

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.sp13.053>

Bioremediation of Molybdenum in Groundwater Using Bacteria Isolated from Rhizosphere Soil

Karthik Govindhan and Geetha Kasi^{ID*}

Department of Microbiology, Annai Violet Arts and Science College, Ambattur, Chennai 600053, Tamil Nadu, India

**Corresponding author*

ABSTRACT

Keywords

Molybdenum; groundwater; bioremediation; rhizosphere bacteria; microbial inoculant; heavy-metal remediation

Article Info

Received:

25 August 2026

Accepted:

30 September 2026

Available Online:

10 October 2026

Molybdenum (Mo) is an essential trace element at low concentrations but may become an environmental concern when present in contaminated water. This study evaluated rhizosphere bacteria and their potential use in molybdenum bioremediation of industrial groundwater collected from the Ambattur area, Chennai. Water samples were examined for preliminary physicochemical characteristics and qualitative heavy-metal reactions. Rhizosphere soil was serially diluted and three isolates (C1, C2 and C3) were screened in groundwater-contaminated medium. Isolate C2 was selected for further characterization because it showed the highest recorded growth response among the three isolates. C2 was Gram-positive, cocci-shaped and non-motile, and was positive for indole, methyl red, Voges–Proskauer, citrate, catalase and oxidase tests. Growth was evaluated over pH 4–9, 25–50 °C and 1–6% inoculum. C2 showed its highest recorded absorbance at pH 7 (0.50), 40 °C (0.50), and 2–4% inoculum (0.52). A microbial inoculant prepared from the selected culture also improved seed germination: at 100 µL/g, germination reached 95% in bhendi and 98% in sunflower compared with 79% and 85% in controls. The findings support further investigation of rhizosphere bacteria as low-cost candidates for molybdenum-affected water, although quantitative residual-molybdenum measurements and molecular identification are required for confirmation.

Introduction

The rapid increase in population and the increased demand for industrial establishments to meet human needs have created problems such as overutilization of accessible resources and increased pollution in the land, air, and water environment (Medfu Tarekegn *et al.*, 2020). The huge amount of organic and inorganic wastes generated annually in the materials cycle and the

improper disposal practices used have also created a worldwide problem (Njoku *et al.*, 2020). Environmental pollutants have been a primary difficulty during the last few years, influencing the high satisfaction of life (Taha *et al.*, 2023).

Heavy metal pollution is a major environmental issue associated with industrial activity and can adversely affect ecosystems (Abideen *et al.*, 2022).

Heavy metal pollution is one of the greatest consequences of industrialization. Heavy metals are metalloids or metallic elements that have relatively high atomic weight and may be poisonous even at low concentrations (Njoku *et al.*, 2020). Contamination with heavy metals is a major concern because it can threaten animals, plants and humans and disturb the environment (Pande *et al.*, 2022). Heavy metals have been removed by various physical and chemical methods, but excessive chemical use can create sludge-disposal difficulties and the possibility of secondary pollution (Akhtar *et al.*, 2020).

Biological remediation uses microorganisms or their products to transform, reduce, immobilize or remove contaminants and has received increasing attention for metal-affected environments (Igiri *et al.*, 2018; Pande *et al.*, 2022). Microbial molybdate reduction has been demonstrated using various fungi and bacteria, including *Escherichia coli* and other environmental isolates (Campbell *et al.*, 1985; Shukor *et al.*, 2007, 2009; Ahmad *et al.*, 2013). Studies have also described bacterial bioremoval of molybdenum from aqueous systems (Mohd Izuan Effendi Halmi *et al.*, 2014) and the isolation of molybdate-reducing strains from soil (AbdEl-Mongy *et al.*, 2015), representing a potential sustainable approach to molybdenum pollution. Thus, the present study focused on analyzing the presence of molybdenum in groundwater and evaluating the potential of rhizosphere bacteria for biological treatment.

