment. A similar pattern was observed for Mg, where only roots exposed to 90 mg kg^{-1} Pb and inoculated with *A. brasilense* showed a 37.24% increase in Mg concentration. Inoculation with both bacteria resulted in significant increases for P and K similar to those observed in the shoot, with values presented in Table 4.

Overall, micronutrients were at higher concentrations in treatments inoculated with *P. fluorescens*, especially at higher Pb doses, with increases of 29.75% for Fe, 16.63% for Cu, and 1.88% for Zn, while Mn did not show significant variations with inoculation of either

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bacterium, as seen in Table 4. Fe in root tissue showed a 64.12% increase with *A. brasilense* inoculation under exposure to 300 mg kg^{-1} Pb, while at 90 mg kg^{-1} Pb, inoculation with *A. brasilense* resulted in a 118.76% increase and *P. fluorescens* promoted a 30.30% increase in Fe content, as shown in Table 4. Cu concentration in root tissue increased progressively with *A. brasilense* inoculation at all tested Pb concentrations, with increments of 416.92%, 327.88%, 35.62%, and 16.68% for treatments without added Pb, 90 mg kg^{-1}, 180 mg kg^{-1}, and 360 mg kg^{-1} Pb, respectively, as detailed in Table 4. Zn and Mn levels in root tissue did not show significant increases due to inoculation in the treatments observed in Table 4. Torres-Torres et al. (2022) reported remarkable improvements in height and overall development of *Brassica napus* after inoculation with *Azotobacter sp.* and *A. brasilense*. Plant growth-promoting microorganisms, such as bacteria and fungi, are essential for optimizing plant development even in adverse environments like soils contaminated with toxic metals. These microorganisms establish a vital symbiosis with plant roots, not only increasing nutrient absorption but also enhancing plant resilience to abiotic stresses, including Pb toxicity. This mechanism stands out as a key element in promoting plant health and mitigating negative impacts from metal contamination, as shown in studies like Alotaibi et al. (2021), Ferrol et al. (2016), and Gong & Tian (2019).

3.5 TOTAL LEAD CONTENT

Pb concentration in the plant shoot showed a significant response to inoculation for all tested concentrations, indicating increased Pb accumulation in the shoot. In the treatment without added Pb but with pre-existing soil levels, non-inoculated plants had 1.15 mg kg^{-1} Pb, while plants inoculated with *A. brasilense* and *P. fluorescens* had 1.28 mg kg^{-1} and 1.37 mg kg^{-1}, respectively. For the 90 mg kg^{-1} Pb treatment, non-inoculated plants contained 2.28 mg kg^{-1} Pb, compared to 4.12 mg kg^{-1} with *A. brasilense* and 3.37 mg kg^{-1} with *P. fluorescens*. This trend continued for higher Pb concentrations, with inoculated plants accumulating significantly more Pb than non-inoculated ones, as detailed in Table 5.

Pb content in root tissue also increased with inoculation of both bacteria, demonstrating that inoculation enhances absorption of this metal. In plants exposed to 90 mg kg^{-1} Pb, roots inoculated with *A. brasilense* and *P. fluorescens* had 73.71% and 48.28% higher Pb content, respectively, than non-inoculated plants. At 180 mg kg^{-1} Pb, root absorption was 88.73% higher with *A. brasilense* and 63.28% higher with *P. fluorescens*, while at 360 mg kg^{-1} Pb, plants inoculated with *A. brasilense* showed an 83.34% increase in

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root Pb concentration, and with *P. fluorescens*, the increase was 49.44%, as observed in Table 5.

Pb uptake by plants predominantly occurs in its divalent form (Pb^{2+}), an essentially passive process via root hairs, as documented by Kabata-Pendias and Pendias (2001). This metal negatively impacts plant nutrition by reducing the availability of several essential macronutrients, an effect largely attributed to competition between Pb and other cations such as Ca, Mg, Cu, Fe, and K (Sharma and Singhvi 2017). Studies with maize indicate that Pb presence significantly reduces Ca and K levels, affecting vital processes such as photosynthesis, mitosis, and water uptake, illustrating the challenges posed by Pb contamination to plant physiology (Malavolta et al. 1997; Huang et al. 2005).

Microorganisms, including *P. fluorescens* and *A. brasilense*, can play a role in Pb solubilization in the soil through the excretion of organic acids and exudates that acidify the rhizosphere, improving the solubility of insoluble Pb compounds. Siderophore production by *Pseudomonas* can be crucial in this process, as these compounds can bind the metal, increasing its mobility in the soil (Battistus, 2019; Jeyalakshmi & Kanmani, 2008; Souza, 2013b). The formation of insoluble complexes with macronutrient ions and the induction of oxidative stress are additional mechanisms through which Pb compromises the absorption and transport of essential nutrients, as described by Collin et al. (2022), Shaari et al. (2024), and Sharma & Dubey (2005). Furthermore, research highlights the beneficial potential of specific microorganisms, such as bacteria and fungi, in converting Pb into less harmful forms—a process that not only mitigates the deleterious impacts of the metal but also promotes mineral nutrition of plants in contaminated environments (Violante et al. 2010; Upadhayay et al. 2020). Nitrogen-fixing or phosphate-solubilizing bacteria play a notable role in improving macronutrient uptake in Pb-contaminated soils (Ma et al. 2013).

The results of this study align with existing literature, underscoring the crucial role of specific groups of microorganisms in facilitating the uptake of toxic metals by plants. Phosphate-solubilizing bacteria of the genus *Pseudomonas* have been shown to chemically transform Pb in the soil into forms more accessible to plants, as evidenced in various studies (Arslan et al. 2017; Khan and Jhung 2017; Ojuederie and Babalola 2017; Wang et al. 2022). Pb presence in the environment causes a range of negative impacts on plant development, from root growth inhibition to metabolic changes and cellular toxicity, due to interference with the absorption and transport of essential nutrients (Sharma and Dubey 2005; Colin et al. 2013; Alkhatib et al. 2019).

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Several factors influence the biosorption capacity of these microorganisms, including metal concentrations, cell physiology and composition, as well as microbial cell structure (Li et al. 2019). Importantly, extracellular polymeric substances produced by bacteria, such as glycoproteins, humic substances, lipids, polysaccharides, proteins, and uronic acid, play a vital role in removing toxic metals from polluted environments (Li and Yu 2014; Gupta and Diwan 2017). Additionally, processes including complexation, ion exchange, and surface precipitation are known as major mechanisms in interactions with metal ions (Li and Yu 2014).

