EXPLORING MICROBIAL APPROACHES FOR THE BIOREMEDIATION OF HEAVY METAL-CONTAMINATED SOILS

Ms. Mini Fernandez
Assistant Professor
Department of Biotechnology,
St. Francis College for Women, Hyderabad

Ms. Archana G
Department of Biotechnology,
St Francis Degree College for Women
Begumpet, Hyderabad

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Abstract

Bioremediation functions as a sustainable microbial-based approach to remove heavy metals from environments. The study examines how bacteria extracted from soil contamination areas of Balanagar (Hyderabad) and Eloor (Kerala) perform in bioremediation processes. Different biochemistry tests using IMViC procedures together with catalase activity tests helped identify multiple bacterial strains. This study examined metal absorption and microbial biomass growth along with heavy metal and Indole-3 acetic acid interrelations to assess microbial efficiency. Spectrophotometric measurements detected heavy metals to track their removal efficiency in the studied system. The laboratory data showed Gram-positive bacteria predominated in Sample 1 from Balanagar while Sample 2 from Eloor was characterized by Gram-negative bacteria. The analyzed samples proved to have catalase enzyme capabilities and presented equivalent outcomes from the IMViC testing method. The biomass quantity in Sample 2 grew significantly higher than Sample 1 particularly when Nickel was present yet Lead diminished biomass levels in both cases. The research demonstrates substantial nickel bioremediation capabilities of Gram-negative bacteria collected from Eloor yet more study needs to be done to optimize their performance. The study demonstrates how bacterial bioremediation handles heavy metal pollution while stressing the need to understand microbial metal uptake processes better to create stronger remediation solutions.

Keywords: *Bioremediation, Heavy metals, Bacteria, Soil contamination, Catalase activity, Metal absorption.*

Introduction

Bioremediation serves as an environmentally friendly and economically feasible technique which provides pollution treatment. The degradation of contaminants relies on living organisms including microbes alongside fungi and algae as well as plants through this method. The process of decontamination takes place at the actual site or in separate locations. Soil pollution reduction through bioremediation operates at a high rate of success in heavy metal removal. Latest research confirms that natural attenuation acts as a tool to let environmental conditions assist contaminant breakdown. (Kulshreshtha et al., 2014)

The process known as bioremediation involves guided biological waste breakdown that leads to pollutant breakdown through microbial operations. The breakdown process

depends on enzymes such as oxidoreductases together with lyases and transferases and hydrolases that help decompose different substrates. Bioremediation requires enzymatic reactions for its success as microbes need ideal environmental factors to multiply and remain active in the process. Repeated adjustments must happen to keep the optimal environmental conditions because they determine efficient pollutant breakdown. (Bala et al., 2022)

In situ bioremediation allows pollution cleanup of soil and groundwater by maintaining conditions in place for treatment. Microorganisms together with plants operate within the site to degrade contaminants through bioremediation procedures. The remediation system requires integration of microorganisms and geology with chemical analysis and engineering principles. Protecting the soil structure becomes possible through this method which also minimizes treatment expenses. (Jørgensen, 2007)

Plants through phytoremediation technology act as biological agents to clean polluted soil together with sediments and groundwater by both absorbing and changing environmental pollutants. The performance rate of phytoremediation relies on contaminant properties which mainly benefits substances that repel water. Plant tissues absorb hydrophilic substances using water transport that allows enzymatic conversion at the tissue level. Various toxicity reactions occur in hybrid poplar trees when exposed to chlorinated compounds yet these responses stem from chlorine atom variations. The scientific community investigates genetically modified plant technology to improve environmental toxin degradation. (Dietz & Schnoor, 2001)

A permeable reactive barrier system has been developed to treat acid sulfate soil leachate through the utilization of alkaline waste products. A laboratory study evaluated 13 different substances starting from recycled concrete materials up to limestone and fly ash to determine their practicality. Soluble compound and pH level measurement occurred through batch testing then acidic water tests (pH 3) assessed the leachable materials. The research identifies suitable materials which can effectively counter soil acidity. (Golab et al., 2006)

Ex-situ bioremediation includes soil excavation followed by separate site treatment which results in accelerated and monitored degradation while remaining more consistent despite its high cost and possible pollutant distribution risks. The use of this method provides better consistency yet the implementation costs are high and leads to possible pollution dissemination. Protective barriers need installation to stop contamination from occurring during treatment. (Da Silva et al., 2020b)

