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CHENNOUF Oum El Khair khadidja

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Theme

Biostimulation of wheat (*Triticum aestivum L.*) during copper stress by *Pseudomonas spp. fluorescents*

Committee Members:

Mrs. MARFOUA Mariem	M CA	President
Mrs. TAKHI Djalila	M AA	reviewer
Mrs. AMEUR Djamila	M AA	Reporter

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Abstract

The study focused on the bioremediation of copper-polluted soil using *Pseudomonas fluorescents*. Bioremediation, which involves the use of living organisms to reduce the concentration of contaminants, is an environmentally friendly approach to soil remediation. In this research, the interaction between copper and *Pseudomonas fluorescents*.was investigated.

The study findings indicated a notable influence of copper concentrations on diverse parameters, encompassing bacterial growth, root length, dry mass, and root volume. The investigation delved into the copper tolerance mechanisms deployed by *Pseudomonas spp.*, discussing factors like metal efflux pumps and metal-binding proteins. These mechanisms enable bacteria to flourish in environments contaminated with copper.

The research also highlighted the potential of *Pseudomonas fluorescents*.in mitigating the adverse effects of copper contamination on plant growth. The bacteria can mobilize, solubilize, or sequester copper, reducing its availability to plants. This has implications for improving plant health and crop yields in copper-contaminated soils.

In conclusion, this study contributes valuable insights into the bioremediation of copper-polluted soil through the use of *Pseudomonas fluorescents*.The findings suggest that these bacteria have the potential to play a crucial role in environmental remediation efforts and sustainable agriculture practices.

Keywords: *Pseudomonas fluorescents*., bioremediation, abiotic stress, copper tolerance, wheat.

Résumé

L'étude s'est concentrée sur la bioremédiation des sols pollués par le cuivre en utilisant des souches de *Pseudomonas fluorescents*. La bioremédiation, qui implique l'utilisation d'organismes vivants pour réduire la concentration de contaminants, est une approche respectueuse de l'environnement pour la remédiation des sols. Dans cette étude, l'interaction entre le cuivre et les souches de *Pseudomonas fluorescents*. a été étudiée.

Les résultats de l'étude ont révélé que les concentrations de cuivre avaient un impact significatif sur divers paramètres, notamment la croissance bactérienne, la longueur des racines, la masse sèche et le volume des racines. Les mécanismes de tolérance au cuivre utilisés par les *Pseudomonas*, tels que les pompes d'efflux de métaux et les protéines de liaison aux métaux, ont été discutés. Ces mécanismes permettent aux bactéries de prospérer dans les environnements contaminés par le cuivre.

L'étude a également mis en lumière le potentiel des souches de *Pseudomonas fluorescents*. dans l'atténuation des effets néfastes de la contamination par le cuivre sur la croissance des plantes. Les bactéries peuvent mobiliser, solubiliser ou séquestrer le cuivre, réduisant ainsi sa disponibilité pour les plantes. Cela a des implications pour l'amélioration de la santé des plantes et des rendements agricoles dans les sols contaminés par le cuivre.

En conclusion, cette étude apporte des informations précieuses sur la bioremédiation des sols pollués par le cuivre grâce à l'utilisation de souches de *Pseudomonas fluorescents*. Les résultats suggèrent que ces bactéries ont le potentiel de jouer un rôle crucial dans les efforts de remédiation environnementale et les pratiques agricoles durables.

Mots clé : *Pseudomonas fluorescents*. , bioremédiation, stress abiotique, cuivre tolérance, Blé.

ملخص

ركزت هذه الدراسة على تقنية المعالجة الحيوية للتربة الملوثة بالنحاس باستخدام سلالات *Pseudomonas fluorescents*. المضيئة. تعتبر المعالجة الحيوية ، التي تنطوي على استخدام الكائنات الحية للحد من تركيز الملوثات، منهجًا صديقًا للبيئة لتنقية التربة. في هذا البحث، تمت دراسة التفاعل بين النحاس وسلالات*Pseudomonas fluorescents.*

أظهرت نتائج الدراسة أن تراكيز النحاس كان لها تأثير كبير على معايير متنوعة، بما في ذلك نمو البكتيريا وطول الجذور والكتلة الجافة وحجم الجذور. تمت مناقشة آليات تحمل النحاس التي تستخدمها سلالاتPseudomonas ، مثل مضخات الإخراج المعدنية وبروتينات الربط المعدني. تسمح هذه الآليات للبكتيريا بالازدهار في البيئات الملوثة بالنحاس.

كما أبرز البحث أيضًا إمكانية سلالات *Pseudomonas fluorescents*. في التخفيف من التأثيرات السلبية لتلوث النحاس على نمو النباتات. يمكن للبكتيريا تحريك النحاس أو تخزينه، مما يقلل من توافره للنباتات. وهذا له تأثيرات على تحسين صحة النباتات وإنتاجية المحاصيل في الترب الملوثة بالنحاس.

في الختام، تقدم هذه الدراسة رؤى قيمة حول تقنية المعالجة الحيوية للتربة الملوثة بالنحاس باستخدام سلالات *Pseudomonas fluorescents*.تشير النتائج إلى أن هذه البكتيريا لها القدرة على أداء دور حاسم في جهود المعالجة الحيوية وممارسات الزراعة المستدامة.

كلمات مفتاحية: *Pseudomonas fluorescents*. ،المعالجة الحيوية ، التوتر اللاحيوي، تحمل النحاس، القمح.

TABLE OF CONTENTS

List of Abbreviations

- °C: Degree Celsius
- cm: Centimeter
- Do: Optical Density
- EDS: Sterile Distilled Water
- G: Gram
- %: Percentage
- H: Hour
- Min: Minute
- Mm: Millimeter
- µm: Micrometer
- PH: Hydrogen Potential
- Pf: *Pseudomonas fluorescents.*
- PGPR: Plant Growth-Promoting
- Cu Copper

List of Figures

List of Tables

General Introduction

Introduction

The global predicament of soil pollution underscores the immediate need to combat the persistent presence of heavy metal contaminants, which pose substantial risks to both ecosystems and human well-being. Among these pollutants, copper (Cu) stands out as a prominent heavy metal that can accumulate in soil due to industrial operations, mining activities, and agricultural practices, attributed to the over application of chemical pesticides, particularly those targeting fungi, resulting in adverse consequences for soil quality and plant health. Traditional methods of remediation often involve invasive and expensive techniques (Alloway, 2013). While contaminated soil can be remediated with phytoextraction, which usesthe natural or induced capacity of plants to uptake and accumulate metals from the soil (Jadia and Fulekar 2009). And also remediated with microorganisms which have attracted much attention due there to potential in enhancing metal uptake and plant growth promotion under heavy metal contamination (Singh et al. 2009). Both offer an ecologically sustainable and environmentally friendly alternative (Alloway, 2013).

Bioremediation capitalizes on the metabolic capabilities of microorganisms to transform or eliminate pollutants from the environment. In this realm, specific strains of fluorescent *Pseudomonas* spp. have garnered attention for their ability to endure and interact with heavy metals, including copper (Kang and Crowley, 2009). These versatile bacteria possess characteristics were initially used in agriculture and forestry to increase productivity and disease resistance and to protect against stress associated with the presence of trace metals or low pH soils, but also due to flooding, organic toxic substances, high salinity, drought, and phytopathogens (Saleem et al. 2007; Glick 2010; Bhattacharyya and Jha 2012) rendering them prime candidates for bioremediation endeavors (Kang and Crowley, 2009).

The potential of fluorescents *Pseudomonas* spp. in bioremediation originates from their distinctive features, such as metal resistance, metal accumulation (Kang and Crowley, 2009). Encompassing their capacity to bind metals, modify redox potentials, and engage in interactionswith plants that bolster both plant development and metal absorption. In addition, fluorescent *Pseudomonas* spp. producing indole acetic acid, siderophores and 1-aminocyclopropane-1- carbox-ylate deaminase and phosphate-solubilizing bacteria are capable of stimulating plant growth and establishing a relationship that aids in creating a stable root environment (Glick et al., 1995; Chabot et al., 1996; Rajkumar et al., 2006).

Our approach centered on evaluating specific non-symbiotic rhizobacteria, fluorescents *Pseudomonas* spp., isolated from the soil of arid environmental conditions, this study aims to

provide insights into the practical application of these microorganisms for the restoration of contaminated soils at various copper concentrations. The core objective of our study was to gauge the efficacy of these bacteria in counteracting the detrimental impacts of heavy metal contamination on wheat plants. The wheat plant was chosen as our research subject.

Ten of our fluorescents *Pseudomonas* spp. isolates collection were examined for their metal tolerance ability to various copper concentrations between 100 and 1000 ppm, four form them were selected to be used in the second part of our research to exploring their capacity to stimulate the growth and development of wheat plants in Contaminated soil with copper, where four concentrations (100,200, 300, 400 ppm) were applied in in vitro essay and three (100,200, 300 ppm) in pots essay.

Chapter one
Theoretical Framework

I.1. Introduction

Microorganisms, particularly plant growth-promoting (PGP) bacteria, have the potential to mitigate various limiting factors associated with phytoremediation technology. These factors include metal solubility, contamination levels, and soil chemistry (Burdg et al., 2000; Zhang et al., 2007; Tangahu et al., 2011; Belimov et al., 2015; Liu et al., 2015). Recently, there has been an increasing emphasis on endophytic microbes due to their non-pathogenic nature and beneficial mutualistic symbiosis (Khan et al., 2015).

Pseudomonas, a diverse genus of bacteria, engages in intricate interactions with copper in various environmental contexts. These bacteria, belonging to the family Pseudomonadaceae, possess unique capabilities to adapt to copper-rich environments, exhibiting both beneficial and detrimental effects. Pseudomonas species play a pivotal role in biogeochemical cycles, including copper cycling, impacting soil health and ecosystem dynamics. The dynamic interplay between Pseudomonas and copper encompasses aspects such as copper resistance, bioremediation, and even potential applications in agriculture (Kang and Crowley ., 2009)

I.1.1. Rhizobacteria non-symbiotic *Pseudomonas fluorescens*

Among non-symbiotic rhizobacteria, *Pseudomonas fluorescens* receives particular attention. This bacterium garners significant interest mainly due to its potential in reducing damages caused by certain fungal diseases attacking cultivable plants (Pant et al., 2001).

Inoculating plants with specific strains of *Pseudomonas spp.* indeed leads to a significant increase in crop yield, and this practice is commercially exploited through seed treatment as a means of plant protection, inducing systemic resistance against various pests and diseases (Mezaache, 2012).

I.1.2. Classification of the genus *Pseudomonas*

Pseudomonas genus is classified into different groups based on various characteristics such as morphology, physiology, and genetic makeup. The classification system typically includes several species and subspecies within the genus, allowing for a more detailed understanding of their diversity and relationships. The genus *Pseudomonas* belongs to the family *Pseudomonadaceae* and is known for its metabolic diversity, bioactive nature, and

aggressive ability to colonize environments (Aberoumand, A. 2010). This genus comprises of Gram-negative bacteria, which are rod-shaped with multiple flagella, and considered chemio-organotrophs.

The fluorescence observed in Pseudomonas is a result of the production of a yellow-green fluorescent pigment called water-soluble pyoverdine, which is insoluble in chloroform (Paulsen et al. 2005; Wong et al. 2012; Gao et al. 2012; Trögl et al. 2012) (Figure 1).

Species within the genus *Pseudomonas* can play various roles, such as phytopathogens (*P. syringae*), animal pathogens (*P. aeruginosa*), agents of bioremediation, food spoilage organisms, or even biocontrol agents (Cappe et al., 1994).Among the species with bioremediation potential, *P. putida* is capable of utilizing toluene, and *Pseudomonas alcaligenes* can degrade polycyclic aromatic hydrocarbons (Dagher, 1997). Regarding the food spoilage trait, *Pseudomonas fragi* and *Pseudomonas ludensis* cause numerous issues in the food industry (Cruden, 1992 and Parker, 1953). As for biocontrol agents, *P. fluorescens* is one of the most studied species for its effectiveness against parasitic micromycetes, particularly *Fusarium sp*. or *Pythium sp*, and certain nematodes (Chin-A-Woeng, 1998).

Bacteria belonging to the group of *fluorescent Pseudomonas spp*. are among the most abundant in the rhizosphere. In some cases, they represent more than 60% of the total bacterial microflora in the soil (Digat and Gardan, 1987). This abundance in natural soils and plant roots makes them suitable candidates for biological control agents (Sands and Rovira, 1971).

These bacteria are excellent competitors against fungal and bacterial microflora in the soil due to their relatively short in situ generation time (Garbaye, 1994). They have the capability to utilize plant exudates as nutrients (Lugtenberg et al., 2002) and chelate ferric ions (Garbaye, 1994). Moreover, they produce antibiotics (Garbaye, 1994; Natsch et al., 1994) and hydrolytic enzymes (Lim et al., 1991; Neilsen et al., 1998; Neilsen and Sorensen, 1999), further enhancing their competitive advantage in the rhizosphere environment.

I.1.3. Mechanisms of action of *fluorescent Pseudomonas spp*.

The antagonistic abilities of PGPR (Plant Growth-Promoting Rhizobacteria) are primarily attributed to both direct and indirect mechanisms.

I.1.4.1. Direct Mechanisms

I.1.4.1.1. Plant Growth Enhancement

The utilization of certain strains of fluorescent *Pseudomonas* spp. capable of colonizing and thriving on the root system leads to significant beneficial effects for the inoculated plants. The ultimate goal of bacterial inoculation is to increase crop yield through plant growth stimulation (Lemanceau, 1992).

Fluorescent *Pseudomonas spp.* is known to promote plant nutrition and growththrough various mechanisms. These include the solubilization of minerals, such as insoluble forms of phosphorus, making phosphorus more available for plant uptake. They also produce siderophores, which are iron-chelating compounds that enhance iron availability for the plants. Additionally, they produce phytohormones like auxins, which are growth-regulating hormones that positively influence plant growth (Lemanceau, 1992).

I.1.4.1.2. Plant Protection against Soil-Borne Diseases

In recent years, research has highlighted the potential of utilizing certain bacteria to control several diseases affecting plant roots. Bacteria capable of producing growth-promoting hormones or reinforcing plant defenses have been isolated from various rhizomes. Many of these fluorescent bacteria also produce numerous compounds with antibiotic activity, contributing to biological control (Lemanceau, 1992).

