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REMOVAL OF HEAVY METALS FROM CETP SLUDGE USING THE PROCESS OF BIOLEACHING

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Abstract: The issue of heavy metals in India due to industrial waste sludge stems from the inadequate management of toxic industrial byproducts. Rapid industrialization generates waste containing heavy metals like lead, cadmium, and mercury, which are often improperly disposed of in open dumps or water bodies. This study encompasses the industrial waste sludge collected from common effluent treatment plant at Kurkumbh MIDC in Pune, to examine presence of heavy metals in the sludge sample and further carry out the process of bioleaching. This study aims to assess the efficiency of microorganisms in removing heavy metals through bioleaching. The metals present in the sludge are Zinc, Copper, and Iron. Following the bioleaching process for 14 days, the total extraction percentages of these heavy metals were determined to be 84.33% for Zinc, 71.03% for Copper, and 25.04% for Iron. In conclusion, bioleaching stands as a promising technology for the removal of heavy metals from industrial waste sludge, offering a sustainable and eco-friendly approach to mitigate the detrimental effects of heavy metal contamination on our environment and public health.

Index Terms -CETP sludge, Heavy metal removal, Aspergillus niger, Bioleaching.

I. INTRODUCTION

Heavy metals are ubiquitous in industrial wastewater, posing a significant threat to environmental health due to their toxicity and persistence in ecosystems. Among the various sources of heavy metal contamination, Common Effluent Treatment Plants (CETPs) are noteworthy contributors, as they receive wastewater from multiple industries, concentrating heavy metals in their sludge. The accumulation of heavy metals in CETP sludge not only jeopardizes soil and water quality but also poses risks to human health and biodiversity.

In recent years, the exploration of eco-friendly and cost-effective methods for the removal of heavy metals from industrial wastewater has gained considerable attention. Bioleaching, a biotechnological approach, has emerged as a promising technique for remediating heavy metal-contaminated environments. Bioleaching utilizes the metabolic activities of microorganisms to solubilize and mobilize metals from solid matrices into a solution, facilitating their subsequent removal.

The significance of employing bioleaching for the remediation of CETP sludge lies in its potential to mitigate the environmental impact of heavy metal contamination while offering a sustainable solution for waste management. By harnessing the natural abilities of microorganisms to metabolize heavy metals, bioleaching presents an environmentally benign alternative to conventional chemical methods, minimizing the generation of hazardous by-products and reducing energy consumption.

However, despite its potential benefits, the application of bioleaching for the removal of heavy metals from CETP sludge is still in its nascent stages. Several challenges such as the optimization of process parameters, selection of suitable microbial consortia, and scale-up feasibility need to be addressed to enhance the efficiency and viability of this approach.

The inefficient management of heavy metal-containing CETP sludge poses a critical environmental challenge, necessitating the development of sustainable remediation strategies. Conventional treatment methods often fall short in achieving complete removal of heavy metals from the sludge, leading to persistent environmental contamination and regulatory non-compliance. Additionally, these methods are often resource-intensive and may generate secondary pollutants, exacerbating the environmental footprint.

Bioleaching holds promise as an eco-friendly and economically viable alternative for the remediation of CETP sludge; however, its practical application is hindered by several technical and logistical hurdles. Addressing these challenges requires a comprehensive understanding of the microbial processes involved in bioleaching, optimization of operational parameters, and evaluation of its scalability for industrial implementation.

