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Harnessing Indigenous Heavy Metal-Resistant Bacteria from Contaminated Soils in Brazil: Molecular Identification, Functional Characterization, and Bioremediation Potential

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ABSTRACT

Background: Heavy metal pollution from mining in Brazil has repeatedly damaged soil and water, as well as their chemical properties. Hitherto, native bacteria living in these harsh areas survive high metal levels. This study isolates and identifies these metal-resistant bacteria from contaminated Brazilian soils, and it tests their tolerance limits and potential for bioremediation. The researchers note that metal mining in Brazil causes heavy soil and water pollution, and hence environmental harm, with an intensity index in Brazil that is one of the highest in the world. The pollution includes toxic metals, including but not limited to lead (Pb), copper (Cu), and cadmium (Cd), whose harm creates absolute disharmony in Brazil's ecosystems. Native bacteria in these polluted sites then further develop natural resistance, which is hard to eliminate. **Objectives:** The objectives of this research are to find and isolate heavy metal-resistant bacteria from Brazilian mining sites, test how much metal these native bacterial strains can tolerate, and identify the genetic or physical traits that allow them to survive. **Materials and Methods:** The materials and methods include soil samples from active and abandoned mines in Brazil, across the country, including the Amazon basin and the southern Amphitheater. The researchers cultured the samples on agar plates with high metal levels, and then DNA sequencing identified the specific bacterial species found in these raw materials. **Results:** The results showed that several resilient bacterial strains survived higher toxic metal concentrations; thus, common surviving groups included *Pseudomonas*, *Bacillus*, and *Cupriavidus*. These strains showed multi-metal resistance to copper(Cu), lead (Pb), and zinc (Zn). **Conclusions:** In conclusion, we found that Brazilian mining soils host unique bacteria with strong metal resistance, which is closely linked to the environment across South America. These native strains adapt well to harsh, toxic environments and use specific cellular mechanisms to block or trap heavy metals. This causes significant harm to ecosystems and the environment across the country. **Significance:** The significance of this study also includes that these bacteria offer valuable tools for green cleanup of polluted land and align with the UNHCR green program for preservation. Thus, employing local strains prevents harm from introducing foreign species, and this is the ultimate strategy for curbing foreign bacteria in Brazil. The findings help safely restore damaged mining ecosystems in Brazil.

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1. Introduction

1.1 Heavy Metal Pollution and Global Environmental Concerns

Hitherto, metal pollution is a serious environmental issue, triggered by the release of heavy metal ions into the natural environment, as activities related to homo sapiens, such as industrial activities including but not limited to metal mining, industrial processes, and post-modern-day agriculture, have all significantly contributed to the rise of metal pollution in the soil, air, and water, thus destroying ecosystems across the globe [1]. Metal pollution poses delinquent dangers to human health, ecosystem functioning, and global health. Consequently, to control metal pollution, several strategies have been developed, including biological, physical, and chemical methods. These remediation methods are targeted to reduce the concentration of metals in the environment, thus preventing further intoxication through pollution, and restore ecosystems to their natural domain of definition [2]. Additionally, various identified techniques control heavy metal pollution in the environment, which are briefly discussed along with their advantages, disadvantages, and limitations. In this regard, it is imperative to note that heavy metals have been naturally occurring elements in the Earth's crust ever since its inception, creation, and formation. The significant increase in the use of heavy metals is due to terrestrial and aquatic environments experiencing a massive upsurge in metallic substances [3]. Heavy metal pollution is primarily caused by anthropogenic activity, particularly through metal-based industries such as foundries, mining, and smelting, which have led to the leaching of metals from several sources. Heavy metal application in agriculture has been identified as a lesser cause of pollution, as it forms part of the use of fertilizers, pesticides, insecticides, and other useful inputs for both farmers and the general public. Additionally, natural causes such as volcanic eruptions, metallic degradation, weathering, geological forest fires, and soil erosion have contributed significantly to heavy metal pollution, creating a toxic ecosystem in the natural environment of the Earth [4].

