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Title of the Paper- Enhancing *Vigna radiata* Growth and Reducing Metal Toxicity with *Pseudomonas* spp. in Hydroponic Systems

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Abstract

Heavy metals in the environment originate from both natural sources and human activity, such as industrial waste and mining. Metals like cadmium, lead, and mercury accumulate in soil, water, and air, which can result in substantial threats to plants and ecosystems. Overexposure to heavy metals in plants leads to regeneration of reactive oxygen species (ROS), causing oxidative stress and disrupting many metabolic pathways, eventually impacting plant growth and productivity. Microbial bioremediation is an approach used to address this issue. This study investigates the effectiveness of the *Pseudomonas* family, specifically *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, in reducing heavy metal toxicity in non-circulating hydroponic systems. The objective is to observe the impact of zinc and cadmium stress on the growth of *Vigna radiata* (mung bean) seedlings. The results indicate that *Pseudomonas aeruginosa* promotes the development of seedlings up to a concentration of 1000 parts per million (ppm) of zinc, as well as cadmium. *Pseudomonas fluorescens*, on the other hand, supports plant growth under cadmium stress of up to 300 ppm and zinc stress of up to 1000 ppm. The results demonstrate the potential of the *Pseudomonas* family, which can serve as an effective method for reducing the presence of heavy metals in soil. Consequently, these bacteria could potentially serve as biofertilizers in heavy metal-contaminated regions, enhancing production while also mitigating the presence of heavy metals in the soil.

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Introduction

The rapid escalation of industrialization and swift economic progress in numerous sectors, including agriculture and industry, has led to increased environmental pollution. This is primarily due to the discharge of diverse forms of toxic effluents into the surrounding areas, thereby causing significant harm to the environment.[1,2] Over the course of the past century, there has been a rapid expansion of industrialization. A surge in demand for the utilization of the Earth's natural resources at an imprudent pace has led to unsustainable exploitation. Consequently, various pollutants, including organic pollutants, radioactive isotopes, gaseous pollutants, inorganic ions, heavy metals, and organometallic compounds, have significantly contaminated the environment.[3,4]

Among these pollutants, heavy metals constitute a substantial threat as pollutants due to their inherent toxicity, persistence, and inability to degrade naturally. Moreover, their tendency to accumulate in the ecosystem and food chain has profound implications for human health and the overall well-being of the ecosystem.[5] Due to urbanization and industrialization surrounding farmland, heavy metal pollution in agricultural land has increased at a speedy rate during the previous few decades making this an urgent issue.[6-8] Heavy metals are toxic and dense metallic chemical components. Lead, mercury, arsenic, zinc, cadmium, and chromium are a few heavy metals. The primary source of heavy metal pollution may be attributed to human activities, such as smelting, mining, metalworking facilities, and other metal-based sectors.[9] Additionally, heavy metals can leach into the surroundings via landfills, disposal facilities, urine, animal, and poultry waste, as well as runoffs originating from automobiles and road construction. Pesticides, insecticides, fertilisers, and other agricultural chemicals have further contributed to secondary heavy metal contamination.[10]

To date, a considerable body of research has been dedicated to investigating the potential hazards and sources of heavy metal pollution globally. Typically, the release of heavy metal pollutants into the environment stems from both human activities and natural processes. These sources encompass many anthropogenic factors such as agricultural and industrial activity, car emissions, as well as natural phenomena such as air precipitation and rock erosion. The industries emit heavy metals by-products, which then accumulate in nearby crop fields and water bodies. This accumulation adversely affects plant development by interfering with the availability of essential nutrients in the soil. Once absorbed by plants, these substances become integrated into food webs, exerting detrimental effects on both humans and animals. The presence of an excessive amount of heavy metals in soil results in competition with other metal ions, leading to a deficiency of essential ions in plants. These heavy metal ions bind to proteins and other targets, resulting in an increase in reactive oxygen species (ROS). Consequently, the malfunctioning of proteins and enzymes occurs, leading to the inhibition of key processes like photosynthesis and respiration. This disruption in metabolic processes induces oxidative stress in plants, causing alterations in cell membrane integrity, DNA damage, protein oxidation, and ultimately, cell death. As a result, plant growth is inhibited, leading to a reduction in crop yield.[11] Figure 1 illustrates the detrimental effects of heavy metal exposure on plant organisms.

