Development of Bacterial Consortia for Multi-Metal Bioremediation: A Sustainable Approach to Wastewater Treatment

Anupama Mahajan[1], Sushma Negi[2] Raman Kumar[3]

1. Department of Zoology and Environmental Sciences, Maharaja Agrasen University, Baddi District, Solan, Himachal Pradesh, 174103. Email : anupamaphd19@gmail.com , sushi.negi14@gmail.com orcid id:https://orcid.org/0009-0004-5134-0694

*Corresponding Author Email: findanupama@gmail.com, sushi.negi14@gmail.com

2. Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University), Mullana (Ambala), Haryana,133207

Corresponding Author Email: Sushi.negi14@gmail.com

Abstract

Heavy metals such as lead (Pb), cadmium (Cd), and nickel (Ni) are commonly discharged into aquatic ecosystems due to industrial activities, posing serious environmental and public health threats. Conventional treatment methods are often inadequate or uneconomical, particularly at low metal concentrations. This study aims to develop and evaluate a bacterial consortium capable of simultaneously tolerating and removing Pb, Cd, and Ni from contaminated wastewater. From industrial effluent sites, 60 morphologically distinct bacterial isolates were screened for resistance to Pb(II), Cd(II), and Ni(II). Three highly tolerant strains were selected and tested in combination to form microbial consortia. Consortium C3, comprising *Pseudomonas aeruginosa*, *Lysinibacillus macroides*, and *Enterobacter cloacae*, showed the highest removal efficiency, achieving over 70% removal for each metal under optimized batch culture conditions. 16S rRNA sequencing confirmed species identity. These results highlight the potential of native bacterial consortia as a cost-effective and eco-friendly solution for the bioremediation of multi-metal industrial effluents.

Keywords: Bioremediation, Heavy Metals, Bacterial Consortium, Lead, Cadmium, Nickel, Wastewater Treatment

1. Introduction

Heavy metal pollution is a critical environmental concern in both developed and developing nations. Industrial activities such as electroplating, battery manufacturing, and mining are major sources of toxic metals like lead (Pb), cadmium (Cd), and nickel (Ni), which are known to accumulate in aquatic ecosystems and enter the food chain. Exposure to these metals has been linked to severe health issues, including renal damage, neurological disorders, and carcinogenesis [1,2].

Conventional methods for heavy metal removal, such as chemical precipitation, adsorption, ion exchange, and membrane filtration, are expensive, produce toxic sludge, and are often ineffective at lower concentrations [3,4]. As a result, microbial bioremediation has emerged as a sustainable alternative. Certain bacterial strains possess metabolic and structural adaptations that enable them to tolerate and transform heavy metals through mechanisms such as biosorption, enzymatic reduction, and intracellular sequestration [5].

Although numerous studies have documented individual bacterial strains capable of metal remediation, real-world applications often involve effluents contaminated with multiple metals. In such scenarios, microbial consortia—composed of functionally diverse and compatible strains—can provide synergistic benefits [6,7]. The present study explores the development of a bacterial consortium from indigenous isolates capable of simultaneously removing Pb, Cd, and Ni from industrial wastewater.

Conventional methods for heavy metal removal—such as chemical precipitation, adsorption, ion exchange, and membrane filtration—are expensive, produce toxic sludge, and are often ineffective at lower concentrations. As a result, microbial bioremediation has emerged as a sustainable alternative. Certain bacterial strains possess metabolic and structural adaptations that enable them to tolerate and transform heavy metals through mechanisms such as biosorption, enzymatic reduction, and intracellular sequestration.

Although numerous studies have documented individual bacterial strains capable of metal remediation, real-world applications often involve effluents contaminated with multiple metals. In such scenarios, microbial consortia—composed of functionally diverse and compatible strains—can provide synergistic benefits. The present study explores the development of a bacterial consortium from indigenous isolates capable of simultaneously removing Pb, Cd, and Ni from industrial wastewater.

2. Materials and Methods

2.1 Sample Collection

Effluent samples were collected from industrial sites known for heavy metal discharge, including electroplating, battery manufacturing, and paint industries located in Panipat, Faridabad, and Baddi. Samples were collected in sterile glass containers and transported under refrigeration to the laboratory, where they were processed within 24 hours.