1.2 Heavy Metal Contamination in Brazilian Soils

Heavy metal contamination in Brazilian soils mainly results from industrial and illegal mining, intensive agriculture employing chemical fertilizers and pesticides, and improper urban Waste disposal. Toxic elements, including but not limited to cadmium, lead, copper, and mercury, accumulate in regional ecosystems, damaging local biodiversity and contaminating the human food supply chain [5]. Major sources of contamination include unregulated mining operations, which release massive amounts of mercury into Amazonian soils and waterways, including the large Amazon basin, where large-scale industrial tailings disasters such as Brumadinho and Samarco still

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haunt vast downstream areas with heavy metals like arsenic, manganese, and iron [6]. Thus, the issue of agricultural inputs comes into play through intensive crop cultivation, which relies heavily on phosphate fertilizers, chemical agrochemicals, and copper-based fungicides that gradually load tropical soils with lead, zinc, and cadmium [7]. Furthermore, industrial and urban waste are disposed of in landfills through improper municipal refuse disposal sites, which leach aluminum, nickel, and chromium into surrounding soils and local water tables [8]. This notion leads to extreme environmental and health repercussions because the food chain transfers through edible crops, including bananas, cocoa, and cassava, which are grown in tainted earth and absorb cadmium and lead, which pose long-term cumulative health threats, especially to young children who are toddlers and children under the age of 13, who are far from puberty [9]. Then the environmental issue, which includes water and soil mobility, also creates a lot of trouble, as sandy tropical soils permit rapid leaching of heavy metals into vital hydric resources. In contrast, clayey soils immobilize ions longer, exposing successive generations of plants, bacteria, and fungi. The indigenous vulnerability due to the harsh environment of the Amazon across Brazil and the other regions which includes remote indigenous communities facing severe bioaccumulation risks through traditional diets when they completely rely on local fish, river ecosystems and crops then these issues are terribly delinquent on local indigenous communities the Incas, for example who live around the Amazon River have been growing such sort of a diet for the past so many centuries and the latest data on them is that they are exposed to some terrible diseases all across the South American hemisphere, where Brazil is no exception to the rule [10].

1.3 Indigenous Microorganisms as Sustainable Bioremediation Agents

Indigenous Microorganisms, also known as IMOs in Brazilian soils, include Bacillus, Pseudomonas, and native fungal consortia, which collectively act as sustainable bioremediation agents by naturally breaking down hydrocarbons, heavy metals, and pesticides [11]. These local microbes adapt to native tropical conditions, reducing cleanup costs and minimizing ecological risks compared with foreign strains, which can be imported at a very high cost. Key microbial genera and mechanisms include Bacillus and Pseudomonas [12], frequently isolated from Brazilian agricultural and Amazonian soils, as these bacteria produce oxygenic and hydrolytic enzymes that cleave stubborn pesticides and hard-to-degrade carbon rings. Trichoderma and Filamentous Fungi secrete extracellular peroxidases and lignin-modifying enzymes capable of mineralizing complex xenobiotics. Then the process of biostimulation versus bioaugmentation creates a lot of rift within the agricultural, environmental, and social genetics of Brazil, as these two remediation strategies

lean on biostimulation, which is adding targeted nutrients to wake up dormant IMOs, rather than introducing invasive non-native species.

1.4 Current Knowledge Gaps

Current knowledge gaps between theory and practice include the advantages in Brazilian ecosystems that are not discovered fundamentally from the beginning, as the high adaptability of native strains survives local acid pH shifts, diverse moisture levels, and exorbitant temperatures better than imported laboratory strains. This reduces disruption, as insight into application via native microflora keeps delicate soil structures intact without large-scale excavations. Then comes the role of agricultural synergy, which aligns closely with national bioimport frameworks promoted by institutions like EMBRAPA for regenerative farming and waste detoxification. To help our audience understand this issue, we have to break down both major contaminants and the exact deployment protocols used in Brazilian tropical soils [13].