Materials and Methods

Sample collection, water analysis and filter prototype

Tap water and groundwater were collected in clean glass containers from an industrial area in and around Ambattur, Chennai. The samples were analyzed for the presence of heavy metal using basic qualitative test using laboratory grade chemical for selective metals like Mercury, Arsenic, Silver and Molybdenum. A simple prototype filter using polyester, polypropylene and cotton materials was also evaluated during preliminary water-treatment work.

Isolation and screening of bacteria

Rhizosphere soil sample was collected from the groundwater contaminated area. Soil harbors numerous

amounts of soil microorganism. The sample were Ten-fold serially diluted and plated for colony enumeration and isolation of rhizosphere microbes. Three isolates, C1, C2 and C3, were screened in minimal salt medium containing collected groundwater. Absorbance at 540 nm was used as a growth indicator.

Test for metal degrading activity using microbes

Three isolates were tested for degradation of molybdenum using Minimal salt medium prepared with the collected ground water sample and distilled water as control and incubated at 37 °C. After incubation the test tubes were evaluated for the growth based on turbidity by measuring the spectrophotometer at 540 nm. The maximum growth occurred strain was considered as effective degradative bacteria and the effective strain was further characterized by morphological, biochemical properties.

Characterization and optimization

The selected isolate was observed for the colony characters like size, shape, colour, margin, elevation and opacity and also morphological characters like Gram staining, motility testing, and further identified using biochemical characteristics such as indole, methyl red, Voges–Proskauer, citrate, catalase and oxidase tests. Growth was assessed at pH 4–9, 25–50 °C and 1–6% inoculum concentration. Absorbance at 540 nm was recorded under the tested conditions.

Optimization

The particular strain was analyzed for optimization of various parameters like pH, temperature and amount of inoculum. This was done using the same procedure by predicting the growth by turbidity.

Also, Optimization is performed for the conditions for the growth determination of microbes on metal concentration through the absorbance recorded in spectrophotometer at 540 nm.

Plant-growth evaluation

A microbial inoculant was evaluated in seed-growth experiments using bhendi and sunflower. Treatments included untreated control, vermicompost, 100 µL/g microbial inoculant and 50 µL/g microbial inoculant.

Germination was recorded after five days and shoot growth was monitored during the experiment.

Results and Discussion

Water characteristics and bacterial isolation

Industrial groundwater was slightly yellowish, very low in turbidity and had a fishy odour. Its temperature was 27.8 °C, TDS 718 ppm and pH 7.26; tap water showed 26.9 °C, 991 ppm TDS and pH 7.05. Mercury, arsenic and silver were negative in both samples, whereas molybdenum was negative in tap water and positive in industrial groundwater.

Colony counts from 10^{-1} to 10^{-6} dilutions were 281, 136, 130, 122, 98 and 42 colonies, respectively. In groundwater-containing medium, absorbance values were 0.49 for C1, 0.50 for C2 and 0.46 for C3. C2 was retained as the selected isolate for subsequent characterization and optimization.

Characterization and optimization

C2 was Gram-positive, cocci-shaped and non-motile. It was positive for indole, methyl red, Voges–Proskauer, citrate, catalase and oxidase tests. The highest recorded C2 growth values were 0.50 at pH 7, 0.50 at 40 °C and 0.52 at 2–4% inoculum.

Plant-growth response

At five days, the 100 µL/g microbial inoculant produced 95% germination in bhendi and 98% in sunflower, compared with 79% and 85% in untreated controls. The same treatment produced the highest recorded 30-day shoot length: 17.5 cm in bhendi and 18.2 cm in sunflower. The 50 µL/g treatment produced 17.1 and 17.6 cm, while vermicompost produced 16.5 and 15.2 cm, respectively.

