Alleviation of Heavy metal toxicity using Plant Growth Promoting Rhizobium (PGPR)

Tapan Gohel[1] Ragini Raghav[1*]

Affiliation
[1]Department of Biotechnology, Atmiya University, Rajkot-360005, India
***Corresponding Author:**
Dr Ragini Raghav, Email: ragini.raghav@atmiyauni.ac.in

ABSTRACT

Rising heavy metal (HM) concentrations as a result of multiple human activities are a serious problem. Plants are adversely affected by HM contamination, particularly in contaminated soils. Plants struggle to live and suffer general health problems while under HM stress. The amount of various heavy metals in the environment has increased and is now dangerously high. The environment and human health have suffered as a result of the industrialization of the fertiliser, pesticide, and metallurgical sectors. Nonetheless, plants encounter a number of challenges in HM-contaminated environments, including nutritional and mineral deficiencies as well as modifications to several physiological and biological processes that reduce the plant's rate of growth. Recent advancements in our knowledge of the variety of PGPR in the rhizosphere, as well as their capacity for colonization and mode of action, should make it easier to use them as a trustworthy component in the management of a sustainable soil environment. The advancements made so far in employing rhizosphere bacteria for a range of purposes relating to agricultural improvement and general soil health

INTRODUCTION

Industrialization has a huge impact on growth and expansion. Without the use of metals, chemicals, or agrochemicals, rapid industrialization is not possible. At the same time, the ecology and agricultural land have suffered as a result of the excessive release of toxic metals and toxins.

Cadmium, Chromium, Copper, Mercury, Lead, Nickel etc are the most typical heavy metal pollutants. Metals are organic components of soil, and some heavy metals serve as essential micronutrients for plants. Yet, since the start of the industrial revolution, the rate at which hazardous metals are polluting the biosphere has increased drastically. due to human activities including the extraction and smelting of metals, electroplating,

gas emissions, production of energy and fuel, application of fertilizers, sewage and pesticides, production of municipal garbage, etc. [14] Due to its extensive peripheral extending from industry to agricultural fields during the past few years, heavy metal pollution has drawn the attention of the entire world. Because of their toxicity and longevity in the environment, inorganic pollutants like heavy metals are a new threat and problem for the scientific community. The beneficial rhizobacteria known as plant growth promoting rhizobacteria (PGPR) develop a partnership with the host plant to stimulate it and reduce the occurrence of different plant-related issues[3]

In their investigation of the rhizosphere root colonization of grasses and legumes in 1888, Hellriegel and Wilfarth postulated that soil bacteria could transform atmospheric N2 into forms that plants could use. Kloepper and Schroth (1978) used the term "rhizo-bacteria" to describe the soil bacterial community that competitively colonized plant roots, encouraged growth, and reduced the incidence of root rot through their research on radishes[1] To create a safe and sustainable environment, it is vital to combat the soil environment's HMs contamination. To remove HMs from the contaminated soil, a number of conventional physical and chemical methods, including burning, aeration, soil washing, surface soil replacement, and oxidation, have been developed. Unfortunately, these technologies are difficult because they have negative economic (because of the high cost) and negative environmental (because they produce secondary wastes) effects.

In this instance, eco-friendly bioremediation methods have drawn a lot of interest for their ability to remove HMs from contaminated locations. The bioremediation approaches use biological products such as plants, bacteria, or other biological agents to remove contaminants from the environment. Phytoremediation is a method of soil remediation that uses plants since they are in intimate touch with the soil and can thus be very helpful. For HM-contaminated soil bioremediation, plants that can withstand challenging HM conditions and accumulate high quantities are ideal. Cost-effective and playing a variety of roles in enhancing soil fertility, stability, soil conservation, and biodiversity is plant-based phytoremediation. However, because the deposited metal enters the food chain, utilizing crop plants for HMs phytoremediation is difficult. Plants have several issues under HM hazardous circumstances, including nutritional and mineral shortages and changes to numerous physiological and biological processes[5] Several industrial locations have accumulated varied amounts of heavy metal in the soil. For example, the soil near the TMT manufacturing industry contains excessive levels of heavy metals such copper, zinc, manganese, nickel, chromium, cobalt, and cadmium. [19]

Different heavy metals have various detrimental impacts on plants. By the production of reactive oxygen species and a reduction in catalase activity, copper causes oxidative stress. altered root development and morphology. Lead alters the permeability of cell membranes to promote phototoxicity. It has a detrimental impact on the seed's shape, physiology, and chloroplasts. Amylase, protease, and ribonucleases are affected by nickel. Plant digestion and food reserve mobilization will be harmed by the adverse effects of nickel. It also reduces the stability of the membrane. Due to excessive Cadmium in soil Germination is delayed. Mineral depletion causes nutritional loss and

high lipoxygenase (LOX) activity, compromising membrane integrity. DNA methylation is induced by cobalt, and active ion transport is inhibited. reduces the number of leaves and shoots. Reduce the water's potential.