The recovery of precious metals from solid waste through bioleaching has become a research hotspot in recent years. Thus, different strategies, such as chemical sulfuric acid leaching and mixed consortium bioleaching, were adopted to extract copper from Copper-Containing Electroplating Sludge. [1] Electro kinetic remediation and bioleaching show promising potential in removing heavy metals; however, challenges such as energy consumption and cost remain. Additional research is required to bridge existing knowledge gaps and enhance the effectiveness and efficiency of these methods. [2] Bioleaching is an environmentally friendly technology for commercial metal recovery from metal ores in mining. There is a growing interest in adapting the technology to remove heavy metals from solid wastes such as fuel ash and sewage sludge. However, bioleaching is not ready for such applications yet because of some technical hurdles. [3] Bioleaching can be effective method of metal recovery for electroplating sludge, which could not only convert metals into products, but also bioleaching residues could be further used as raw building materials. [4] A. ferrooxidans are effective to bioleaching heavy metals from PCB sludge, electroplating sludge, and stainless steel sludge. [5] Acidithiobacillus ferrooxidans are effective in bioleaching treatment of digested sludge for heavy metal removal and dewaterability improvement. Increasing ferrous iron loading improved sludge dewaterability but had little effect on heavy metal removal. Bioleaching treatment resulted in the reduction of heavy metals, especially at higher ferrous iron loadings.[6] Electroplating sludge is classified by environmental agencies as a hazardous waste, the disposal of which can be a serious environmental concern. the filtrated culture of Aspergillus niger has the ability to recover metals from electroplating sludge and detoxify it.[7] pH-controlled fermentation and anaerobic metal bioleaching. Alkaline fermentation resulted in the highest metal solubilization and best performance. Alkaline fermentation allowed for the solubilization and extraction of metals, resulting in an alkaline biosolid with lower metal concentrations and bioavailability. [8] Anaerobically digested (AD) sludge is widely applied to agricultural land as fertilizer. However, heavy metals in AD sludge potentially pose a significant threat to environment. Bioleaching enables the economical and safe reuse of excess sludge generated during biological wastewater treatment without adding external chemicals.[9] Sludge after bioleaching can be used for agricultural fields such as gardens, pastures, and other land for nonedible crops. The bioleaching method is feasible for the environmentally friendly treatment of waste sludge. [10]

Optimizing parameters like pH, temperature, and microbial activity is critical to improving extraction efficiencies in bioleaching processes. Additionally, comparing bioleaching with traditional chemical extraction methods can offer valuable insights into its economic and environmental advantages. However, scaling up bioleaching for industrial application requires careful consideration of factors such as scalability, cost-effectiveness, and regulatory compliance. Despite the importance of iron removal efficiency, this aspect has not been thoroughly researched, and existing studies have primarily focused on specific types of sludge. Therefore, our study seeks to address this gap by investigating the removal efficiency of metals like copper, zinc, and iron from CETP sludge from Kurkumbh MIDC, Pune using A. niger.

Therefore, this project aims to investigate the efficacy of bioleaching in removing heavy metals from CETP sludge. By addressing these research objectives, this study seeks to contribute to the development of sustainable solutions for the management of heavy metal-contaminated wastewater generated by industrial activities.

II. LITERATURE REVIEW

Table 1 Summary of Literature Paper.

Sr. No.	Author Name	Title of paper	Outcomes
1	Ying Xu et al.	Comparison of bioleaching and electrokinetic remediation processes for removal of heavy metals from wastewater treatment sludge.	Electrokinetic remediation and bioleaching show promise in removing heavy metals, challenges such as energy consumption and cost remain. The article also provides a list of references covering various techniques and methods related to heavy metal removal from sewage sludge.
2	Tingyue Gua et al.	Advances in bioleaching for recovery of metals and bioremediation of fuel ash and sewage sludge.	Highlights the importance of optimizing operating parameters and selecting effective microbes and pretreatment methods. Discusses the potential impact of ultrasound on bacterial metabolic activity in bioleaching and its ability to enhance enzymatic and microbial activities. It mentions the use of pretreatment methods like mutation breeding to increase metal extraction efficiency.
3	Wenbo Zhou et al.	Cleaner utilization of electroplating sludge by bioleaching with a moderately thermophilic consortium: A pilot study.	A microbial consortium consisting of three strains of microorganisms was cultivated. Optimal pH -1.5. Temperature - 45 °C. Bacterial liquid ratio - 40%. Liquid-solid ratio - 4:1. Leaching time - 5 hours. The total removal rate of heavy metals was over 95% for Zinc, Copper, Nickel and Chromium.
4	Jianfeng Bai et al.	Bioleaching for extracting heavy metals from electronic waste sludge.	A. ferrooxidans are used to extract heavy metals. PCB sludge - Bioleaching rates of 76%, 74%, and 72% for Cu, Ni, and Zn respectively. Electroplating sludge - Bioleaching rates of 81.43%, 75.32%, and 78.97% for Cu, Ni, and Zn respectively. Stainless steel sludge - bioleaching rate of 78.16% for Zn.