The study provides a laboratory-scale workflow linking detection of molybdenum in industrial groundwater with isolation of rhizosphere bacteria and evaluation of a selected culture. The positive qualitative molybdenum reaction in groundwater and negative reaction in tap water identify a clear difference between the two sampled waters, although the qualitative assay does not establish the actual molybdenum concentration. Such

confirmation is important because environmental risk and treatment efficiency depend on concentration and chemical form.

All three isolates grew in the groundwater-containing medium, with isolate C2 showing the highest recorded absorbance (0.50) of the qualitative molybdenum reaction after inoculation. Previous studies have demonstrated microbial molybdate reduction and molybdenum-blue formation, including work with *E. coli* and environmental bacteria (Campbell *et al.*, 1985; Shukor *et al.*, 2007, 2009; Ahmad *et al.*, 2013). Molybdenum bioremoval from aqueous solution has also been reported (Mohd Izuan Effendi Halmi *et al.*, 2014), supporting the relevance of investigating microbial systems.

The growth profile of isolate C2 was strongest around neutral pH and at 40 °C, while 2–4% inoculum produced the highest recorded absorbance. These observations are consistent with the general importance of pH, temperature and inoculum density in microbial metal-remediation systems (Jayaram *et al.*, 2022; Sreedevi *et al.*, 2022). The plant experiment additionally showed improved germination and shoot growth with 100 µL/g microbial treatment. Microbial remediation studies commonly describe beneficial interactions between microorganisms and contaminated soils, but the present plant results should be interpreted as a growth-response observation rather than proof of molybdenum removal from plant tissues (Fauziah *et al.*, 2017; Njoku *et al.*, 2020; Pande *et al.*, 2022).

The main limitation is that residual molybdenum was not quantified after treatment. Consequently, a percentage-removal efficiency cannot be calculated from the reported data. In addition, C2 was not identified to species level by molecular methods, and replicate-level variance was not provided. Future work should quantify molybdenum before and after treatment using an appropriate analytical method, identify the isolate by 16S rRNA sequencing, include biological replicates and statistical analysis, and evaluate treatment performance across different initial molybdenum concentrations.

These additions would allow direct comparison with established microbial bioremediation studies and strengthen the case for practical application (Akhtar *et al.*, 2020; Medfu Tarekegn *et al.*, 2020; Yakasai *et al.*, 2021).

Table.1 Preliminary water characteristics and qualitative metal reactions.

Parameter	Tap water	Industrial groundwater
Appearance	Normal	Slightly yellowish
Odour	No unusual odour	Fishy
Temperature (°C)	26.9	27.8
TDS (ppm)	991	718
pH	7.05	7.26
Hg / As / Ag	Negative	Negative
Mo	Negative	Positive

Table.2 Degrading activity of micro – organisms at absorbance of 540 nm.

Isolated strain	Absorbance at 540 nm		
	Distilled water containing minimal salt medium	Metal Contaminated Ground water sample containing minimal salt medium	Selection
C1	0.40	0.49	Screened
C2	0.35	0.50	Selected
C3	0.44	0.46	Screened

Table.3 Seed germination and 30-day shoot length.

Treatment	Bhendi germination (%)	Sunflower germination (%)	Bhendi shoot at 30 d (cm)	Sunflower shoot at 30 d (cm)
Control	79	85	15.6	14.2
Vermicompost	83	89	16.5	15.2
Microbial inoculant 100 µL/g	95	98	17.5	18.2
Microbial inoculant 50 µL/g	84	84	17.1	17.6