In the context of Pb bioremediation, bacteria resistant to this metal use various mechanisms for its detoxification, including biosorption, efflux mechanisms, induced precipitation, extracellular sequestration, and intracellular bioaccumulation of Pb (Jarosławiecka and Piotrowska-Seget 2014), reiterating the versatility and effectiveness of microorganisms in treating soils contaminated with toxic metals.

3.6 NUTRIENT LEVELS FOUND IN THE SOIL

For both roots and soil, total Ca content was higher in non-inoculated plants. Analyzing the harmonic mean across all Pb concentration treatments, which also represents the isolated mean values, it was found that the Ca content in non-inoculated soil was 22.52 g kg^{-1}. In soils treated with *A. brasilense*, the Ca content was 16.99 g kg^{-1}, and in soils inoculated with *P. fluorescens*, it was 19.86 g kg^{-1}. The available Ca content for plants in non-inoculated soil was 7.53 cmolc/dm^3. In soils with *A. brasilense*, it was 7.15 cmolc/dm^3, and with *P. fluorescens*, 6.89 cmolc/dm^3, as shown in Tables 6 for total levels and Table 7 for available levels.

Mg followed a similar pattern, with total contents of 6.02 g kg^{-1} for non-inoculated soils, 4.90 g kg^{-1} for soils treated with *A. brasilense*, and 4.69 g kg^{-1} for soils with *P. fluorescens*. Available Mg levels were 3.76 cmolc/dm^3 for non-inoculated soils, 3.32 cmolc/dm^3 for soils with *A. brasilense*, and 3.30 cmolc/dm^3 for soils with *P. fluorescens* (Tables 6 and 7).

There was a significant difference in total and available P levels in inoculated soils. Non-inoculated soil had a total P content of 52.25 mg kg^{-1} and an available level of 12.33 mg kg^{-1}. In soils with *A. brasilense*, levels were 63.12 mg kg^{-1} (total) and 19.62 mg kg^{-1} (available), and in soils with *P. fluorescens*, 66.34 mg kg^{-1} (total) and 24.20 mg kg^{-1} (available) (Tables 6 and 7).

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K showed a pattern similar to P, with non-inoculated soils showing a total concentration of 289.34 mg kg^{-1} and 0.48 cmolc/dm^3 available. In soils with *A. brasilense*, levels were 552.23 mg kg^{-1} (total) and 0.80 cmolc/dm^3 (available), and in soils with *P. fluorescens*, 547.25 mg kg^{-1} (total) and 0.79 cmolc/dm^3 (available) (Tables 6 and 7).

Among micronutrients, *P. fluorescens* provided a 4% increase in Fe levels. Cu had increases of 11.70% in soils with *A. brasilense* and 31.32% with *P. fluorescens*. No significant increases were observed in Zn levels with inoculation. For Mn, only *P. fluorescens* promoted an increase of 9.43% compared to non-inoculated plants (Tables 6 and 7).

Measurements of Pb concentration in the soil after the experiment revealed remarkable results. Soils that did not receive added Pb displayed a naturally low concentration of this metal. At the end of the experiment, non-inoculated soil showed a Pb concentration of 7.55 mg kg^{-1}, while soils inoculated with *P. fluorescens* dropped to 6.67 mg kg^{-1}, and with *A. brasilense*, even lower at 6.03 mg kg^{-1}. In soils exposed to 90 mg kg^{-1} Pb, the difference was more pronounced compared to soils without bacteria, recording 89.41 mg kg^{-1} for non-inoculated soils, 42.44 mg kg^{-1} for soils treated with *A. brasilense*, and 58.86 mg kg^{-1} for soils with *P. fluorescens*. For the Pb concentration of 180 mg kg^{-1}, results were 155.81 mg kg^{-1} for non-inoculated soils, 89.99 mg kg^{-1} for soils inoculated with *A. brasilense*, and 111.66 mg kg^{-1} for soils with *P. fluorescens*. At the highest concentration tested, 360 mg kg^{-1} Pb, non-inoculated soils presented 326.11 mg kg^{-1}, while soils with *A. brasilense* showed 186.16 mg kg^{-1} and with *P. fluorescens*, 247.28 mg kg^{-1}, indicating a significant reduction with *A. brasilense* (Tables 6 and 7).

A fundamental aspect of plant growth-promoting bacteria is their ability to solubilize phosphates, essential for plant development since P acts as a regulator in metabolic pathways and biochemical reactions (Taiz and Zeiger 2017). In Red Latosols of tropical regions like the Cerrado, where clay soils are rich in Fe and Al oxides, much of the P becomes fixed, rendering it inaccessible to plants. Malavolta (1980) noted that despite its abundance, P is often found in insoluble forms.

Microorganisms promote phosphate solubilization mainly through the exudation of a variety of organic acids such as gluconic, citric, and oxalic acids, among others (Marciano Marra et al. 2012). Additionally, microorganisms like *P. fluorescens* and *A. brasilense* are crucial for increasing Pb uptake by plants, resulting in lower metal concentrations in the substrate. Such studies suggest that these microorganisms can directly increase metal mobility and bioavailability in soil by producing chelating agents or modifying soil pH (Ma et

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al. 2013; Glick 2014; Wu et al. 2016). The significant reduction in soluble Pb concentrations observed after inoculation with *A. brasilense* and *P. fluorescens* demonstrates the effectiveness of these microorganisms in solubilizing Pb, reducing its environmental availability (De et al. 2008; Naik and Dubey 2013; Heidari and Panico 2020).

The effectiveness of bacterial strains in improving the phytoextraction of toxic metals suggests a promising approach to combine soil remediation with sustainable agricultural production, boosting plant growth while also increasing contaminant uptake (Pilon-Smits 2005; Vangronsveld et al. 2009). However, due to the limitations of phytoextraction for Pb recovery, phytostabilization emerges as a viable alternative, with plant growth-promoting bacteria effective at reducing Pb mobility, making them useful for its stabilization in soil (Egendorf et al. 2020; Shabaan et al. 2021).