For instance strains of *P. fluorescens* have been implicated in controlling *Phytophthora* in soybeans, *Thielaviopsis basicola* in tobacco (Keel, 1989), *Erwinia carotovora* in potatoes, *Fusarium* in various plants, and other fungal diseases in citrus fruits, oranges, and even certain ornamental plants (Schnider-Keel, 2000; Kloepper, 1980; Lifshitz, 1986; Gardner, 1984; Xu and al, 1986). These bacteria have shown promising potential in managing soil-borne diseases and protecting plant health.

I.1.4.1.3. Stimulation of Germination

Certain bacterial strains, particularly those belonging to the group of *fluorescent Pseudomonas spp.*, appear to improve seed germination when environmental conditions are unfavorable. For example, Kloepper et al. (1986) demonstrated that the germination rate of rapeseed seeds sown in cold and compacted soil could be significantly increased through inoculation with specific bacterial strains. Similarly, Hofte et al. (1991) recorded a significant increase in the germination rate of corn seeds exposed to cold temperatures after inoculation with two strains of *Pseudomonas fluorescens*. One of these strains even maintained the germination percentage of a batch of seeds aged two years at the same level as those aged only one year. These strains are referred to as "Emergence Promoting Rhizobacteria" (EPR) (Kloepper et al., 1986).

Digat et al. (1990) also demonstrated that certain *Pseudomonas* strains can significantly stimulate the germination of tomato seeds, even under seemingly unfavorable environmental conditions. These findings suggest that these bacteria play a role in promoting seed germination and aiding plants during challenging conditions.

I.1.4. Stimulation of Interactions between Symbiotic Microflora and theHost Plant

Certain strains of fluorescent *Pseudomonas* spp. stimulate legume nodulation. For instance, Grimes and Mount (1987) showed that a strain of *Pseudomonas putida* significantly enhances the nodulation of beans by *Rhizobium*. Similarly, Polonenko et al. (1987) demonstrated that certain rhizobacteria can improve soybean nodulation by *Bradyrhizobium*. These strains are referred to as "Nodulating Promoting Rhizobacteria (NPR)." All of these strains promote root growth, produce indole acetic acid, and possess pectinolytic activity, resulting in an increase in the mass of nodules rather than their number (Lemanceau, 1992).

Furthermore, some bacterial strains positively influence the root colonization of the host plant by endomycorrhizae (von Alten et al., 1991) or ectomycorrhizae (Garbaye and Bowen, 1987). Mamoun and Olivier (1992) demonstrated that certain strains of *fluorescent Pseudomonas spp.* improve the longevity of the symbiotic association between the ectomycorrhizal fungus *Tuber melanosporum* and hazelnut. The association of

endomycorrhizae and *fluorescent Pseudomonas spp.* leads to greater stimulation of plant growth compared to bacterial or fungal inoculation alone (Meyer and Linderman, 1986; Oliveira et al., 1987). These interactions contribute to the beneficial effects of *Pseudomonas spp.* in promoting plant health and growth.

I.1.5. Antibiosis

Antibiosis is defined as "the inhibition of one organism by the metabolic product of another organism" (Cook and Baker, 1974), "a metabolic production with antifungal and/or antibiotic properties" (De Souza et al., 2003). The production of antibiotics by *Pseudomonas* is now recognized as an important factor in controlling several diseases. This bacterial genus produces a myriad of compounds with antimicrobial activities, including phenazines (Thomashow and Weller, 1988), pyoluteorine (Howell and Stipanovic, 1979), pyrrolnitrine (Howell and Stipanovic, 1980), tropolone (Lindberg, 1981), and 2,4-diacetylphloroglucinol (Keel et al., 1990). Their spectrum of action varies greatly from one molecule to another. These metabolites, produced in low concentrations, can inhibit the germination, mycelial growth, and/or sporulation of pathogenic agents (Montesinos et al., 2009).

Bacteria of the *Pseudomonas* genus are known for their antagonistic activity against several phytopathogens (Haas and Defago, 2005). Their ability to produce various antibiotics contributes to their role in controlling plant diseases and protecting plants from harmful pathogens.

I.1.4.2. Indirect Mechanisms

I.1.4.2.1. Induced Systemic Resistance (ISR)

Certain strains of PGPR can protect plants indirectly by stimulating inducible defense mechanisms in the plant, making the host much more resistant to future attacks by pathogens. This phenomenon is known as "Induced Systemic Resistance" (ISR) (Van Loon et al., 1998; Pieterse et al., 2002). It has also been demonstrated that *fluorescent Pseudomonas spp.* can act as elicitors, triggering the activation of plant defense genes (Soylu et al., 2002).

I.1.4.2.2. Trophic Competition

Trophic competition mainly occurs for root exudates and iron. The bioavailability of iron in an environment like the rhizosphere is often a limiting factor for microbial growth, leading to competition for the acquisition of this limited metal (O'Sullivan and O'Gara, 1992). Over time, several organisms have developed strategies for iron acquisition by synthesizing molecules called siderophores, which serve to capture iron, making it more soluble and available for their growth. Siderophores are characterized by their low molecular mass, high specificity for Fe+3, and their biosynthesis controlled by iron availability (Neilands, 1981).

Control of harmful organisms is achieved through the sequestration of iron in siderophore-iron complexes, which can only be assimilated by an organism possessing specific receptors for the complex. *Fluorescent Pseudomonas spp.* produces siderophores with a high affinity for iron, known as pyoverdines or pseudobactins (Lemanceau, 1992). These siderophores facilitate iron chelation and support the growth of Pseudomonas, which are harmless to neighboring plants. Consequently, *Pseudomonas spp.* outcompete other organisms in the environment through trophic competition (Cline, 1982, and Cline, 1984). Iron deficiency in the environment tends to inhibit the germination of spores of certain pathogenic agents. It is worth noting that some plants can obtain their iron by scavenging bacterial siderophores (Elad and Baker, 1985).

4.2.3. Secondary Metabolites

On the other hand, secondary metabolites play various roles since they are produced in large numbers and possess diverse molecular structures. These molecules, exhibiting antimicrobial activity, are secreted by certain *P. fluorescens* strains, such as 2,4-diacetylphloroglucinol, pyoluteorine, pyrrolnitrin, rhamnolipids, mupirocin, hydrogen cyanide (HCN), certain siderophores, and phenazines (Whatling et al., 1995; Dwivedi and Johri, 2003; Lee, 2003). For example, the production of HCN by *Pseudomonas* is involved in suppressing pathogens like *Thielaviopsis basicola*, *Septoria tritici*, and *Puccinia recondita* (Ramette et al., 2003). The compound acts directly on the pathogen cells by blocking cytochrome oxidase in the respiratory chain.

Regarding adaptation to the rhizosphere, HCN production can be advantageous for acquiring nutrients as it causes an increase in nutrient exudation by plant tissues. It may also contribute to the acquisition of certain metallic ions by forming complexes with them (Blummer et al., 2000). Moreover, in vitro culture, HCN production can inhibit the growth of several phytopathogenic fungi (Blummer et al., 2000). The presence of these secondary metabolites allows Pseudomonas spp. to compete with and suppress pathogenic microorganisms in the rhizosphere, contributing to plant health and growth.

I.2. The Biological Functions of Copper

Copper (Cu) stands as a pivotal trace element essential for maintaining the normal growth and developmental processes of plants. Operating as a cofactor, Cu assumes a dynamic role as the active center for a range of enzymes, participating extensively in diverse biological functions such as protein transportation, cell wall metabolism, electron transfer in respiration and photosynthesis, and signaling pathways for hormone responses (Gong et al., 2021). Inadequate Cu levels give rise to stunted plant growth, distortion, or the yellowing of young leaves (chlorosis), as well as the curling of leaf margins, impairment of apical meristems, and decreased seed-setting rates (Marschner,1995, Epstein and Bloom.,2005). Forests grappling with Cu deficiency experience considerable impacts on wood production (Ruiter., 1969). Further repercussions of Cu deficiency can lead to insufficient water transport due to compromised cell wall formation and lignification in specific tissues, including the xylem (Marschner., 1995). Moreover, Cu deficiency exerts a profound influence on pollen and embryonic development, pollen and seed viability, and the production of seeds and fruits (Burkhead et al., 2009). Integral to the electron transport chain of chloroplasts and mitochondria,Cu-associated proteins play a crucial role. Cu also engages in the photosynthetic reaction of PSII, stimulating oxygen-evolving activity independent of plastocyanin (Barr and Crane., 1976,Lightbody and Krogmann., 1967in Chen et al., 2022).

Cu plays a pivotal role in Cu/Zn-SOD (Festa and Thiele., 2011), ethylene receptor (Rodriguez et al 1999), laccase, polyphenol oxidase, and other multicopper oxidases (Choi and Davidson., 2011). Among these, certain copper oxidases, including amine oxidase enzymes that bind to cell walls, facilitate the oxidation of putrescine, producing essential hydrogen peroxide (H_2O_2) required for lignification, cross-linking cell wall proteins, and programmed cell death (Møller and Mcpherson., 1998). H_2O_2 functions as a signaling molecule engaged in various

physiological and biochemical processes, regulating plant growth and development, enhancing stress resistance and tolerance, fortifying cell walls, boosting photosynthesis, retarding senescence, and influencing stomatal movement (Nazir et al.,2019).

I.2.1. Copper characteristics

Copper is a naturally occurring element and an essential micronutrient for plants. However, excessive levels of copper in soil can have detrimental effects on plant growth and soil health. Here are some characteristics of copper in soil:

I.2.1.1. Copper Toxicity in Plants: Insights from Alloway (2013)

Copper, vital yet potentially toxic in soil, presents a dichotomy for plant health. Galloway's work (2013) sheds light on this complex interplay. Copper excess disrupts nutrient absorption, impacting plant equilibrium and root growth. Alloway's research underscores these repercussions. Chlorosis, leaf yellowing, is a copper toxicity sign. Alloway's study pinpoints it as a visible indicator of stress. Plants differ in copper sensitivity. Some withstand, others suffer. Alloway's reference highlights this diversity. Copper's dual role—essential micronutrient and potential toxin—is illuminated by Alloway's insights.

I.2.1.2. Copper's Soil Binding Complexities: McBride's Insights (1994)

McBride's research unveils copper's intricate interactions with soil components—clay, organic matter, and minerals. These bindings intricately modulate copper's availability to plants. McBride delves into how soil characteristics, particularly pH, sway copper's solubility and uptake. His reference illuminates the intricate balance between copper and soil, enriching our comprehension of its journey into plant ecosystems.

I.2.1.3. Copper's Soil Mobility: Delving into Sposito's Analysis (2008)

Sposito's examination delves deeper into copper's behavior within the soil matrix, spotlighting its mobility intricacies—a topic of paramount importance. Copper typically exhibits limited mobility, manifested by its reluctance to percolate extensively through soil

profiles. Sposito's comprehensive study highlights this phenomenon, underlining how copper's movement is restrained, particularly in most soil types. A pivotal aspect emerges in the context of soil type. In sandy soils, characterized by their coarse texture and porous nature, copper's mobility experiences a shift. Sposito's insights underscore how these conditions can potentially facilitate copper's downward migration into deeper layers, which raises concerns about groundwater contamination. The intricate balance between soil structure, copper's affinity to soil particles, and environmental factors becomes a focal point in understanding the risk of copper's mobility. Sposito's reference not only deepens our understanding of copper's movement but also underscores the significance of soil composition and prevailing conditions in shaping its behavior. This comprehensive exploration serves as a valuable guide in assessing the potential for copper migration and its implications on soil and water quality.

I.2.1.4. Copper's Bioaccumulation Dynamics: Insights from Baker and Walker (1990)

Baker and Walker's research delves into copper's intricate bioaccumulation within soil ecosystems. They highlight its propensity to gather in organisms and plants, tracing its journey through the ecological cycle. Bioaccumulation extends beyond individual levels, affecting higher trophic levels in the food chain. Their reference underscores the intricate interplay of soil, organisms, and plants, enriching our understanding of copper's ecological impact.

I.2.1.5. Unraveling Copper's Environmental Ripples: Insights from Kabata-Pendias and Mukherjee (2007)

Kabata-Pendias and Mukherjee's exploration delves deep into copper's environmental resonance, spotlighting how its elevated presence within soil reverberates through intricate systems—a phenomenon of paramount significance. The profound implications of heightened copper levels echo in their work, particularly concerning soil microorganisms and the delicate balance of soil ecosystems. Central to their analysis is the revelation that copper's impact extends beyond its direct interaction with plants. Kabata-Pendias and Mukherjee emphasize how copper's elevated concentrations can act as a disruptive force on soil microorganisms. Beneficial soil bacteria and fungi, vital for nutrient cycling and essential soil processes, aren't immune to copper's influence. The duo's reference offers a comprehensive exploration of how copper's presence can inhibit the growth and activity of these crucial microorganisms,

potentially unsettling the equilibrium within soil ecosystems. The interplay between copper, soil organisms, and ecological processes underscores Kabata-Pendias and Mukherjee's work. By illuminating how copper reaches spans beyond plants, they enrich our understanding of the intricate web of interactions that constitute soil ecosystems. This understanding resonates as a cornerstone in assessing the far-reaching repercussions of copper presence on environmental balance.

I.2.3. Morphological symptoms of copper toxicity in plants

Toxicity arising from elevated levels of copper becomes apparent primarily within the root system. With prolonged exposure to copper, the root system's vitality diminishes, leading to darkened and thickened roots, ultimately resulting in suppressed growth. Above ground, plants affected by copper toxicity exhibit distinct morphological signs. Leaves manifest chlorosis, accompanied by a significant decline in overall growth parameters such as leaf area, height, and stem diameter. In advanced stages, leaf edges may succumb to necrosis. As depicted in Figure 2, the comprehensive picture of copper toxicity impact on plants encompasses disruptions in photosynthetic, antioxidant, and mineral metabolism, ultimately culminating in compromised plant growth (Cruz et al .,2022).