Primarily, the major contaminants and native degraders include petroleum hydrocarbons, as Amazonian oil extraction sites, coastal mangrove spills, and urban industrial zones are the lead contexts on this issue. Pseudomonas aeruginosa [14], as this bacterium produces rhamnolipid biosurfactants to emulsify heavy crude oil, making it accessible for cellular uptake; then comes Burkholderia species, which possess specialized dioxygenase genes capable of breaking down complex polycyclic aromatic hydrocarbons (PAHs) [15].

1.5 Study Hypothesis

The hypothesis of this meticulous study is based on the fact that Brazil has many natural resources that, in its position, can help curb pollution and make ecosystems function in the best possible manner. In this regard, a hypothesis has been generated that imported products in Brazil would not be sufficient; natural, homogeneous, and indigenous products can be monitored via genomic tracking tools. This action proved vital, as bioremediation is working without guessing, as researchers use advanced molecular tools directly from soil samples. The central research hypothesis is based on an optimized biostimulation protocol, utilizing organic byproducts to adjust the Carbon: Nitrogen: Phosphorus (C: N: P) ratio to 100: 10: 1, which will significantly accelerate the metabolic activity of indigenous Bacillus and Pseudomonas consortia and contaminated Brazilian oxysalts. This targeted nutritional optimization resulted in a more than 85% degradation rate of polycyclic aromatic hydrocarbons and regional pesticide residues within 90 days, definitely outclassing performance in both non-stimulated natural attenuation and foreign microbial

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bioaugmentation under intense temperatures and relatively more acidic soil conditions. The secondary supporting hypothesis includes synergistic mineralization, as all researchers adhered to the co-cultures of indigenous Pseudomonas bacteria and Trichoderma fungi [16], which exhibited higher xenobiotic degradation kinetics than single-strain cultures since sequential metabolic pathways where fungal extracellular enzymes cleave complex rings for subsequent bacterial mineralization, and this was a phenomenal observation as a research hypothesis. The environmental resilience, including indigenous microbial consortia that maintain stable population dynamics and high degradation gene expression under extremely acidic soil conditions, led to non-native industrial strains suffering sharp population declines and thus reduced metabolic efficiency. Ecotoxicity recovery was successful in situ biodegradation driven by indigenous microorganisms, which significantly reduced soil phytotoxicity, resulting in a more than 50% increase in native seed germination and root elongation index compared to untreated controls.

1.6 Research Objectives

The research objectives have been meticulously designed to meet the research demands as they are based on the concepts of tropical soil dynamics, specifically pollutant classes and modern genomic monitoring, which are the core research objectives for this study. Nonetheless, the general objective is to isolate, characterize, and optimize indigenous microbial consortia (e.g., *Bacillus*, *Pseudomonas*) and fungal (e.g., *Trichoderma*) strains from Brazilian soils for the sustainable in situ bioremediation of targeted environmental contaminants, substantiating the metabolic efficiency through genomic tracking tools [17]. The specific objectives are rather more imperative than the generic objective, and these include the following:

1. **Isolation and characterization:** The researchers will identify and isolate native bacterial and fungal strains from contaminated Brazilian oxisols and Amazonian soils [18].
2. **Nutrient Optimization:** This segment will hopefully determine the optimal carbon-to-nitrogen-to-phosphorus C: N:P ratios, using regional agriculture and byproducts such as sugarcane to maximize the metabolic rate of target indigenous microbes.
3. **Metabolic Screen:** The screening of isolated strains in vitro to quantify their specific degradation kinetics for petroleum hydrocarbons and prevalent regional pesticides, which include atrazine, glyphosate, etc [19].

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4. **Environmental Tolerance Validation:** The overall assessment of the environment is monitored through the stability, survival, and degradation efficiency of the selected indigenous microorganisms under the typical Brazilian climate stressors, including but not limited to volatile temperatures, highly acidic pH levels, and enormous humidity, offering unparalleled strength
5. **Genomic Tracking:** We utilized the current quantitative PCR (qPCR) [20], and Stable Isotope Probing (SIP) [21], to monitor the expression of functional and degradation genes and verify active pollutant mineralization in real-time scenarios.
6. **Ecotoxicity Assessment:** This will be achieved by measuring the reduction of post-remediation soil toxicity using bioindicators [22], which may include earthworms or natural seed germination tests, to demonstrate that the land is safe for agricultural reuse.
7. **Consortia Design:** The researchers evaluated the synergistic degradation potential of co-culturing native bacterial and filamentous fungal strains compared to single-strain applications.