The economic importance of phytoremediation for cleaning contaminated agricultural areas is highlighted by recent studies in China, showing it as a preferred option among farmers (Yan et al. 2022). The possibility of integrating phytoremediation with crop production not only increases the economic appeal of the approach but also requires careful consideration of combinations of soil types, plant species/varieties, and agronomic practices, along with diligent contaminant monitoring (Haller and Jonsson 2020).

Therefore, the interaction between plants and microorganisms plays a critical role in increasing the effectiveness of phytoremediation, offering significant practical applications for the recovery of soils contaminated with toxic metals and promoting more sustainable agriculture (Gladkov et al. 2023). This synthesis of mechanisms and impacts of microbe-assisted phytoextraction emphasizes the importance of ongoing research to optimize effective bioremediation strategies, helping to mitigate environmental challenges and promote resilient, sustainable farming practices.

Soil pH remained stable, ranging between 7.20 and 7.90 in all evaluated treatments, indicating that variations in Pb concentration did not significantly affect soil acidity as observed in Figure 10.

Maintaining soil pH stability in contamination and recovery studies is a crucial indicator, suggesting that variations in the availability and uptake of toxic metals are not directly influenced by changes in pH. This indicates that the observed benefits in Pb absorption modulation should be attributed to factors beyond mere changes in soil acidity or alkalinity.

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This phenomenon can be explained through specific biochemical and physiological mechanisms induced by the activity of inoculated microorganisms. Among these mechanisms are biosorption, bioprecipitation, and the chemical transformation of Pb. These processes are often mediated by enzymes or metabolites produced by the microorganisms. For example, certain microorganisms can secrete chelating substances that bind Pb, reducing its mobility and toxicity by forming stable complexes. Additionally, these microorganisms can alter Pb valence, making it less soluble and, consequently, less available to plants, contributing to lower plant absorption of the metal (Domingos 1997; Chavez Apare 2019; Florida Rofner et al. 2019).

3.7 ACCUMULATION AND TRANSLOCATION INDEX

Inoculation notably increased the Pb accumulation index observed in Figure 11 for crambe plants, with the increase being more pronounced as the Pb concentration in the soil rose. *A. brasilense* stood out, considerably boosting the accumulation index by 34.04%, 258.20%, 225%, and 227.27% for Pb concentrations of 0 mg kg^{-1}, 90 mg kg^{-1}, 180 mg kg^{-1}, and 360 mg kg^{-1}, respectively. In contrast, *P. fluorescens* showed lower accumulation indices of 23.40%, 150%, 125%, and 100% for the same Pb concentrations, respectively.

Without significant variation, the translocation index observed in Figure 12 remained stable among treatments. Phytoextraction, using root uptake and accumulation in aerial parts to remove inorganic contaminants, is reaffirmed as an effective technique for soil decontamination (Vamerali et al. 2010).

Plants with accumulation and translocation factors greater than one are recognized for their potential as hyperaccumulators of toxic metals due to their ability to concentrate the contaminant mainly in their aerial parts. This feature makes them ideal for phytoremediation processes, as highlighted by Boechat (2014) and Souza et al. (2013). The accumulation factor evaluates a plant's ability to absorb metals from the soil and store them in its biomass, classifying them as accumulators, indicators, or excluders. Meanwhile, the translocation factor measures the efficiency with which a plant transports the metal from roots to shoots, with a factor greater than one indicating a species efficient in phytoextraction, as pointed out by Tiwari et al. (2011).

In the bacterial context, species like *Pseudomonas* spp. have been isolated and successfully applied in bioremediation strategies for toxic metals, demonstrating the effectiveness of these microorganisms in soil and water decontamination processes. Recent

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studies, such as those by Chen et al. (2017), Dabir et al. (2019), Kalita & Joshi (2017), and Pramanik et al. (2018), illustrate this application. Metal-resistant bacterial strains use various mechanisms, including extracellular and intracellular sequestration, as well as surface biosorption, to mitigate the toxicity of metal ions in contaminated environments, as evidenced by Huang et al. (2018) and Kushwaha et al. (2018).

4 CONCLUSION

This study highlights bioremediation as a promising and sustainable strategy for treating soils contaminated with Pb. The use of *Crambe abyssinica* in combination with specific microorganisms demonstrated not only the ability to improve soil quality and plant health but also an effective method to increase the absorption and accumulation of this element by plants.

P. fluorescens proved highly effective in promoting crambe plant growth, significantly increasing total length, fresh and dry biomass, and Pb accumulation in aerial parts. This microorganism also improved phosphate solubilization, contributing to better plant nutrition in contaminated soils.

A. brasilense was equally effective in promoting plant development. This microorganism contributed to biological nitrogen fixation and phytohormone synthesis, resulting in more robust growth and generating plant resistance to stress caused by Pb contamination.

Both microorganisms were effective in reducing Pb concentration in the soil, making it less available and toxic to plants. Inoculation with *P. fluorescens* and *A. brasilense* resulted in lower Pb concentrations in the soil after the experiment, indicating these microorganisms' efficiency in remediating contaminated soils.

The combination of *Crambe abyssinica* with *P. fluorescens* proved particularly promising for Pb phytoextraction due to the high accumulation of the metal in the plant's aerial parts. This study reinforces the potential of plant growth-promoting microorganisms as allies in the bioremediation of contaminated soils, promoting more sustainable agriculture.

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ATTACHMENT

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

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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 Pb doses