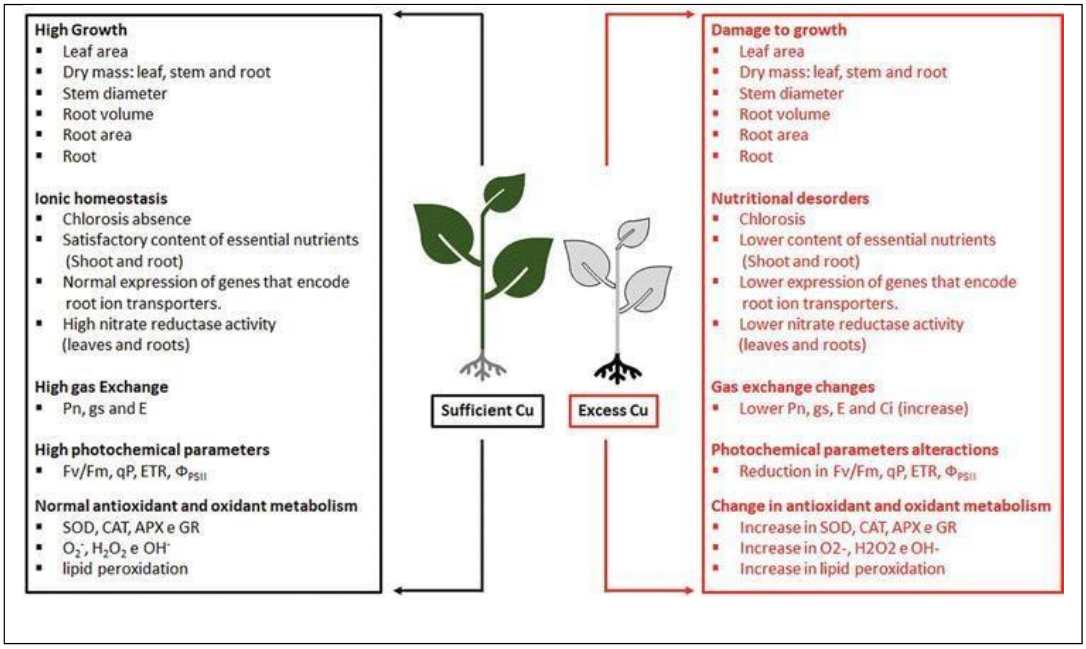


Figure 1. An overview of copper toxicity in plants at the level of gas exchange, oxidant-to-antioxidant metabolism, mineral metabolism, and growth.
(https://cdnintech.com/media/chapter/82460/1512345123/media/F2.png)

I.2.3. 1 In wheat

Copper toxicity symptoms in wheat can vary depending on the severity of the toxicity, the stage of plant growth, and the specific environmental conditions. However, there are general symptoms associated with copper toxicity in wheat. These symptoms include yellowing of leaves, particularly the younger ones, due to disrupted chlorophyll synthesis. Leaves might also show upward rolling or curling along their edges. Stunted growth, reduced plant height, fewer tillers, and overall diminished growth are common. Additionally, browning or death of leaf tissue, starting from leaf margins and tips and progressing towards the center of the leaf, is observed. Impaired root development, which can negatively impact water and nutrient uptake, is another symptom. Moreover, lower grain yield with fewer grains per spike and decreased seed weight due to disrupted reproductive development is a prevalent issue (Marschner.,2011).

I.2. 3. 2. Toxicity

Copper toxicity in soil refers to the negative impact of elevated copper levels on soil health, plant growth, and overall ecosystem balance. While copper is essential for plant growth in trace amounts, excessive concentrations can lead to various adverse effects. Here are some aspects of copper toxicity in soil along with a reference for further reading:

a. Plant Growth Inhibition: Kabata-Pendias and Mukherjee's Insight (2007)

Elevated copper's intricate impact on plant growth, specifically nutrient uptake like iron and zinc, sparks a domino effect of imbalance. Copper's interference in nutrient pathways triggers crucial nutrient compromise, hindering growth and development. Kabata-Pendias and Mukherjee reference underscores the far-reaching influence of copper on nutrient dynamics, casting light on the vulnerability of plants in copper-enriched environments. The study enriches our understanding of the challenges plants face in ecosystems marked by heightened copper levels.

b. Root Damage (Chen et al ., 2022)

Copper (Cu) toxicity initiates within the roots and subsequently extends to the aboveground portions of plants, disrupting a range of physiological processes. Elevated Cu levels in the soil impose constraints on root growth, impeding nutrient and water uptake. This limitation in root growth is typically linked to the rupture of the root epidermis and exodermis. The detrimental effects of Cu toxicity manifest as root cuticle rupture, decreased root hair proliferation, darkened appearance, stunted growth, and pronounced deformation of root architecture (Marques et al., 2019).Research has indicated that Cu ions have the capacity to modulate the pace of root meristem cell proliferation by governing plant root cell hormones such as melatonin, auxin, and abscisic acid. This regulatory action influences root development dynamics (Park and Back, 2012; Cui et al., 2019). Studies by (Batool et al., 2015) underscore that the inhibition of root growth correlates with diminished cell division, resulting in an increase in cell wall thickness. Similarly, Marques et al. (2019) demonstrated that elevated Cu concentrations (1000 mg L−1) led to alterations in the root structure, root volume, and the density of root hairs in Siberian cypress (Microbiota decussata).

c. Inhibition of Photosynthesis

An overabundance of copper (Cu) disrupts the structure of chloroplast and thylakoid membranes, initiating oxidative stress within plant cells. This disturbance leads to diminished levels of photosynthetic pigments and electron carriers, subsequently impeding the electron transfer process in photosynthesis (Vassilev et al., 2003, GonMendoza et al-ezláz.., 2019) A prominent symptom of Cu toxicity is leaf yellowing, and Hossain et al (2020) noted a substantial decline in essential photosynthetic pigments—chlorophyll a, chlorophyll b, and carotenoids— following the treatment of lentils (Lens culinaris) with 3 mM Cu sulfate (CuSO4). Similarly, Panou-Filotheou et al (2001) documented that Cu toxicity (17–25.5 mM) drastically reduced both the size and count of chloroplasts. Moreover, the grana thylakoids exhibited deterioration and swelling under Cu stress, often accompanied by an elevated globulin content within plastids.

Cu holds significance in the electron transport process mediated by plant photosystem II (PSII), pivotal in the water molecule photolysis within photosynthetic cells. Nevertheless, excessive Cu concentrations can undermine the efficiency of light-harvesting complex II (LHCII) or PSII. In sea buckthorn (Hippophae rhamnoides), elevated Cu levels primarily

impacted photosynthesis by obstructing the PSII reaction center. Application of 23 mM Cu directly suppressed PSII activity through chlorophyll content reduction, leading to inefficient photosynthesis (Cambrollé et al., 2015). Cu hindered PSII electron transport within the range of 75–150 μM, thus influencing the composition of the thylakoid membrane in black algae (Audouinella spp.) (Xu et al., 2013). Consequently, an excess of Cu detrimentally impacts plant photosynthesis by curbing chlorophyll biosynthesis and hindering PSII function. Consequently,maintaining a stringent Cu equilibrium within plants is imperative.

d. Altered Soil PH

Copper toxicity consequences extend to soil pH, triggering acidification. The excessive copper ions, displacing cations such as calcium and magnesium from soil particles, create a domino effect. This cascade alters the delicate balance, ultimately impacting soil pH and nutrient accessibility. The interplay between copper's presence and soil chemistry is unveiled through this process. This phenomenon underscores the complex interactions between copper and soil composition, amplifying our comprehension of the multifaceted impacts of copper toxicity on soil health and the intricate web of environmental processes.

I.4. Soil Contamination

Copper (Cu) is found in diverse soil forms, including oxide, carbonate, sulfate, and sulfide compounds. In natural settings, soil typically contains an average Cu concentration of 6–80 mg kg−1. However, human activities, particularly industrial and agricultural practices, have significantly escalated soil Cu levels, designating it as a prominent pollutant. The widespread use of agricultural chemicals containing Cu, like fertilizers, fungicides, herbicides, and pesticides, further exacerbates soil Cu accumulation (Adrees et al., 2015).Accumulated Cu in soil remains resistant to both biological and chemical degradation, imposing considerable risks on the environment, food security, and human well-being. Elevated Cu concentrations ranging from 20 to 100 mg kg−1 exert toxic effects on soil microorganisms, impeding the mineralization of essential nutrients such as phosphorus (P) and nitrogen (N). This surplus Cu content also diminishes the availability of phosphorus (Azeez et al., 2015). Additionally, the accumulation of Cu can lead to reduced levels of other trace elements, including iron and zinc. The inherent low mobility and solubility of Cu in soil contribute to persistent soil pollution that endures for extensive periods (Yrurla, 2009).

I.5. Tolerance

Copper tolerance in soil refers to the ability of plants to withstand and grow in soils with elevated copper concentrations. Some plants have developed mechanisms to tolerate higher levels of copper, allowing them to thrive in environments that might be toxic to other plant species. so certain plant species are naturally adapted to copper-rich environments, such as copper mines or areas with naturally high copper content in the soil. These plants have developed physiological and biochemical mechanisms to tolerate and accumulate copper without suffering from toxicity. And Copper-tolerant plants often produce metal-binding peptides called metallothioneins that help sequester and detoxify excess copper ions within plant cells. They also produce compounds called phytochelatins that can chelate copper ions, reducing their harmful effects. And some copper-tolerant plants have specialized transporters that facilitate the movement of copper ions out of sensitive tissues, such as roots or shoots, helping to prevent copper accumulation in vital plant parts. Copper-tolerant plants might develop specialized root structures that prevent the uptake of excess copper ions. This can involve restricted entry points or root exudates that bind to copper in the soil solution (Baker and Brooks., 1989)

Chapter two
Material and methods

In this study, rhizospheric fluorescent *Pseudomonas* spp. has been studied for tolerance to copper (Cu) and for plant growth-promoting activity on wheat (*Triticum aestivum*) in soil contaminated with copper.

II.1. Biological Materials

II.1.1 Plant Materials

The *in vivo* tests were conducted on durum wheat (*Triticum turgidum*) variety of (Vitron) and grown locally in Algeria

II.1.2. Bacterial strains

In our experimental work, we used four (P212; P12; P429; R2) strains of fluorescent *Pseudomonas* spp to study their activities, in vivo and in vitro, in copper contaminated soil. These strains have been isolates from spontaneous plants rhizospheres and identified in Amar Telidj university laboratories – Laghouat.

II. 2. Tolerance of strains to Copper

The tolerance of fluorescent *Pseudomonas* spp. selected strains to copper, was determined in King B (KB) broth amended with ($CuSO_2$,$5H_2O$) in ten increasing concentrations ranging from 100 to 1000 ppm of Cu; in addition one was kept as negative control (0 ppm of Cu). Finally, the tubes were incubated in a shaking incubator at 150 rpm and 28°C for 24 to 48 hours. During this incubation period, the interaction between the bacteria and copper, at different concentrations, was closely monitored at different concentrations of copper, to evaluatetheir growth and response to the heavy metal stress. This test allowed us to assess the tolerance levels of the bacterial strains to copper and identify which strains demonstrated the most promising potential for mitigating copper toxicity in the soil. Bacterial strains surviving the maximum level of metals were recorded as metal tolerant isolates. The minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of heavy metal compounds were evaluated against selected heavy metals for heavy metal tolerance. The optical density at 600 nm, pH and electrical conductivity (EC) was also recorded at the same time.

II.3. Plant growth-promoting activity of fluorescent *Pseudomonas* spp. on wheat (*Triticum aestivum*) in soil contaminated with copper.

In this section plant growth-promoting activity on wheat (*Triticum aestivum*), in copper contaminated soil, was evaluated in two essay, the first was carried *invitro,* the second was performed in pot soils contemned with Cu. For both essays we have used thesame seeds disinfection and soil sterilization protocols.

II.3.1. Seed disinfection

Uniform and healthy wheat seeds were surface disinfected with 0,1% mercury chloride for 3 min. The seeds were then rinsed with five changes of sterile distilled water for five minutes each (Ostrovskii *et al.*, 2000).

II.3.2. Soil sterilization

The soil preparation phase involves the sterilization of soil, the soil was sterilized twice at 120°C for 45 min, at 24 hours 'intervals,

II.3.3. First essay: Plant growth-promoting activity of fluorescent *Pseudomonas* spp. on wheat (*Triticum aestivum*) in soil contaminated with copper *in vitro*

This essay was carried *in vitro* in aseptic condition using sterile glass jar of 500 ml.

II.3.3.1. Soil preparation

The sterilized soil was packaged as 40g / jar. Two days before the seeds are sown, different sterile concentrations of copper solution (0, 100, 200, 300 and 400 ppm) were added to soil. All copper solutions were sterilized by microfiltration using 0.22µm membrane filters.

II.3.3.2. Seed bactrisation

Bacterial inoculum was prepared in sterile distilled water to get an inoculum density of 10^8 cell /ml. Disinfected seeds were immersed in respective bacterial suspension (P212, P12, R2, P429) for 3 hours, moreover the non-inoculated seeds that were immersed in sterile water served as a control.

II.3.3.3. Experimental design

The experiment included two factors, arranged in a randomized design, the first factor with five levels (four PGPR stains and a non-bacterized), second factor with five levels (0, 100, 200, 300, 400 ppm of Cu), each in 3 replicates, and five sterilized wheat seeds are planted within each prepared soil jar. The jars were placed in controlled photoperiod of 8 hours' dark/ 16 hours' light at ambient temperature, and arranged in a randomized design, the experiment was performed with daily.

The overall experimental design encompasses distinct treatments each one includes:

1. The negative control group, consisting of soil without copper exposure and without bacterial inoculation. T1, Control (un-inoculated).
2. The bacterial group, involving soil inoculated with bacteria but not exposed to copper.
   - T2, T3, T4, T5 corresponds to soil inoculated respectively with P212, P12, R2. P429.
3. The copper group, where plants are grown in copper-spiked soil but without bacterial inoculation.
   - T6, T7, T8, T9 corresponds to soil exposed respectively to 100, 200, 300, 400 ppm.
4. The combined group, wherein plants are both inoculated with bacteria and exposed to copper-spiked soil.
   - T10, T11, T12 T13corresponds to soil inoculated with P212 and exposed respectively to 100, 200, 300, 400 ppm.
   - T14, T15, T16 T17 corresponds to soil inoculated with P12 exposed and respectively to 100, 200, 300, 400 ppm.

- T18, T19, T20 T21 corresponds to soil inoculated with R2 and exposed respectively to 100, 200, 300, 400 ppm.
- T22, T23, T24 T25 corresponds to soil inoculated with P429 and exposed respectively to 100, 200, 300, 400 ppm.

II.3.3.4. Parameters measurements

II.3.3.4.1. Biometric parameters

a. Morphological parameters

For morphological study, the roots fresh biomass and roots dry biomass were recorded upon reaching a growth period of 28 days. Additionally, measurement of the length of roots was performed using millimeter paper after harvesting three plants per replicate. Dry weight (DW) of roots, was determined at 70°C until constant weight was reached.

b. Relative water content (RWC)

Relative water content (RWC, %) of roots was evaluated by using fresh and dry weight of roots, and was carried out according to the following equation:

$$\mathbf{RWC\ (\%) = (FW - DW)/\ (TW - DW) \times 100}$$

RWC %: relative water content
FW: fresh weight,
DW: dry weight,
TW: total plant weight.