Figure.1 Filter-layer arrangement

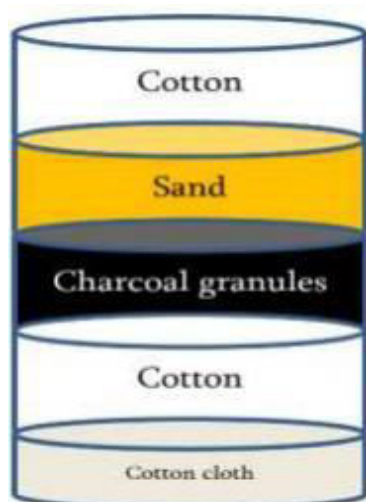
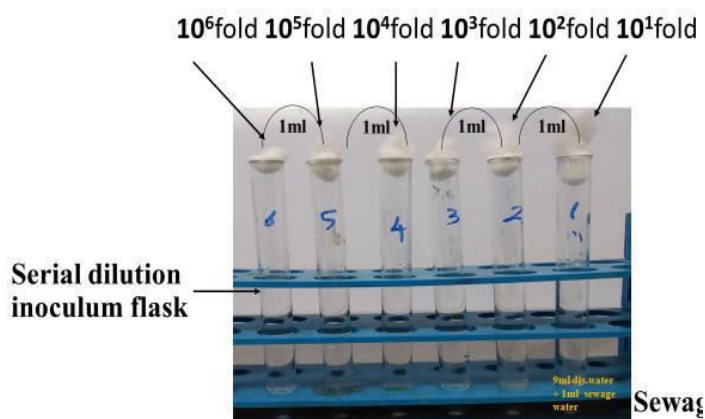


Figure.2 Serial-dilution procedure



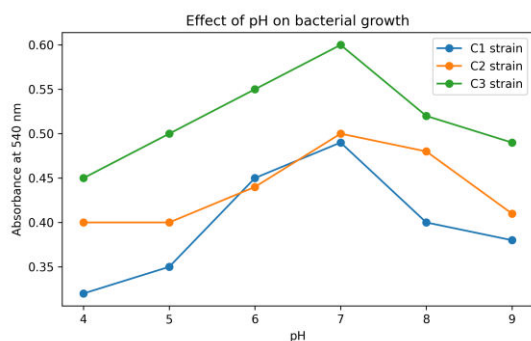


Figure.3 Effect of pH.

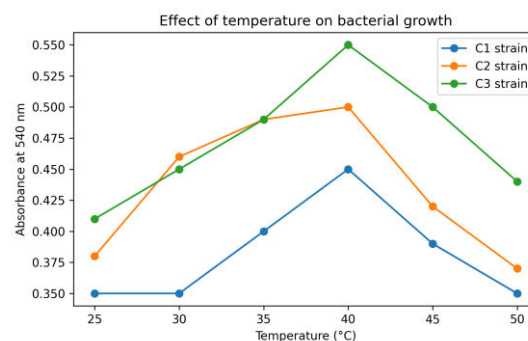


Figure.4 Effect of temperature.

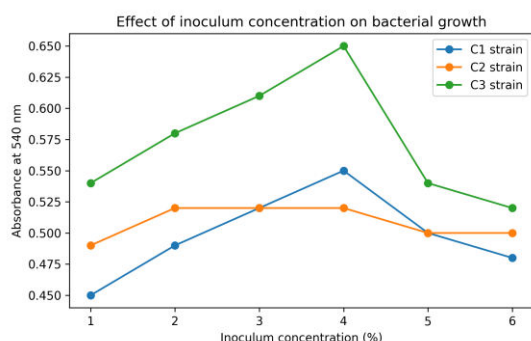


Figure.5 Effect of inoculum.

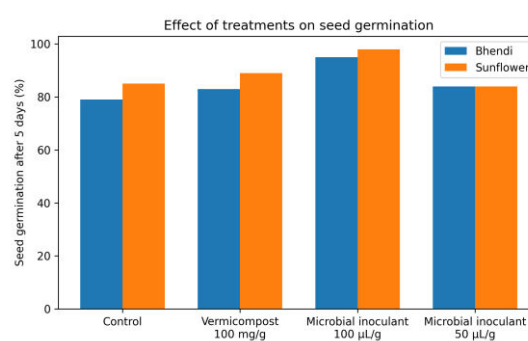


Figure.6 Seed germination.