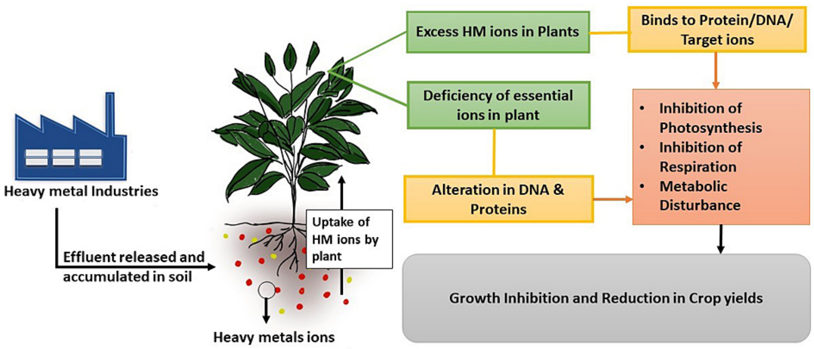

Fig. 1: Effect of heavy metals exposure on plant

The widespread deposition of heavy metals in soils, particularly near aquatic habitats has been studied extensively. Concentrations of Copper (Cu), lead (Pb), cadmium (Cd), zinc (Zn), and chromium (Cr) were found to be significantly higher in the Water-level-fluctuation zone (WFLZ) compared to nearby bank soils.[12] In another study, the presence of heavy metal toxicity in sheep grazing near a smelting facility has been identified. The research investigation revealed that sheep who consumed plants near the smelter had significantly elevated levels of Cadmium (Cd) and Lead (Pb) concentrations, above the fatal threshold for fatality.[13]

This study focuses on the effects of all heavy metals Zinc and Cadmium toxicity on plants' health. Long-term fertilizer usage contributes to heavy metals accumulation in agricultural soils, which in turn reduces soil fertility and hampers plant growth. Heavy metal contamination makes soil recovery difficult. Metals such as Cu, Zn, and Cd accumulate more in agricultural soil owing to prolonged fertilizer usage.[14,15] In previous research, both zinc and cadmium stress affected mung seedlings *in vitro.* In the water agar seedling symptom test, cadmium had the greatest impact on seedlings, followed by zinc. During metabolic analysis, photochemical contents increased at low metal concentration but dropped sharply at high concentrations.[16] Excessive Zinc exposure caused inward-rolled leaf margins, damaged and brownish root systems, and reduced lateral root growth. High Zn levels disrupt nutrient uptake, decreasing Nitrogen, Magnesium, Potassium, and Manganese levels in plant tissue while increasing Phosphorous and Calcium in shoots.[17] Zinc although essential for plant growth, becomes harmful when accumulated in high concentrations. After Fe, Zn is the second most abundant transition metal in organisms and strongly affects all enzymatic functions. Roots carry Zn^{2+} from the soil to the plant. Zinc is essential for photosynthetic redox processes. Zn accumulation in roots or shoots hurts plants severely. Plant cells with too much Zn experience extreme physiological turbulence, and eventually, the plant dies.[18,19] Whereas, Cd (Cadmium), a non-essential and hazardous heavy metal, threatens ecosystems due to human activity. Due to mobility, bioavailability, and concentration, plant roots absorb most Cd ions, while the rest can be obtained from the environment. Ca (Calcium) channels and other transporters let Cd into plant cells and accumulate in roots, shoots, and edible parts. Plants with high Cd levels alter physiologically and biochemically. Mineral transport, photosynthetic system, and nutritional absorption are harmed by Cd accumulation in plants. It also prevents Fe (Iron) transfer to plant shoots. The adverse effects of Cadmium influences plant metabolism, cytotoxicity, and phenotype.[20-23] Therefore, the need for remediation of heavy metals arises due to their potential harmful effects on both human health and the environment. The remediation efforts are crucial to reduce the exposure and prevent further contamination of heavy metals to safeguard ecosystems.

There are many conventional methods of degrading heavy metals but there is a need of bioremediation instead of conventional method of degrading heavy metals because bioremediation offers a more sustainable and environmentally friendly approach. Unlike conventional methods that often involve the use of harsh chemicals or physical removal,[24] bioremediation utilizes natural processes and microorganisms to degrade or immobilize heavy metals from contaminated sites. This not only reduces the use of harmful substances but also promotes the restoration and regeneration of ecosystems.[25] Bacterial remediation, also known as bioremediation, is gaining recognition as a sustainable approach for heavy metal remediation. This method utilizes the natural abilities of certain bacteria to degrade or transform heavy metals into less toxic forms, reducing their environmental impact. There are various bacteria that aid in heavy metal bioremediation such as *Pseudomonas*, *Bacillus*, *Rhizobium* species etc.[26,27] These bacteria possess unique genetic traits that enable them to metabolize and detoxify heavy metals such as lead, cadmium, and mercury. Additionally, they can also improve soil fertility by fixing nitrogen and enhancing nutrient availability for plants.