Heavy metals demonstrate ductility alongside tolerance toward cations and conductivity in addition to their malleability. Their numerous protons and heavy elemental weight distinguishes heavy metals as ingredients that exceed atomic number 20. A handful of such heavy metals including Co, Cu, Fe, Mn, Mo, Ni, V, and Zn serve essential biological functions while large quantities harm organisms. The toxic metals Pb, Cd, Hg and As provide no biological values while creating substantial risks to living beings that lead to both environmental destruction and health-related risks. (Chibuike & Obiora, 2014)

Industrial waste along with mining operations and sewage together with fertilizers and pesticides release heavy metals into the soil along with airborne particles that settle by rain

and sedimentation. Industrial activities together with transportation sector and farming produce pollution that leads to heavy metal contamination. Toxic metals in phosphoric fertilizers are known to exceed those present in nitrogen and potash fertilizers. The contamination levels of metals increase in vegetables situated close to industrial sites or roadways which disrupts their nutrient maintenance. Metallurgical operations together with paints and vehicle exhaust produce main pollutants of chromium, nickel, cadmium, and lead. (Kumari & Mishra, 2021)

Lead functions as one of the most dangerous environmental pollutants that results primarily from mining operations and fuel combustion as well as manufacturing practices. Inhalation of lead along with consumption of food and water allows entrance into the body whereas organic lead absorption occurs through the skin (Mehri, 2020). Cadmium exposure takes place through contaminated food and water and air because this metal exists in batteries and plastics and alloys. The main causes of human intake of cadmium stem from industrial emissions in addition to smoking habits while skin absorption remains minimal (Balali-Mood et al., 2021). Nickel functions as a required trace element in small doses however natural volcanic emissions together with industrial operations including electroplating and battery production introduce this element as an environmental contaminant. Excessive consumption of nickel leads to dangerous health effects while it serves as an activator for enzymes. (Al-Lami et al., 2020) Research utilizing photobioreactors has established that particular microalgal species eliminate multiple pesticides from agricultural runoff to reduce environmental toxicity. (García-Galán et al., 2020)

Biochemical procedures enabled by microorganisms convert pollutants to nontoxic substances such as water and carbon dioxide which reduces environmental damage. The procedure saves costs while needing minimal tools and power which enables treatment at the site location thus reducing transportation-related hazards. The cleanup technique enhances soil fertility additionally improves water quality to create sustainable environmental benefits. (Sharma, 2020)

A main drawback of bioremediation includes extended treatment times and secondary product formation together with requirements for constant environmental steadiness. The process success depends on finding sufficient microorganisms and some remediation procedures generate pollutants that exceed safe levels. Project complexity along with increased costs requires extended monitoring procedures. (Ayilara & Babalola, 2023)

Materials and Methods

1. Sample Collection

Soil samples were collected from two industrial areas: Balanagar, Hyderabad, and Eloor, Kerala. These locations were selected due to their high levels of industrial pollution and heavy metal contamination.

2. **Isolation of Microorganisms**

The isolation of heavy metal-resistant microorganisms from contaminated soil is critical for bioremediation applications. The method involves collecting soil samples, stepwise reduction techniques, and culturing microbes on heavy metal-containing substrates.

Identification is performed using morphological, biochemical, and molecular characterization. The chemicals used for the nutrient medium included potassium dihydrogen phosphate (KH_2PO_4) - 0.4 g, ammonium sulphate ($(NH_4)_2SO_4$) - 3.0 g, sodium citrate ($Na_3C_6H_5O_7$) - 3.0 g, copper sulphate ($CuSO_4$) - 0.01 g, zinc sulphate ($ZnSO_4$) - 0.005 g, ferrous sulphate ($FeSO_4$) - 0.001 g, cadmium chloride ($CdCl_2$) - 0.2 g, magnesium sulphate ($MgSO_4$) - 0.5 g, manganese sulphate ($MnSO_4$) - 0.1 g, peptone - 8.0 g, maltose - 5.0 g, agar-agar - 20.0 g (for solid medium), and distilled water up to 1 L. The chemicals were dissolved in 800 mL of distilled water, adjusted to a final volume of 1 L, and autoclaved at 121°C for 15-20 minutes. One gram of soil was weighed and added to 100 mL of liquid medium, followed by incubation at 25°C with continuous shaking at 100 rpm for 24 hours. Microbial growth was assessed via turbidity and plated onto solid medium for colony isolation.