Total nutrient content in shoot											
		Zero (0 mg kg^{-1} de Pb)		Half (90 mg kg^{-1} de Pb)		Dose (180 mg kg^{-1} de Pb)		Double (360 mg kg^{-1} de Pb)	
Ca (g kg^{-1})	*P. fluorescens*	15,45	(b)	17,07	(a)	12,47	(c)	10,78	(d)
	A. brasilense	11,61	(c)	15,35	(a)	12,98	(b)	10,80	(c)
	Controle	12,66	(b)	12,89	(b)	14,33	(a)	12,73	(b)
Mg (g kg^{-1})	*P. fluorescens*	2,29	(ab)	2,78	(a)	1,71	(b)	2,13	(ab)
	A. brasilense	1,76	(b)	2,75	(a)	2,37	(ab)	1,85	(b)
	Controle	2,39	(ab)	3,08	(a)	2,55	(a)	1,75	(b)
N (g kg^{-1})	*P. fluorescens*	52,71	(a)	54,49	(a)	53,55	(a)	52,53	(a)
	A. brasilense	48,69	(a)	58,03	(a)	52,08	(a)	52,34	(a)
	Controle	50,78	(a)	49,70	(a)	47,70	(a)	49,49	(a)
Fe (mg kg^{-1})	*P. fluorescens*	999,59	(c)	848,79	(d)	2089,8	(a)	1169,11	(a)
	A. brasilense	1222,82	(b)	439,47	(d)	1053,00	(c)	1247,80	(a)
	Controle	573,53	(c)	389,64	(d)	1332,4	(b)	1640,46	(a)
Cu (mg kg^{-1})	*P. fluorescens*	14,25	(d)	18,35	(c)	42,96	(a)	31,28	(b)
	A. brasilense	14,05	(d)	16,65	(c)	40,60	(a)	20,30	(b)
	Controle	21,50	(b)	14,16	(c)	72,73	(a)	20,13	(b)
Zn (mg kg^{-1})	*P. fluorescens*	0,41	(d)	0,71	(a)	0,49	(c)	0,57	(b)
	A. brasilense	0,43	(b)	0,52	(a)	0,55	(a)	0,53	(a)
	Controle	0,48	(b)	0,53	(a)	0,55	(a)	0,55	(a)
Mn (mg kg^{-1})	*P. fluorescens*	78,82	(a)	73,76	(b)	67,06	(c)	70,66	(b)
	A. brasilense	57,43	(c)	70,75	(bc)	81,72	(a)	72,78	(b)
	Controle	81,02	(a)	73,19	(b)	77,76	(a)	62,65	(c)

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.

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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 Pb doses

Total nutrient contents in roots					
		Zero (0 mg kg^{-1} de Pb)	Half (90 mg kg^{-1} de Pb)	Dose (180 mg kg^{-1} de Pb)	Double (360 mg kg^{-1} de Pb)
Ca (g kg^{-1})	*P. fluorescens*	111,89 (a)	84,01 (b)	82,83 (bc)	81,65 (c)
	A. brasilense	82,04 (d)	100,68 (a)	88,40 (c)	92,95 (b)
	Control	110,23 (c)	118,30 (b)	86,13 (d)	157,89 (a)
Mg (g kg^{-1})	*P. fluorescens*	14,96 (a)	14,96 (a)	6,54 (b)	14,01 (a)
	A. brasilense	12,71 (c)	22,22 (a)	14,71 (b)	8,67 (d)
	Control	24,00 (a)	16,19 (b)	14,60 (c)	16,70 (b)
Fe (mg kg^{-1})	*P. fluorescens*	192,54 (b)	147,01 (c)	340,08 (a)	196,92 (b)
	A. brasilense	388,31 (a)	246,81 (d)	317,96 (c)	327,94 (b)
	Control	915,07 (a)	112,82 (d)	735,38 (b)	199,81 (c)
Cu (mg kg^{-1})	*P. fluorescens*	15,51 (d)	77,18 (c)	117,36 (a)	98,68 (b)
	A. brasilense	175,03 (b)	138,85 (d)	153,31 (c)	394,06 (a)
	Control	33,86 (c)	32,46 (c)	112,91 (b)	337,7 (a)
Zn (mg kg^{-1})	*P. fluorescens*	39,65 (b)	36,72 (c)	42,47 (a)	43,69 (a)
	A. brasilense	45,90 (d)	53,17 (b)	55,31 (a)	50,28 (c)
	Control	62,33 (c)	50,23 (d)	68,13 (b)	84,51 (a)
Mn (mg kg^{-1})	*P. fluorescens*	128,17 (a)	39,78 (d)	71,97 (b)	56,65 (c)
	A. brasilense	63,20 (b)	64,64 (b)	66,90 (b)	77,98 (a)
	Control	129,24 (c)	67,98 (d)	142,10 (b)	156,84 (a)

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

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

Total Pb Content (mg kg^{-1})					
		Zero (0 mg kg^{-1} de Pb)	Half (90 mg kg^{-1} de Pb)	Dose (180 mg kg^{-1} de Pb)	Double (360 mg kg^{-1} de Pb)
Soil	*P. fluorescens*	6,67 (d)	58,86 (c)	111,66 (b)	247,28 (a)
	A. brasilense	6,03 (d)	42,44 (c)	89,99 (b)	186,16 (a)
	Control	7,55 (d)	89,14 (c)	155,81 (b)	326,11 (a)

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Roots	*P. fluorescens*	2,49	(d)	5,19	(c)	14,01	(b)	36,24	(a)
	A. brasilense	2,55	(d)	6,08	(c)	16,08	(b)	44,46	(a)
	Control	2,42	(d)	3,50	(c)	8,58	(b)	24,25	(a)
Shoot	*P. fluorescens*	1,37	(d)	3,79	(c)	6,48	(b)	19,30	(a)
	A. brasilense	1,28	(d)	4,12	(c)	7,99	(b)	23,87	(a)
	Control	1,15	(d)	2,28	(c)	4,18	(b)	12,59	(a)

Notes: Limits of quantification (LQ): Pb = 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 6

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

Total Nutrient Contents in Soil										
		Zero (0 mg kg^{-1} de Pb)		Half (90 mg kg^{-1} de Pb)		Dose (180 mg kg^{-1} de Pb)		Double (360 mg kg^{-1} de Pb)		
Ca (g kg^{-1})	*P. fluorescens*	22,30	(b)	24,42	(a)	14,51	(d)	18,17	(c)
	A. brasilense	17,97	(a)	16,70	(b)	17,72	(a)	16,60	(c)
	Control	22,25	(c)	26,80	(a)	25,32	(b)	15,72	(d)
Mg (g kg^{-1})	*P. fluorescens*	5,32	(a)	4,89	(b)	4,02	(d)	4,52	(c)
	A. brasilense	5,34	(a)	5,21	(a)	4,61	(b)	4,47	(b)
	Control	6,25	(b)	7,42	(a)	5,76	(c)	4,67	(d)
Fe (mg kg^{-1})	*P. fluorescens*	1012,49	(c)	916,75	(d)	1118,12	(a)	1083,63	(b)
	A. brasilense	1046,65	(b)	853,97	(d)	965,00	(c)	1105,92	(a)
	Control	1061,23	(a)	987,24	(b)	977,94	(c)	1063,81	(a)
Cu (mg kg^{-1})	*P. fluorescens*	82,27	(b)	56,75	(c)	141,5	(a)	81,75	(b)
	A. brasilense	82,89	(a)	83,75	(a)	59,00	(b)	82,50	(a)
	Control	64,37	(c)	77,75	(a)	64,25	(c)	69,50	(b)
Zn (mg kg^{-1})	*P. fluorescens*	1,77	(a)	0,56	(bc)	0,88	(b)	0,45	(c)
	A. brasilense	0,57	(b)	0,83	(b)	1,37	(a)	1,50	(a)
	Control	0,94	(b)	0,97	(b)	2,71	(a)	0,37	(c)
Mn (mg kg^{-1})	*P. fluorescens*	13,26	(a)	9,52	(b)	13,47	(a)	13,40	(a)
	A. brasilense	11,79	(b)	10,65	(c)	10,52	(c)	12,40	(a)
	Control	9,57	(c)	11,68	(b)	11,72	(b)	12,40	(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 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.