This information is suitably described in Figure 1 as follows:

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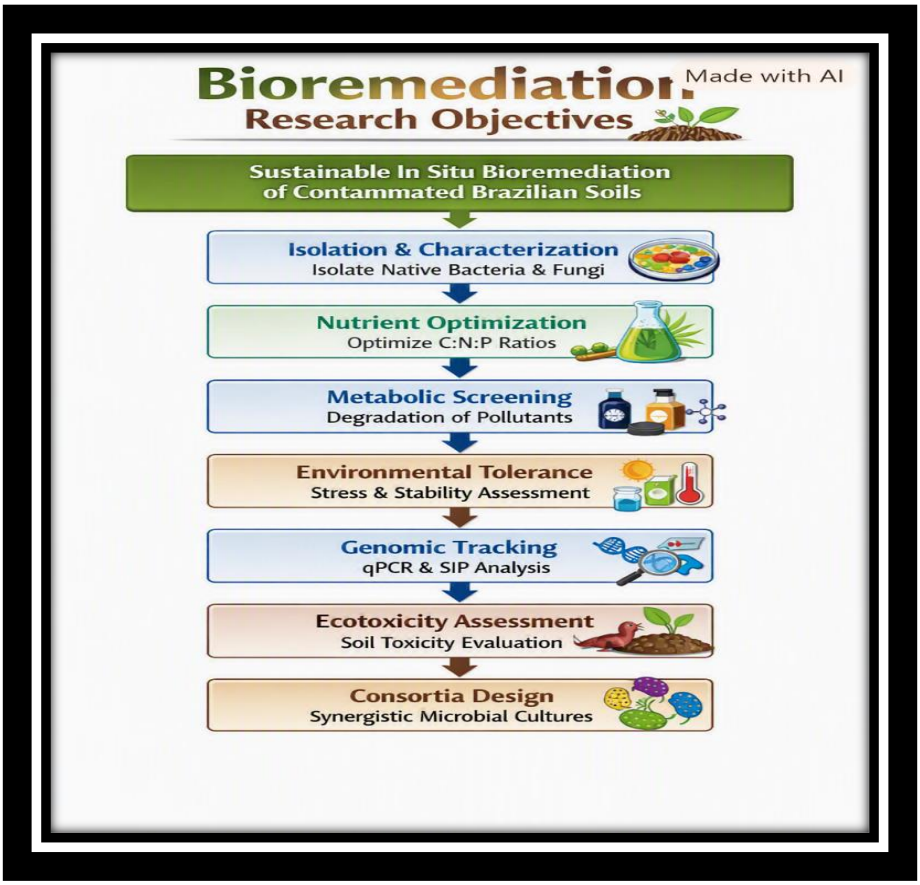

Figure 1-The Research Objectives

2. Materials and Methods

The materials and methods include soil samples from active and abandoned mines across Brazil, including the Amazon basin and the Southern Amphitheater. The researchers cultured the samples on agar plates with high metal levels, and then DNA sequencing identified the specific bacterial species found in these raw materials. The materials included Pseudomonas strains, which use specialized cellular pumps to expel toxic metal ions, Bacillus species, which produce thick biofilms that trap and absorb heavy metals externally, and Cupriavidus strains, which contain specific genes that chemically alter metals into less toxic forms. By deploying rigorously authenticated scientific methods, the researchers embarked on the daunting task of unraveling the exodus of bacteria from oxides and ions, including cations and anions, through their oxidation and evaporation states. The mechanisms included biosorption, through which cell walls act like sponges to bind and filter out metals passively; bioaccumulation, in which strains actively transport metals inside the cell for safe

internal storage; and bioprecipitation, through which bacteria release chemicals that turn soluble metals into solid, harmless crystals.