Pseudomonas spp., a versatile bacterium, detoxifies heavy metals and promotes plant development. These bacteria secrete metal-chelating chemicals and enzymes that bind and neutralize heavy metals from the environment, reducing their hazardous effects on plants and other species. Moreover, by generating phytohormones, these bacteria boost plant growth and nutrient absorption.[28] *Pseudomonas aeruginosa*, *Pseudoalcaligenes*, and *fluorescens* have been found to reduce metal toxicity in various ways. These bacteria use biochemical, genetic, and physiological methods to mitigate heavy metals, making them useful in bioremediation. *Pseudomonas aeruginosa* decreases heavy metal concentrations in contaminated environments using biochemical mechanisms. They convert metals into less harmful forms, as demonstrated in tannery wastewater studies.[29] *Pseudomonas* species also have enzymatic pathways that aid in the redox modification of metals, mitigating toxicity. These mechanisms allow bacteria to adapt to different environmental situations.[30] *Pseudomonas* species have adaptive genes that confer resistance to heavy metals, including membrane transporters and regulatory systems. Quorum sensing, a process involving upregulation of genes, is used to resist metal toxicity from noble metal-based nanomaterials. This process reduces reactive oxygen species production and enhances biofilm formation, providing a protective barrier against metal ions.[31]

In this study, we aim to assess the efficacy of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* in reducing zinc and cadmium concentrations in a non-circulating hydroponic system. We will evaluate the morphological and biochemical characteristics of mung beans seedlings as indicators of the bacteria's capability. *Vigna radiata*, often known as Mung Bean, is an ideal model plant for investigating the effects of heavy metal stress and remediation due to its compact stature, short life cycle, and adaptability to hydroponic cultivation methods. The attributes facilitate the ease may carrying out tests and analysing the impact of heavy metal stress on plant development within a controlled setting. In addition, the genetic attributes of the mung bean provide it a very suitable specimen for investigating the capacity of plants to mitigate heavy metal contamination in soils by means of its inherent detoxifying mechanisms.[32]

Although several studies have examined the role of *Pseudomonas* in mitigating heavy metal stress, limited research exists on their impact in crops such as mung bean cultivated in contaminated regions of Rajasthan, India. This study aims to evaluate how *Pseudomonas* spp. can influence the growth, biochemical, and morphological responses of mung bean (*Vigna radiata*) under zinc and cadmium stress in hydroponic systems. The research will assess parameters such as chlorophyll content, total proline content, total reducing sugar, total soluble sugar, and protein estimation, with the goal of exploring biological interventions for improving crop resilience in heavy metal-contaminated soils.

Materials and Methods

Seed Source for Study

Mung bean seeds of the Jaigrow-winner (JS-22-SK-300) variety were used, and obtained from a reputable source. All seeds in this study are categorized as research grade and have consistent size attributes to ensure consistency in experimental conditions.

Aseptic Transfer of Seeds

The seeds in the non-circulating hydroponics system were sterilized by washing them with 70% ethanol, followed by 0.1% mercuric chloride ($HgCl_2$), soaking in 5 % sodium hypochlorite (NaOCl) for 5 minutes, followed by rinsing with sterile distilled water 2-3 times to reduce the risk of infection.[33]

Hydroponic Apparatus

The study was carried out utilizing a non-circulating hydroponics system. Knop's salt solution served as a growing medium for plants with a pH ranging between 5.8-6. It provides essential nutrients and minerals necessary for plant development, maintaining balanced pH levels to optimize plant growth.[34] Various concentrations of Knop's salt solutions were prepared by adding zinc and cadmium ranging from 100 ppm to 1000 ppm.

Several sets of boiling tubes each holding 55ml of Knop’s salt solution were set up for growing mung beans in a non-circulating hydroponic system. Each set included boiling tubes with different concentrations (100ppm, 200ppm, 300ppm, 500ppm, and 1000ppm) of two heavy metals, zinc, and cadmium. The heavy metals were mixed with two types of bacteria, specifically *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. The ideal parameters for the hydroponic chamber were a temperature maintained between 21°C and 26°C. LEDs with a light intensity of approximately 10,000 lux were utilized. The objective was to study how the *Pseudomonas* family affects plant development under heavy metal stress. Each set was grown in triplicates to avoid chances of error.