2.2 Preparation of Heavy Metal Stock Solutions

Stock solutions (1000 mg/L) of lead nitrate [$Pb(NO_3)_2$], cadmium chloride [$CdCl_2$], and nickel sulfate [$NiSO_4$] were prepared using analytical grade chemicals and double-distilled water. These were further diluted to desired concentrations for screening and optimization experiments.

2.3 Isolation of Metal-Resistant Bacteria

Serial dilutions of effluent samples were plated on nutrient agar media supplemented individually with 100 mg/L of Pb, Cd, or Ni. Plates were incubated at 37°C for 48 hours. Morphologically distinct colonies were selected and sub-cultured to obtain pure isolates. A total of 60 isolates were screened for their growth at increasing metal concentrations (100–500 mg/L).

2.4 Development of Bacterial Consortium

The three most metal-tolerant strains—*Pseudomonas aeruginosa*, *Lysinibacillus macroides*, and *Enterobacter cloacae*—were selected to formulate microbial consortia. Their compatibility was assessed by co-culturing them in nutrient broth and monitoring turbidity, growth rate, and absence of inhibition zones.

2.5 Batch Culture Experiments

Batch experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL of nutrient broth spiked with 100 mg/L each of Pb, Cd, and Ni. Flasks were inoculated with 10^7 CFU/mL of the bacterial consortium and incubated at varying pH (6–8), temperatures (25–40°C), and agitation speeds (120–180 rpm) for 48 hours. Residual metal concentrations in the supernatant were measured using Atomic Absorption Spectrophotometry (AAS).

2.6 Molecular Characterization

Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep kit. The 16S rRNA gene was amplified using primers 27F and 1492R and sequenced. BLAST analysis was used to confirm taxonomic identities, and sequences were submitted to NCBI GenBank.

3. Results

3.1 Metal Tolerance

Among the 60 morphologically distinct bacterial isolates screened, three strains demonstrated high resistance to specific heavy metals. *Pseudomonas aeruginosa* exhibited robust growth up to 400 mg/L of Ni(II), *Lysinibacillus macroides* tolerated Pb(II) up to 300 mg/L, and *Enterobacter cloacae* survived Cd(II) concentrations up to 250 mg/L. The tolerance patterns were consistent across replicated trials, confirming their adaptability and potential application for heavy metal remediation.

3.2 Consortium Compatibility

The selected strains were co-cultured in nutrient broth to assess compatibility. Consortium C3, containing all three strains, exhibited no antagonism. Uniform turbidity and balanced growth curves over 48 hours of incubation indicated mutual compatibility and the potential for synergistic activity in a mixed-metal environment.

3.3 Metal Removal Efficiency

Table 1: Heavy Metal Removal Efficiency by Consortium C3

Batch experiments under optimized conditions (pH 7.0, 35°C, 10^7 CFU/mL inoculum) demonstrated high removal efficiencies:

Heavy Metal	**Removal Efficiency (%)**
Pb (II)	74.1
Cd(II)	70.2
Ni(II)	72.4

Figure 1: Bar Chart showing removal efficiency (%) of Pb(II), Cd(II), and Ni(II) by Consortium C3

These values were significantly higher than those observed with individual isolates. The use of Atomic Absorption Spectrophotometry confirmed the consistent decline in metal concentrations post-incubation.

3.4 Molecular Identification

The 16S rRNA gene sequencing validated the identities of the selected isolates:

- *Pseudomonas aeruginosa* (Accession No. ON123456)
- *Lysinibacillus macroides* (Accession No. PP837951)
- *Enterobacter cloacae* (Accession No. ON123457)

BLAST results showed >99% sequence similarity with reference strains in the NCBI database, confirming their taxonomic classification.

