

Microbial Alleviation: Harnessing Microorganism for Sustainable Heavy Metal Bioremediation in Agriculture Soil

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ABSTRACT

Heavy metal contamination in agricultural soils significantly threatens food safety, crop productivity, and environmental health. This work explores the potential of microbial alleviation as a sustainable approach to mitigate heavy metal pollution in agricultural soils. Soil samples were collected from an onion field in Thoothukudi, India, and analyzed for microbial content. The predominant microorganism identified was *Pseudomonas aeruginosa*, confirmed through MALDI-TOF analysis. To evaluate the heavy metal bioremediation capability of *Pseudomonas aeruginosa*, metal solutions of varying concentrations were prepared and incubated with the bacterial colonies. The uptake of heavy metals by *Pseudomonas aeruginosa* was quantitatively assessed using Atomic Absorption Spectroscopy (AAS). Results indicate that *Pseudomonas aeruginosa* effectively absorbs significant amounts of heavy metals, highlighting its potential as a viable agent for bioremediation in contaminated agricultural soils. This study underscores the importance of utilizing naturally occurring microorganisms to enhance soil health and promote sustainable agricultural practices.

Keywords: Bioremediation, Agriculture soil, heavy metals, detoxification, and resistance.

INTRODUCTION

Earth bestows us with a wealth of natural resources such as forests, wildlife, land, soil, air, water, wind, plants, and animals, but now the misuse of them has led to the decline of natural resources to the extent that today half of our natural wealth is either depleted or at the verge of depletion. Over-exploitation of natural resources has now become common practice in current development initiatives. Many anthropogenic actions contribute to the degradation of natural resources like the use of chemical fertilizers in agriculture, the release of industrial waste, and the burning of fossil fuels (Wilson and Kevin 1993). The global population has grown dramatically increasing food needs (Tilman *et al.*, 2002). To satisfy the food needs, farmers of all countries have implemented green revolution technology. However, the green revolution has provoked several adverse effects on the environment due to the indiscriminate use of pesticides, herbicides, and nitrogen fertilizers; the use of improved varieties and transgenic, among others (Tilman, 1998). A wide variety of synthetic organic compounds are used in agricultural practices. These compounds are used mainly to control pests and increase crop productivity. However, after utilization,

these agricultural compounds enter the soil, water, air, and plant tissues (McGuinness and Dowling 2009). Numerous pesticides and herbicides have the potential to cause cancer.

Hence, the application of various chemical fertilizers and pesticides is a demanded need to maximize the yield. However, pesticides in the soil environment, concerning pest control efficacy, have become a matter of environmental concern because of the hazardous effects of pesticidal chemicals on soil microorganisms that ultimately affect soil fertility. As the use of pesticides becomes more vigorous and continuous, significant quantities of pesticides and their degraded products may accumulate in the soil ecosystem. The potential of pesticides causing harmful effects is so hazardous that it may cause an accumulation of toxic material in the organisms. Moreover, hence influences and controls health (Virendra and Pandey 2005). Pesticide that disrupts the activities of the soil microorganisms could be expected to affect the nutritional quality of soils and would, therefore, have ecological consequences (Chowdhury Ashim *et al.*, 2008). Thus, the use of pesticides not only degrades the soil quality but also reaches to water table and hence enters the aquatic environment, so it can be inferred that the fate of pesticides is often uncertain, thus decontamination of pesticide-polluted areas is a very complex process (Uqab Baba *et al.*, 2016). Farmers used more fertilizer inputs as a result of the positive benefits of chemical fertilizers on yield and production. The result of utilizing chemicals excessively and beyond what plants can consume is that the toxins are absorbed by the soil, which has subsequent impacts on the soil itself on plant products as well as groundwater.

Moreover, microbes and plants are among the most critical biological agents that remove and degrade waste materials to enable their recycling in the environment. Soil microflora, mainly bacteria, algae, fungi, and protozoa, make a valuable contribution to making the soil fertile through their primary catabolic role in the degradation of plants and animal residues in the cycling of the organic, inorganic nutrient content of the soil. Hence, to remediate the above-concerned problem, bioremediation is a sustainable approach for ameliorating contaminated soil, which causes an uncertain effect on the soil and human health. Bioremediation is a biological process for recycling wastes into a form that can be used and reused by organisms. It is the process of cleaning up environmental sites contaminated with chemical pollutants by using living organisms to transform organic compounds into less/non-toxic substances or even essentially remove them from soil water, or air. It is a cost-effective and eco-friendly technique that can destroy or render harmless contaminants using natural biological activity. It has proved to be an efficient tool for decontaminating polluted sites in the prevailing environment (Niti Chawla *et al.*, 2013).