Morphological and Biochemical Studies

The root and shoot lengths of each plant were measured and then these measurements were correlated with biochemical analysis, which included determining the total protein content,[35] estimation of total phenolic content,[36] estimation of total chlorophyll[37] determination of total soluble sugar content,[38] and analysis of total reducing sugar content.[39] Linear Regression Analysis was employed to evaluate spectrophotometer readings with a standard curve to quantify the specified biochemical parameters, including protein, phenol, total sugar, and reducing sugar concentrations.

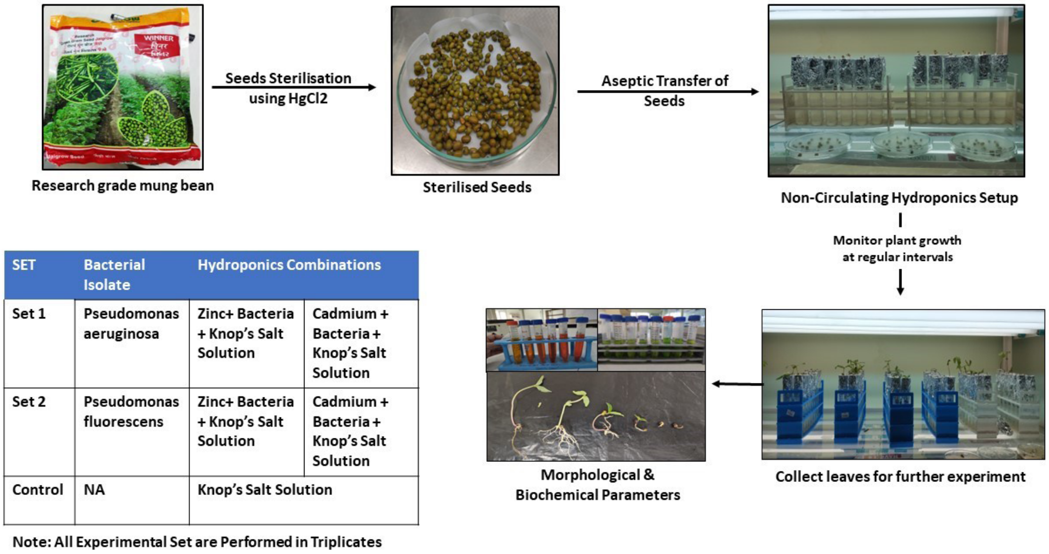

Fig. 2: Experimental Methodology to assess the impact of the Pseudomonas family on plant development in mitigating the presence of heavy metals in a controlled hydroponic environment.

Figure 2 depicts the methodological steps employed to assess the impact of the *Pseudomonas* family on plant development as a means of mitigating the presence of heavy metals.

Results and Discussion

A previous study investigated the impact of zinc and cadmium stress on the growth of *Vigna radiata* at various concentrations. A clear correlation was observed between increasing heavy metals concentrations and a decline in both plant morphology and biochemical parameters of plants.

To do this study, we looked at how the heavy metal-resistant bacteria P. *fluorescens* and *P. aeruginosa* affected the growth of *Vigna radiata* when zinc and cadmium stress levels were changed. The experiment was conducted using a non-circulating hydroponic system, and the concentrations ranged from 100 ppm to 1000 ppm. The study aimed to observe the efficacy of these bacteria in mitigating heavy metal stress and its effects on the plant's growth.

Figure 3 illustrates the influence of *P. aeruginosa* on the morphological characteristics of mung bean plants, such as root length and shoot length, subjected to heavy metal stress. When seedlings were subjected to zinc stress, plants showed minimal differences in root and shoot length compared to the control (without stress and *P. aeruginosa*). However, a marked reduction in growth was evident when the concentration of zinc reached 500 ppm. In seedlings exposed to cadmium stress, a notable decrease in growth occurred after reaching a concentration of 300 ppm. However, plant growth continued, unlike in the absence of resistant bacteria, plant development completely ceased after reaching a concentration of 100 ppm, leading to seed withering. Similarly, Figure 4 showed when P. *fluorescens* was introduced into a hydroponic system that is under heavy metal stress, like *P. aeruginosa*, it was observed that good seedling growth occurs up to a zinc concentration of 1000 ppm. However, in the case of a cadmium-stressed system, no seedling growth was observed after reaching a concentration of 500 ppm. Additionally, stunted root growth was noted at 300 ppm of cadmium.