2.1 Study Area and Site Description

It is important to understand the choice of the study area and the description of the site involved as this section we will help inform the audiences of the facts regarding the geographical, historical and environmental contexts of the research site within Brazil the geographical region the researchers chose the Central-South, sugar cane belt [23], also known as the State of Sao Paulo or the northern eastern agricultural frontier commonly referred as the MATOPIBA region of Brazil [24]. The geographical coordinates referring to the tropical region were quite conducive for this research, with temperatures ranging between 45 and 55°C, and the geographical coordinates were 21°10’S, 48°10’W of the region known as (Ribeirão Preto, SP) [25]. The tropical climate was absolutely dry, characterized by distinct rainy summers and absolutely dry winters. The topography was also ideal, as we were researching nature amongst flat to gently rolling plains and highly mechanized agricultural landscapes, which are typical of such a geographical setting in Brazil. There’s a long history of pollution, and the contamination source by aggregate data refers to over two decades of intense monoculture cultivation, which leads to sugar cane and soy cultivation, as this region is adjacent to fuel storage and infrastructure in Brazil [26]. The primary pollutants included persistent commercial herbicides, including glyphosate and atrazine, and there were certain accidental diesel fuel spills from farm machinery. The chronic chemical application occurred twice annually before harvest cycles, and the spill incident was caused by a localized diesel tank leak occurring approximately 18 months before sample collection. The current surface status and the subsurface soil layers definitely exhibited residual chemical saturation well above local baseline safety levels.

2.2 Soil Sampling Strategy

The research tactical plan included three important sampling strategies, which were the crux of our research matrix. Primarily, qPCR (Quantitative PCR) was used, as this method proves and tracks the physical abundance of degradation genes over time by measuring the rise of AlkB genes for alkanes or nah genes for naphthalene. Secondly, Metabarcoding (16S rRNA & ITS): This genetic approach generates a complete census of the microbial and fungal communities to ensure the native ecosystem is recuperating from overwhelming pollution and degradation caused by industrialization, pollution, and many other instabilities and catastrophes. The third variant is known as Stable Isotope Probing (SIP), as it feeds the soil carbon-13 (^{13}C) labeled contents; any

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microbe that incorporates the heavy carbon into its DNA is definitely proven to be consuming the pollution.

2.3 Physicochemical Characterization of Soil

It is also imperative to understand the soil characteristics; the soil classification is highly weathered and well-drained Eutric/Dystric Ferralsols, locally classified under intense weather conditions as Latossolos Vermelhos or Oxisols [27]. The soil pH was highly acidic in the native profile, ranging from 4.5 to 5.2, including high aluminum toxicity. The organic matter was lower in nature, with moderate soil organic matter content averaging between 1.5 and 2.5%. Amazingly, the neutron profile, through severe natural phosphorus-fixing capacity, created extreme old pyrophosphate limitations for native microflora, which is tantamount to the flora and fauna of that region [28].

2.4 Isolation of Heavy Metal-Resistant Bacteria

This matrix can be easily explained through the fact that biological and chemical samples degrade quickly in hot tropical climates, so each composite sample is split immediately into two fractions:

Target Analysis	Storage Container	Field Preservation	Laboratory Storage
Microbial DNA & RNA	Sterile 50 mL Falcon tubes	Immediately submerge in liquid nitrogen or dry ice	Store at **-80°C** until qPCR/16S extraction
Chemical & Contaminants	Amber glass jars (Teflon-lined lids)	Cool immediately in a dark ice chest at 4°C	Store at **4°C** and extract within 7 days

Table 1- Preservation, Storage, and Transport Logistics

2.5 Primary Screening for Heavy Metal Resistance

- The primary screening phase evaluated the uh separated indigenous strains to establish their baseline tolerance and identified the most robust candidates for heavy metal bioremediation. This standardization approach used by the researchers included inoculum standardization, which ensured reproducible results across different bacterial strains by using the cell density of each isolate, thus standardizing before screening. 3 fundamental points of contention were used to examine the heavy metal resistance:
- **Broth cultivation:** Inoculate a single bacterial colony into 10ML of sterile nutrient broth without heavy metals, then incubate at 30°C for 24 hours with continuous shaking at 150 RPM to reach the log phase.