Thus, there is a need to remove these hazardous heavy metals from the environment. To seek a solution to this problem, bioremediation is applied as a tool. The term bioremediation implies the use of microorganisms and plants to degrade environmental contaminants to less toxic forms (Mani and Kumar 2014; Upadhyay *et al.*, 2016). The reason that bioremediation is used as a potential tool for this problem is because it helps to restore the natural state of the polluted environment. It has long-term environmental benefits and is cost-effective (Dixit *et al.*, 2015).

Bacteria such as *P. aeruginosa* produce surfactants that aid in biodegradation. A recent study has found a *P. aeruginosa* strain that supports plant growth. This characteristic, along with the fact that *P. aeruginosa* can transform polycyclic aromatic hydrocarbons, suggests the future uses of *P. aeruginosa* for environmental detoxification of synthetic chemicals and pesticides and industrial purposes (Botzenhardt *et al.* 1993). There is great potential for reducing environmental contamination while enhancing soil health and crop output by using microbial alleviation for sustainable heavy metal bioremediation in agricultural soil. We can efficiently target and degrade heavy metal contaminants, lessening their detrimental impacts

on ecosystems and human health, by utilizing the natural skills of microbes. By pursuing more studies and employing remediation solutions based on microbes, we may create a more sustainable and eco-friendly agriculture sector.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The agricultural soil sample was collected from the onion farm of Namashivayapuram, Thoothukudi, Tamil Nadu. The sample was collected from the top 6 to 8 inches (15-20cm) of soil. The collected soil samples are then transferred gently into a Polythene zip-lock sample cover. Then the soil samples were quickly taken to a laboratory for further studies.

BACTERIAL ISOLATION AND IDENTIFICATION

Enrichment of soil sample

Bacteria are isolated from the agricultural soil sample. 2gm of soil sample was weighed in a beaker and mixed with 100 ml distilled and filtered through the Whatman filter paper. After filtration 100µl solution was added to Luria-Bertani (LB) liquid medium and incubated for 16-18 hours at 37°C.

Isolation of Bacteria

After the enrichment of the soil sample, the serial dilution technique is performed to isolate the pure culture of bacteria. 1 ml of the soil sample is added to 9 ml of Dilution Blank Tube. This is then followed by the same procedure, where 1 ml from Tube 1 is added to 9 ml of Tube 2, 1 ml from Tube 2 is added to 9 ml from Tube 3, and so on until the desired concentration is reached. 1ml of diluted samples were transferred into cetrimide agar plates and incubated at 37°C for 24 hours.

MICROSCOPIC EXAMINATION

Gram Staining Technique

One loopful of culture was taken and spread on a clean glass slide. And allowed the smear to air dry and then heat fixed flooded the smear with crystal violet and waited for 1 minute. Wash the slide in a gentle and indirect stream of tap water for 2 seconds. Flood slide with the mordant: Gram's iodine. Wait 1 minute. Wash the slide in a gentle and indirect stream of tap water for 2 seconds. Flood slide with a decolorizing agent. Wait 15 seconds or add drop by drop to slide until the decolorizing agent running from the slide runs clear. Flood slide with a counterstain, and safranin. Wait 30 seconds to 1 minute. Wash slide in a gentle and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper. Observe the results of the staining procedure under oil immersion using a Brightfield microscope.