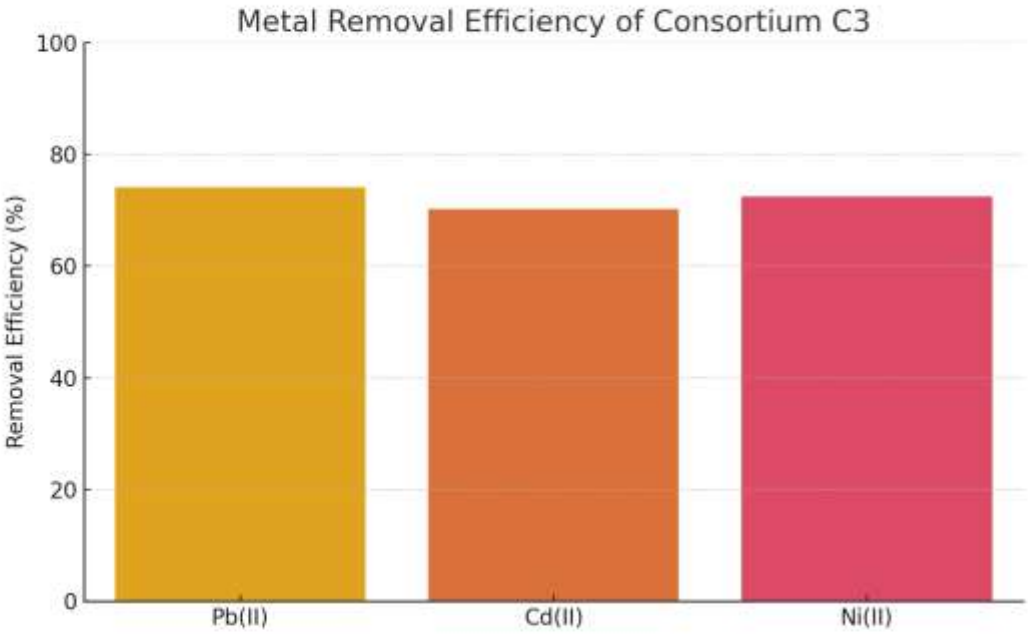
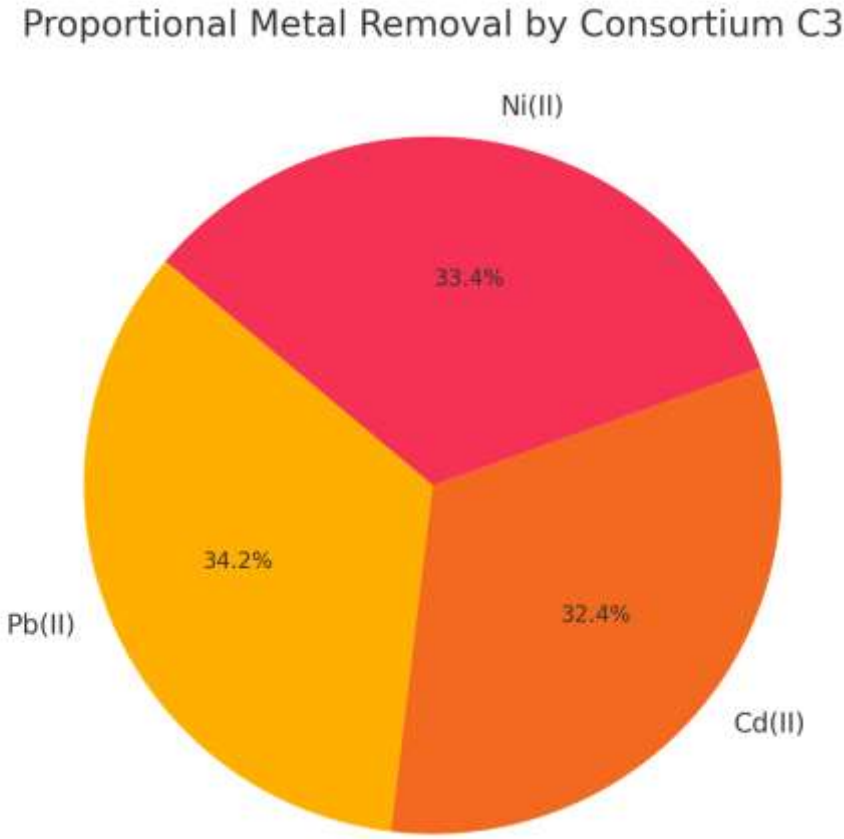