II.3.4. Second essay: Plant growth-promoting activity of fluorescent *Pseudomonas* spp. on wheat (*Triticum aestivum*) in soil contaminated with copper in pot

II. 3.4.1. Seed Sterilization

Seeds disinfection was carried out using the same protocol as was described earlier (see II. 3...1).

II.3.4.2. Soil contamination

Soil sterilization was carried out using the same protocol as was described earlier (see II. 3..2)., then this soil was packaged as 100g / pot Two days before the seeds are sown, different concentrations of non-sterile copper solution (0, 100, 200 ,300 ppm) were added to respective soil.

II.3.4.3. Soil bactrisation

Bacterial inoculum was prepared in sterile distilled water to get an inoculum density of 10^8 cell /ml of respective bacterial suspension (P212. P12. R2. P429). 24 hours before sowing those suspensions were added to soil by irrigation, control, Non-inoculated treatments, soil was irrigated by water.

II. 3.4.4. Experimental design

The experiment included two factors, arranged in a randomized design, the first factor with five levels (four PGPR stains and a non-bacterized), second factor with five levels (0, 100, 200, 300, 400 ppm of Cu), each in 3 replicates, and sterilized wheat seeds inserted into the pots to a depth of 0.5 cm. Pots were placed a controlled photoperiod of 8 hours' dark/ 16 hours' light at ambient temperature, The plants were watered three times a week at 15 mL per pot the experiment was carried out with daily observations

The overall experimental design encompasses distinct treatments each one includes:

5. The negative control group, consisting of plants without copper exposure and without bacterial inoculation. T1. Control (un-inoculated)
6. The bacterial group, involving plants inoculated with bacteria but not exposed to copper.
7. T2, T3, T4, T5 corresponds to soil inoculated respectively with P212, P12, R2. P429.
8. The copper group, where plants are grown in copper-spiked soil but without bacterial inoculation.
9. T6, T7, T8, corresponds to soil exposed respectively to 100, 200, 300 ppm.

The combined group, wherein the plants are inoculated with both the bacterial suspension and copper.

10. T9, T10, T11 corresponds to soil inoculated with P212 and exposed respectively to 100, 200, 300 ppm
11. T12, T13, T14 corresponds to soil inoculated with P12 exposed and respectively to 100, 200, 300 ppm
12. T15, T16, T17 corresponds to soil inoculated with R2 and exposed respectively to 100, 200, 300 ppm
13. T18, T19, T20 corresponds to soil inoculated with P429 and exposed respectively to 100, 200, 300 ppm

II.3.5. Parameters measurements

II.3.5.1. Biometric parameters

Both morphological parameters and relative water content (RWC) were assessed out usingthe same protocol as was described earlier (see **II.3.3.4.1. (a)** and **(b)**).

II.3.5.2. Biochemical parameters

a. Sugar extraction

Sugar extraction was carried out according to Dubois s (1956) method, 0.1 g fresh leaves and roots samples were added into test tubes and homogenized with 3 mL of an 80% ethanol solution. The samples tubes were thoroughly closed and put it in a dark place for 48 hours, after the completion of this period, the tubes were opened and put in the incubator at 80°C until completely dry, and then20 ml of distilled water were added to tubes. Afterwards, 2 ml of sugar solution were put in a clean tube and to which 1ml of an aqueous phenol solution (5%) and 5ml of concentrated sulfuric acid were added. Then the tubes were allowed to stand 10 minutes, and then they were shaken and placed for 10 to 20 minutes in a water bath at 30° C. Using a spectrophotometer, the absorbance of the samples was measured at wavelength of 485 nm.

c. Chlorophyll Extraction

Chlorophyll extraction was carried out with a mixture of acetone and water at a ratio of 80% (v/v). 0.1g of fresh wheat plant leaves was placed in 2ml acetone. The samples were kept in the dark.

Chlorophyll concentration (a, b and total) was expressed as µg / ml and determined by the following formulae:

$$\text{Chlorophyll a (µg / ml)} = 12.7 \times A_{663} - 2.7 \times A_{665}$$
$$\text{Chlorophyll b (µg / ml)} = 22.9 \times A_{665} - 4.7 \times A_{663}$$
$$\text{Total chlorophyll = Chl a + Chl b (µg / ml)} = 20.2 \times A_{665} + 8.02 \times A_{663}$$

Where: A_{665}=absorption value at 665nm,
A_{663}=absorption value at 663nm,

II.4. Statistical analysis

The data were analyzed statistically for ANOVA.) The analysis was performed using the STATBOXVEGETAL essay version7.6. Differences between treatment mean values were determined following LSD test at 0.05 probability levels. Newman-Keuls test (α = 5%) grouped. The meanvalues were compared test at $p < 0.05$.

Chapter three
Results and discussion

In this chapter, we summarize the results of our study, which aimed to highlight the beneficial effect of *Pseudomonas fluorescens spp*. on wheat growth in copper-contaminated soil.

This work consists of two parts, the first is a test of their tolerance of bacterial capacity *in vitro* on and the second is a study of the effect of *Pseudomonas fluorescens spp.* identified on plant growth biostimulation in copper contained soil. Four bacterial strains (P12, R2, P212, P429) usingtow essay, one was conducted in glass jar *in vitro*, the other on pots.

III. 1. Results

III. 1. 1. The results relating to the tolerance test

Minimal inhibitory concentrations (MIC), bactericidal (MBC) and bacterial growth (OD600), pH and electrical conductivity (EC) of the growth medium are present in the first part of our results in the screening for Copper tolerant stains.

a. CMB and CMI for Copper Tolerance

For the Minimum Inhibitory Concentration (MIC), we have observed that P212 has the highest MIC which is equal to 800 ppm of Cu, followed respectively by P429 and R2 which have MIC equal to 700 ppm and 600 ppm. Subsequently, P12 and P108 at 400 ppm. Finely AS02, P704, AZ, T32 and RS21 have the lowest MIC equal to 200 ppm of Cu **(Table 01**)

Table 01: Minimum Inhibitory Concentration (CMI) of Copper against Studied *Pseudomonas fluorescens spp.* strains

	0	100	200	300	400	500	600	700	800	900	1000
AS02	+	+	-	-	-	-	-	-	-	-	-
P12	+	+	+	+	-	-	-	-	-	-	-
P108	+	+	+	+	-	-	-	-	-	-	-
P212	+	+	+	+	+	+	+	+	-	-	-
P429	+	+	+	+	+	+	+	-	-	-	-
R2	+	+	+	+	+	+	-	-	-	-	-
P704	+	+	-	-	-	-	-	-	-	-	-
AZ	+	+	-	-	-	-	-	-	-	-	-
T32	+	+	-	-	-	-	-	-	-	-	-
RS21	+	+	-	-	-	-	-	-	-	-	-

(-): Negative growth (+): Positive growth

As for the Minimum Bactericidal Concentration (CMB), P12, P212, P108, R2 don't have CMB to any copper concentration tested in our study (until 1000 ppm), furthermore P429 has CMB equal to 900 ppm, and AS02 equal to 500 ppm, however the others strains (P704, AZ,T32, and RS21) have showed fluctuating responses **(Table 02)**.

Table 02 Minimum Bactericidal Concentration (CMB) of Copper against Studied *Pseudomonas fluorescens spp.* strains

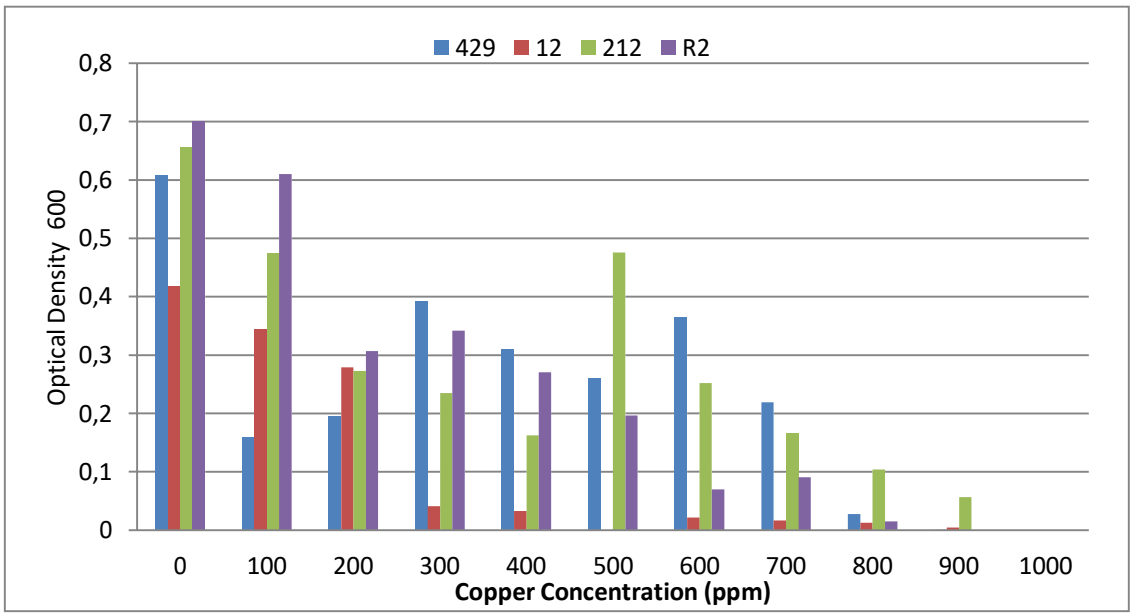
	0	100	200	300	400	500	600	700	800	900	1000
AS02	\	\	+++	++	+	-	-	-	-	-	-
P12	\	\	\	\	+++	+++	+++	+	++	++	++
P108	\	\	\	\	+++	+++	+++	+	++	+++	+
P212	\	\	\	\	\	\	\	\	+++	++	+
P429	\	\	\	\	\	\	\	+++	+	-	-
R2	\	\	\	\	\	\	\	++	+	+	+
P704	\	\	++	-	-	-	-	-	-	+	+
AZ	\	\	+++	+	++	-	+	-	-	-	-
T32	\	\	+++	++	+	-	-	+	+	++	++
RS21	\	\	+++	+++	+	-	-	-	-	+	++

(-) Absence of growth; (+): Low growth; (++): Abundant growth; (\) Non realized

b. Bacterial growth (OD600)

To assess the effect of copper on the growth of fluorescent *Pseudomonas* spp. cultures were conducted in a KB liquid medium. Growth was determined by measuring optical density at 600nm**.**

The results obtained indicate that growth is slightly inhibited in the medium containing copper. This slowdown is likely due to the bacteria adaptation phase to the culture conditions. While copper at lower concentrations does not inhibit the bacteria, it does decreases growth (200 to 400ppm). This explains how the bacteria defend themselves against toxic agents (Figure2).

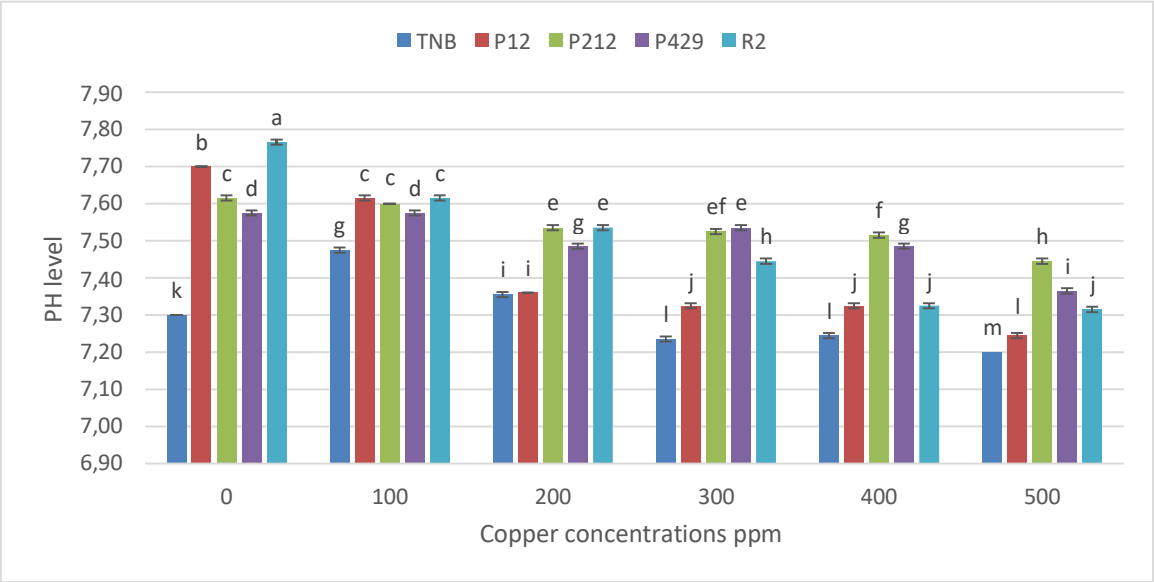


P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; 300; 400; 500;600;700;800;900; and 1000 ppm Copper concentrations

Figure2. Effect of the interaction between the bacterization factor and the concentration factor of the optical density

c. PH of the growth medium

The analysis of variance revealed a highly significant difference for the interaction between the concentration factor and the bacterization factor (*P*=0.000). We observed that the treatment R2 at copper concentration 0 ppm yielded an average of approximately 7.765 and was classified in group (a). Following closely, the treatment P12 at copper concentration 0 falls into the intermediate group (b) with an average around 7.7. Treatments R2, P12, P212 at copper concentration 100 ppm, and P212 at copper concentration 0 ppm, all with an average of 7.6, are classified in Group (c). The treatment P429 at copper concentrations 0 and 100 ppm, with an average of 7.575, is categorized in group (d), followed by treatments P212 at 200 ppm, P429 at 300 ppm, and R2 at 200 ppm, all classified in Group (e). The treatment P212 at copper concentration 300 ppm is placed in group (ef), and finally, P212 at copper concentration 400 ppm, with an average of approximately 7.515, is also classified in group (f) (Figure3).