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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 Pb doses

Available Macronutrient Contents in Soil									
		Zero (0 mg kg^{-1} de Pb)		Half (90 mg kg^{-1} de Pb)		Dose (180 mg kg^{-1} de Pb)		Double (360 mg kg^{-1} de Pb)	
Ca (cmolc/dm^3)	*P. fluorescens*	7,36	(b)	6,56	(c)	6,07	(d)	7,94	(a)
	A. brasilense	7,74	(a)	6,50	(c)	7,25	(b)	7,13	(b)
	Control	7,49	(b)	7,34	(b)	0,39	(b)	7,89	(a)
Mg (cmolc/dm^3)	*P. fluorescens*	2,89	(c)	3,85	(a)	2,96	(c)	3,51	(b)
	A. brasilense	2,80	(c)	3,83	(a)	3,46	(b)	3,19	(b)
	Control	3,34	(b)	4,46	(a)	4,26	(a)	2,97	(b)
K (cmolc/dm^3)	*P. fluorescens*	0,63	(d)	0,81	(c)	0,87	(a)	0,84	(b)
	A. brasilense	0,63	(c)	0,84	(b)	0,89	(a)	0,82	(b)
	Control	0,52	(c)	0,68	(a)	0,65	(b)	0,63	(b)
P (mg/dm^3)	*P. fluorescens*	24,46	(a)	24,6	(a)	24,23	(a)	23,52	(b)
	A. brasilense	20,43	(a)	20,31	(a)	19,51	(b)	18,23	(c)
	Control	14,94	(a)	14,30	(b)	12,23	(c)	9,00	(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.

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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 $^{-1}$ Pb; B - Half = 90 mg kg $^{-1}$ Pb; C - Dose = 180 mg kg $^{-1}$ Pb; D - Double = 360 mg kg $^{-1}$ Pb, 1 – No inoculation, 2 – Inoculation with A. brasiliense, 3 – Inoculation with P. fluorescens)