BACTERIAL CONFIRMATION TEST

VITEK MS PRIME is a benchtop, high-throughput, automated microbial identification system that uses a mass spectrometry system using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of microorganism's culture. (Sarkar *et al.* 2019)

EVALUATION OF THE BIOREMEDIATION POTENTIAL OF *Pseudomonas aerogenosa*

Stock solutions of Cadmium, Iron, Barium, and Copper (1000 mg/L) were prepared from corresponding metal salts (i.e. CdCl₂, FeSO₄.7H₂O, BaCl₂.2H₂O, CuSO₄.5H₂O). The glassware used for this purpose was

leached in 2N HNO₃ and rinsed several times with distilled water before use to avoid metal contamination. Fe²⁺ is oxidized to Fe³⁺ in the presence of nitric acid. 1ltrs of a stock solution of each metal ion was prepared in distilled water and acidified with HNO₃ (10-20 ml of 2% HNO₃) to prevent precipitation and was sterilized at 121°C for 15 min.

SAMPLE PREPARATION FOR METAL ABSORPTION

Various concentrations of heavy metals i.e. 100-1000 (mg/L) were prepared in a final volume of 10 ml in Luria-Bertani (LB) broth. 1 ml of 24 hours isolated bacterial cultures were inoculated at 37°C for 24 hours.

DETERMINATION OF METAL UPTAKE BY *PSEUDOMONAS AEROGENOSA*.

Atomic absorption spectroscopy (AAS):

Various concentrations of heavy metals i.e. 100-1000 (mg/L) were prepared in a final volume of 10 ml in Luria-Bertani (LB) broth. To which 1 ml of 24 hours isolated bacterial cultures were inoculated at 37°C for 24 hours. The broth was subjected to atomic absorption spectrophotometry (at PSG College, Coimbatore) to determine the uptake of metal ions. Atomic Absorption Spectroscopy (AAS) was employed to quantify the concentrations of heavy metals (barium, copper, cadmium, and ferrous) in samples before and after bioremediation treatments. The AAS analysis was conducted using a highly equipped analyzer. Calibration standards for each metal were prepared by diluting commercially available standard solutions to concentrations of 100 ppm, 200 ppm, 500 ppm, and 1000 ppm (Sammur *et al.*, 2010).

RESULT

COLLECTION OF SAMPLES

The agricultural soil sample was collected from the onion farm of Namashivayapuram, Thoothukud, Tamil Nadu.



Figure. 1: Soil Sample

BACTERIAL ISOLATION AND IDENTIFICATION

Enrichment of Soil Sample

The enrichment of the soil sample in Luria-Bertani (LB) broth and incubated for 16-18 hours at 37°C. Show turbidity, which indicates microbial growth.



Figure 2: Enrichment of the sample.

Isolation of Bacteria

After the enrichment of the soil sample, the serial dilution technique is performed to isolate the pure culture of bacteria. 1 ml of diluted samples were transferred into cetrimide agar plates and incubated at 37°C for 24 hours. After the incubation period, a growth of yellow-green colonies was indicated on a cetrimide agar plate.

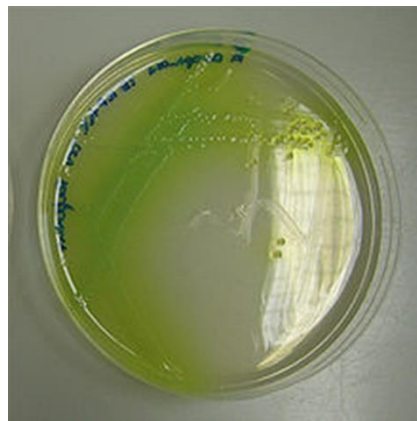


Figure 3: Bacterial growth on cetrimide agar

MICROSCOPIC EXAMINATION

Gram Staining Technique

In the gram-staining method, gram-negative, rod-shaped bacteria were observed.