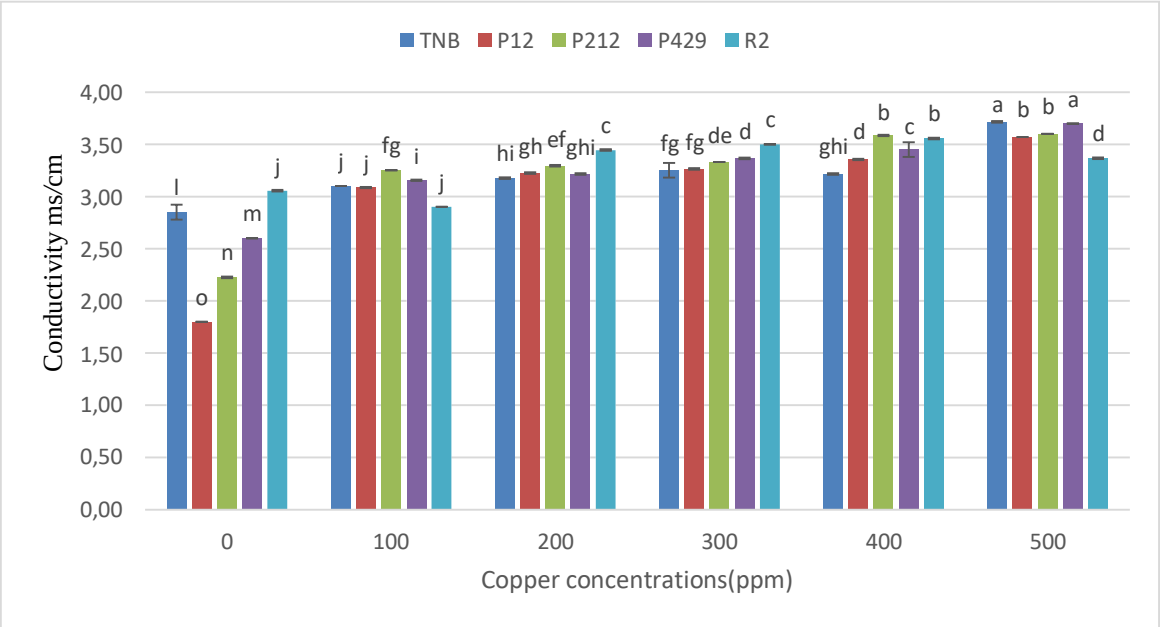


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; 300; 400; and 500 ppm Copper concentrations

Figure 3. Effect of the interaction between the bacterization factor and the concentration factor of the pH level

d. Electrical conductivity (EC) of the growth medium

The analysis of variance revealed a highly significant difference for the interaction between copper concentration factor and bacterization factor (P=0.000). In Group (a), TNB treatment at 500 ppm copper concentration was classified with an average of 3.715 ms/cm, and P429 at 500 ppm copper concentration with an average of 3.700 ms/cm. Following them, treatments P212 at 500 ppm, P212 at 400 ppm, P12 at 500 ppm, and R2 at 400 ppm were classified in the second homogeneous group (b) with averages ranging from 3.600 to 3.555 ms/cm. In Group (c), treatments R2 at 300 ppm, P429 at 400 ppm, and R2 at 200 ppm had averages of 3.500, 3.450, and 3.440 ms/cm, respectively. The treatments TNB at 100 ppm, P12 at 100 ppm, and R2 at 0 ppm were classified in the homogeneous group (j) with averages ranging from 3.100 to 3.055 ms/cm. R2 at 0 ppm, with an average of 2.900 ms/cm, was placed in Group (k). Finally, TNB at 0 ppm was classified in Group (l) (Figure 4).



The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; 300; 400; and 500 ppm Copper concentrations

Figure 4. Effect of the interaction between the bacterization factor and the concentration factor of the conductivity ms/cm

III. 1. 2. Plant growth-promoting activity of *Pseudomonas fluorescens spp.*on wheat (*Triticum aestivum*) in soil contaminated with copper.

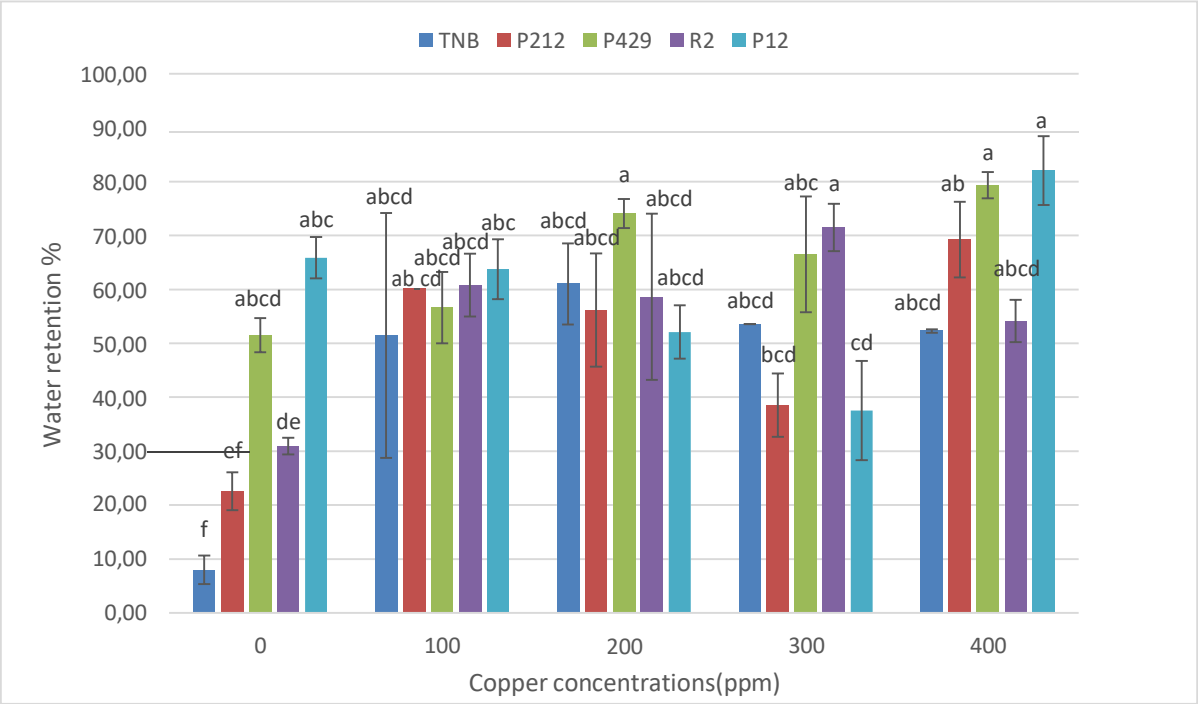
In the second part of our study, for both essays, we have analyzed only the parameters related to root growth and Relative water content (RWC), in addition for second essay we have added the sugar and total chlorophyll level.

III. 1. 2. 1 First essay: Plant growth-promoting activity of *Pseudomonas fluorescens spp.* On wheat (*Triticum aestivum*) in soil contaminated with copper *in vitro*

a. Water Retention on root *in vitro*

The analysis of variance revealed a highly significant difference for the interaction between the bacterization factor and the concentration factor (P=0.000). It was observed that treatments P12 at 400 ppm, P429 at 400 and 200 ppm, and R2 at 300 ppm of copper had the

highest average values ranging from 81.999% to 71.497% and were classified in group (a). Following this, the treatment P212 at 400 ppm of copper was placed in the intermediate group (ab) with an average around 69.216%. Treatments P429 at 300 ppm and P12 at 0 and 100 ppm were grouped under (abc) with averages ranging from 66.480% to 63.758%. Finally, TNB at 0 ppm of copper, with an average of 7.987%, was classified in group (f) (Figure 5).



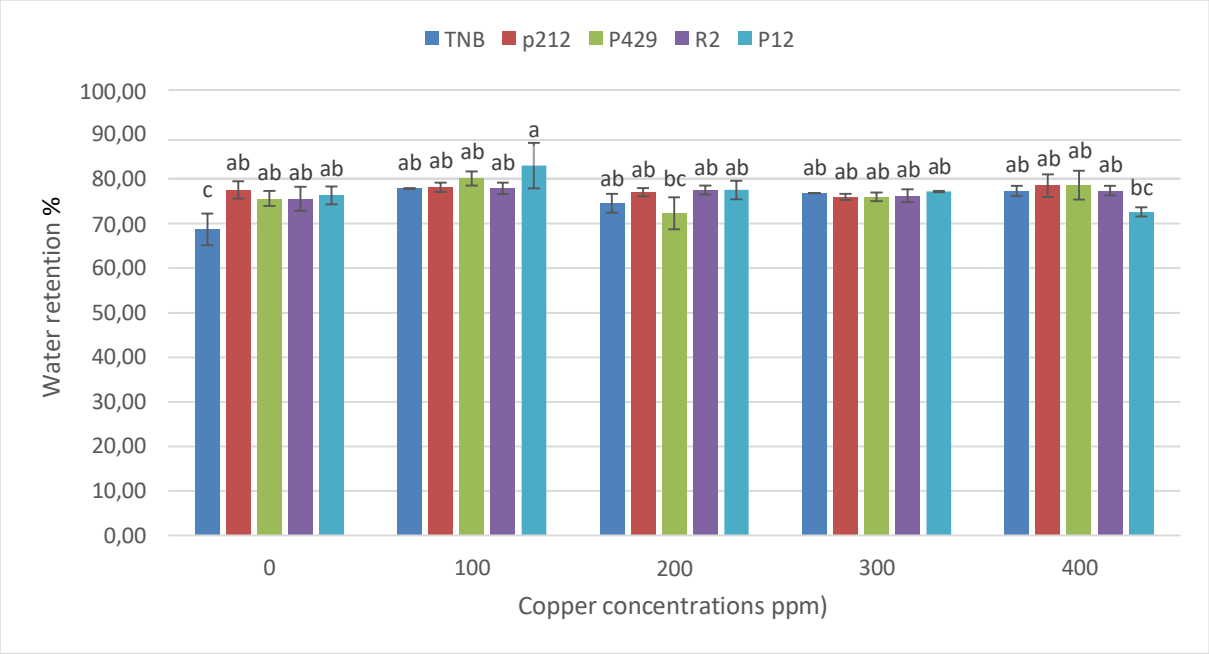
The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; 300 and 400 ppm Copper concentrations

Figure 5. Effects of the interaction between the bacterization factor and copper concentration on the water retention of roots

b. Water Retention on leaves *in vitro*

The analysis of variance revealed a highly significant difference for the interaction between the bacterization factor and the concentration factor (*P*=0.000). It was observed that treatment P12 at 100 ppm of copper had the highest average, around 83.151%, and was classified in group (a). Most of the other treatments were classified in the second intermediate group (ab) with averages ranging from 80.152% to 74.604%. Treatments P12 at 400 ppm and P429 at 200 ppm were grouped under (bc) with averages ranging from 72.643% to 72.306%.

Finally, TNB at 0 ppm of copper, with an average of 68.678%, was classified in group (c) (Figure 6).



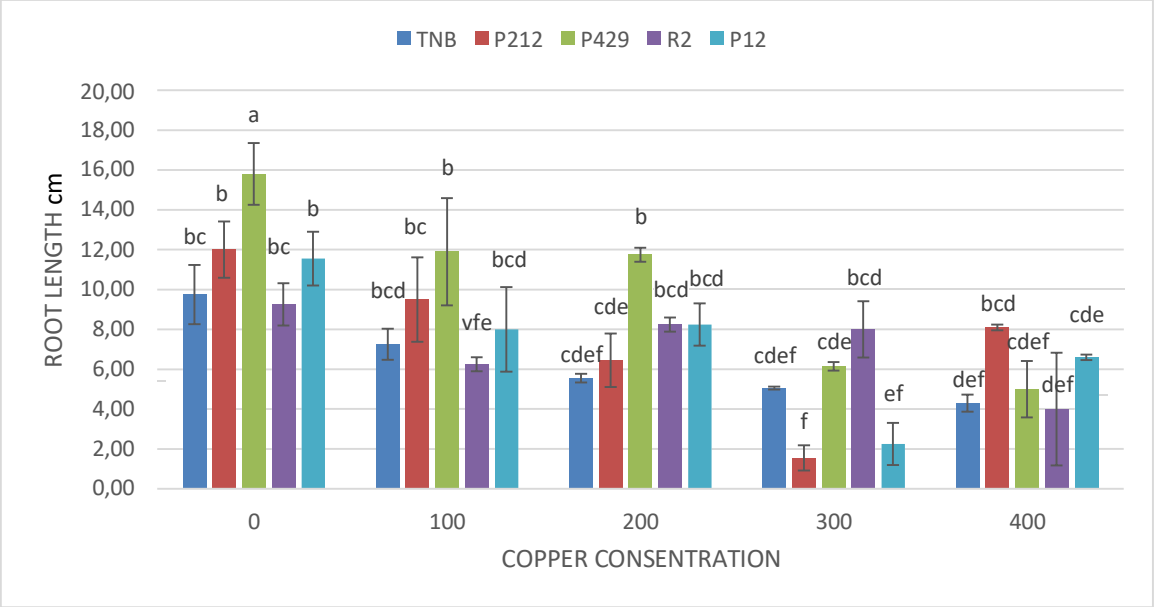
The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure 6 Effects of the interaction between the bacterization factor and copper concentration on the water retention of leaves

c. Effect on root length *in vitro*

The analysis of variance revealed a highly significant difference for the interaction between copper concentration and bacterization factor (*P*=0.000). The best treatment, P429 at 0ppm of copper, is classified in group (a) with an average of 15.800 cm. In the second group (b), we find treatments P212 at 0 ppm; P429 at 100 and 200 ppm; and P12 at 0 ppm of copper with an averages ranging from 12.000 to11.550 cm, treatments TNB at 0 ppm; P212 at 100 ppm; and R2 at 0 ppm; of copper with an averages ranging from 9.750 to 9.250 cm classified in group (bc). Following them, treatments P12 at 100 and 200 ppm; R2 at 200 and 300 ppm; P212 at 400ppm and TNB at 100ppm of copper is with averages ranging from 8.250 cm to

7.250 cm. They are subsequently classified in group (bcd). The shortest root length P12 at 300 ppm had a length of 2.250 cm and was classified in groups (ef) successively (Figure 7).