5	Sima Nikfar et al.	Enhanced bioleaching of Cr and Ni from a chromium-rich electroplating sludge using the filtrated culture of Aspergillus niger.	Aspergillus niger is used. Ni and Cr recoveries were 95.7% and 53% respectively. Optimal condition - 10 g/L pulp density, 66 C leaching temperature, and minimum leaching duration of 1 day using fungal filtrated culture.
6	Hang Liu et al.	Study on bioleaching of heavy metals and resource potential from tannery yard sludge.	This study examined the use of acidophilic sulfur-oxidizing bacteria. The removal efficiencies of the heavy metals Cr, Cu, Cd, Pb, Zn, and Mn were 96.36%, 88.49%, 99.24%, 93.73%, 99.71%, and 98.11%, respectively.
7	Zhiyao Wang et al.	Bioleaching of toxic metals from anaerobically digested sludge without external chemical addition.	Candidatus Ni- trosoglobus bacteria is used. Cu and Zn, were solubilized with high efficiencies of 88 ± 4% and 96 ± 3%, respectively.
8	Hatice Yesil et al.	Extent of bioleaching and bioavailability reduction of potentially toxic heavy metals from sewage sludge through pH-controlled fermentation.	The dominant bacterial phyla in the reactors were Chloroflexi, Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, TM7 (Sacchariabacteria), and Acidobacteria. Compared to raw sludge, bioavailable fractions of Cr, Cu, Ni, and Zn were decreased from 47.0% to 32.3%, 75.0% to 40.3%, 90.6% to 54.4%, and 61.0% to 34.9%, respectively, through alkaline fermentation.
9	Jianxing Sun et al.	Bioleaching of Copper-Containing Electroplating Sludge.	Bioleaching of copper-containing electroplating sludge using a mixed microbial consortium (Leptospir- illum ferriphilum CS13, Acidithiobacillus caldus S2 and Sulfobacillus acidophilus TPY) was investigated. Copper bio- leaching efficiency reached 94.3% on day 7 (21.1% higher than that of chemical leaching).
10	G. Cai et al.	Effect of ferrous iron loading on dewaterability, heavy metal removal and bacterial community of digested sludge by Acidithiobacillus ferrooxidans.	Acidithiobacillus ferrooxidans are used. Increasing ferrous iron loading improved sludge dewaterability but had little effect on heavy metal removal. In the presence of A. ferrooxidans, the removal of Ni, Mn and Zn reached 93%, 88% and 80%, respectively, at a ferrous iron loading of 21%.

III. Methods and methodology

3.1 Study Area:

The sludge utilized for our research was sourced from the CETP located in Kurkumbh MIDC, Pune. Kurkumbh has come up as a Pharmaceutical and Chemical hub centre on the Pune Sholapur Highway at about 65 Kms. from Pune in Taluka – Daund, District–Pune in Maharashtra. MIDC has constructed Common Effluent Treatment Plant of 1 MLD capacity and the plant is fully operational. New industrial units with zero discharge are only allowed to be set up the industries in Kurkumbh. However, subsequently the units can apply and take the membership of CETP and may discharge the effluent in CETP once permission is granted.

3.2 Characterization of the sludge:

Energy-dispersive X-ray spectroscopy (EDS) is a powerful analytical technique used to identify the elemental composition of materials. It works by detecting the characteristic X-rays emitted by a sample when it is bombarded with a focused electron beam in a scanning electron microscope (SEM). The elemental composition of the sample was done by energy dispersive X-ray spectroscopy (EDS) which is as follows.