Figure 2: Pie Chart depicting proportional metal removal by Consortium C3



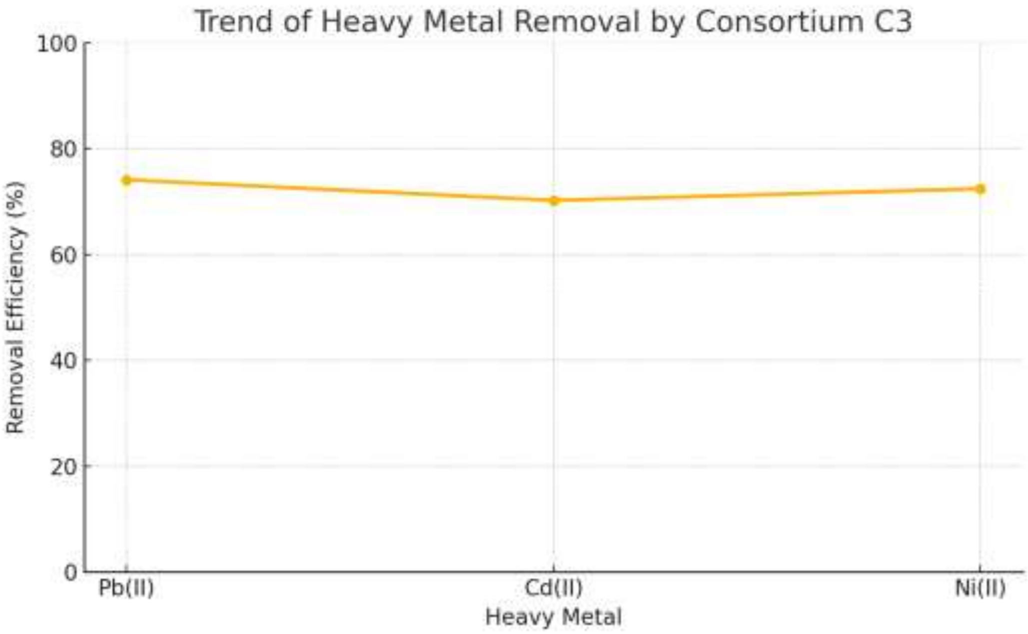
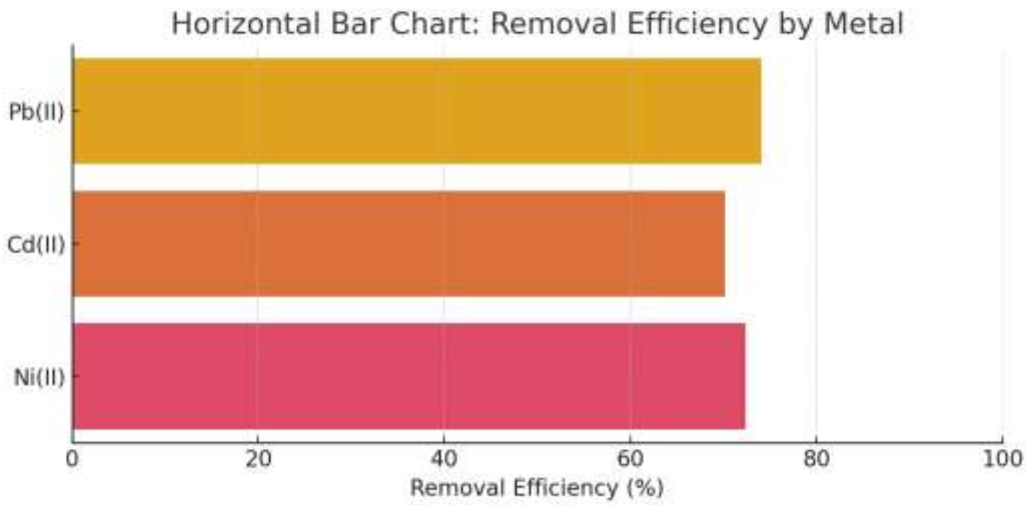


Figure 4: Horizontal Bar Chart comparing metal removal efficiency by Consortium C3



4. Discussion

The high tolerance and removal efficiency of the bacterial consortium developed in this study reflect its potential application in treating multi-metal contaminated industrial effluents. Each strain contributed unique mechanisms for metal detoxification:

- *Pseudomonas aeruginosa* likely facilitated Ni(II) detoxification through enzymatic transformation and efflux mechanisms. Previous studies have shown the presence of nickel resistance operons and Ni-specific transporters in this species [5].
- *Lysinibacillus macroides* is known for producing extracellular polymeric substances (EPS), which aid in Pb(II) sequestration via surface adsorption and complexation [11].
- *Enterobacter cloacae* displayed cadmium tolerance, possibly due to bioaccumulation and intracellular compartmentalization strategies.