Methods & Materials

1) Collection of samples

Agricultural land near industrial zones was sampled for soil at two sites:

A) Sihor (district of Bhavnagar, Gujarat), Longitude 21.7302598268257, Latitude 71.95499967344803. Trigonella foenum-graecum (fenugreek) was collected. B) metoda (district of rajkot, Gujarat), Longitude 22.241595879409843, Latitude 70.67172280559807. Coriandrum sativum (coriander) & Spinacia oleracea (spinach) was collected.

Plants were selected randomly and were rooted out from the soil. Soil Samples from root were collected in sterilized plastic bags.[8][11][12]

2) Serial dilution

Adhering soil was suspended in 1 ml of sterile distilled water for the production of the rhizospheric sample, and dilutions were produced. For the rhizoplane sample, soil that was adhering was jerked off by hand, leaving only the soil that was adhered to the roots. To make dilutions, 1 gram of connected dirt was collected. Collected soil samples were serially diluted in sterile test tubes with sterile distilled water from 10^{-1} to 10^{-7} Concentrations.

3) Isolation by Spread Plating Method:

Serial dilutions No. 1, 2, 3, 4, 5, 6, and 7 were employed to obtain bacterial colonies. A micropipette with a 0.1ml volume tip was used to take 0.1ml of the solution from the already shaken bottle No. 1, drop it on the nutrient agar media plate, and then spread it with a glass spreader on the nutrient agar plates .incubation of 24 hours at 37 °C in the Incubator. After 24 Hours of incubation different strains were isolated and streaked on nutrient agar plates. Incubation was done at 37 °C for 24 hours pure isolates were obtained from the soil sample. Multiple isolates were screened from the different soil samples.

4) seclusion of pure isolates & morphological Colony characterization

When colonies formed on the plates, they were picked up as separate, single colonies using a sterile loop and streak. Before and after each streak, this loop on a media plate that had already been prepared was rinsed with spirit and made red hot using a spirit lamp for sterilization. These plates were then streaked, and after streaking, were placed inverted in the incubator at 37 °C for 24 hours.
Microbial colonies were characterized on the basis of Size, Shape, Margin, Elevation, Opacity, Colour & Gram Staining.

5) Gram Staining

The procedure for Gram staining was carried out. A drop of the algal broth was inserted and heated on a microscope slide to fix it. After 1 minute of primary staining with crystal violet, the slide was washed with water. The slide was then given a one-minute mordant treatment with iodine solution before being thoroughly cleaned with water. After quickly decolorizing the slide with alcohol, it was washed with water. The slide was then cleaned with water, counterstained for 1 minute with Safranin, and inspected under a microscope. [21]

6) Bacterial growth at different heavy metal concentrations

Screening for heavy metal resistance was carried out using standard heavy metal solutions of Zinc ($ZnSO_4$ H2O) lead ($Pb(No_3)_2$), Cadmium($3CdSO_4$ 8H2O) , Copper($CuSO_4$5H2O) & Nickel ($NiCl_2$6H2O) Were added to Nutrient agar medium. The concentration of the standard heavy metal salts solutions ranged from 1 ppm to 1000 ppm according to the maximum permissible limits of different metals in soil. The salt solutions were prepared with Distilled water.

The bacterial growth limits were determined by a plating method. Cadmium resistance was determined by the appearance of growth of bacteria after 24 hours at 37 °C of incubation. [15]

- Different concentration (in ppm) of heavy metal taken for experiment given below in table 1

Heavy Metal	Salt	PPM	PPM	PPM	PPM	PPM
lead	Pb(No3)2	50	100	150	200	
Zinc	$ZnSO_4$ H2O	200	400	600	800	
Cadmium	3CdSO4 8H2O	1	3	5	100	200
Copper	CuSO45H2O	30	100	500		
Nickel	$NiCl_2$6H2O	100	150	200		

(PPM = Parts per millon)

Table 1 : Different Concentrations of heavy metals taken in to experiment

Result and Discussion

A total of 14 strains, [NM1, NM2, NM3, NM4, NM5, NM6, NBA1, NBA2, NBA3, NB1, NB2, NB3, NB4, NB5] were isolated from the rhizosphere of Fenugreek spinach & coriander . It was found that all the colonies have different morphological characteristics.

Those strains which had irregular forms were NB2 & NBA3 while all others were Circular. On the basis of elevation, the strains were divided into groups. Strains NM6, NB2, NB4 & NBA1 were raised while all others were flat. The strains with opaque opacity were NM2, NM5, NM4, NB1, NB2, NB3, NB4, NBA1 & NBA2 whereas strains NM1, NM3, NM6, NB5 & NBA3 were translucent. The results demonstrate that the strains NM5 & NBA2 were yellow , NM1, NM2, NM4, NB1, NB5, NBA3 were white & NM3, NM6, NB2 & NBA1 were off white & NB3 was reddish yellow in colour.