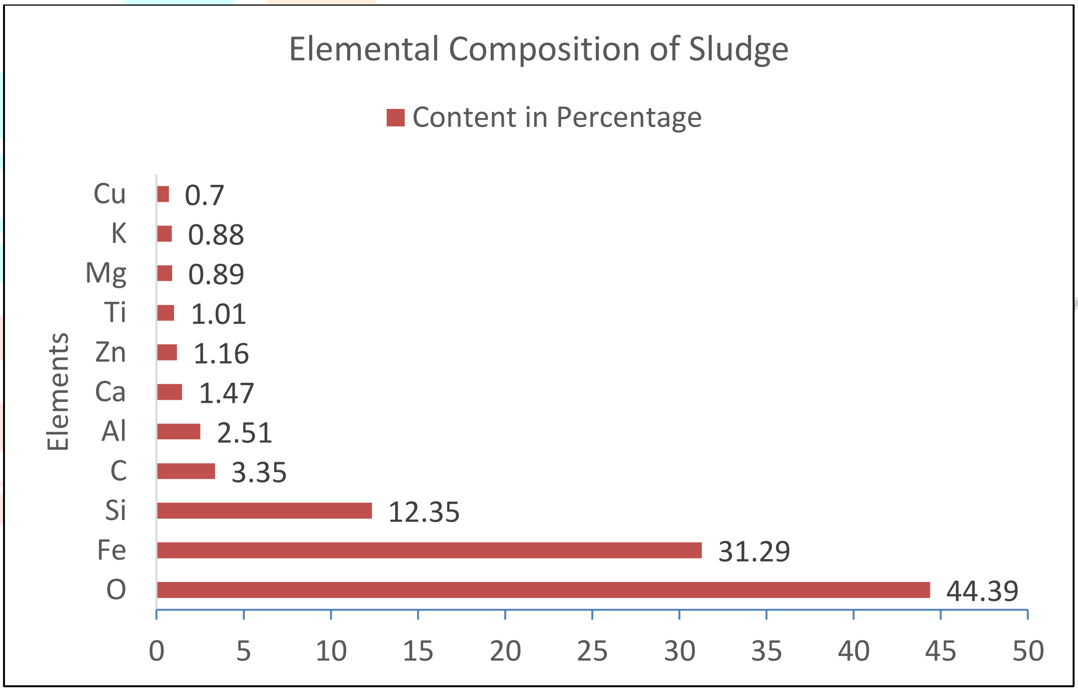

Fig No.1. Representation of elemental composition of sludge sample

3.3 Determination of heavy metals:

Inductively coupled plasma mass spectrometry :-

Presence of heavy metals in the sludge was determined by inductively coupled plasma mass spectrometry (ICP-MS). ICP (Inductively Coupled Plasma) Spectroscopy is an analytical technique used to measure and identify elements within a sample matrix based on the ionization of the elements withing the sample. Mass Spectrometer (MS) separates the ions out by their mass-to-charge ratio after going through the ICP, and the detector counts the number of selected ions per second which allows the instrument to determine the concentration of each chosen element. Here is a breakdown of how ICPMS works:

1) **Sample Introduction**: A small amount of the sample, often in liquid form, is introduced into the ICP source. This can be done using a nebulizer, which converts the liquid into a fine aerosol, making it suitable for analysis.
2) **Inductively Coupled Plasma (ICP):** The sample aerosol is then introduced into an extremely hot (~10,000°C) and ionized argon plasma. This high-temperature plasma source ensures that the sample is completely atomized and ionized.
3) **Ionization:** In the ICP, the high temperature causes the atoms and molecules in the sample to break down into their constituent ions. This process results in the formation of positively charged ions
4) **Mass Spectrometry (MS):** The ionized particles from the ICP are then directed into the mass spectrometer. In the MS, these ions are separated based on their mass-to-charge ratio (m/z). The ions are accelerated and then passed through a magnetic field, which causes them to follow curved paths. The degree of curvature is directly related to the mass-to-charge ratio, allowing for separation of different ions.
5) **Detection and Quantification:** As the ions of different elements pass through the mass spectrometer, they are detected by a detector, such as an electron multiplier or a time-of-flight detector. The intensity of the ion signals is proportional to the concentration of each element in the sample. This information is then processed to quantify the elemental composition.

ICP-MS is highly sensitive and can detect trace elements at very low concentrations (parts per trillion or even lower), making it valuable in various fields, including environmental monitoring, geochemistry, metallurgy, pharmaceuticals, and more. It offers the ability to analyze a wide range of elements simultaneously, making it a versatile tool for elemental analysis in research and industry.

Acid digestion :-

ICP analysis requires the use of liquified sample solutions, so solid samples and biological samples are often digested prior to analysis. Sample is prepared by making aqua regia. The explanation of the process is as follows:

1) 1ml of Nitric acid (HNO_3) added to 3ml of Hydrochloric acid (HCl).

2) Sludge weighing 0.1 grams is added to the solution and allowed to sit for 5 to 10 minutes.

3) Above procedure is repeated until the sludge is thoroughly digested.