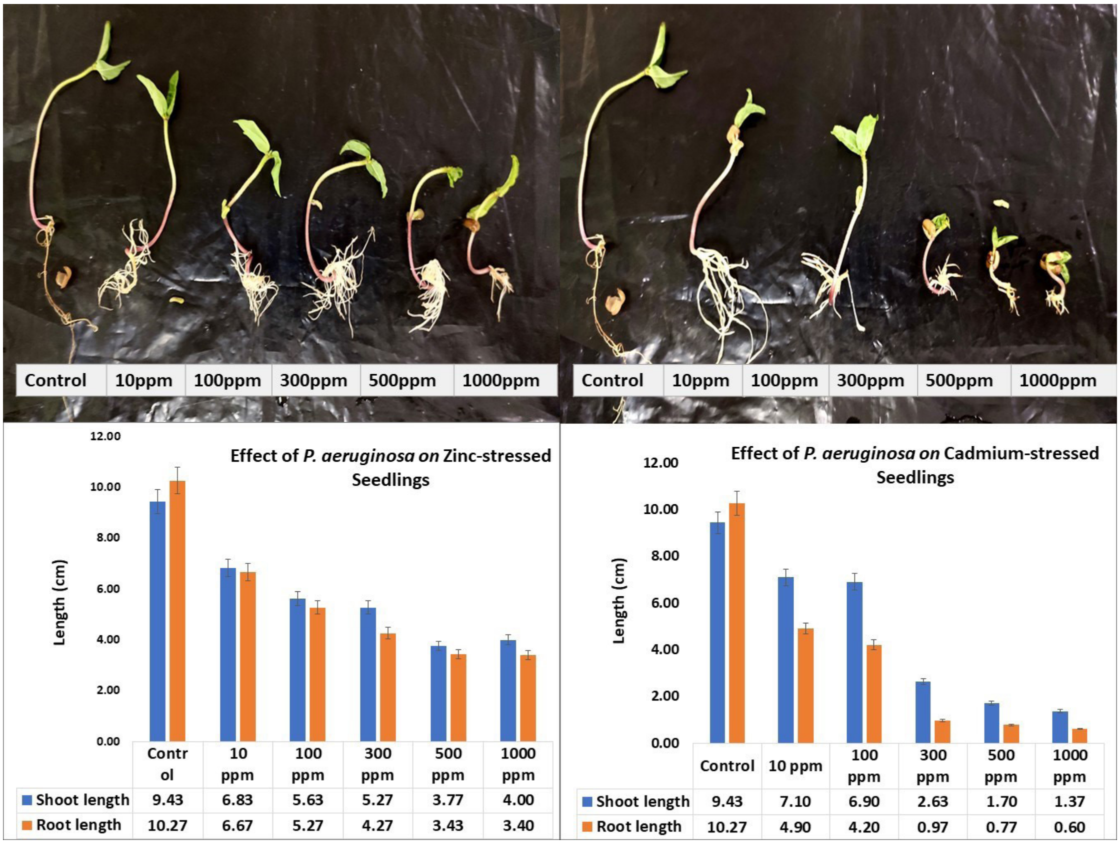

Fig. 3: Impact of *P. aeruginosa* on the growth of *Vigna radiata* seedlings under heavy metal stress. (a) Effect of *P. aeruginosa* on root and shoot length in seedlings under zinc stress at various concentration (b) Effect of *P. aeruginosa* on root and shoot length in seedlings under cadmium-induced stress at different concentrations.