	Size	Shape	Margin	Elevation	Texture	Opacity	Color
NM1	Small	Circular	Entire	Flat	Viscid	Translucent	White
NM2	Medium	Circular	Entire	Flat	Viscid	Opaque	White
NM3	Small	Circular	Entire	Flat	Viscid	Translucent	Off-White
NM4	Medium	Circular	Entire	Flat	Viscid	Opaque	White
NM5	Medium	Circular	Entire	Flat	Viscid	Opaque	Yellow
NM6	Small	Circular	Entire	Raised	Viscid	Translucent	Off-White
NB1	Medium	Circular	Entire	Flat	Viscid	Opaque	White
NB2	Medium	Irregular	Entire	Raised	Viscid	Opaque	Off-white
NB3	Small	Circular	Entire	Flat	Viscid	Opaque	Reddish-yellow
NB4	Small	Circular	Entire	Raised	Viscid	Opaque	Yellow
NB5	Medium	Circular	Entire	Flat	Viscid	Transparent	White
NBA1	Small	Circular	Entire	Flat	Viscid	Opaque	Off-White
NBA2	Medium	Circular	Entire	Raised	Viscid	Opaque	yellow
NBA3	Small	Irregular	Entire	Flat	Viscid	Translucent	White

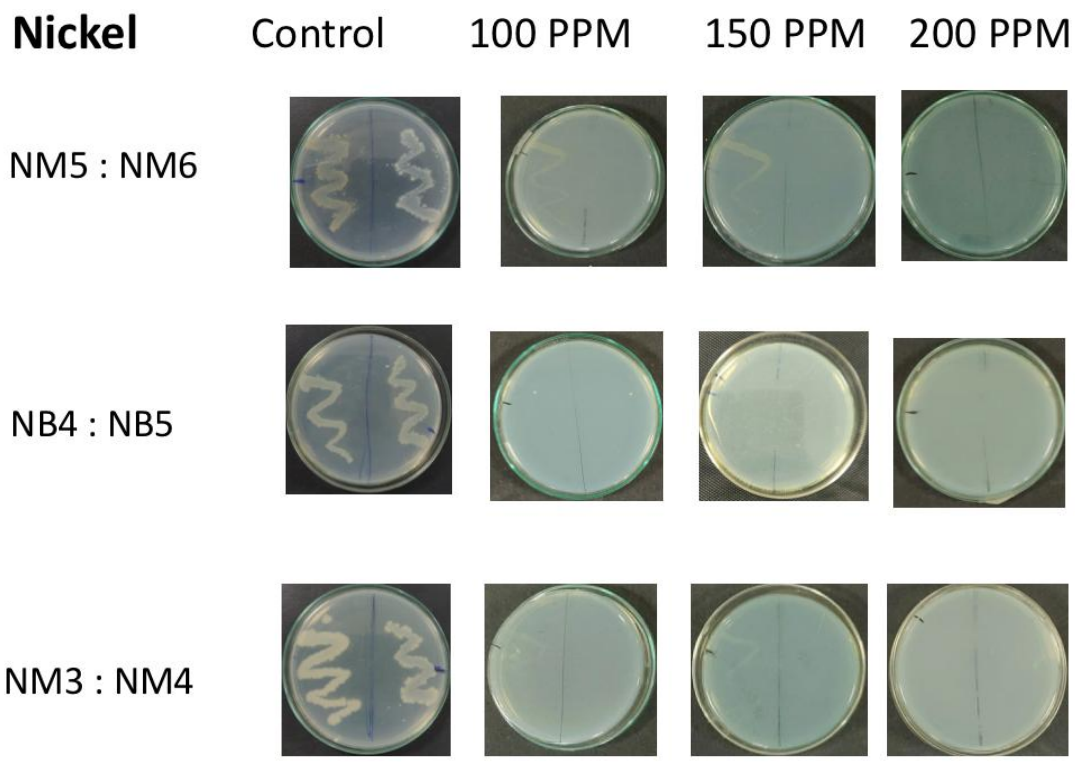
Table 2 : Colonies with different morphological characteristics