4) Once the sludge is digested, distilled water is added to bring the total solution volume to 100ml. Sample prepared by above method is sent for the testing by ICP-MS method to check the concentration of heavy metal.

3.4 Identification of fungi species:

Identification and characterization of fungi species was done by Serial Dilution method and microscopic observation. Identifying fungi species through Serial dilution method and microscopic observation involves several steps.

1) 1) Petri dishes and culture media are autoclaved for half an hour at a temperature of 121°C and a pressure of 15 psi to prevent contamination.
2) Weighed 1 gram of sludge sample and placed it in a test tube containing 10 ml of distilled water (DW) (stock), and mixed it thoroughly.

3) Prepared 6 test tubes, each containing 9 ml of distilled water (DW).
4) Added 1 ml of the stock solution to the first tube, resulting in the first dilution of 1/10 (10-1).
5) Added 1 ml of the first dilution to the second tube, resulting in the second dilution of 1/100 (10-2).
6) Added 1 ml of the second dilution to the third tube, resulting in the third dilution of 1/1000 (10-3).
7) Added 1 ml of the third dilution to the fourth tube, resulting in the fourth dilution of 1/10000 (10-4).
8) Added 1 ml of the fourth dilution to the fifth tube, resulting in the fifth dilution of 1/100000 (10-5).
9) Added 1 ml of the fifth dilution to the sixth tube, resulting in the sixth dilution of 1/1000000 (10-6).
10) Placed 1 ml of each diluted sample in a Petri dish and then poured the culture media, ensuring thorough mixing.
11) Allowed the Petri dishes to solidify undisturbed, then incubated them for 7 days at 30-35°C.
12) Observed fungal growth daily.

3.5. BIOLEACHING

3.5.1 Colony isolation and pure culture :-

1) **Examine Colonies**: After the incubation period, the agar plates are visually inspected for the presence of colonies. Aspergillus niger colonies typically exhibit distinct characteristics such as being black in color, with a powdery texture.
2) **Select Well-Isolated Colonies:** Colonies are selected that appear to be characteristic of Aspergillus niger and are well-separated from neighboring colonies.
3) **Transfer to Fresh Plates:** Using a sterile inoculating loop or needle, the selected colonies are transferred to petri dishes which are filled with culture media. The transfer is done by streaking the loop across the surface of the petri dish to pick up a portion of the colony and then streaking it onto the surface of the fresh petri dish.
4) **Incubation:** Incubated the petri dishes for 30 to 35^{0}C for seven days

3.5.2 Preparation of liquid culture and performing bioleaching :-

1) **Preparation of Dextrose Solution**: 1 gram of dextrose is dissolved in 600 ml of distilled water (DW) ensuring thorough dissolution.
2) **Mixing and Filtration: A** magnetic stirrer is utilized to completely disperse the dextrose in the solution. The mixture is filtered to remove any impurities.
3) **Sterilization:** The filtered solution is transferred into a jar with an airport lid. The jar is autoclaved at 121°C for 20 minutes under 15 psi pressure to sterilize the contents.
4) **Inoculation with A. niger:** After allowing the sterilized solution to cool for 12 hours, the culture of A. niger is introduced into the jar.
5) **Incubation Period**: The jar is sealed and incubated for 7 days to facilitate the growth of the fungus.
6) **Preparation of Bioleaching Flask**: A 500 ml conical flask is taken having A. niger culture and 10 grams of sludge is added to it.
7) **Sealing and Incubation**: The flask is sealed with aluminum foil to prevent contamination and incubated for 14 days.
8) **Post-Incubation Processing:** After the incubation period, the mixture is filtered to separate the sludge. Test is performed on the collected sludge to determine the final content of heavy metals.

3.6 Check removal efficiency:

Following the completion of the bioleaching process, the concentration of metals in the sludge was assessed through ICP-MS. A thorough comparative analysis was conducted to evaluate the levels of heavy metals present in the original untreated sludge versus the treated sludge. This comparison aims to quantify the removal efficiency achieved through the bioleaching process, providing valuable insights into the effectiveness of the treatment in reducing heavy metal content in the sludge.

IV. RESULT ANALYSIS

In our study, we looked at how well our sludge treatment process works by comparing the amount of harmful heavy metals in untreated sludge with treated sludge.