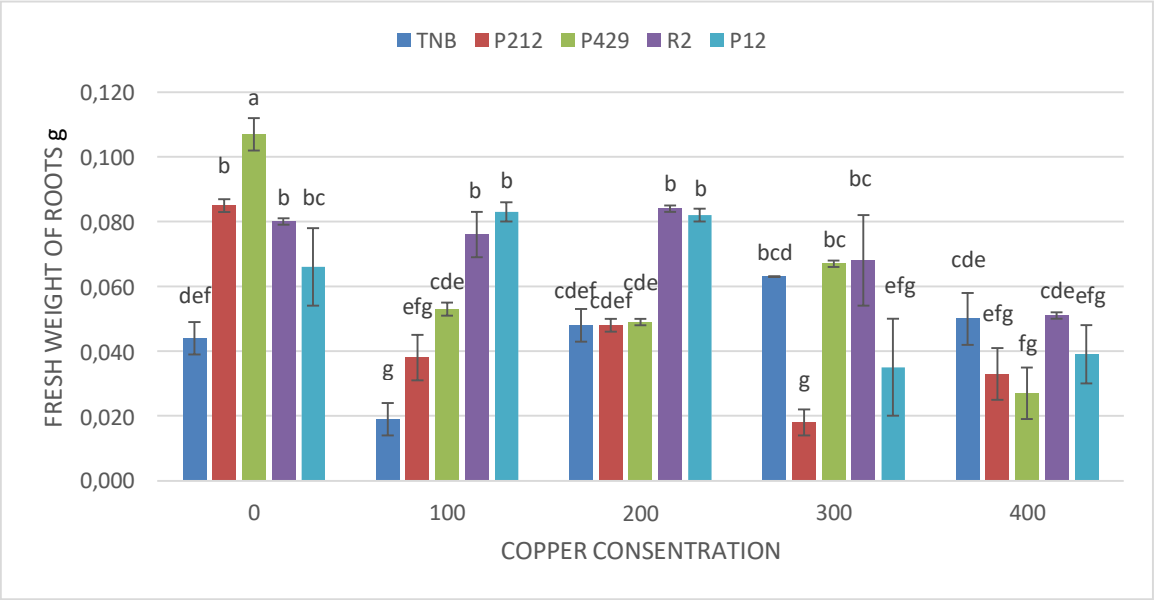


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp*.
TNB: Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure 7 Effects of the interaction between the bacterization factor and copper concentration on root length

d. Fresh weight of roots

The analysis of variance revealed a highly significant difference for the interaction between copper concentration and bacterization factor (P=0.000). The best-performing treatment, P429 at 0 ppm of copper, is classified in Group (a) with an average of 0.107 g. In the second group (b), we find treatments P212 at 100 ppm; R2 at 200, 0 and 100 ppm and P12 at 100 and 200 ppm of copper with average from 0.082 to 0.076 g. The treatments R2 at 300 ppm; P429 at 300 ppm and P12 at 0 ppm of copper, with an average from 0.068 to 0.066 g, is classified in group (bc). Following them, treatment TNB at 300 ppm of copper are classified in group (bcd) with an average of 0.063g. The lowest weight is recorded for the TNB treatment at 100 ppm and P212 at 300 ppm of copper, classified in Group (g) with an average of 0.019 g and 0.018g (Figure 8).

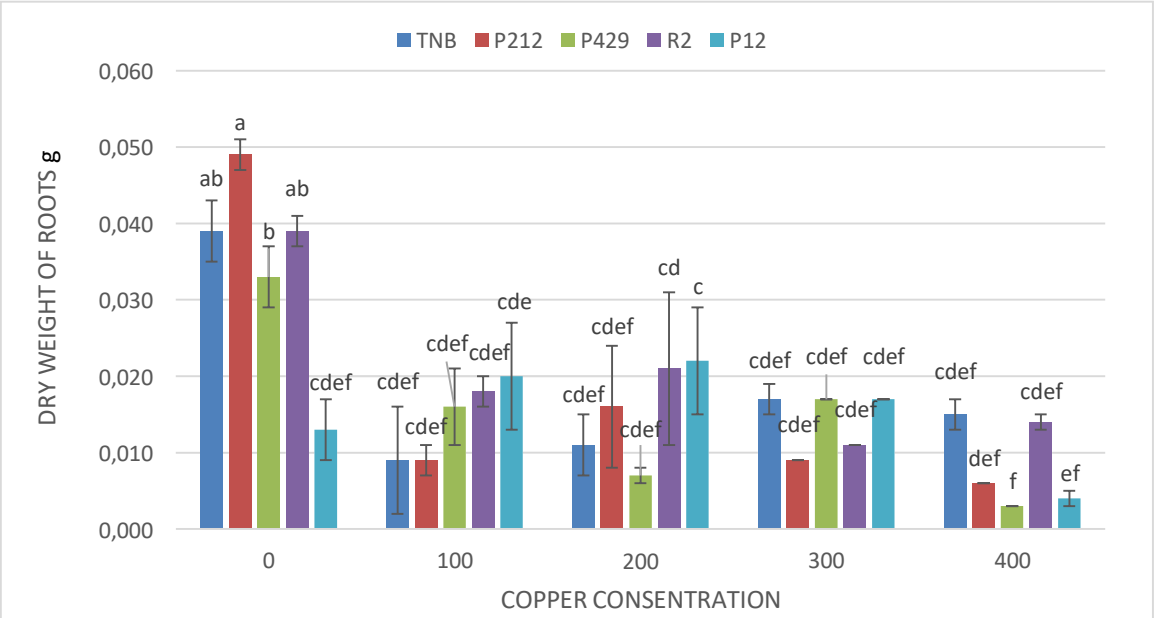


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
TNB: Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure 8. Effects of the interaction between the bacterization factor and copper concentration on fresh weight of roots

e. Dry weight of roots *in vitro*

For the dry weight of roots, the analysis of variance revealed an extremely significant difference (P=0.000) for the interaction between copper concentration and bacterization factor. The top-performing treatment, P212 at 0 ppm with an average of 0.049g, is classified in group (a). Following them in group (ab) is treatments R2 at 0 ppm and TNB at 0 ppm of copper with averages of 0.039 g. The treatment P429 at 0 ppm is classified in group (b) with an average of 0.033 g. Next, treatment P12 at 200 ppm of copper, with an average of 0.022 g, is classified in group (c). Lastly, treatment P12 at 400 ppm is classified in group (ef) with an average of 0.004 g (Figure 9).



The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
TNB: Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

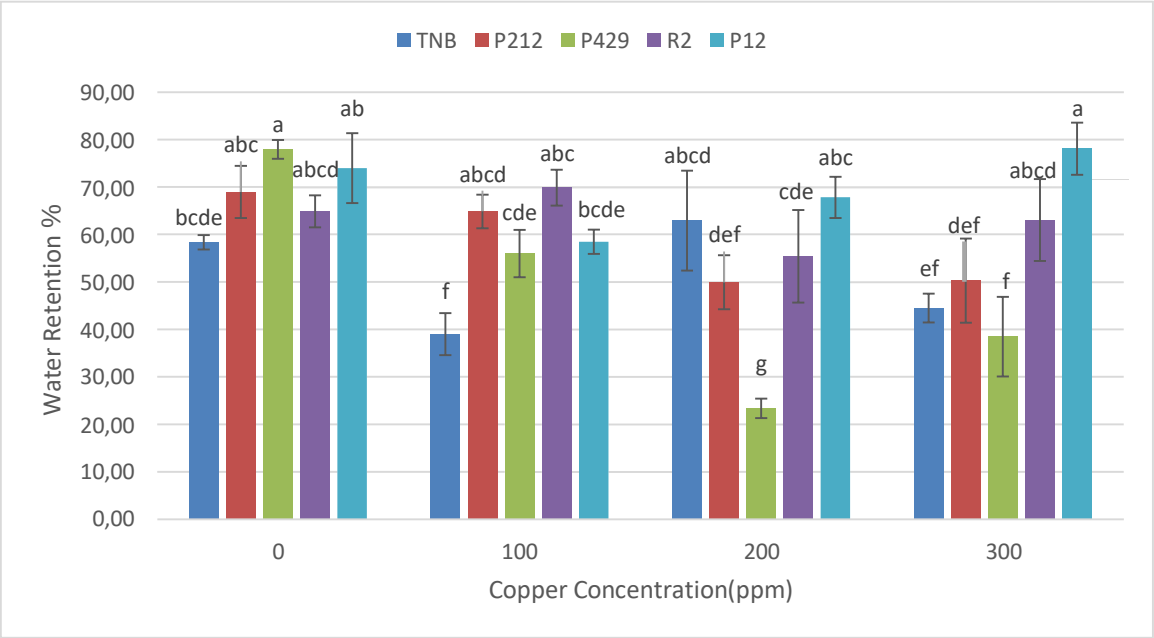
Figure 9. Effects of the interaction between the bacterization factor and copper concentration on dry weight of roots

III. 1. 2. 2 Second essay: Plant growth-promoting activity of *Pseudomonas fluorescens spp.* on wheat (*Triticum aestivum*) in soil contaminated with copper in pot.

In section of our work

a. Water Retention on root *in vivo*

The analysis of variance revealed a highly significant difference for the interaction between the bacterization factor and the concentration factor (P=0.000). It was observed that treatments P12 at 300 ppm and P429 at 0 ppm of copper had the highest averages, approximately 78.080% and 77.946%, respectively, and were classified in group (a). Following that, treatment P12 at 0 ppm of copper was placed in the intermediate group (ab) with an average around 74.007%. Treatments R2 at 100 ppm, P212 at 0 ppm, and P12 at 200 ppm of copper were grouped under (abc) with averages ranging from 69.886% to 67.825%. Finally, TNB at 100 ppm and P429 at 300 ppm of copper, with averages around 39.033% and 38.508%, respectively, were classified in group (f) (Figure10).

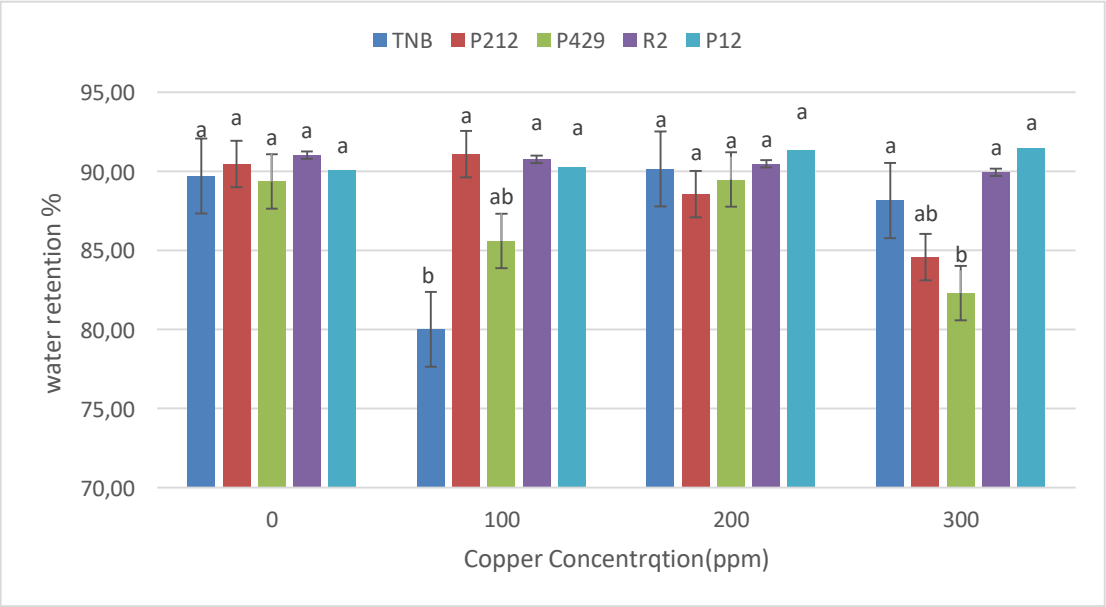


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
TNB: Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure10. Effects of the interaction between the bacterization factor and copper concentration on the water retention of roots

b. Water Retention on leaves

The analysis of variance revealed a highly significant difference for the interaction between the bacterization factor and the concentration factor (P=0.000). It was observed that most of the treatments provided the best average, ranging from 91.492% to 88.143%, and were classified in group (a). Following that, treatment P429 at 100 ppm and P12 at 300 ppm of copper were placed in the intermediate group (ab) with averages around 85.583% and 84.558%, respectively. Finally, P429 at 300 ppm and P212 at 100 ppm of copper, with averages approximately 82.284% and 80.005%, respectively, were classified in group (b) (Figure 11).

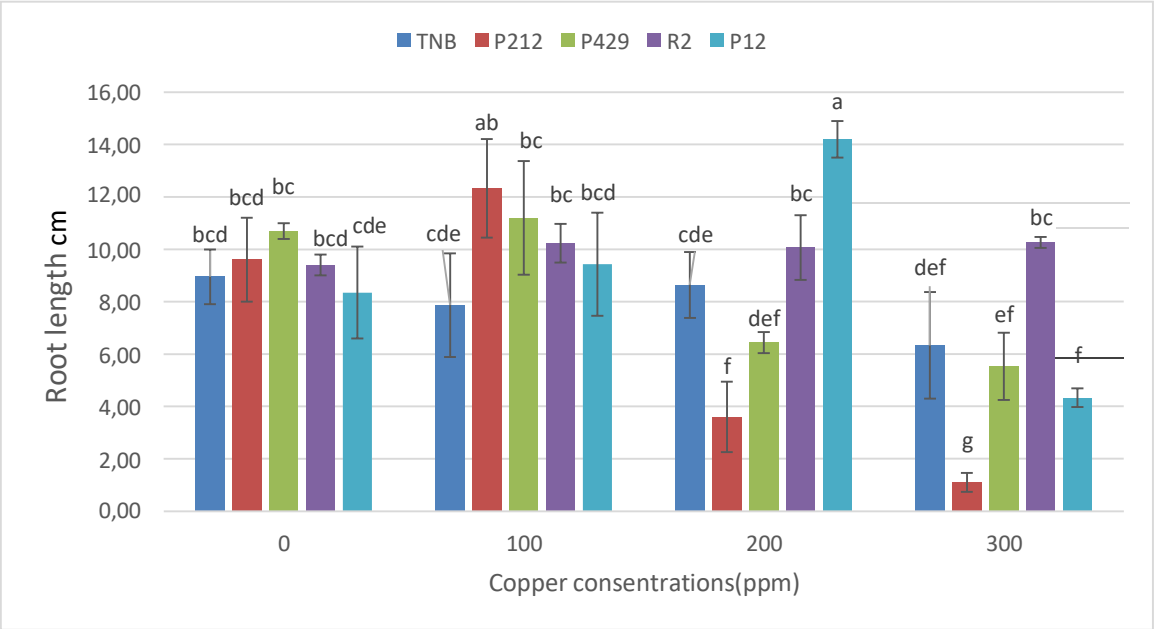


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
TNB: Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure11. Effects of the interaction between the bacterization factor and copper concentration on the water retention of leaves

c. Effect on root length

The analysis of variance revealed a highly significant difference for the interaction between copper concentration and bacterization factor (P=0.000). The best treatment, P12 at 200 ppm of copper, is classified in group (a) with an average of 14.200 cm. In the second group (ab), we find treatment P212 at 200 ppm of copper with an average of 12.333 cm. Following them, treatments P429 at 100 and 0 ppm, and R2 at 300, 100, and 200 ppm of copper are classified in Group (b c) with averages ranging from 11.200 cm to 10.067 cm. They are subsequently classified in group (bcd). The shortest root lengths were recorded at 300 ppm and 200 ppm of copper for P12 and P212, measuring 4.333 cm and 3.600 cm, respectively. P212 at 300 ppm had a length of 1.100 cm and was classified in groups (f) and (g) successively (Figure12).