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Figure 3

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

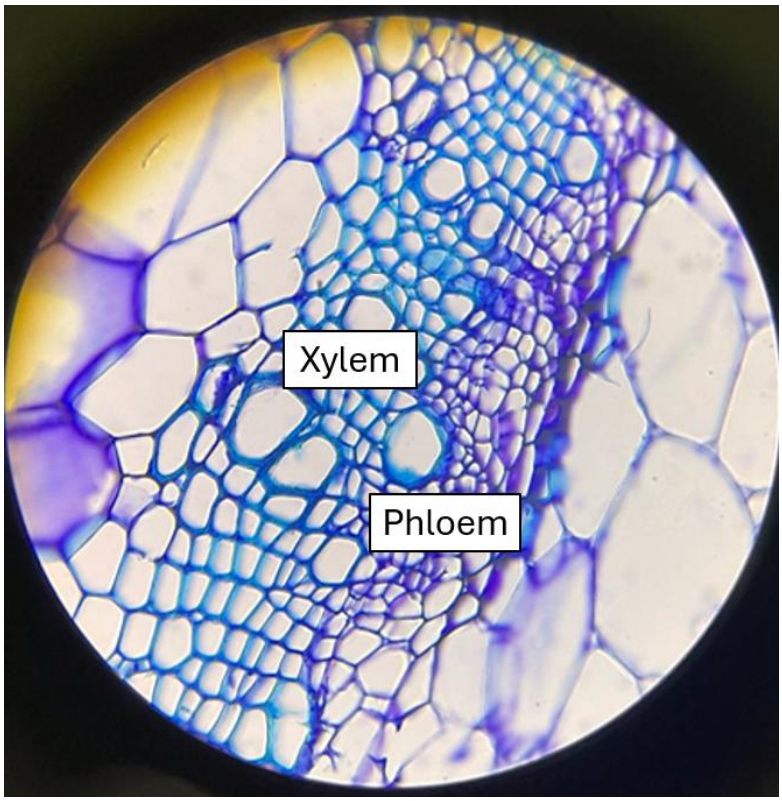

Figure 4

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

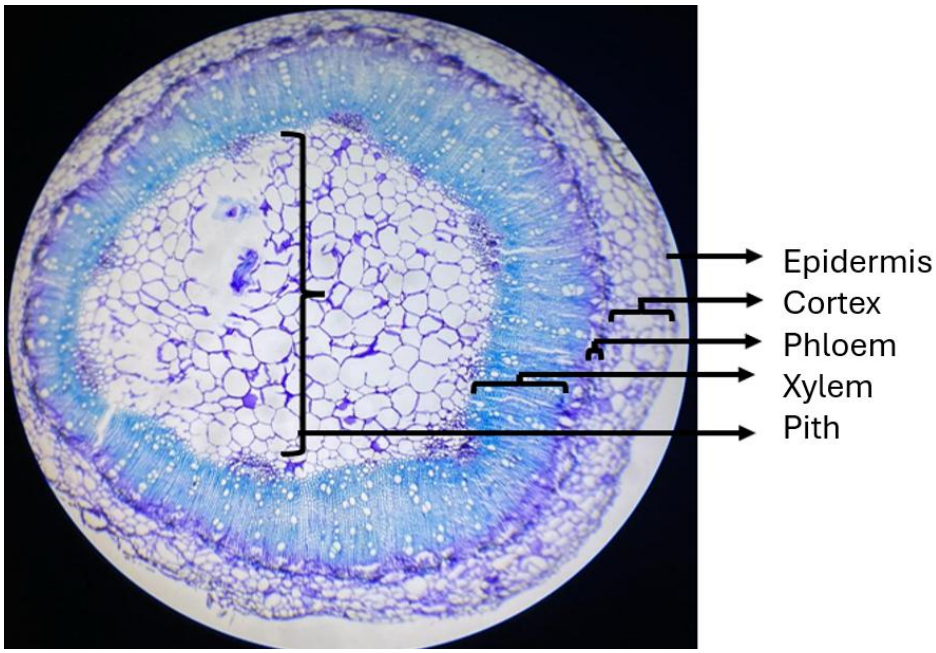

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Figure 5

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

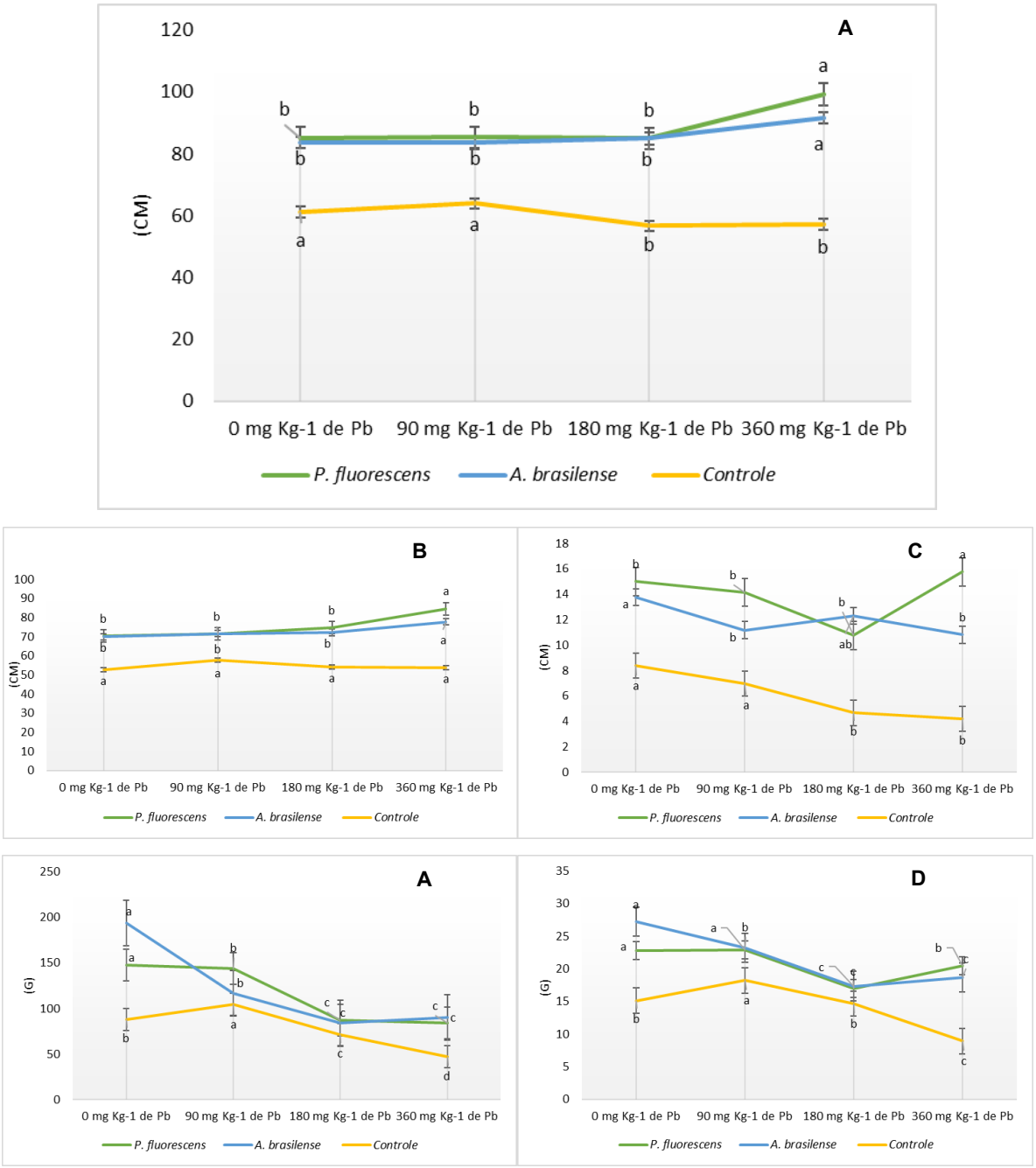

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Figure 6

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 Pb 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.