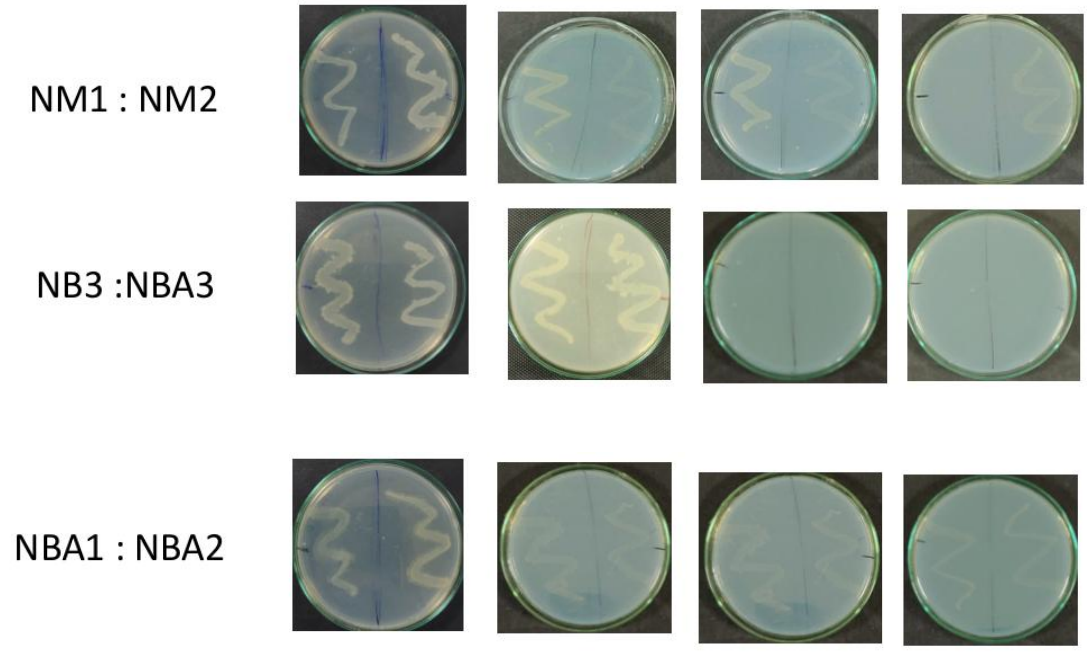
By the gram staining of the pure isolates morphology of microbes can be identified. Gram staining was done for 14 strains NM1, NM2, NM3, NM4, NM5, NM6, NBA1, NBA2, NBA3, NB1, NB2, NB3, NB4, NB5. Result of gram staining shows that NM2, NM6, NM1, NM4, NB3, NBA3, NB2, NB4 & NM3 were gram negative while others were gram positive. Most bacteria are gram-negative.

(A) Nickel has been considered to be an essential trace element for human and animal health. The permissible limit of Nickel in plants recommended by WHO is 50 PPM. all isolated microbes easily tolerate the & grow in the 50 PPM concentration. Different isolates have different amounts of growth at various concentrations which is mentioned below.

Isolates	100 PPM	150 PPM	200 PPM
NM1	✓	✓	✓
NM2	✓	✓	✓
NM3	✓	✓	✗
NM4	✓	✗	✗
NM5	✓	✓	✗
NM6	✓	✗	✗
NBA1	✓	✓	✓
NBA2	✓	✓	✓
NBA3	✗	✗	✗
NB1	✓	✓	✗
NB2	✓	✗	✗
NB3	✗	✗	✗
NB4	✓	✗	✗
NB5	✓	✗	✗