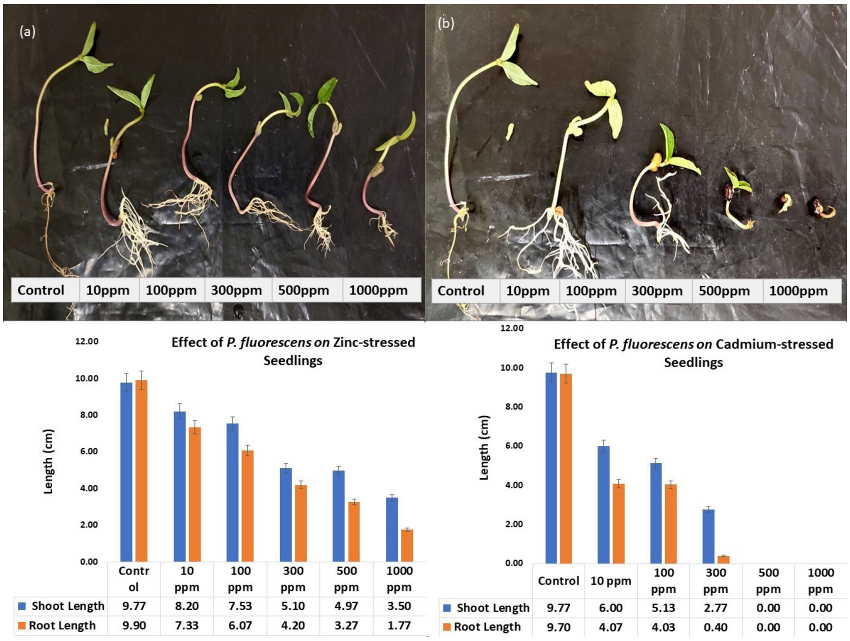

Fig. 4: Impact of P. fluorescens on the growth of *Vigna radiata* seedlings under heavy metal stress. (a) Effect of *P. fluorescens* on root and shoot length in seedlings under zinc stress at varying concentrations (b) Effect of *P. fluorescens* root and shoot length in seedlings under cadmium stress at varying concentration.

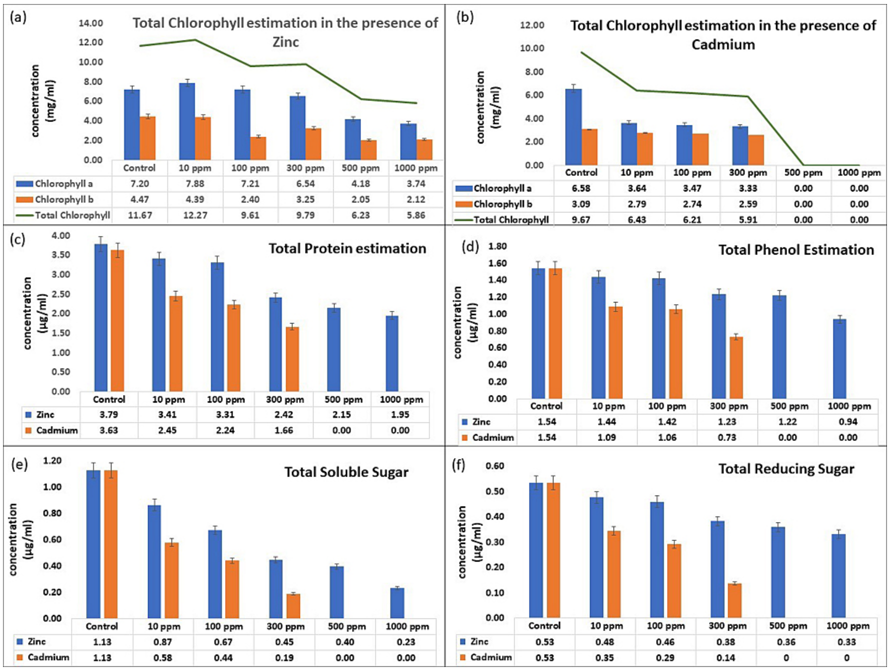

Fig. 5: Effect of *P. fluorescens* on the biochemical parameters of mung bean seedlings under a zinc and cadmium-stressed environment. (a) Total Chlorophyll estimation of zinc-stressed seedlings; (b) Total Chlorophyll estimation of cadmium- stressed seedlings (c) Total Protein Estimation (d) Total Phenol Estimation (e) Total Soluble Sugar (f) Total Reducing Sugar

Figure 5 demonstrates similar results for the biochemical parameters of mung bean seedlings in a hydroponics system as shown in morphological parameters under heavy metal stress with P. *fluorescens*. Initially, there is a slight increase in the overall chlorophyll content at a zinc concentration of 10 ppm. However, it begins to decrease slightly after 500 ppm. Nonetheless, the chlorophyll content stayed generally steady compared to the control setup until it reached 500 ppm. Under zinc stress, all other biochemical measures, such as the total protein estimate, total phenol, reducing sugar, and soluble sugar, exhibited a similar pattern as the estimation of total chlorophyll. Seedlings with a concentration of up to 500 ppm exhibited a consistent level of concentration like the control setup, although a little decline was seen after reaching 500 ppm. However, no biochemical data were recorded for cadmium concentrations over 300 ppm, since no seedling development was detected, and up to 300 ppm growth was observed but it was very slow.