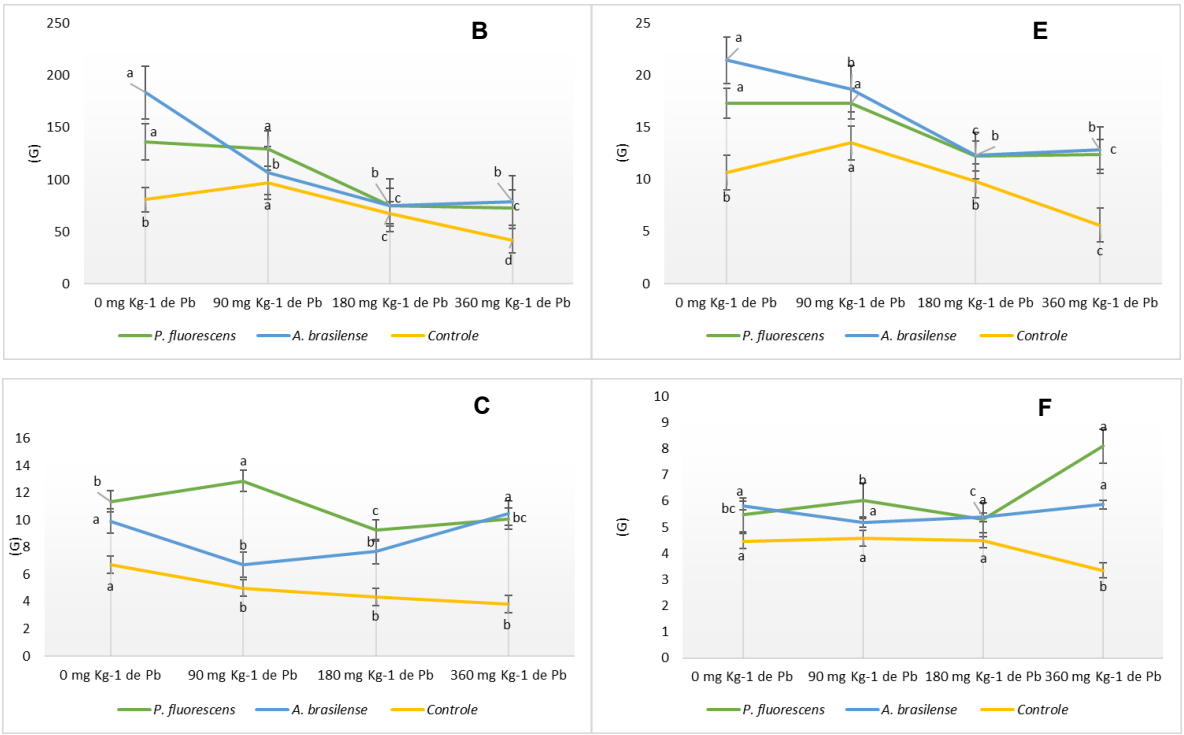

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Figure 7

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

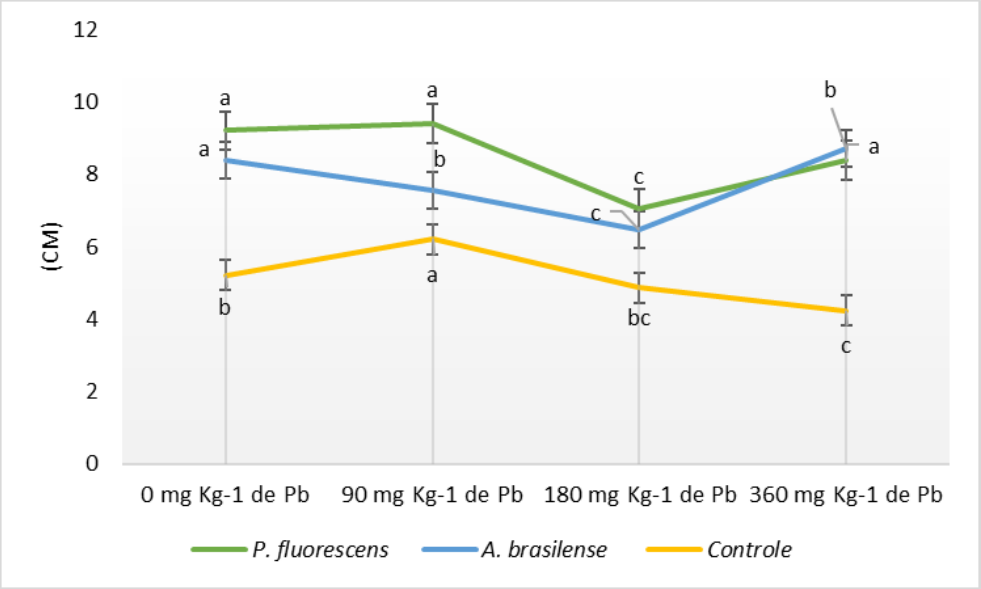
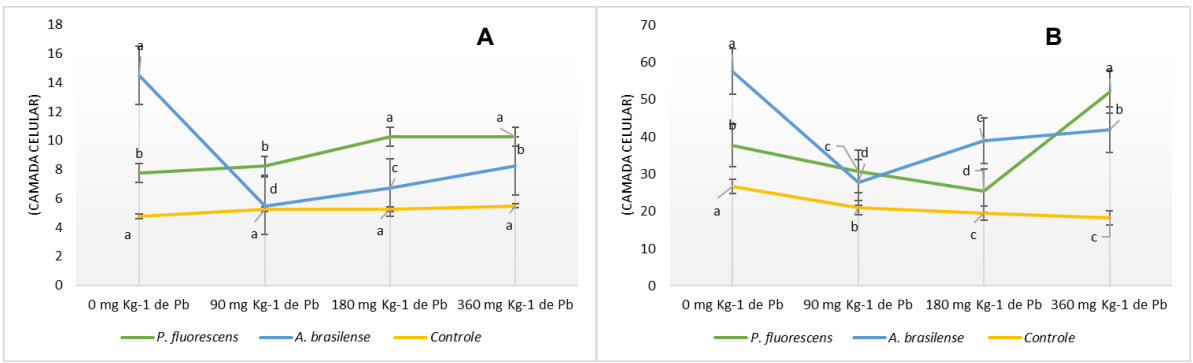


Figure 8

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 Pb 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.



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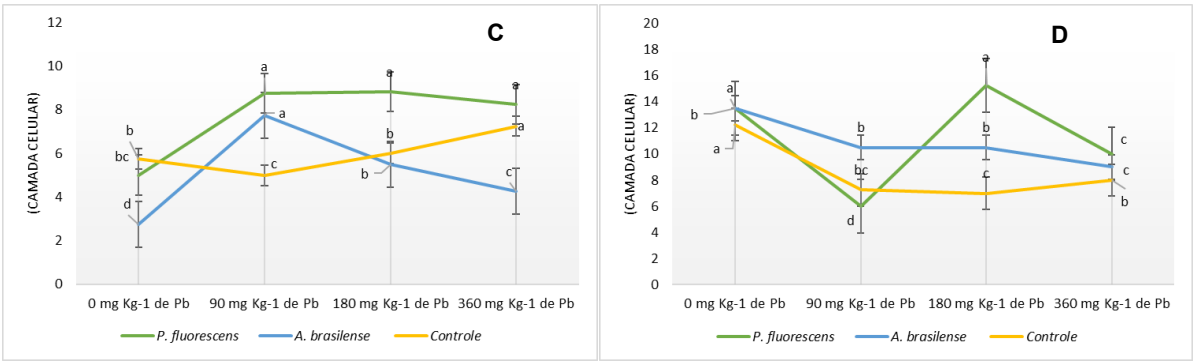

Figure 9

Histological section of the collar of crambe plants, (a) plants without inoculation and exposed to double the maximum Pb dose (360 mg kg ⁻¹), (b) plants inoculated with P. fluorescens and exposed to double the maximum Pb dose (360 mg kg ⁻¹); both observed at 40× magnification.