Table 3 : growth of isolates at different concentrations of nickel

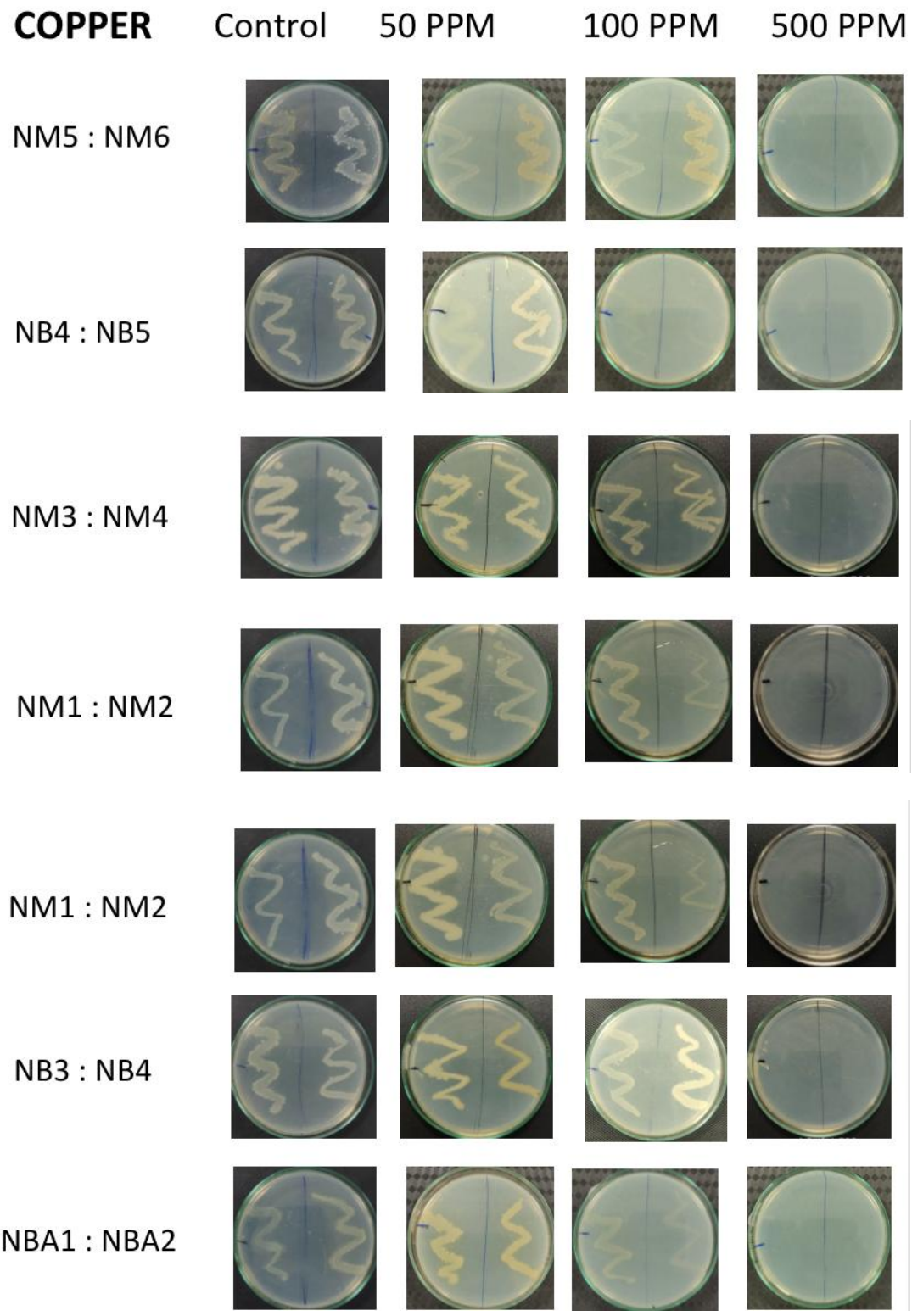




(B) The permissible limit of copper for plants is 150 PPM recommended by WHO. all isolates easily tolerated the 150 PPM concentration but there was no growth in 500 PPM plates.

Isolates	50 PPM	100 PPM	500 PPM
NM1	✓	✓	✗
NM2	✓	✓	✗
NM3	✓	✓	✗
NM4	✓	✓	✗
NM5	✓	✓	✗
NM6	✓	✓	✗
NBA1	✓	✓	✗
NBA2	✓	✓	✗
NBA3	✓	✓	✗
NB1	✓	✓	✗
NB2	✓	✓	✗
NB3	✓	✓	✗
NB4	✓	✓	✗
NB5	✓	✗	✗

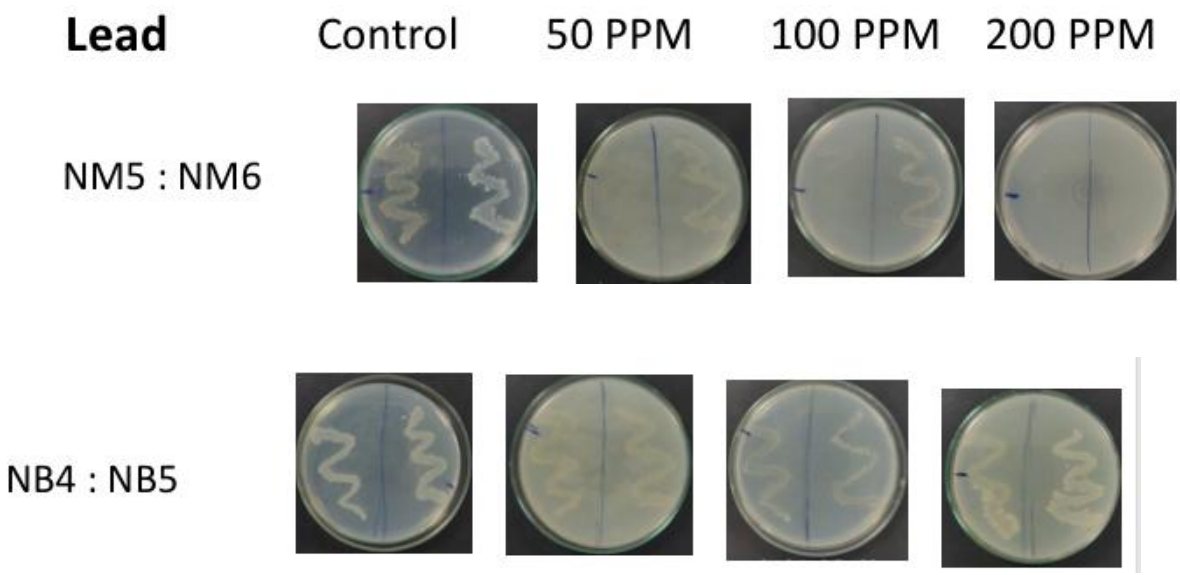
Table 4 : growth of isolates at different concentrations of Copper