3. Obtaining Pure Culture

A 1 mL aliquot of the liquid culture was streaked on an agar plate and incubated at 25°C for 24 hours. Colonies were selected based on morphology and further purified using the streak plate method.

4. Gram Staining

A differential staining method was used to classify bacteria based on cell wall composition. The procedure involved preparing a smear, air-drying, and heat-fixing. The smear was stained with crystal violet for one minute, rinsed, and treated with Gram's iodine. After another rinse, ethanol was used for decolourization, followed by counterstaining with safranin for 30 seconds. The stained smear was observed under an oil immersion microscope.

5. Biochemical and Microbiological Tests

The biochemical tests performed included the catalase test, which determined the presence of the catalase enzyme by adding hydrogen peroxide (H_2O_2), with a positive result indicated by bubble formation. The IMViC tests included the Indole Test, which confirmed Indole production with a red colour after adding Kovac's reagent; the Methyl Red (MR) Test, which showed a red colour for stable acid production; and the Voges-Proskauer (VP) Test, which indicated acetoin production by a red/pink color development.

6. Determination of Heavy Metal Uptake by Bacteria

The materials used included heavy metal salts ($Pb(NO_3)_2$, $Ni(NO_3)_2$, $Cd(NO_3)_2$), beef extract broth, and isolated bacterial cultures. The procedure involved preparing beef extract broth and sterilizing it. A solution containing 2.5 mg of metal salt in 2.5 mL distilled water was added to 25 mL of beef extract broth, followed by bacterial inoculation. The cultures were incubated at 25°C with shaking at 100 rpm for five days. The residual metal concentration was measured spectrophotometrically.

7. Relationship of Heavy Metals with Indole-3-Acetic Acid (IAA)

The materials used included a 0.1% L-Tryptophan solution, beef extract broth, and bacterial cultures. The bacterial cultures were mixed with beef extract broth and L-Tryptophan, followed by incubation at 25-30°C for 48 hours. The presence of IAA was tested using Salkowski reagent, and absorbance was measured at 535 nm.

8. Determination of Biomass Accumulation

Bacterial cultures were centrifuged at 10,000 rpm for 5 minutes. The presence of IAA was tested using Salkowski reagent. The bacterial biomass was dried and weighed. Biomass accumulation was calculated using the formula: Biomass Accumulation = V (media volume) / m (dry biomass weight).

9. Effect of Temperature and pH on Bacterial Growth

The pH was adjusted using 1N HCl (for pH 3-7) or 1N NaOH (for pH 7-9). Bacterial cultures were incubated at varying temperatures. Optical density (OD) was measured at 600 nm to assess bacterial growth.

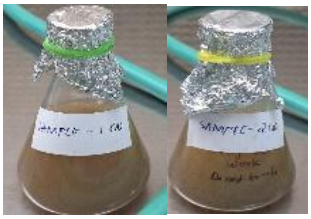
Results and Discussions

1. Characteristics of Soil Samples

Sample 1 (Balanagar Industrial Site) is located in an area with granitic rocks, pegmatite, and dolerite dykes. The soil has a yellowish to reddish-brown color with moderate permeability. Heavy rainfall may cause surface runoff and erosion. Sample 2 (Eloor Industrial District) appears dark or reddish-brown due to industrial contamination. It has a clayey or silty texture with poor aeration and is often compacted. Chemical residues result in a noticeable acidic odour and hardened surface.

2. Isolation of Microorganisms

Microbial growth was observed after incubating soil samples in a liquid nutrient medium for 24 hours.Sterile media was inoculated and incubated, leading to bacterial growth, confirming the presence of metal-resistant strains

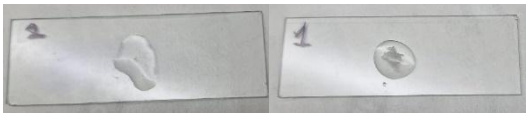


(a) (b)

Figure 1: Samples isolated in nutrient medium (a) Sample 1 (b) Sample 2

3. Obtaining Pure Cultures

Streak plating was performed, and microbial colonies were observed after 24 hours at 37°C.The use of nutrient agar medium allowed the isolation of bacterial colonies, ensuring pure cultures for further analysis.