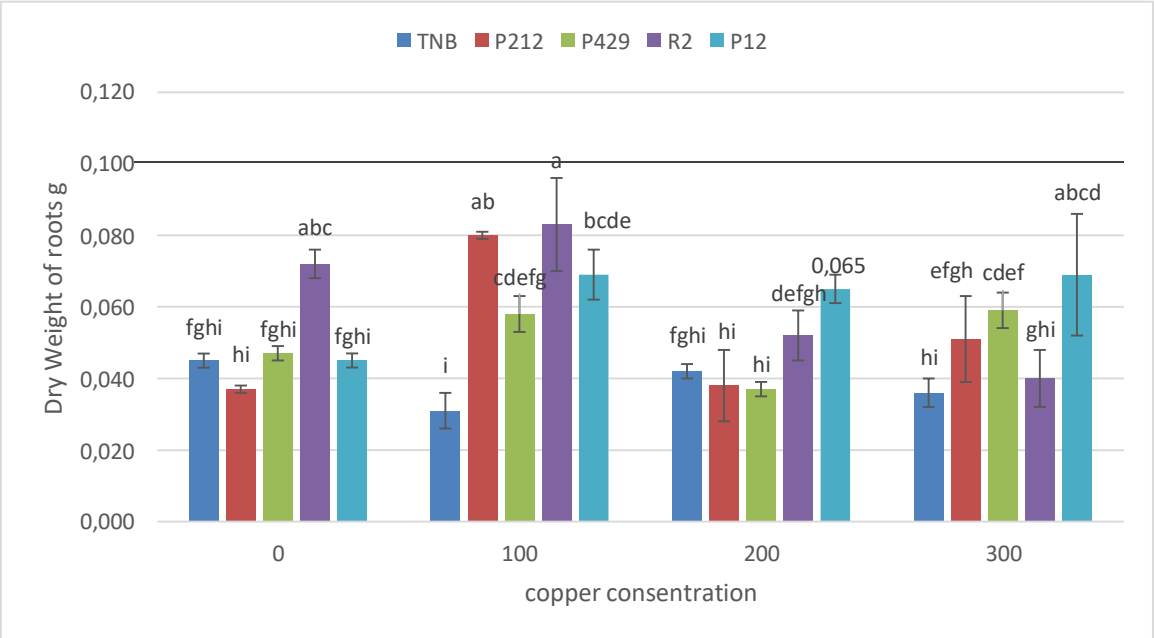


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure12. Effects of the interaction between the bacterization factor and copper concentration on root length.

d. Fresh weight of roots

The analysis of variance revealed a highly significant difference for the interaction between copper concentration and bacterization factor (P=0.000). The best-performing treatment, R2 at 100 ppm of copper, is classified in group (a) with an average of 0.083 g. In the second group (ab), we find treatment P212 at 100 ppm of copper with an average of 0.080 g. The treatment R2 at 0 ppm of copper, with an average of 0.072 g, is classified in group (abc). Following them, treatments P12 at 100 and 300 ppm of copper are classified in group (abcd) with an average of 0.069 g. The lowest weight is recorded for the TNB treatment at 200 ppm of copper, classified in group (i) with an average of 0.031 g **(Figure13)**.

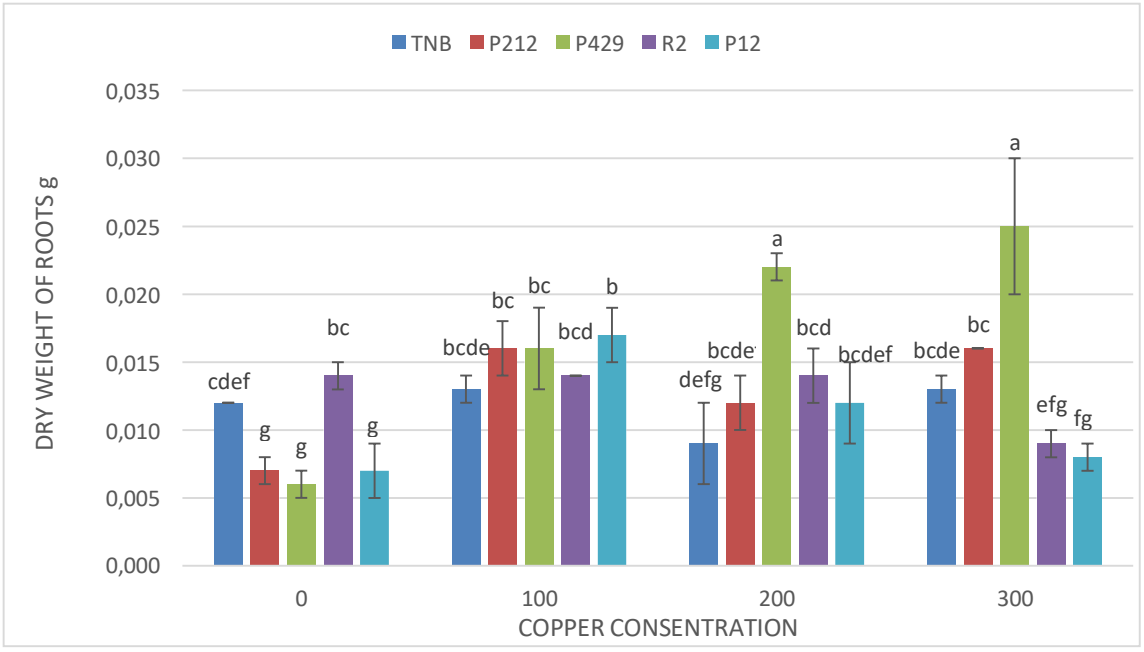


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of fluorescent Pseudomonas spp.
Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure13. Effects of the interaction between the bacterization factor and copper concentration on the fresh weight of roots

e. Dry weight of roots *in vivo*

For the dry weight of roots, the analysis of variance revealed an extremely significant difference (P=0.000) for the interaction between copper concentration and bacterization factor. The top-performing treatments, P429 at 300 and 200 ppm, with averages of 0.025 and 0.022 g, are classified in group (a). Following them in group (b) is treatment P12 at 100 ppm of copper with an average of 0.017 g. The treatments P212 at 100 ppm, P429 at 100ppm, and P212 at 300ppm are classified in group (bc) with an average of 0.016 g. Next, treatment R2 at 200 and 100ppm of copper, with an average of 0.014 g, is classified in group (bcd). Afterward, the treatment TNB at 300 and 100 ppm, with an average of 0.013, is classified in group (bcde).P212 and P12 at 200 PPM of copper, with an average of 0.012 g, are classified in group (bcdef). TNB at 0ppm of copper, with an average of 0.012 g, is classified in group (cdef). Lastly, treatment P12 at 300ppm is classified in group (fg) with an average of 0.008 g. **(Figure14)**

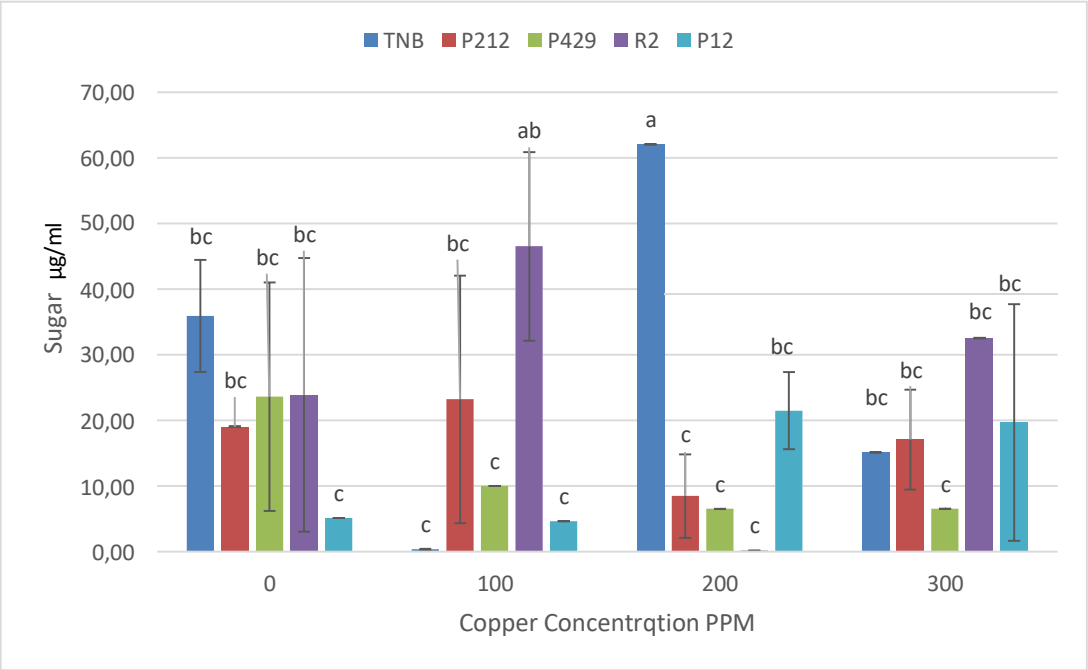


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure14. Effects of the interaction between the bacterization factor and copper concentration on the dry weight of roots

f. Sugar level in leaves

The analysis of variance revealed a highly significant difference for the interaction between the bacterial factor and the concentration factor (P=0.000). It was observed that the TNB treatment at 200ppm of copper yielded the highest average, approximately 62.051, and was classified in group (a). This was followed by the R2 treatment at 100ppm of copper, which was placed in the intermediate group (ab) with an average around 46.510. The treatments TNB at 0 and 100ppm; R2 at 300 and 0ppm; P429 at 0ppm; P212 at 100, 0, and 300ppm; and P12 at 200 and 300ppm of copper were classified in group (bc) with averages ranging from approximately 35.897 to 15.119. Finally, P429 at 100, 300, and 200ppm; P212 at 200ppm; P12 at 0 and 100ppm; TNB at 100ppm; and R2 at 200ppm of copper, with averages ranging from approximately 10.000 to 0.128, were classified in group (c) (Figure 15).

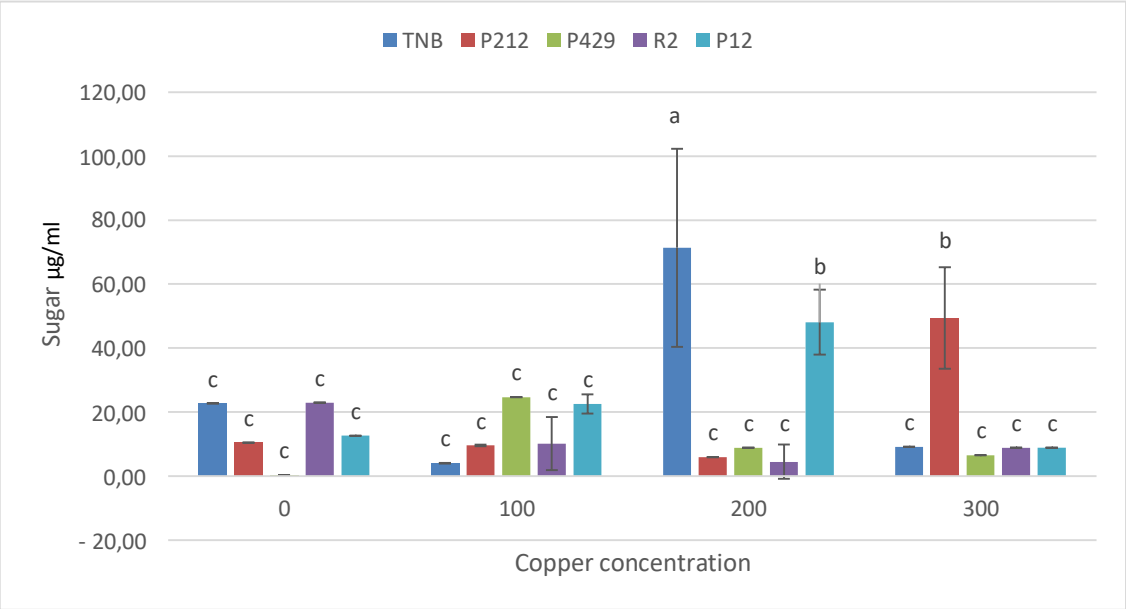


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure15. Effects of the interaction between the bacterization factor and copper concentration on the sugar in leaves

a. Sugar level in roots

The analysis of variance revealed a highly significant difference for the interaction between the bacterial factor and the concentration factor (*P*=0.000). It was observed that the TNB treatment at 200 ppm of copper yielded the highest average, approximately 71.314, and was classified in group (a). This was followed by the P212 treatment at 300 ppm and P12 treatment at 200ppm of copper, which were placed in the intermediate group (b) with averages ranging from approximately 49.423 to 48.077. Finally, the others were classified in the last group (c) with averages ranging from approximately 24.615 to 0.256 (Figure 16).