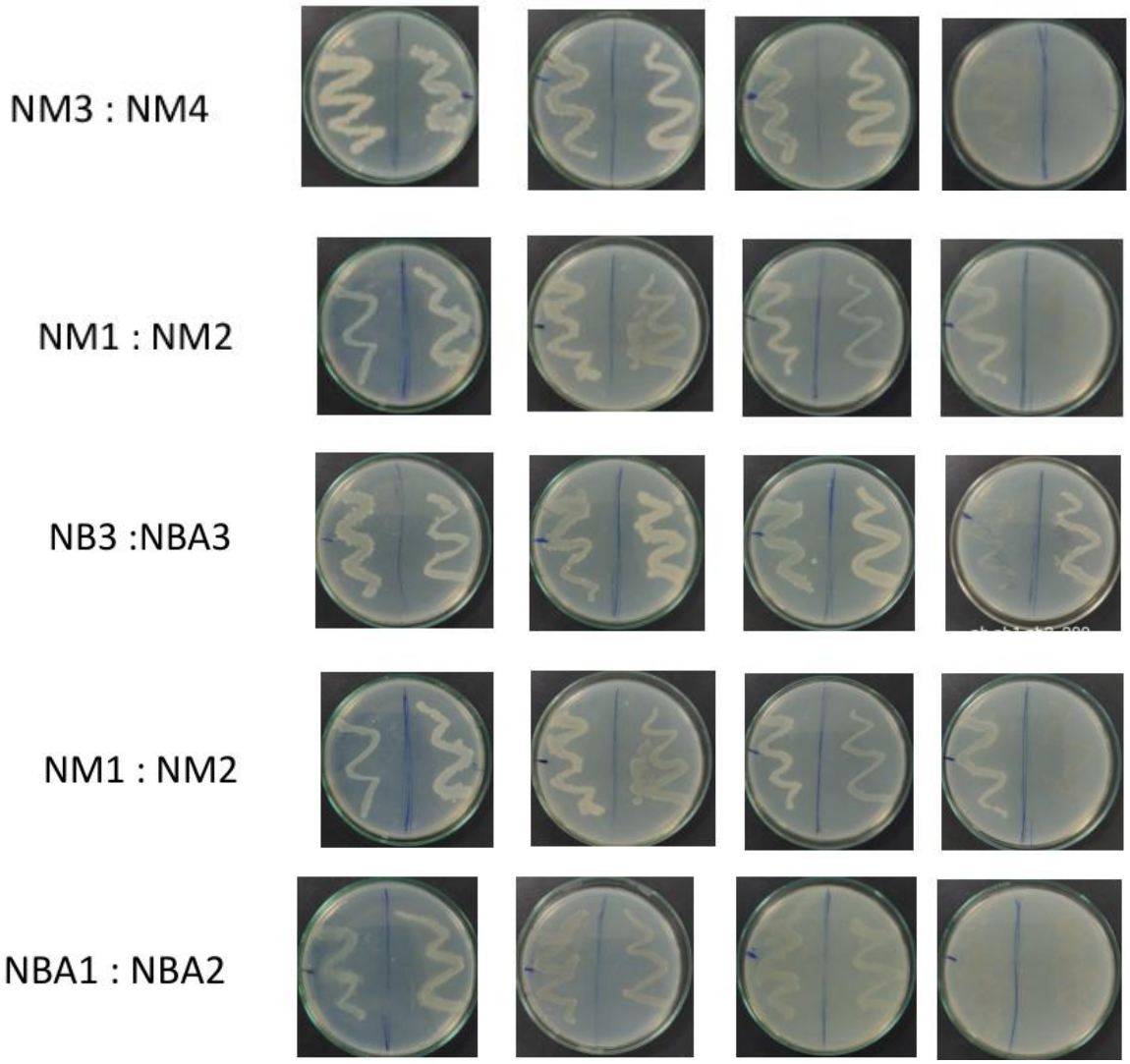

COPPER
Control
50 PPM
100 PPM
500 PPM
NM5 : NM6
NB4 : NB5
NM3 : NM4
NM1 : NM2
NM1 : NM2
NB3 : NB4
NBA1 : NBA2

(C) In case of lead permissible limit recommended by WHO is 100 ppm in soil. Most Isolates tolerate the concentration of 200 PPM. Growth was slow and less as concentration of lead increases gradually.

Isolates	50 PPM	100 PPM	200 PPM
NM1	✓	✓	✓
NM2	✓	✓	✓
NM3	✓	✓	✓
NM4	✓	✓	✓
NM5	✓	✓	✗
NM6	✓	✓	✗
NBA1	✓	✓	✓
NBA2	✓	✓	✗
NBA3	✓	✓	✗
NB1	✓	✓	✗
NB2	✓	✓	✓
NB3	✓	✓	✗
NB4	✓	✓	✗
NB5	✓	✓	✓

