Fungal and Bacterial-Infused Fiber Mats: A Sustainable Solution for Heavy Metal Pollution

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***Abstract*- The research looks into a biodegradable fiber mat approach towards heavy metal contamination with an infusion of bacteria and fungi. Pseudomonas aeruginosa and Aspergillus niger were specifically used as they have been found to effectively remove heavy metals such as cadmium, lead, and manganese from contaminated water. The mat, sterilized jute, was inoculated with the microorganisms and tested by submerging it in heavy metal solutions. During a span of a few days, the metal concentrations were tracked by Atomic Absorption Spectroscopy (AAS). The results indicated a definite decrease in the levels of metals, particularly lead, validating the efficacy of the microbial mat. The technique is cost-effective, green, and possesses great promise for the remediation of contaminated environments.**

I. INTRODUCTION

Bioremediation entails harnessing the metabolic processes of naturally occurring degrader microorganisms to remove, biodegrade, or convert toxic substances into non-toxic or harmless products.

Microorganisms Utilized in the Project:

The project utilizes the bacterium Pseudomonas aeruginosa and the fungus Aspergillus niger for heavy metal (HM) sequestration.
Both the organisms are well established for their potential in bioremediation of heavy metals. Heavy Metal Sequestration by Microorganisms
Pseudomonas aeruginosa is efficient in sequestering heavy metals like cadmium (Cd) and lead (Pb). Aspergillus niger is capable of sequestering heavy metals like cadmium (Cd) and Manganese (Mn).

Method for Quantifying Heavy Metal Concentrations:

Atomic Absorption Spectroscopy (AAS): Used for precise quantification of the concentration of heavy metals both prior to and following the remediation treatment.

Heavy metal pollution is among the largest environmental issues we are dealing with today, inflicting tremendous damage on ecosystems and serious harm to human health. Ironically, most rehabilitation approaches are based on algae and bacteria when, in fact, funginatures own decomposerscan actually be more effective at sequestering heavy metals. Conventional cleanup techniques continue to be expensive and unsustainable, creating room for change. The objective of this project is to find out the possibility of integrating a fungi and bacteria on a biodegradable fiber mat, developing a tool to tackle heavy metal pollution in an environmentally friendly manner.

Objectives

Primary Objectives
Incubate biodegradable mat to bioremediate Test sequestration capacity
Optimize co-culture conditions
Heavy metal analysis

Secondary Objectives
Recommend disposal/reuse options
Optimize mat design
Long term performance study

II. LITERATURE SURVEY

Gul, Isma, et al. "Microbial Strategies for Lead Remediation in Agricultural Soils and Wastewater: Mechanisms, Applications, and Future Directions." Frontiers in Microbiology, vol. 15, 2024, Article 1434921.
Frontiers, doi:10.3389/fmicb.2024.1434921

Summary-The paper discusses microbial technology for eliminating lead (Pb) contamination from water and soil, and emphasizes environmentally friendly processes such as biosorption, bioprecipitation, biomineralization, and bioaccumulation. Microorganisms such as bacteria (Bacillus, Pseudomonas), fungi (Aspergillus niger) convert toxic Pb to stable, less toxic forms. Genetic modification increases microbial resistance and efficacy.

Chellaiah, Edward Raja Cadmium (heavy metals) bioremediation by Pseudomonas aeruginosa: A Minireview Applied Water Science, vol. 8, no. 154, 2018, pp. 110. Springer, https://doi.org/10.1007/s13201-018-0796-5.

Summary-This article presents an overall overview of the contribution of Pseudomonas aeruginosa to cadmium bioremediation. It points out the bacterias extreme resistance to Cd as a result of its genetic acclimatizations, including plasmid-encoded and chromosomal mechanisms. P. aeruginosa exhibits multiple abilities like biosorption, bioaccumulation, biofilm development, and biosurfactant production, which enable it to be a good agent in cadmium elimination from water, soil, and other polluted media. Its pathogenicity, though, is a safety issue for its application in practice.