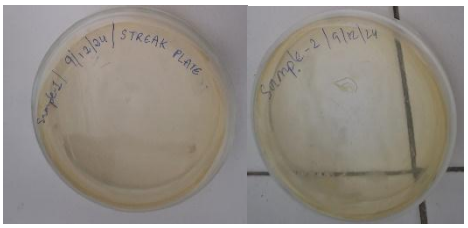


(a) (b)

Figure 2: Results of streak plating (a) Sample 1 (b) Sample 2

4. Gram Staining:

Gram staining confirmed distinct bacterial types, aiding in species characterization and further biochemical testing. Sample 1 exhibited purple-coloured cells (Gram-positive bacteria), while Sample 2 showed red-coloured cells (Gram-negative bacteria).



(a) (b)

Figure 3: Results of Gram staining (a) Sample 1 (b) Sample

5. Biochemical and Microbiological Tests

Catalase Test: Oxygen bubble formation in both samples indicated the presence of catalase-positive bacteria (Enterobacteriaceae, Vibrionaceae, or Pseudomonadaceae).

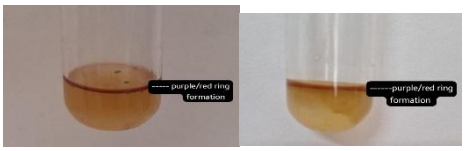


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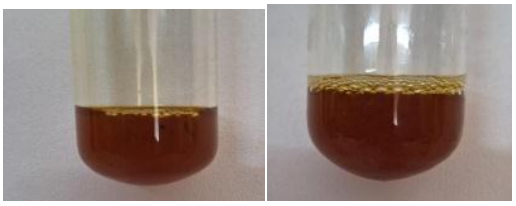
Figure 4: Results of catalase activity (a) Sample 1 (b) Sample 2

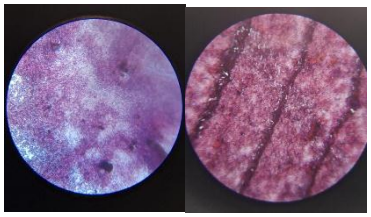
Indole Test: Positive result indicated potential presence of *Escherichia coli* or *Vibrio cholerae*.



(a) (b)

Figure 5: Results of Indole test (a) Sample 1 (b) Sample 2

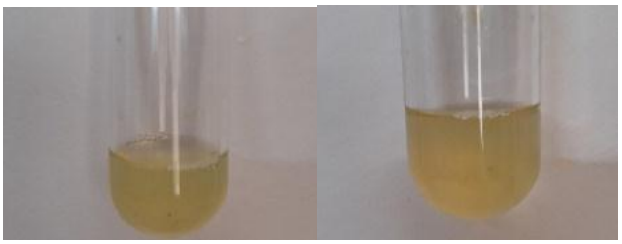
Methyl Red Test: Red colour confirmed the presence of acid-producing bacteria belonging to Enterobacteriaceae.



(a) (b)

Figure 6: Results of Methyl Red test (a) Sample 1 (b) Sample 2

Voges-Proskauer Test: Negative result (yellow top layer) indicated the absence of glucose fermentation via acetoin production.



(a) (b)

Figure 7: Results of Voges-Proskauer test (a) Sample 1 (b) Sample 2

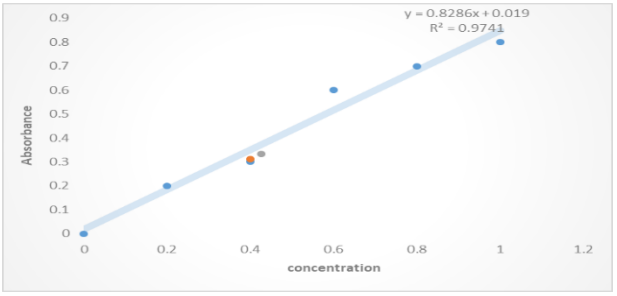
6 Heavy Metal Uptake Analysis

Heavy Metal	Sample 1	Sample 2	Interpretation
Lead Nitrate	0.273	0.111	Lower in sample 2, suggesting higher uptake.
Nickel Nitrate	0.312	0.334	Slightly higher in sample 2, indicating lower uptake.
Cadmium Nitrate	0.144	0.434	Significantly higher OD in sample 2, meaning lower uptake.