Table 5 : growth of isolates at different concentrations of lead



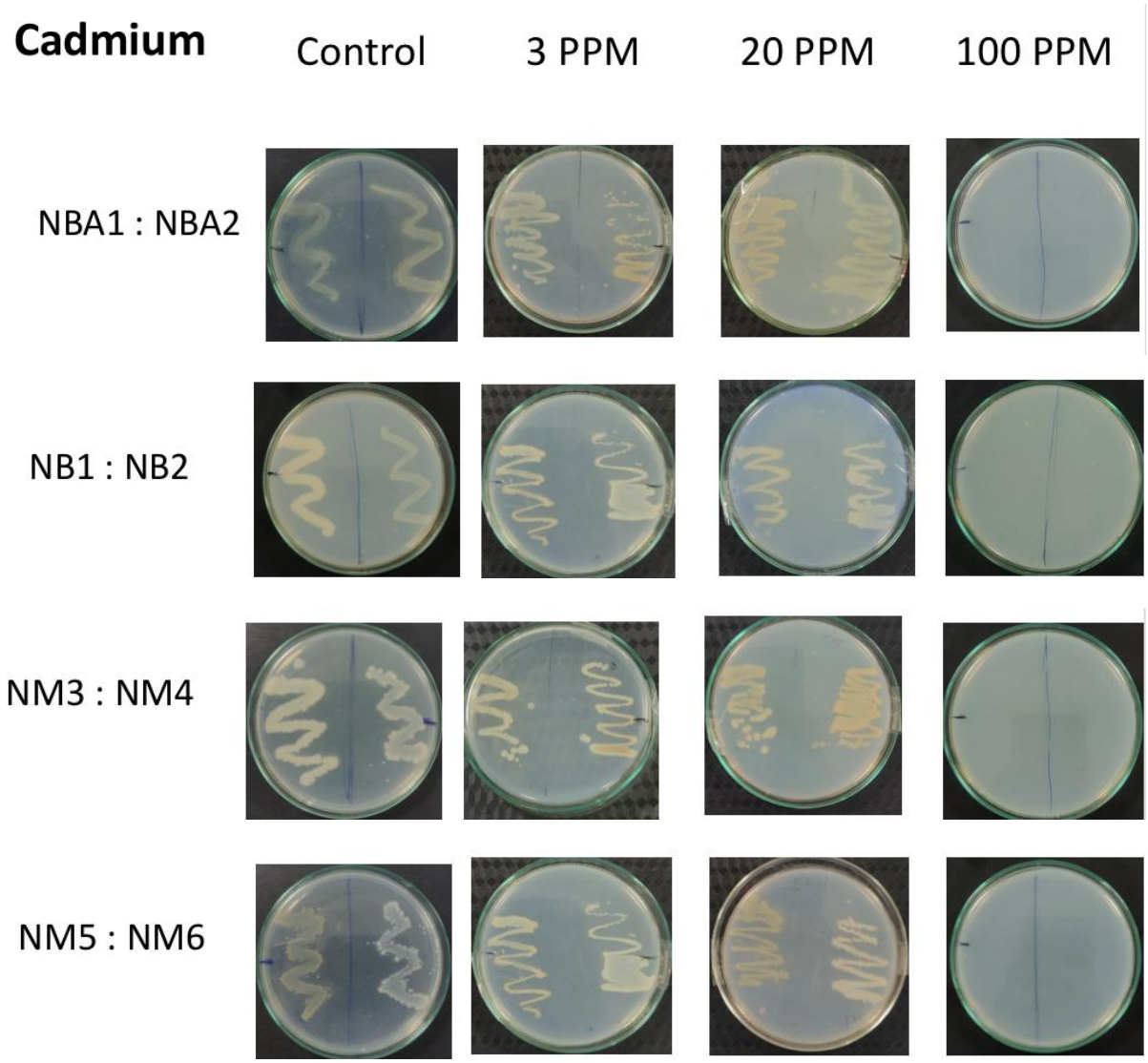


(D) Cadmium has 3maximum permissible limit of 3 PPM. Growth of different isolates were obtained in 20 PPM concentration also.

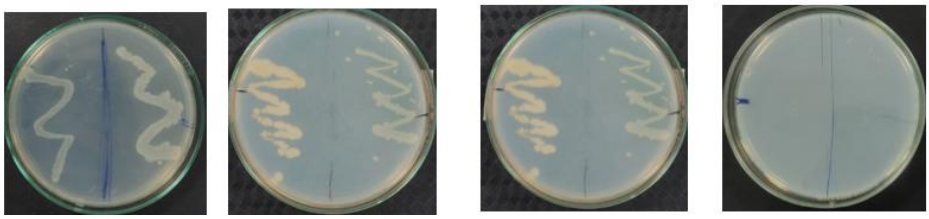
Isolates	3 PPM	20 PPM	100 PPM
NM1	✓	✓	✗
NM2	✓	✓	✗
NM3	✓	✓	✗
NM4	✓	✓	✗
NM5	✓	✓	✗
NM6	✓	✓	✗
NBA1	✓	✓	✗
NBA2	✓	✓	✗

NBA3	✓	✓	✗
NB1	✓	✓	✗
NB2	✓	✓	✗
NB3	✓	✓	✗
NB4	✓	✓	✗
NB5	✗	✗	✗

Table 6 : Growth of isolates at different concentrations of Cadmium



NM1 : NM2



Conclusion

In this work, plant growth-promoting rhizobacteria from a crop of fenugreek, coriander, and spinach are isolated and screened. Each of the 14 isolates tested positive for heavy metal tolerance. Further research on plant growth promoting traits will be conducted. Further studies about siderophore production, ACC Deaminase Production , Phosphate Solubilization will be carried out with these isolates. Potent isolate can be use in In-Situ as well as Ex-Situ bioremediation.