Ghosh, Soumya, et al. Filamentous Fungi for Sustainable Remediation of Pharmaceutical Compounds, Heavy Metal, and Oil Hydrocarbons; Frontiers in Bioengineering and Biotechnology, vol. 11, 2023, article 1106973

Summary-This review article presents an extensive overview of bioremediation mechanisms utilized by filamentous fungi, emphasizing their application in mycoremediation via non-enzymatic, extracellular enzymatic, and intracellular enzymatic processes. Sequestration of heavy metals is largely achieved by non- enzymatic mechanisms, including adsorption onto fungal cell walls.

Javaid, Amna, Rukhsana Bajwa, and Tariq Manzoor. Biosorption of Heavy Metals by Pretreated Biomass of Aspergillus niger. Pak. J. Bot., vol. 43, no. 1, 2011, pp. 419425.

Summary-The research explores the utilization of chemically pretreated Aspergillus niger biomass for the removal of heavy metals, Cu(II) and Ni(II), from aqueous solution. Of all the pretreatments used, sodium carbonate (NaCO) was found to effectively improve the biosorption efficiency, while acid treatments significantly decreased it. The study brings to the fore A. niger as a promising heavy metal biosorbent, with pretreatment conditions being a key factor to enhance its adsorption efficiency.

Qader, Muzhda Qasim, and Yahya Ahmed Shekha. Bioremediation of Heavy Metals by Using Aspergillus niger and Candida albicans. ; ZANCO Journal of Pure and Applied Sciences, vol. 35, no. 3, 2023, pp. 180-186

Summary-The efficiency of fungi Candida albicans and Aspergillus niger in removing heavy metals (cadmium and lead) from water was examined in this study. Both fungi exhibited high percentages of removal, particularly at low concentrations (5 ppm). A. niger had a slightly higher efficiency, with lead removal percentages up to 85.6% and cadmium up to 80%. The study identifies the promise of this fungi as an eco-friendly means for detoxifying heavy metals.

Abo-Alkasem, Mohamed I., et al. Microbial Bioremediation as a Tool for the Removal of Heavy Metals.; Bulletin of the National Research Centre, vol. 47, no. 31, 2023. doi:10.1186/s42269-023-01006-z. Summary-A paper on microbial bioremediation, which is related, talks about the definition and impacts of heavy metals on organisms. It gives a comprehensive description of bioremediation, including its principles, types, factors that influence it, advantages, disadvantages, and the challenges of using it.

Petkovi, Katarina, et al. Potential of Aspergillus niger in Bioremediation of Contaminated Soils.; Acta Mycologica, vol. 50, no. 1, 2015, pp. 1-10.

Summary-Microscopic fungi, such as Aspergillus niger, are useful for eliminating arsenic and other harmful metals from the environment. In laboratory experiments, these fungi emitted almost 80% of arsenic via processes such as absorbing, releasing gases, and leaching. This indicates that soil fungi are also effective at decontamination. The research indicates that these fungi may be able to assist in cleaning contaminated soils and sediments. Fungi may prove to be a useful tool for bioremediation of contaminated zones.

Abdullahi, M., and Machido, D. J. Heavy Metals Resistance Potential of Some Aspergillus spp. Isolated from Tannery Wastewater.; Nigerian Journal of Basic and Applied Sciences, vol. 25, 2017, pp. 120-129.

Summary-Fungi is capable of degrading materials in the environment and can also endure heavy metal ions. Fungi in the research were more efficient at removing lead compared to cadmium. The removal of both metals was greater using Aspergillus niger than it was for Candida albicans. Maximum removal occurred at a concentration of 5 ppm. The removal rate was lower when the concentrations of the metals were higher.

Abbas, S. Z., Rafatullah, M., Ismail, N., and Lalung, J. Isolation, Identification, and Characterization of Cadmium Resistant Pseudomonas sp. M3 from Industrial Wastewater.; Journal of Waste Management, 2014, https://doi.org/10.1155/2014/160398.
Summary-In this article, P. aeruginosa has several characteristics that are suitable for cadmium cleanup, such as metal resistance, removal ability, and yielding helpful compounds such as biofilms and surfactants.
These characteristics render it a solid option for bioremediation. Nevertheless, P. aeruginosa is also a dangerous bacterium that can infect in hospitals. This complicates the use of it for bioremediation. Additional studies must be conducted to utilize it safely while controlling its dangers.