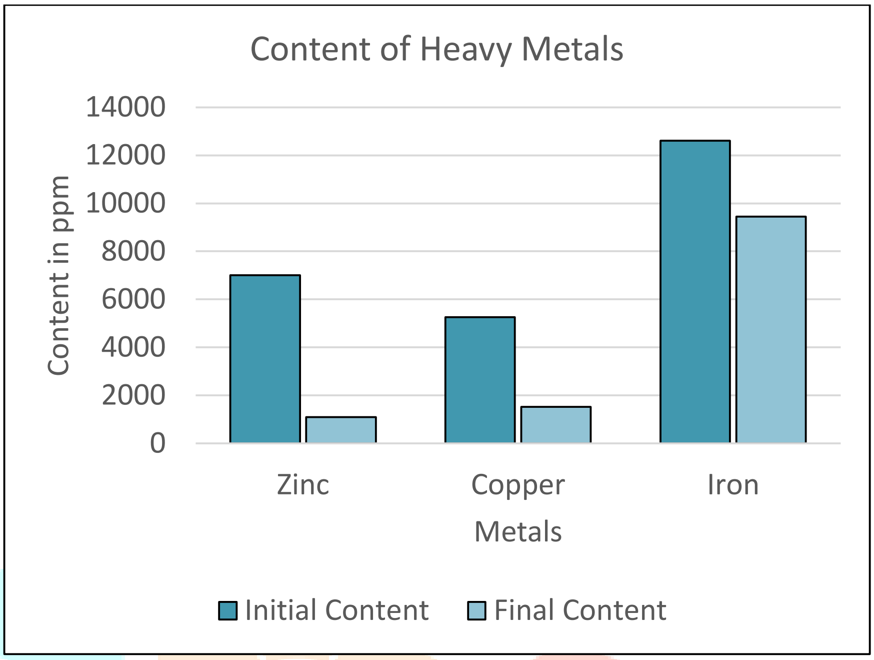

Fig No 2. Representation of Removal Efficiency of Heavy Metals

The bioleaching process effectively reduced the concentration of heavy metals in the untreated sludge. The initial concentrations of zinc, copper, and iron were 7009 ppm, 5252 ppm, and 12606 ppm respectively. After the bioleaching process, the concentrations decreased significantly. The total extractions of heavy metals at the end of 14th day were found to be 84.33% for zinc, 71.03% for copper, and 25.04% for iron.The bioleaching process demonstrated significant effectiveness in reducing the concentration of heavy metals, particularly zinc and copper. With extraction rates of 84.33% and 71.03% respectively, it highlights the efficiency of the selected bioleaching method in removing these metals from the sludge.

V. CONCLUSION

The bioleaching process applied to untreated sludge has demonstrated significant potential for the remediation of heavy metal contamination. Through this study, we have observed substantial reductions in the concentrations of zinc and copper, with extraction rates of 84.33% and 71.03% respectively. While the extraction of iron proved to be more challenging, achieving a rate of 25.04%, it highlights the complexity involved in treating certain metal species within the sludge. These findings underscore the effectiveness of bioleaching as a viable alternative to traditional chemical extraction methods for heavy metal remediation. The environmental implications of reducing heavy metal concentrations in sludge are substantial, contributing to the protection of soil and water quality and enhancing overall environmental sustainability. In conclusion, this study lays the groundwork for continued research and development in the field of bioleaching for heavy metal remediation, with the ultimate goal of providing effective and sustainable solutions for environmental protection and resource recovery.

Fine-tuning parameters such as pH, temperature, and microbial activity could potentially enhance extraction efficiencies further, warranting future research in this area. It would be insightful to compare the efficiency of bioleaching with traditional chemical extraction methods. Such a comparative analysis could provide valuable insights into the economic viability and environmental sustainability of bioleaching as a preferred remediation technique for heavy metal-contaminated sludge. Scaling up the bioleaching process from laboratory scale to industrial application warrants consideration. Factors such as scalability, cost-effectiveness, and regulatory compliance need to be evaluated to assess the feasibility of implementing bioleaching on a larger scale for real-world applications. Future research avenues may include exploring alternative microbial strains or bioleaching techniques to enhance the extraction efficiencies of heavy metals, particularly iron.

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