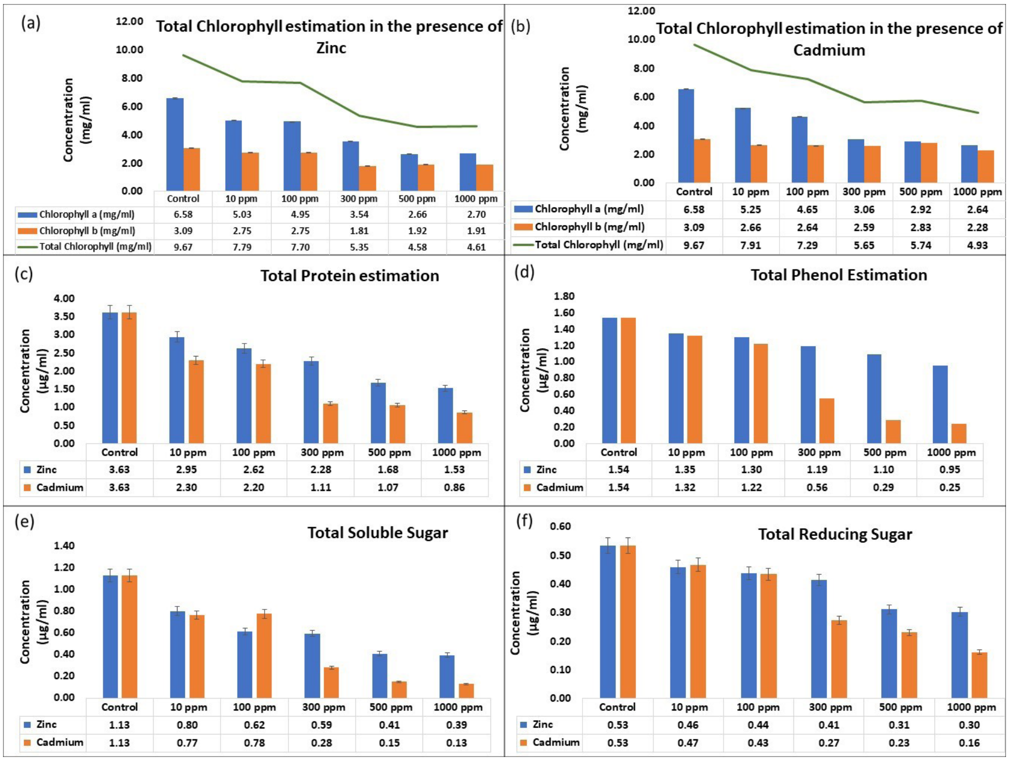

Fig. 6: Effect of *P. aeruginosa* on the biochemical parameter of mung bean seedlings under Zinc and cadmium-stressed environment. (a) Total Chlorophyll estimation of zinc-stressed seedlings (b) Total Chlorophyll estimation of Cadmium- stressed seedlings (c) Total Protein Estimation (d) Total Phenol Estimation (e) Total Soluble Sugar (f) Total Reducing Sugar

When *P. aeruginosa* was introduced into mung bean to mitigate zinc and cadmium stress in non-circulating hydroponics, the influence on mung bean biochemical parameters was investigated. As depicted in Figure 6, all the biochemical parameters showed healthy growth up to 1000 ppm in zinc-stressed non-circulating hydroponics in comparison to the control setup without *P. aeruginosa* and zinc in the medium. On the other hand, in the cadmium-stressed medium, the concentration of the biochemical parameters drops quickly from 300 ppm to 1000 ppm, but growth persists up to 1000 ppm. This was different from P. *fluorescens*, where seedling development ceased at 300 ppm cadmium concentration.