Awasthi, Garima, Anjali Chester, Rachna Chaturvedi, and Jyoti Prakash. Study on Role of Pseudomonas aeruginosa on Heavy Metal Bioremediation. ; International Journal of Pure and Applied Bioscience, vol. 3, no. 4, 2015, pp. 92-100.
Summary-The study evaluated Pseudomonas aeruginosa for decontaminating heavy metals (Cu, Cr, Fe, and Zn) from water. In the laboratory tests, it removed copper by 79.2% and chromium by 41.6%. In practical tests with industrial wastewater, it took away 44.6% of chromium from effluent of the leather industry and 9.2% of copper from effluent of the steel industry. The bacterium is tolerant to a significant extent of the metals and can, therefore, be applied in cleaning contaminated areas. More studies are necessary to know how it metabolizes these metals at a molecular level.
Wei, Yuqiu, et al. Ecological Risk Assessment of Heavy Metal Pollutants and Total Petroleum Hydrocarbons in Sediments of the Bohai Sea, China.; Marine Pollution Bulletin, vol. 184, 2022, p. 114218.
Summary-This research article gives us the information regarding how human actions are harming coastal marine ecosystems, particularly by pollution from heavy metals and chemicals such as TPHs in sediments. These are toxic pollutants, bioaccumulative, and biopersistent. We carried out research in the Bohai Seas for a period of 3 years and discovered most pollutants were within safety levels, apart from cadmium, which would be hazardous to ecosystems. Our study calls for better environmental management. The aim is to save these areas' environments in the future.

III. DESIGN

Methodology

Fungi and bacterial media selection and procurement
Preparation of fibre mat
Inoculation of the co-culture fungi and bacteria on the fibre mat Maximization of the co-culture conditions to maximize the growth of microbes Heavy metal sequestration analysis

Preparation of media and inoculation of microbes Bacteria
1 g of Luria Broth(LB) dissolved in 50 ml of distilled water and autoclaved. Approximately 20 ul of pure bacteria was added to this flask to culture P. aeruginosa and left in a shaking incubator at 37C for a day.
Preparation of 2 g of LB in 100 ml of distilled water for nutrient medium preparation on mat for bacterial growth.
Fungi 1.2 g of Potato Dextrose Agar(PDA) in 50 ml of distilled water and autoclaved. A Some spores of A.niger were inoculated in the nutrient medium and grown at room temperature for approximately 3 days.
Preparation of 2.4 g of PDA in 100ml of distilled water for mat preparation of nutrient medium for fungi growth.

Preparation of Jute mat for Bioremediation
Sterilization of the jute mat was done by autoclaving and later cut into suitable pieces. These mats were utilized as substrates for promoting the growth of bacteria and fungi. For this, 100 ml of both LB and PDA media were spread on the mats and then inoculated with 50ml of cultured Pseudomonas aeruginosa and Aspergillus niger. The inoculated mats were left to incubate at room temperature for about two days to enable microbial growth on their surfaces.

The formation of Aspergillus niger was verified by the development of black spores, and that of Pseudomonas aeruginosa was confirmed through a partially spreading method conducted on two distinct regions of the mat.

Preparation of metal solution and immersion of mat

The heavy metals were made up as solutions from their respective salts to prepare a 0.01N solution in 2 liters of distilled water. 3.526 g of cadmium sulfate, 3.253 g of lead(II) acetate trihydrate, and 1.69 g of manganous sulfate were weighed precisely and dissolved in distilled water. The salts were selected and blended on the basis of solubility and their capability to yield the desired metal ions in solution. The solution was then applied to check for the sequestration capacity of the microbial mat. The cultivated microbial mat was gently submerged in the heavy metal solution under controlled conditions. Baseline levels were measured using the initial readings of the concentrations of the metal ions before submerging them for baselines to be used for comparison later. Post-readings were conducted at regular intervals to track the decrease in the concentration of metal ions over time, giving indication of the efficiency of sequestration by the microbial-infused mat. This enabled the testing of the removal capability of the mat for cadmium, lead, and manganese from the solution.