REFERENCES

1. Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, *28*, 1327-1350.
2. Fahsi, N., Mahdi, I., Mesfioui, A., Biskri, L., & Allaoui, A. (2021). Plant Growth-Promoting Rhizobacteria isolated from the Jujube (Ziziphus lotus) plant enhance wheat growth, Zn uptake, and heavy metal tolerance. *Agriculture*, *11*(4), 316.
3. Singh, S., Kumar, V., Sidhu, G. K., Datta, S., Dhanjal, D. S., Koul, B., ... & Singh, J. (2019). Plant growth promoting rhizobacteria from heavy metal contaminated soil promote growth attributes of Pisum sativum L. *Biocatalysis and agricultural biotechnology*, *17*, 665-671.
4. Osmani, M., Bani, A., & Hoxha, B. (2015). Heavy metals and Ni phytoextractionin in the metallurgical area soils in Elbasan. *Albanian Journal of Agricultural Sciences*, *14*(4), 414.
5. Gulzar, A. B. M., & Mazumder, P. B. (2022). Helping plants to deal with heavy metal stress: the role of nanotechnology and plant growth promoting rhizobacteria in the process of phytoremediation. *Environmental Science and Pollution Research*, *29*(27), 40319-40341.
6. Singh, S. K., Singh, P. P., Gupta, A., Singh, A. K., & Keshri, J. (2019). Tolerance of heavy metal toxicity using PGPR strains of Pseudomonas species. In *PGPR amelioration in sustainable agriculture* (pp. 239-252). Woodhead Publishing.
7. Mishra, J., Singh, R., & Arora, N. K. (2017). Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. *Frontiers in microbiology*, *8*, 1706.
8. Jarosławiecka, A. K., & Piotrowska-Seget, Z. (2022). The effect of heavy metals on microbial communities in industrial soil in the area of Piekary Śląskie and Bukowno (Poland). *Microbiology Research*, *13*(3), 626-642.
9. Daulta, R., Prakash, M., & Goyal, S. (2023). Metal content in soils of Northern India and crop response: a review. *International Journal of Environmental Science and Technology*, *20*(4), 4521-4548.

10. Chiroma, T. M., Ebewele, R. O., & Hymore, F. K. (2014). Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. *International refereed journal of engineering and science*, *3*(2), 01-09.
11. Verma, C., Singh, P., & Kumar, R. (2015). Isolation and characterization of heavy metal resistant PGPR and their role in enhancement of growth of wheat plant under metal (cadmium) stress condition. *Arch. Appl. Sci. Res*, *7*(7), 37-43.
12. Nazir, R., Khan, M., Masab, M., Rehman, H. U., Rauf, N. U., Shahab, S., ... & Shaheen, Z. (2015). Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb, Zn, Fe) in the soil, water and plants and analysis of physico-chemical parameters of soil and water collected from Tanda Dam Kohat. *Journal of pharmaceutical sciences and research*, *7*(3), 89.
13. Sobhanardakani, S., Tayebi, L., & Hosseini, S. V. (2018). Health risk assessment of arsenic and heavy metals (Cd, Cu, Co, Pb, and Sn) through consumption of caviar of Acipenser persicus from Southern Caspian Sea. *Environmental Science and Pollution Research*, *25*, 2664-2671.
14. Vocciante, M., Grifoni, M., Fusini, D., Petruzzelli, G., & Franchi, E. (2022). The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant's environmental stresses. *Applied Sciences*, *12*(3), 1231.
15. Meliani, A., & Bensoltane, A. (2016). Biofilm-mediated heavy metals bioremediation in PGPR Pseudomonas. *J Bioremediat Biodegrad*, *7*(5), 2.
16. Mishra, J., Singh, R., & Arora, N. K. (2017). Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. *Frontiers in microbiology*, *8*, 1706.
17. Asati, A., Pichhode, M., & Nikhil, K. (2016). Effect of heavy metals on plants: an overview. *International Journal of Application or Innovation in Engineering & Management*, *5*(3), 56-66.
18. Badawy, I. H., Hmed, A. A., Sofy, M. R., & Al-Mokadem, A. Z. (2022). Alleviation of cadmium and nickel toxicity and phyto-stimulation of tomato plant l. by endophytic micrococcus luteus and enterobacter cloacae. *Plants*, *11*(15), 2018.
19. Bhayani, D. (2021). STUDY OF PHYSICO CHEMICAL PROPERTIES OF SOIL IN METODA AND SHAPAR INDUSTRIAL ZONE OF RAJKOT REGION. *Vidhyayana-An International Multidisciplinary Peer-Reviewed E-Journal-ISSN 2454-8596*, *6*(6).
20. Akbar, K. F., Hale, W. H., Headley, A. D., & Athar, M. (2006). Heavy metal contamination of roadside soils of Northern England. *Soil Water Res*, *1*(4), 158-163.
21. Bartholomew, J. W., & Mittwer, T. (1952). The gram stain. *Bacteriological reviews*, *16*(1), 1-29.