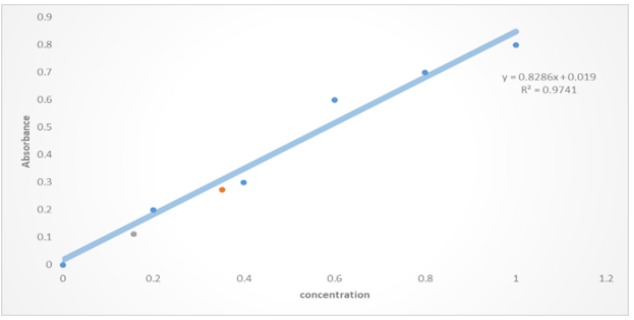
Table 1: Heavy Metal Analysis

S.no	Lead Nitrate	Nickel Nitrate	Cadmium Nitrate
Sample 1	0.273	0.312	0.144
Sample 2	0.111	0.334	0.434

Table 2: OD Values for Each Sample with Respect to Metals



(a)



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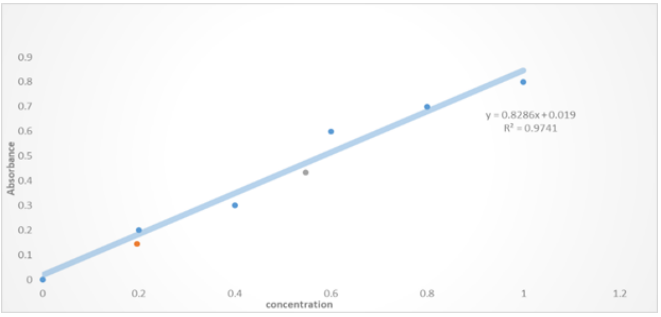

(c)

Figure 8: Spectrophotometric Analysis (a) Lead Nitrate Uptake (b) Cadmium Nitrate Uptake (c) Nickel Nitrate Uptake

7. Relationship of Heavy Metals with IAA Production

IAA Production by Bacteria: Sample 1: OD = 0.40 Sample 2: OD = 0.41

IAA production was significant in both samples, indicating their potential role in plant growth promotion under heavy metal stress. Gram-negative bacteria (Sample 2) exhibited slightly higher IAA production, suggesting better adaptability to metal stress.

HEAVY METAL	SAMPLE 1	SAMPLE 2
Nickel Nitrate	O.3692	0.7453
Cadmium Nitrate	0.0299	0.0747
Leaed Nitrate	0.1236	0.1009

Table 3: Biomass Accumulation

8. Biomass Accumulation

Sample 2 accumulated more biomass, particularly in Nickel and Cadmium treatments, indicating better metal tolerance. Lead exposure reduced biomass accumulation in both samples, confirming its toxicity.

9. Effect of pH on Bacterial Growth

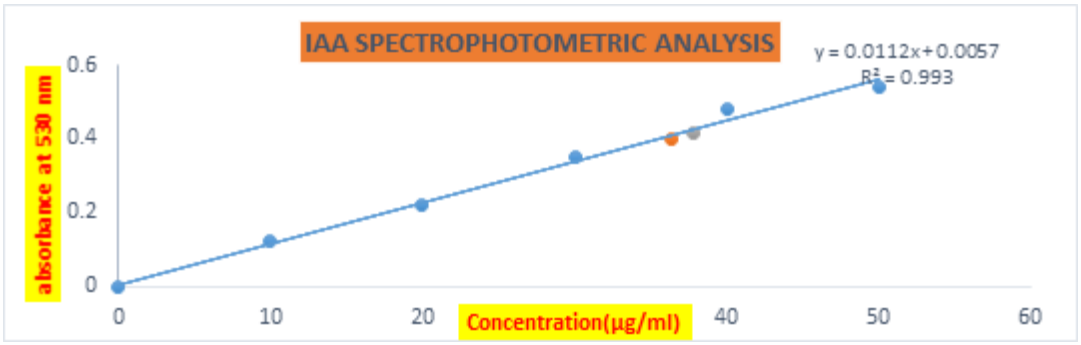

Figure 9: Spectrophotometric Analysis of Relation between Heavy Metals and Bacteria