IV. DESIGN AND IMPLEMENTATION DESIGN

Brainstorming and Problem Analysis: Recognized the necessity for an effective and sustainable heavy metal remediation method to overcome the inefficiencies of traditional processes such as chemical precipitation and ion exchange.

Concept Design: Developed a biodegradable fiber mat loaded with Pseudomonas aeruginosa and Aspergillus niger, harnessing their complementary metal sequestration mechanisms (adsorption and enzymatic pathways).

Material Selection: Chose jute as the substrate fiber because of its biodegradability, low cost, and compatibility for microbial growth.

Implementation

Preparation of Fiber Mat: Sterilized jute mat and made it ready for inoculation with bacterial and fungal cultures.

Microbial Culturing: Plated Aspergillus niger and Pseudomonas aeruginosa in nutrient-enriched media.

Inoculation: Co-inoculate the fiber mat with microbial cultures in aseptic conditions.

Experimental Setup: Exposure of the inoculated mat to synthetic heavy metal solutions to assess sequestration efficiency.

Monitoring and Analysis: Measuring heavy metal removal efficiency through Atomic Absorption

Spectroscopy (AAS) and conclusions are made.

Tools and Techniques Used Tools

Autoclave: Used for sterilizing the fiber mat and media to ensure a sterile environment. Incubator: To ensure temperature and humidity conditions optimal for bacterial growth.

Shaking incubator: Ensures a controlled environment with conditions of regulated temperature and agitation to ensure even growth of microorganisms or cells.

Atomic Absorption Spectroscopy (AAS): Used for determining heavy metal concentrations prior to and following remediation.

Laminar Air Flow Cabinet: Sterile working area to ensure a contamination-free environment.

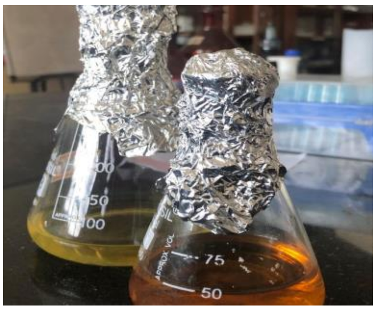
Techniques

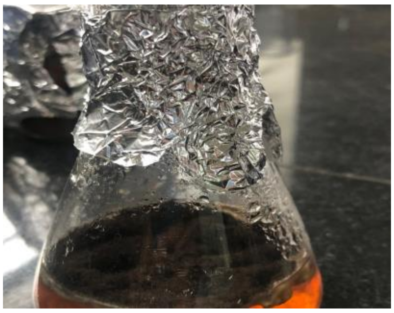
Sterilization: Assured asepsis by autoclaving all materials.

Co-Culturing: Maximize the growth conditions (temperature, pH, and nutrients) for ensuring compatibility of P. aeruginosa and A. niger.

Heavy Metal Analysis: Measuring sequestration efficiency by AAS.

V. RESULTS & DISCUSSIONS





The result from the Atomic Absorption Spectrometry (AAS) analysis was utilized for plotting a graph, where the serial number of the readings was taken along the x-axis and the concentration of the heavy metal along the y-axis.

Concentration of lead (Pb) vs number of readings: The trend on the graph shows a decline in the concentration of lead with successive readings. This reflects efficient sequestration of lead over time by

the jute mat microbes. The final concentration reached on 22nd may 2025 is 1.397ppm (or 1.397mg/L).

Concentration of cadmium (Cd) against the number of readings: The graph shows a decreasing concentration of cadmium with successive readings. This indicates that more time is needed for sequestration of cadmium. The final concentration of cadmium reached on 22nd may 2025 is 7.217ppm (or 7.217mg/L).