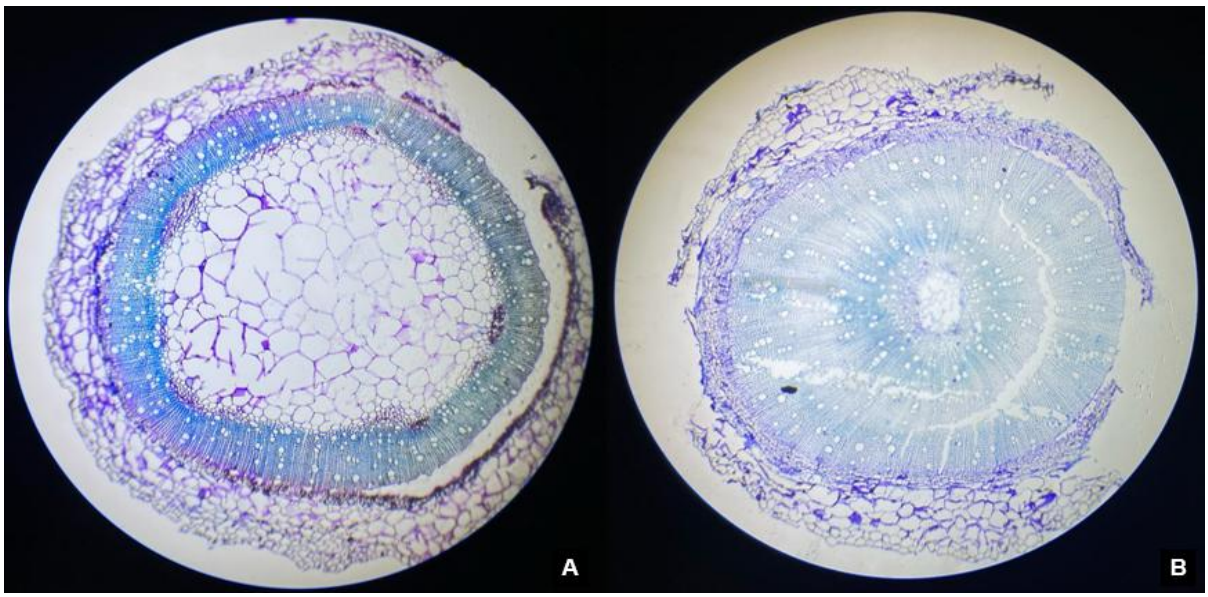

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Figure 10

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

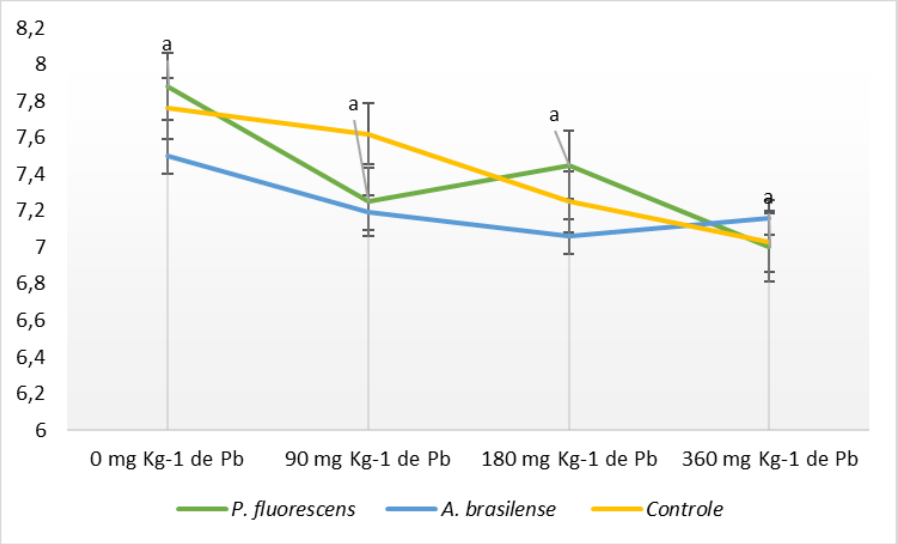

Figure 11

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

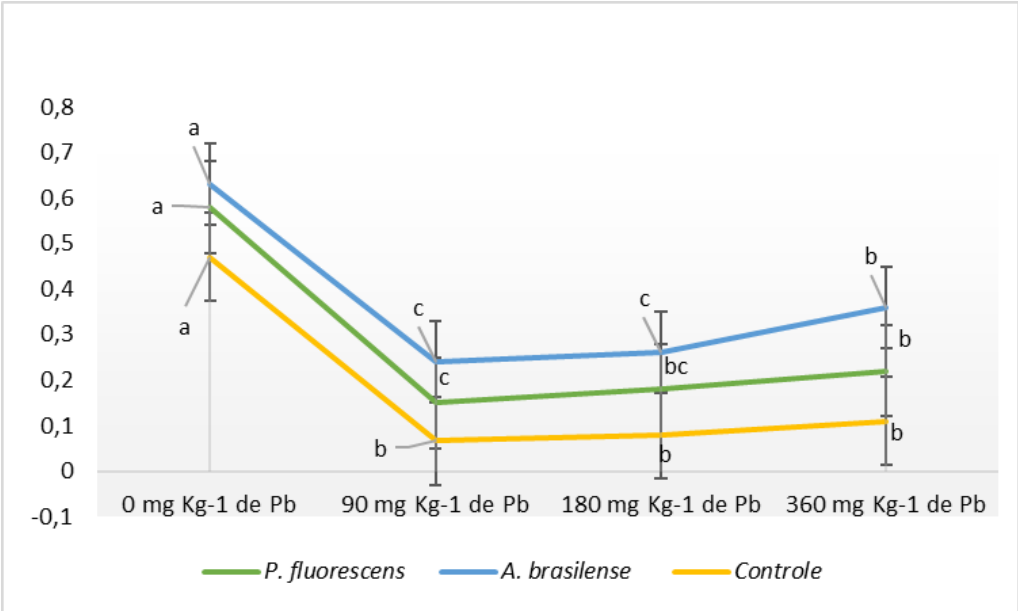

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Figure 12

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