Limitations

The present study has several limitations. The sample size was small and based on convenience sampling, limiting the generalizability of the findings. Incomplete clinical and demographic data restricted assessment of the association between FHV-1 DNA detection and clinical disease. Conventional PCR did not allow estimation of viral load or differentiation between active infection and latent infection or low-level shedding.

Furthermore, amplification was obtained only for the gI and TK targets, resulting in partial molecular characterization, and sequencing and phylogenetic analysis were limited to a small number of specimens.

Future studies should include larger, systematically selected populations, standardized clinical data, quantitative PCR, paired specimens from multiple sites, and multilocus or whole-genome sequencing.

In conclusion, Industrial groundwater collected near Ambattur showed a positive qualitative reaction for molybdenum, and three rhizosphere isolates were obtained and screened. C2 was selected for further study

and showed the strongest recorded growth under neutral pH, 40 °C and 2–4% inoculum conditions. The microbial inoculant also improved seed germination and shoot growth in bhendi and sunflower.

The results indicate potential for rhizosphere bacteria in molybdenum-affected water treatment, but quantitative molybdenum measurements and molecular identification are necessary before treatment efficiency or practical application can be established.

Acknowledgments

The authors acknowledge Dr. P. Dhasarathan, Global bio invention centre, Avadi, Chennai, for providing laboratory facilities and support for the work and the Principal, Dr. C. Initha Lebanon Ebency, and Mrs. B. Angel Jenefa, Head of the Department of Microbiology, Annai Violet Arts and Science College, Ambattur, Chennai for their encouragement and academic support.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this study.

Funding

No external funding was reported for the study.

Author Contributions

Karthik. G: Sample processing, heavy metal degradation, plant growth response and manuscript preparation.
Geetha. K: Supervision, scientific guidance and manuscript review.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Abideen, A. Adekanmi, Okewole, Akinwale Moses, Oluwadare, Olusegun Emmanuel, Lawal, Kola Ahmad, Awodiran, Tunji Paul, Owolabi Oluwafemi, Akinkunmi. (2022, August). Remediation of Heavy Metals in the Environment by Microorganisms. *International Journal of Academic and applied research*, 6(8), 201-209.

AbdEl-Mongy, M.A., Shukor, M.S., Hussein, S., Ling, A.P.K., Shamaan, N.A. and Shukor, M.Y. (2015). Isolation and characterization of a molybdenum-reducing, phenol- and catechol-degrading *Pseudomonas putida* strain Amr-12 in soils from Egypt. *Science Study and Research: Chemistry and Chemical Engineering & Biotechnology & Food Industry*, 16, 353–369.

Ahmad, S.A., Shukor, M.Y., Shamaan, N.A., Mac Cormack, W.P. and Syed, M.A. (2013). Molybdate reduction to molybdenum blue by an

Antarctic bacterium. *BioMed Research International*, 2013, 1–10.

Akhtar, F.Z., Archana, K.M., Krishnaswamy, V.G. and Rajagopal, R. (2020). Remediation of heavy metals (Cr, Zn) using physical, chemical and biological methods: a novel approach. *SN Applied Sciences*, 2(2).

Akpor, O.B. and Muchie, M. (2010). Remediation of heavy metals in drinking water and wastewater treatment systems: processes and applications. *International Journal of Physical Sciences*, 5(12), 1807–1817.

Campbell, A.M., Del Campillo-Campbell, A. and Villaret, D.B. (1985). Molybdate reduction by *Escherichia coli* K-12 and its *Chl* mutants. *Proceedings of the National Academy of Sciences USA*, 82, 227–231.

Carolyn, C.F., Kumar, P.S., Saravanan, A., Joshiba, G.J. and Naushad, M. (2017). Efficient techniques for the removal of toxic heavy metals from aquatic environment: a review. *Journal of Environmental Chemical Engineering*, 5(3), 2782–2799.