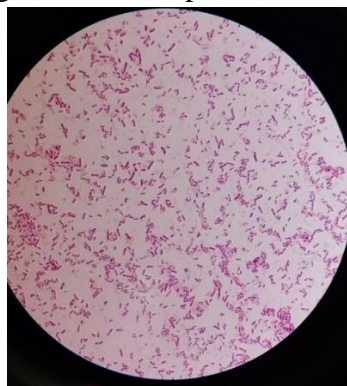


Figure 4: Microscopic view of the culture

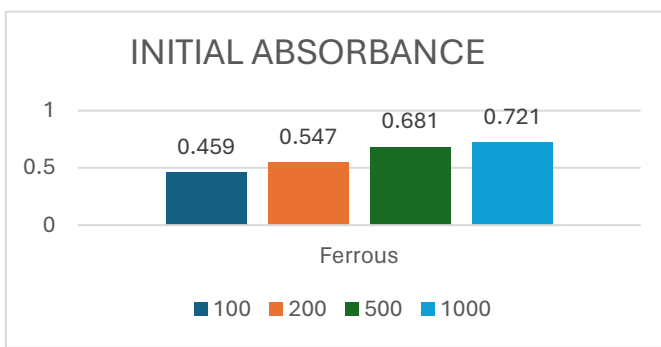
BACTERIAL CONFIRMATION TEST

The culture was identified in the Royal Care Hospital by the VITEK® MS PRIME method. The culture was identified as a *Pseudomonas aerogenosa*. The organism identified shows 99.9 % Probability in VITEK MS PRIME (which is a mass spectrometry system using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of microorganism’s culture).

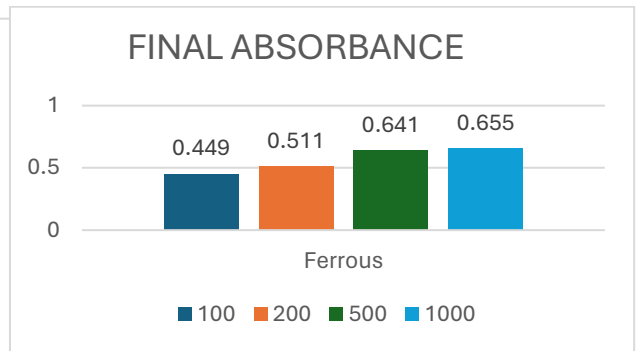
DETERMINATION OF METAL UPTAKE BY *Pseudomonas aerogenosa*.

Atomic absorption spectroscopy (AAS):

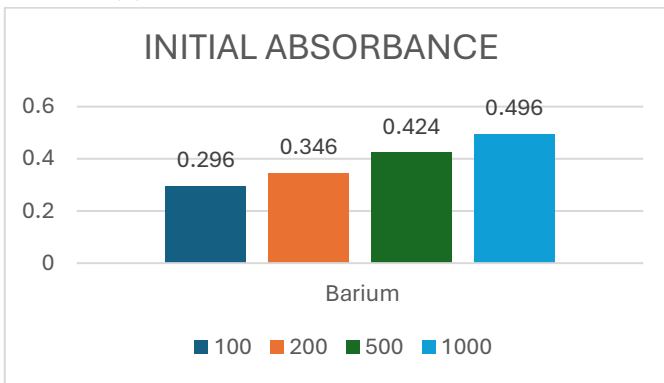
The heavy metals (Barium, Copper, Cadmium, and Ferrous) were treated with *Pseudomonas aerogenosa* to determine the degrading capacity of *Pseudomonas aerogenosa* towards the heavy metals. Heavy metals were taken for atomic absorption spectroscopy before and after bioremediation for comparative studies.