Recent research highlights the significant potential of *Pseudomonas* species in the bioremediation of heavy metals, showcasing their ability to detoxify and remove various toxic metals from contaminated environments. In one of the studies, it was found that when *Pseudomonas* species S7 was combined with *Chlorella,* it exhibited both tolerance and reduction capabilities towards Cr (VI) highlighting its potential for effective bioremediation of heavy metals.[40] Another study indicated that *Pseudomonas* putida uses chromate reductase to remediate chromium contamination, showing its effectiveness in cleaning heavy metal pollution, especially in groundwater that was contaminated with chromium.[41] Moreover, two *Pseudomonas* species from Iraq's sludge and sewage showed resistance against heavy metals and some antibiotics, with Ps-1(M9) and Ps-2(M19) showing resistance at concentrations ranging from 50 to 180 µg/ml.[42] Additionally, efficient bioremediation of heavy metals in Kpo-Fire-impacted soil was demonstrated by *Bacillus* flexus and *Pseudomonas aeruginosa*. These microorganisms showed significant removal rates for Mercury, Cadmium, Boron, and Arsenic. This suggests a clear relationship between bacterial biomass and the removal of heavy metals, suggesting that microbial processes play a significant role in the detoxification of contaminated environments.[43] Thus, the *Pseudomonas* family plays a crucial role in the bioremediation of heavy metals, demonstrating tolerance and uptake capabilities that surpass those of other bacterial strains. These bacteria also enhance plant growth under stress, making them suitable candidates for bioremediation efforts in contaminated environments. *Pseudomonas aeruginosa* has been widely examined for its capacity to resist and break down heavy metals including chromium, nickel, and aluminium. Strong resistance and in situ remediation capability are shown by its high minimum inhibitory concentration (MIC) for certain metals. *Pseudomonas aeruginosa* degrades nickel better than *Bacillus* cereus.[44] Other study highlights *Pseudomonas aeruginosa* HIB11, obtained from the Hindon River, effectively eliminates cadmium, nickel, and lead, exhibiting biodegradation capabilities like *Bacillus* subtilis, makes it a powerful degrader of heavy metals.[45] *Pseudomonas species* play a crucial role in heavy metal detoxification through mechanisms like metallothionein protein production and biosorption. In *Pseudomonas aeruginosa*, metallothionein proteins bind metals like cadmium and lead, with key residues such as Ala33, Ser34, and Glu59 aiding in this process.[46] Similarly, *Pseudomonas putida* shows strong metal-binding capabilities. In addition, *Pseudomonas aeruginosa* and *Pseudomonas* nitroreducens utilize biosorption and exopolysaccharide production for lead removal.[47] The co-application of biochar with these bacteria enhances microbial activity, reducing heavy metal concentrations while improving soil health.[48] While *Pseudomonas* species exhibit significant potential in heavy metal bioremediation, their effectiveness can vary depending on environmental factors and metal concentrations. Future research should focus on optimizing these strategies, potentially through genetic engineering or developing synergistic microbial consortia for enhanced performance. Additionally, studying interactions between *Pseudomonas* strains and other agents like biochar may yield more sustainable solutions to heavy metal pollution. A key limitation of this research is that while hydroponic systems provide valuable insights, they do not fully reflect the potential of *Pseudomonas* species in more complex soil environments. To fully understand the bacteria's capability in heavy metal bioremediation, future studies should explore metal toxicity reduction in the presence of these bacteria under more realistic conditions. This includes conducting pot and field trials to evaluate how *Pseudomonas* interacts with soil, plants, and other environmental factors. Conducting pot trials using these bacteria with mung bean plants could further clarify their efficiency in natural environments, offering insights into their dual role in mitigating heavy metals and promoting plant growth, positioning them as potential biofertilizers.

Conclusion

After examining the findings and existing research, it is evident that bacteria belonging to the *Pseudomonas* family, known for their resistance to heavy metals, play a vital role in reducing the levels of zinc and cadmium in the hydroponic medium. This, in turn, supports the growth of mung seedlings in a non-circulating hydroponics system, up to a certain concentration of heavy metal stress. *Pseudomonas fluorescens* enhances seedling development under zinc stress up to a concentration of 1000 ppm and under cadmium stress up to a concentration of 300 ppm. Although seedling development decreases after reaching a zinc concentration of 300 ppm in a zinc-stressed environment, some evidence of negative root geotropism was observed. It has been observed that the implementation of *Pseudomonas aeruginosa* promoted seedling growth in both zinc and cadmium-stressed hydroponics, supporting plant growth even at heavy metal concentrations of up to 1000 ppm. The research findings indicate that the *Pseudomonas* family has the significant potential to act as a biofertilizer, helping to reduce zinc and cadmium levels in soil and promote plant growth in environments with high levels of heavy metals. Additional experiments can be conducted in pot trials to assess the effectiveness of these bacteria in promoting plant growth in heavy metal-contaminated field areas within a natural environment. These trials would provide critical insights for further research.

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Conflict of Interest

The author(s) assert(s) that there are no conflicts of interest pertaining to the publishing of this manuscript. We assert that the study outlined in this publication has been undertaken with the highest level of honesty, and no financial or personal affiliations with individuals or organisations could have exerted any effect on its content.

Data Availability Statement

All data generated or analysed during this study are included in this published article [and its supplementary information files]

Ethics Statement

This research has been deemed exempt from ethical approval as it does not involve human or animal subjects in any aspect of the study. The manuscript does not contain experiments using animals as well as human beings.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author Contributions

- **Nidhi Saini:** led the experimental design, execution, data analysis, and manuscript preparation, ensuring the study's foundation and scientific rigour.
- **Dr. Sumer Singh:** provided crucial supervision, guidance, and mentorship throughout the project, steering it in alignment with research goals.
- **Dr. Parul Chowdhur:** contributed significantly to result analysis and offered valuable guidance and insights, manuscript editing enhancing the study's depth and robustness.

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