Concentration of manganese (Mn) against the number of readings: The graph shows a slight rise and then fall in the concentration of manganese over the readings. We can conclude that more time is taken in sequestration but takes relatively less time compared to cadmium. The last concentration achieved on 22nd may 2025 is 12.587ppm (or 12.587mg/L).

VI. TESTING

Preparation of Heavy Metal Solutions

Made ready synthetic heavy metal solutions of known lead (Pb), cadmium (Cd), and manganese (Mn) concentrations to mimic actual field contamination conditions.

Made precise measurements with analytical-grade reagents and confirmed concentrations by initial Atomic Absorption Spectroscopy (AAS) measurements.

Experimental Setup
Soaked the microbial-inoculated jute mats in individual containers of heavy metal solutions.

Manipulated environmental factors like temperature, pH, and aeration to provide favorable conditions for microbial growth.
Ensured sterile conditions during the beginning of the experiment to avoid contamination from outside sources. Sampling Protocol

Sampled solution samples at fixed intervals (Every 24 hours) to monitor changes in heavy metal content over time.

Ensured repeated sampling was done with fixed volumes to prevent bias and ensured aseptic conditions to maintain sample integrity.
Measurement and Analysis

Measured the content of heavy metals within the sampled solutions using Atomic Absorption Spectroscopy (AAS) to provide accurate quantification.
Measured pH changes in the solutions to track possible changes in chemical dynamics caused by microbial activity.
Data Recording and Visualization

Tabulated AAS readings and graphed plots for:

Lead (Pb) concentration vs. Reading (number of observations) Cadmium (Cd) concentration vs. Reading (number of observations) Manganese (Mn) concentration vs. Reading (number of observations)
Trend observed in the graphs to ascertain sequestration efficiency for every heavy metal: Lead (Pb): A declining trend clearly, showing efficient sequestration.
Cadmium (Cd): A downward trend gradually, reflecting a decelerated rate of sequestration.
Manganese (Mn): Increase initially followed by a fall, may indicate microbial acclimatization over time.

VII. CONCLUSION

This project was to evaluate the potential of jute mat as a microbial substrate, its environmental sustainability, and efficiency in heavy metal remediation. From our research, some important conclusions were reached:

Efficient substrate for microbial growth: The jute fiber mat was found to be an efficient substrate for the growth of Pseudomonas aeruginosa and Aspergillus niger, facilitating microbial colonization and stability.

Biodegradability and flexibility: The biodegradable property of the jute mat provides minimal environmental disturbance, thereby a healthy option for bioremediation.

Efficiency of heavy metal sequestration: The microbial-impregnated mat exhibited the ability to sequester

several heavy metals, with both fungi and bacteria exhibiting certain affinities towards different metals.

Practical significance and scope for the future: The mat presents a promising means of heavy metal removal in polluted environments and indicates scope for scalability and field application.

Personal Reflection

Dhruthi-"I learned how to tackle intricate problems methodically in this project. Learning about the interactions of microbes and heavy metals exposed me to thinking creatively and testing different solutions. It was satisfying to observe how even slight changes, such as optimizing growth conditions, could result in profound outcomes, and it gave me more confidence in managing scientific obstacles."

Rachana-"Applying theoretical knowledge to a real-world problem was eye-opening. I learned how foundational concepts, like microbial bioremediation and metal sequestration, can have tangible environmental benefits. This experience deepened my appreciation for how science can directly impact and improve lives."

Samuel-"Closely working with colleagues on this project taught me improved communication and cooperation skills. We all had varying strengths, and I learned how the integration of viewpoints can develop innovative solutions. This further reiterated the power of collective work towards fulfilling intricate objectives."

Omkar-"This project really enhanced my technical skills, especially in the management of microbiological methods and analytical equipment such as atomic absorption spectroscopy. These practical experiences enhanced my knowledge of the methodologies and their relevance to research and industry."

Vaishali-"Apart from the technical skill, this project opened my eyes to a new vision of sustainability and care of nature. It encouraged me to consider creative, green approaches to solving world problems and to be responsible for making significant contributions to the environment."