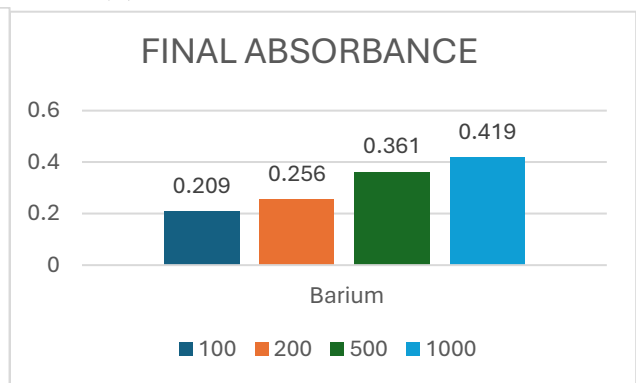
(a) Initial absorbance of Ferrous



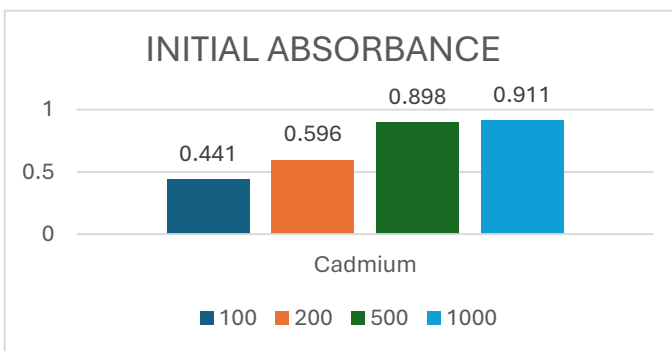
(b) Final absorbance of Ferrous



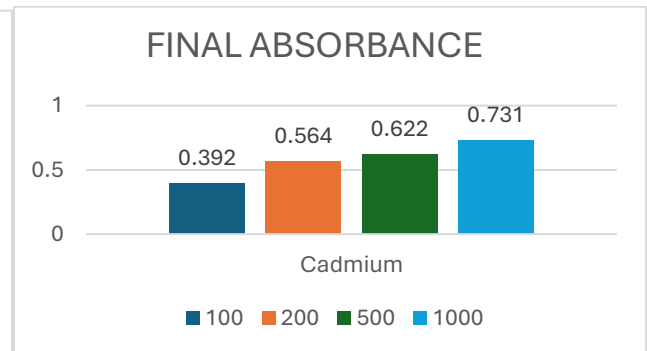
(c) Initial absorbance of Barium



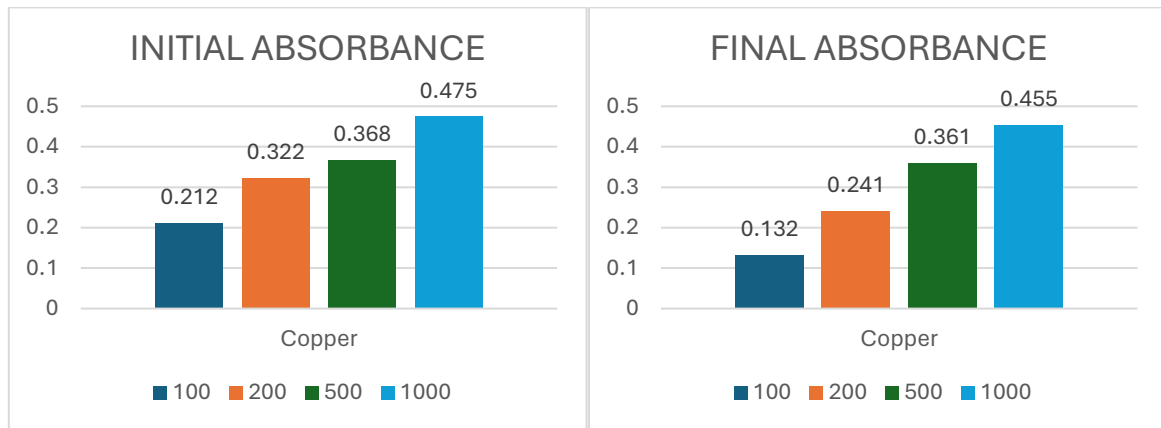
(d) Final absorbance of Barium



(e) Initial absorbance of Cadmium



(f) Final absorbance of Cadmium



(g) Initial absorbance of Copper

(h) Final absorbance of Copper.

Figure 5: Initial and final absorbance of heavy metals.

The results of the AAS analysis revealed a significant reduction in the concentrations of heavy metals in the samples following bioremediation treatment. Specifically, there was a decrease in the levels of Cadmium < Barium < Ferrous < Copper ions, indicating the efficacy of the bioremediation process in mitigating heavy metal contamination in the soil.

CONCLUSION

This study confirms the potential of *Pseudomonas aeruginosa* in alleviating heavy metal contamination in agricultural soils, thereby contributing significantly to food safety and sustainable agricultural practices. The ability of *Pseudomonas aeruginosa* to absorb substantial amounts of heavy metals, as demonstrated through Atomic Absorption Spectroscopy, highlights its role in reducing soil toxicity. By integrating such naturally occurring microorganisms into bioremediation strategies, it is possible to enhance soil health, safeguard crop productivity, and ensure the production of safe, uncontaminated food. These findings advocate for the broader adoption of microbial bioremediation techniques as part of sustainable agriculture, promoting environmental health and long-term agricultural viability. Future research should focus on field implementation and the comprehensive benefits of this approach on food security and ecosystem health.

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