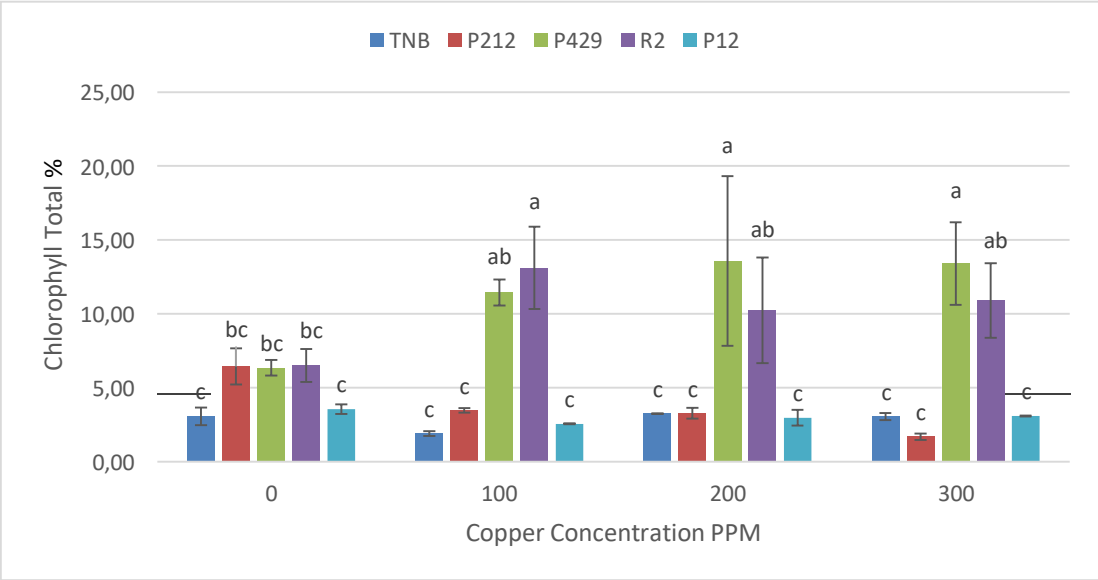


The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure16. Effects of the interaction between the bacterization factor and copper concentration on the sugar in root

b. Total Chlorophyll level

The analysis of variance revealed a highly significant difference for the interaction between the bacterial factor and the concentration factor (*P*=0.000). It was noted that the treatment P429 at 200 and 300ppm and R2 at 100ppm of copper yielded the highest average, ranging from approximately 13.575 to 13.106, and were classified in group (a). This was followed by the treatment P429 at 100ppm and R2 at 300 and 200ppm of copper, which were placed in the intermediate group (ab) with averages ranging from approximately 11.446 to 10.241. The treatments R2 at 0ppm, P212 at 0ppm, and P429 at 0ppm of copper were classified in the group (bc) with averages ranging from approximately 6.512 to 6.348. Finally, the others were classified in the last group (c) with averages ranging from approximately 3.542 to 1.678 (Figure 17).



The values followed by the same letter belong to the same homogeneous group,
according to the Newman-Keuls test (α = 5%).
P12, P212, P249, R2: Strains of *Pseudomonas fluorescens spp.*
Non-bacterized treatment (control)
0; 100; 200; and 300 ppm Copper concentrations

Figure 17. Effects of the interaction between the bacterization factor and copper concentration on the chlorophyll

III. 2. Discussion

The results obtained in the test tolerance, the Minimum Inhibitory Concentration (MIC) for living organisms were determined at concentrations ranging from 300 to 500 ppm. The MIC values indicate that the strains of *Pseudomonas fluorescents*. have shown significant resistance to copper sulfate, with these bacteria tolerating copper sulfate concentrations up to 500 ppm. However, the actual MIC value for the studied isolates is approximately 600 ppm. Other studies have shown that *Pseudomonas putida* CZ1 recorded an MIC value of 1 mM for copper (Chen et al., 2006). According to Virender et al. (2010), the copper MIC for the tested Pseudomonas strains falls between 180 and 300 µg/ml. This same value was also observed by Rajbhansi (2008).

In our findings, the soil pH ranged from 7.1 to 7.8, which aligns with the results observed by Abou-Shanab et al. (2008). They reported slightly acidic soil pH (6.7) in tannery-effluent-polluted soils and alkaline soil pH (7.2) in soils collected from the Tourney smelter

site. It's worth noting that soil pH plays a pivotal role as one of the key factors influencing the transformation of metals from relatively immobile solid forms to more mobile and potentially bioavailable forms in the soil solution (Tessier and Campbell, 1988; Chlopecka et al., 1996).

Richards et al. (1983) previously reported the heightened toxicity of pollutants at lower soil pH levels. Their study demonstrated that maintaining a soil pH above 7 led to a reduction in the cadmium (Cd) content of herbage compared to plants grown in soil with a pH of 5. Consequently, gaining a deeper insight into the interactions between elements such as aluminum (Al) and copper (Cu) is essential (Guo et al., 2007).

Copper doses also had a quadratic effect on dry mass and root volume. The maximum values were observed at 300 ppm of copper, resulting in 0.025 g of dry mass. Lower copper levels tended to increase the number of secondary roots and dry root mass. However, excessive copper concentrations had a toxic effect, leading to a linear decline in root volume and dry mass (Rossi et al., 2008), limiting the plant's ability to absorb water and nutrients necessary for growth and development (Taiz and Zeiger, 2017).

The highest root parameters (length fresh dry weigh) were recorded in our first essay at bacterized treatment, also we have recorded that they decrease in soil contained with Cu.

Optimum concentration of Cu in soil ensures normal growth and development of plant. However, slightly higher than normal concentration of Cu in soil is toxic for crops which in-turns adversely affects the plant growth by influencing biochemical and physiological processes such as respiration, photosynthesis, nutrients uptake, DNA and membrane integrity and stability (Ovečka and Takáč, 2014; Qin et al. 2015)

About 88% of the copper accumulates in the roots, resulting in minimal copper translocation to the shoots. As a result, factors such as chlorophyll content, net photosynthesis rate, carbon assimilation, dry biomass, root system development, and nodulation remained unaffected by the presence of copper (Da Silva et al., 2022).

According to Mirjankar et al (2023), exposure to copper (Cu) led to a significant reduction in leaf chlorophyll content, decreasing it from the standard total chlorophyll level of 300 to 0.25. This reduction resulted from the inhibition of photosynthetic pigment biosynthesis in the leaves. There are works that report that inoculation of stressed plants with plant growth–promoting microorganisms, e.g., *Rhizobium sp., Bacillus subtilis,* and *Pseudomonas fluorescents*., resulted in an increase in chlorophyll and carotenoid content (Wani and Khan 2013;

2015). Bacterized treatment noted total chlorophyll level more than non-bacterized treatment(control), at soil contaminated with Cu, P429 and R2 recorded highest total chlorophyll level. As shown by Upadhyay et al. (2012), wheat inoculation increases the level of dry biomass, total soluble sugar, and proline content.

The water content in the contaminated samples decreased and exhibited distinct decomposition patterns compared to the control group. Unlike the control plants, those subjected to Cu stress exhibited unique weight loss characteristics. These observed variations can be attributed to the restructuring processes induced by Cu stress at the cell wall level, which in turn affected water distribution and plant interactions (Mirjankar et al (2023), in our study water content of root in Bacterized treatment are higher than the control for both essay

The study results indicate no significant interaction among various plant parameters in response to varying copper doses. Instead, these parameters exhibited individual responses. Copper doses had a notable impact on wheat plant height, showing a positive quadratic effect with a maximum height of 53,3 cm achieved at 300ppm copper concentration. Despite being required in small quantities, micronutrients like copper play a crucial role in plant growth, and their deficiency can reduce crop yields (Malavolta, 2006). However, elevated copper levels in the soil can hinder plant growth, as reported by Zortéa et al. (2016), indicating the potential for phytotoxicity.

Besides that, lack of mobility and solubility of such metals additionally synergized the toxic effects on plant growth. Cu toxicity symptoms in plants appear as chlorosis and necrosis in leaves as well as abnormal root morphology, all of which results into retard growth and development (Adrees et al., 2015) Also Cu stress causes photosynthetic inhibition in tomatoes, which decreases the percentage of transpiration and stomatal conductance (Wang et al., 2015). In our case we noticed a chlorosis of the edges of leaves

Moreover, increasing copper doses led to a linear reduction in root length, with a 23% decrease compared to the control (zero dose) when 400 mg of copper per kg of soil was applied. High copper concentrations are known to be toxic to plants and can hinder root development (Amarante and Fleig, 2008). The root system's role in plant productivity, including water and nutrient absorption efficiency, especially for phosphorus (P), is crucial (Reinert et al., 2008; Pimentel, 2016).

Among rhizosphere microorganisms, plant growth-promoting rhizobacteria (PGPR) hold significance for their role in enhancing phytoremediation. They influence metal bioavailability through mechanisms like pH modification, chelator secretion, phytohormone production, and more (Ma et al., 2011).

Pseudomonas fluorescents. has been explored as a PGPR by several authors. Gomez et al. (2019) observed significant improvements in banana plants (Musa acuminata) when these bacteria were introduced into the rhizosphere. Additionally, these strains have shown effectiveness in enhancing plant growth in soils with high salinity levels (Ullal et al., 2015). Field trials by Sivasakthi et al. (2014) demonstrated yield improvements of up to 44% due to their rapid root colonization ability. Furthermore, *Pseudomonas fluorescents*. tolerance to high concentrations of mercury (Hg) has been investigated, highlighting its potential for bioremediation through biosorption and biotransformation of this heavy metal (MacLean et al., 2020; Wang et al., 2017). Sandhya et al. (2010) also observed that in PGPR-inoculated plants there is an increase in biomass, relative water content, leaf water potential, and mean stem diameter and a higher level of proline, sugars, and free amino acids. Such plant beneficial bacteria species can increase metal tolerance in plant such as *Pseudomonas putida* which their abilities to alleviate the abiotic stress (Armada et al. 2015; Ortiz et al. 2015). *P. putida* effectively enhanced the shoot length and fresh weight of soybean plants suffered at salt and drought stress, *P. putida* application reprograms the chlorophyll, stress hormones, and antioxidants expression in abiotic stress affected soybean plant and improves their growth under stress environment (Kang et al., 2014).

Bacteria can influence the mobility and bioavailability of trace elements through various mechanisms, including the release of chelating agents such as organic acids, phenolic compounds, and siderophores. They can also induce changes in soil pH and redox conditions in the rhizosphere (Lloyd, 2003; Glick, 2010). Sessitsch et al. (2013) reviewed potential mechanisms through which microbes can impact bioavailability in the rhizosphere environment. Trace elements that are sorbed, precipitated, or occluded in the soil can be solubilized through acidification, chelation, and ligand-induced dissolution.

Microbial biomass provides a metal sink, by biosorption to cell walls, pigments and extracellular polysaccharides, intracellular accumulation, or precipitation of metal com-pounds in and/or around cells, hyphae or other structures (Gadd, 2007; Baldrian, 2003; Fomina et al., 2007)

Metal interactions with specific cell-surface groups may also enhance or inhibit metal transport, metal transformations and biomineralization processes (Barkay & Schaefer, 2001) HCN-forming bacteria, such *Pseudomonas fluorescents*. , can mobilize Ni, Au, Pt and Cu as various cyanide complexes and compounds from solid materials such as copper-containing ores, electronic scrap, and spent auto-mobile catalytic converters (Brandl and Faramarzi, 2006)

Siderophores, for instance, can form high-affinity complexes with Fe(III) and, to varying degrees, with other trace elements such as Al, Cd, Cu, Ga, In, Ni, Pb, and Zn. This complex formation affects the bioavailability of these elements (Schalk et al., 2011; Rajkumar et al., 2012; Sessitsch et al., 2013). Inoculating soils with siderophore-producing bacteria, such as *P. aeruginosa*, has been shown to significantly increase the concentrations of bioavailable Cr and Pb, with enhanced heavy metal uptake correlating with increased siderophore production (Braud et al., 2009).

Modern agriculture faces challenges in maintaining productivity while minimizing synthetic fertilizer and pesticide use. Soil inoculation with plant-growth-promoting rhizobacteria (PGPR), such as *Pseudomonas* spp., offers an eco-friendly alternative. *Pseudomonas* spp. play a vital role in sustainable agriculture by controlling plant pathogens, fixing atmospheric nitrogen, solubilizing nutrients, and producing beneficial compounds. They enhance plant growth, induce systemic resistance, and protect plants during various stress conditions. While there are challenges and variability among Pseudomonas spp., exploring their potential as biocontrol agents and biopesticides supports sustainable agriculture.

In the realm of environmental challenges, bioremediation emerges as a sustainable solution for copper-contaminated soil. Recent research, exemplified by studies like 'Bioremediation of Copper-Contaminated Soils Using *Pseudomonas fluorescents*. : A Contemporary Approach' (Smith et al., 2022), underscores the pivotal role of *Pseudomonas fluorescents*. These bacteria, armed with copper tolerance mechanisms, are effective agents against copper-induced soil pollution. Their ability to extrude copper, bind metals, and immobilize them revolutionizes the field. Bioremediation's eco-friendly nature, as highlighted in 'Sustainable Approaches in Environmental Bioremediation' (Johnson and Brown, 2021), makes it an attractive choice. Challenges remain, such as optimizing bacterial strains and accounting for varying soil conditions, but ongoing research and practical applications of *Pseudomonas fluorescents*. offer hope for restoring contaminated soils and ensuring a healthier environment.

Conclusion

Conclusion

In conclusion, this study represents a significant contribution to the field of bioremediation, particularly in the context of addressing soil pollution resulting from copper contamination through the utilization of *Pseudomonas fluorescents*. Our research has unveiled several critical findings and knowledge enhancements. Firstly, we have demonstrated the remarkable copper tolerance exhibited by various *Pseudomonas fluorescents*. strains, including P12, P212, P429, and R2, with some strains showcasing resistance to copper concentrations of up to 700 ppm. This insight is pivotal for selecting appropriate strains in copper-contaminated soil bioremediation.

Additionally, our study underscores the vital role of rhizosphere microorganisms, especially plant growth-promoting rhizobacteria (PGPR) like *Pseudomonas fluorescents.* , in augmenting phytoremediation processes through mechanisms such as pH modulation, chelator secretion, and phytohormone production. Overall, our study emphasizes the environmentally friendly and sustainable nature of bioremediation as an effective approach to address soil contamination, offering promising solutions for soil restoration, ecosystem preservation, and a healthier environment.

In summary, our research underscores the significance of *Pseudomonas fluorescents*.as valuable tools in the bioremediation of copper-contaminated soils, contributing to the optimization of their practical application and advancing strategies for effective and sustainable soil remediation, ultimately aiding in the global efforts to fight soil pollution and its adverse impacts on the environment and human health.

Thus, suggesting a possible future on bioremediation of copper-contaminated soils using *Pseudomonas fluorescents*. , we made several valuable contributions. However, there are some aspects and potential areas for further research and knowledge enhancement that was not fully addressed in our study.

These include the need for genetic and molecular analysis to uncover the specific genes responsible for copper resistance, investigating the ecological impacts of introducing these microorganisms into soils, assessing the long-term effects and sustainability of the bioremediation approach, transitioning from controlled experiments to real-world field applications, evaluating potential risks associated with genetically modified strains, addressing scenarios of multiple contaminants, optimizing the bioremediation process and assessing

economic viability, and considering regulatory and policy implications. These unexplored dimensions offer opportunities for future research, contributing to a more comprehensive understanding of the technology's potential and its broader implications for environmental restoration and sustainability.

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