REFERENCES

[1] Gul, Isma, et al. "Microbial Strategies for Lead Remediation in Agricultural Soils and Wastewater: Mechanisms, Applications, and Future Directions." Frontiers in Microbiology, vol. 15, 2024, Article 1434921. Frontiers, doi:10.3389/fmicb.2024.1434921

[2] Chellaiah, Edward Raja. "Cadmium (heavy metals) bioremediation by Pseudomonas aeruginosa: A Minireview." Applied Water Science, vol. 8, no. 154, 2018, pp. 110. Springer

[3] Ghosh, Soumya, et al. "Filamentous Fungi for Sustainable Remediation of Pharmaceutical Compounds, Heavy Metal, and Oil Hydrocarbons." Frontiers in Bioengineering and Biotechnology, vol. 11, 2023, article 1106973

[4] Javaid, Amna, Rukhsana Bajwa, and Tariq Manzoor. "Biosorption of Heavy Metals by Pretreated Biomass of Aspergillus niger." Pak. J. Bot., vol. 43, no. 1, 2011, pp.419425.

[5] Qader, Muzhda Qasim, and Yahya Ahmed Shekha. "Bioremediation of Heavy Metals by Using Aspergillus niger and Candida albicans." ZANCO Journal of Pure and Applied Sciences, vol. 35, no. 3, 2023, pp. 180-186

[6] Abo-Alkasem, Mohamed I., et al. "Microbial Bioremediation as a Tool for the Removal of Heavy

[7] Abdullahi, M., and Machido, D. J. "Heavy Metals Resistance Potential of Some Aspergillus spp. Isolated from Tannery Wastewater." Nigerian Journal of Basic and Applied Sciences, vol. 25, 2017, pp. 120-129.

[8] Abbas, S. Z., Rafatullah, M., Ismail, N., and Lalung, J. "Isolation, Identification, and

Characterization of Cadmium Resistant Pseudomonas sp. M3 from Industrial Wastewater." Journal of Waste Management, 2014,https://doi.org/10.1155/2014/160398.
[9] Awasthi, Garima, Anjali Chester, Rachna Chaturvedi, and Jyoti Prakash. "Study on Role of Pseudomonas aeruginosa on Heavy Metal Bioremediation." International Journal of Pure and Applied Bioscience, vol. 3, no. 4, 2015, pp. 92-100.
[10] Wei, Yuqiu, et al. "Ecological Risk Assessment of Heavy Metal Pollutants and Total Petroleum Hydrocarbons in Bohai Sea Sediments, China." Marine Pollution Bulletin, vol. 184, 2022, p. 114218.
[11] Metals." Bulletinof the National Research Centre, vol. 47, no. 31, 2023. doi:10.1186/s42269-023-01006-z.
[12] Petkovi, Katarina, et al. "Potential of Aspergillus niger in Bioremediation of Contaminated Soils." Acta Mycologica, vol. 50, no. 1, 2015, pp. 1-10.
[13] Abdullahi, M., and Machido, D. J. "Heavy Metals Resistance Potential of Some Aspergillus spp. Isolated from Tannery Wastewater." Nigerian Journal of Basic and Applied Sciences, vol. 25, 2017, pp. 120-129.
[14] Abbas, S. Z., Rafatullah, M., Ismail, N., and Lalung, J. "Isolation, Identification, and Characterization of Cadmium Resistant Pseudomonas sp. M3 from Industrial Wastewater." Journal of Waste Management, 2014,https://doi.org/10.1155/2014/160398.
[15] Awasthi, Garima, Anjali Chester, Rachna Chaturvedi, and Jyoti Prakash. "Study on Role of Pseudomonas aeruginosa on Heavy Metal Bioremediation." International Journal of Pure and Applied Bioscience, vol. 3, no. 4, 2015, pp. 92-100.
[16] Wei, Yuqiu, et al. "Ecological Risk Assessment of Heavy Metal Pollutants and Total Petroleum Hydrocarbons in Sediments of the Bohai Sea, China." Marine Pollution Bulletin, vol. 184, 2022, p. 114218.