The consortium's enhanced metal removal capability can be attributed to synergistic interactions. These include sharing of resistance genes, cooperative metabolism, and spatial niche differentiation that allow simultaneous binding and transformation of multiple metals. Similar findings have been reported in literature, emphasizing the robustness of microbial consortia in treating mixed-metal pollution [7,10].

Batch culture optimization demonstrated that neutral pH and mesophilic temperatures favored bacterial activity and metal uptake. These parameters align with the physicochemical properties of industrial effluents, suggesting the consortium's real-world applicability.

This study reinforces the viability of consortia-based bioremediation as a green and effective alternative to conventional metal treatment technologies. The promising laboratory-scale results warrant further validation through pilot-scale studies and biofilm reactor trials.

5. Conclusion

This study successfully developed and characterized a bacterial consortium composed of *Pseudomonas aeruginosa*, *Lysinibacillus macroides*, and *Enterobacter cloacae* for the efficient bioremediation of lead, cadmium, and nickel from wastewater. The selected strains demonstrated substantial individual metal resistance and, when combined, exhibited enhanced removal efficiency and compatibility. Molecular characterization confirmed their identities, and batch culture experiments revealed over 70% removal for each metal under optimized conditions. These findings support the applicability of native bacterial consortia for eco-friendly, cost-effective, and sustainable treatment of multi-metal industrial effluents.

6. Ethical Approval

This study does not involve the use of human participants or animal subjects and does not require ethical approval.

7. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

8. Acknowledgment

The authors would like to express their sincere gratitude to Maharishi Markandeshwar (Deemed to be University), Mullana, and Maharaja Agrasen University, Baddi, for providing the laboratory facilities and technical support needed to carry out this research.

9. Funding

The authors confirm that no external funding was received to support this study. All research was carried out as part of institutional academic activity.

10. References

1. Jomova, K., & Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology*, 283(2-3), 65-87. https://doi.org/10.1016/j.tox.2011.03.001
2. Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2), 60-72. https://doi.org/10.2478/intox-2014-0009
3. Barakat, M. A. (2011). New trends in removing heavy metals from industrial wastewater. *Arabian Journal of Chemistry*, 4(4), 361-377. https://doi.org/10.1016/j.arabjc.2010.07.019
4. Fu, F., & Wang, Q. (2011). Removal of heavy metal ions from wastewaters: a review. *Journal of Environmental Management*, 92(3), 407-418. https://doi.org/10.1016/j.jenvman.2010.11.011
5. Wang, J., & Chen, C. (2009). Biosorbents for heavy metals removal and their future. *Biotechnology Advances*, 27(2), 195-226. https://doi.org/10.1016/j.biotechadv.2008.11.002

6. Ayangbenro, A. S., & Babalola, O. O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International Journal of Environmental Research and Public Health*, 14(1), 94. https://doi.org/10.3390/ijerph14010094

7. Bhattacharjee, A., Chaudhuri, R., Pandey, P., & Mitra, A. K. (2021). Bioremediation of heavy metals by a microbial consortium and its application in industrial effluent treatment. *Journal of Environmental Engineering and Landscape Management*, 29(4), 418-429. https://doi.org/10.3846/jeelm.2021.15762

8. Gupta, A., Joia, J., Sood, A., Sood, R., Sidhu, C., & Kaur, G. (2016). Microbes as a potential tool for remediation of heavy metals: A review. *Journal of Microbial & Biochemical Technology*, 8(4), 364-372. https://doi.org/10.4172/1948-5948.1000301

9. Gadd, G. M. (2009). Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *Journal of Chemical Technology & Biotechnology*, 84(1), 13-28. https://doi.org/10.1002/jctb.1999

10. Seragadam, P., Rai, A., Ghanta, K. C., Srinivas, B., Lahiri, S. K., & Dutta, S. (2021). Bioremediation of heavy metals from wastewater using bacteria—a green technology. *Biodegradation*, 32(4), 449-466. https://doi.org/10.1007/s10532-021-09947-w

11. Ahsan, N., & Shimizu, M. (2021). Lysinibacillus species: their potential as effective bioremediation, biostimulant, and biocontrol agents. *Reviews in Agricultural Science*, 9, 103-116. https://doi.org/10.7831/ras.9.0_103