PH	OD(SAMPLE 1)	OD(SAMPLE 2)
3	0.2	0.22
4	0.5	0.57
5	1.5	1.52
6	1.85	1.9
7	2.5	2.6
8	1.45	1.5
9	1.2	0.8

Table 4: Effect of pH on Bacterial growth

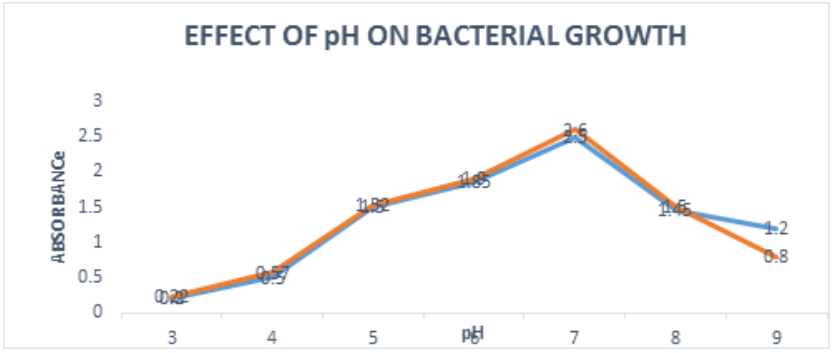

Figure 10: Effect of pH on Bacterial Growth

Optimal growth at pH 7; significant growth at pH 6 and 8. Reduced growth at pH 3 and 4 suggests low tolerance to acidic conditions. Sample 1 showed better tolerance to alkaline conditions (pH 9)

10. Effect of Temperature on Bacterial Growth

TEMPERATURE	OD(SAMPLE 1)	OD)SAMPLE 2)
20	1.4	1.3
25	2.6	2.5
30	3.0	3.2
35	2.8	2.5
40	1.7	1.5
45	0.8	0.5

Table 5: Effect of Temperature on Bacterial Growth

Optimal growth at 30°C, indicating mesophilic nature. Reduced growth at 20°C and 40°C, with significant decline at 45°C, suggesting heat sensitivity. Suitable for bioremediation in moderate temperature environments.

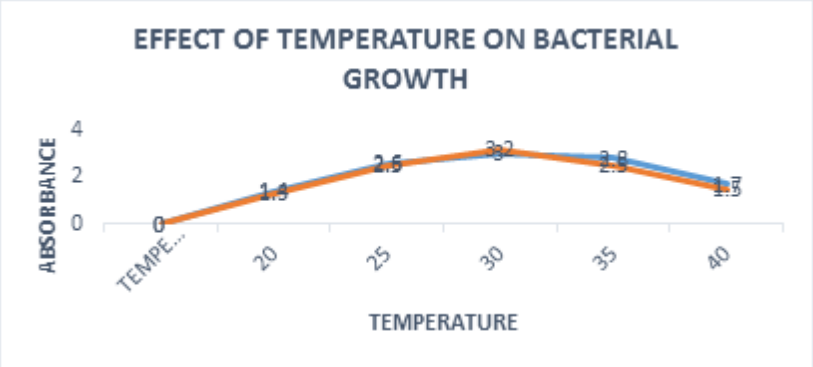

Figure 41: Effect of Temperature on Bacterial Growth

Conclusion

In conclusion, this research analyzed the bioremediation capacities of bacteria derived from Balanagar (Hyderabad) along with those from Eloor (Kerala). The isolates obtained from Balanagar displayed Gram-positive characteristics but Eloor showed Gram-negative identification while both tested positive for Methyl Red and Indole tests to confirm their metabolic capabilities in pollution sites. Tests on biomass accumulation demonstrated that Gram-negative bacteria isolated from Eloor grew best in nickel-containing environments thus indicating improved tolerance to metals. Both bacteria strains demonstrated a restricted capacity to deal with lead contamination which indicates the harmful effect of lead exposure. This research shows that bacteria from Eloor effectively remediate nickel contamination yet additional studies are required to boost their metal extraction capabilities.

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