Fauziah, S.H., Jayanthi, B., Emenike, C.U. and Agamuthu. (2017). Remediation of heavy metal contaminated soil using potential microbes isolated from a closed disposal site. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 7(4), 230–237.

Igiri, B.E., Okoduwa, S.I.R., Idoko, G.O., Akabuogu, E.P., Adeyi, A.O. and Ejiogu, I.K. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. *Journal of Toxicology*, 2018, 1–16.

Jayaram, S., Ayyasamy, P., Aiswarya, K., Devi, M.P. and Rajakumar, S. (2022). Mechanism of microbial detoxification of heavy metals: a review. *Journal of Pure and Applied Microbiology*, 16(3), 1562–1574.

Medfu Tarekegn, M., Zewdu Salilih, F. and Ishetu, A.I. (2020). Microbes used as a tool for bioremediation of heavy metal from the environment. *Cogent Food & Agriculture*, 6(1), 1783174.

Mohd Izuan Effendi Halmi, M.I., Wasoh, H., Sukor, S., Ahmad, S.A., Yusof, M.T. and Shukor, M.Y. (2014). Bioremoval of molybdenum from aqueous solution. *International Journal of Agriculture and Biology*, 16(4), 848–850.

Njoku, K.L., Akinyede, O.R. and Obidi, O.F. (2020). Microbial remediation of heavy metals

- contaminated media by *Bacillus megaterium* and *Rhizopus stolonifer*. *Scientific African*, 10, e00545.
- Pande, V., Pandey, S.C., Sati, D., Bhatt, P. and Samant, M. (2022). Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem. *Frontiers in Microbiology*, 13.
- Rahman, M., Rusnam, M., Gusmanizar, N., Masdor, N., Lee, C., Shukor, M., Roslan, M. and Shukor, M. (2016). Molybdate-reducing and SDS-degrading *Enterobacter* sp. strain Neni-13. *Nova Biotechnologica et Chimica*, 15(2), 166–181.
- Shukor, M.Y., Adam, H., Ithnin, K., Yunus, I., Shamaan, N.A. and Syed, A. (2007). Molybdate reduction to molybdenum blue in microbe proceeds via a phosphomolybdate intermediate. *Journal of Biological Sciences*, 7, 1448–1452.
- Shukor, M.Y., Rahman, M.F., Suhaili, Z., Shamaan, N.A. and Syed, M.A. (2009). Bacterial reduction of hexavalent molybdenum to molybdenum blue. *World Journal of Microbiology and Biotechnology*, 25, 1225–1234.
- Shukor, M.Y., Rahman, M.F.A., Shamaan, N.A., Lee, C.H., Karim, M.I.A. and Syed, M.A. (2008). An improved enzyme assay for molybdenum-reducing activity in bacteria. *Applied Biochemistry and Biotechnology*, 144, 293–300.
- Sreedevi, P., Suresh, K. and Jiang, G. (2022). Bacterial bioremediation of heavy metals in wastewater: a review of processes and applications. *Journal of Water Process Engineering*, 48, 102884.
- Taha, A. *et al.*, (2023). Bioremediation of heavy metals in wastewaters: a concise review. *Egyptian Journal of Aquatic Biology and Fisheries*, 27(1), 143–166.
- Yakasai, H.M., Rahman, M.F., Manogaran, M., Yasid, N.A., Syed, M.A., Shamaan, N.A. and Shukor, M.Y. (2021). Microbiological reduction of molybdenum to molybdenum blue as a sustainable remediation tool for molybdenum: a comprehensive review. *International Journal of Environmental Research and Public Health*, 18(11), 5731.

How to cite this article:

Karthik G. and Geetha K. 2026. Bioremediation of Molybdenum in Groundwater Using Bacteria Isolated from Rhizosphere Soil. *Int.J.Curr.Microbiol.App.Sci*. Special Issue-13: 508-514.

doi: <https://doi.org/10.20546/ijcmas.2026.sp13.053>