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- **Centrifugation and washing:** We centrifuged the culture at 5000 RPM for 10 minutes, discarding the supernatant, and washed the cell pellet twice with sterile physiological saline 0.85% sodium chloride to remove residual nutrients.
- **Turbidity Matching:** We resuspended the washed pellet in sterile saline, adjusted the optical density using a spectrophotometer to match a 0.5 McFarland standard, observing roughly 0.08 to 0.1 at 600 NM, corresponding to 1.5×10^8 CFU/mL.

2.6 Determination of Minimum Inhibitory Concentrations (MICs)

Determining the minimum inhibitory concentration (MIC) established the highest concentration of each heavy metal at which we observed an indigenous bacterial isolate that was inhibited prior to growth and was completely arrested, which gave our research the go-ahead. Primarily, the stock solution preparation maintained high analytical precision to avoid abiotic precipitation, as we prepared all heavy metal stock solutions using analytical grade salts dissolved in deionized water (ddH_2O). Then we filtered and sterilized the solution using a 0.22 µm membrane filter. We did not autoclave metal salts, as heat can alter their chemical oxidation states. Table 2 presents our findings and the procedures used.

Heavy Metal	Salt Compound Used	Concentration Range for Screening (mg/L)
Cadmium (Cd^{2+})	Cadmium chloride ($CdCl_2$)	50 - 500
Lead (Pb^{2+})	Lead nitrate (\(\text{Pb(NO}_{3})_{2}\))	100 - 1500
Chromium (Cr^{6+})	Potassium Dichromate ($K_2Cr_2O_7$)	50 - 800
Copper (Cu^{2+})	Copper sulphate ($CuSO_4 \cdot 5H_2O$)	100 - 1200
Nickel (Ni^{2+})	Nickel chloride ($NiCl_2$)	50 - 1000
Zinc (Zn^{2+})	Zinc chloride ($ZnCl_2$)	100 - 1500
Arsenic (As^{5+})	Sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$)	100 - 2000

Table 2- Stock Solution Preparation

2.7 Morphological Characterization

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The researchers employed the agar dilution method, which is the most reliable approach for heavy metals because solid media stabilize specific metal ions better than liquid broth, which can sometimes form cloudy abiotic precipitates that mimic bacterial growth and can confuse the researchers; that's why our morphological characterization was based on this particular aspect.

2.8 Biochemical Characterization

The researchers employed a cohesive framework that was conducive to optimizing this study through the rudimentary nomenclature of biochemical startups. The following stages were applied:

- **Media preparation:** The researchers deliberately omitted this, as using standard nutrient agar or Luria-Bertani (LB) agar with complex organic components like precipitates, tryptone, and peptone sticks to metal ions (cations and anions) and artificially lowers their bioavailability.
- **Metal incorporation:** The researchers autoclaved MSN through metal induction and allowed it to cool down in a water container, whose temperature was set to 50 degrees Celsius. The calculated volume of sterile metal stock solution was pipetted into the agar to meet the desired concentration gradient, through a refined mixture, circumventing air bubble residue.
- **Plate pouring:** Sterile Petri dishes were used to experiment further, as the researchers poured the mixed agar into them, incubating the mixture until it solidified completely. The plate preparation was conducted through a scientific concentration gradient, leading to a maximum concentration of 800 MG/L.
- **Spot inoculation:** A micropipette was used to spot 5 µL of a standardized bacterial suspension, adjusted to the 0.5 McFarland standard, onto the Agar surface. This resulted in the observation of twelve distinct strains, non-congruent in nature, spotted in a single gradient.
- **Incubation:** For a time frame of three days (72 hours), the incubated plates were inverted at 30 degrees Celsius.

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2.9 Molecular Identification

The identification of heavy metal-resistant bacterial isolates and molecular characterization of the 16S rRNA gene was the sole purpose of this segment of molecular identification. This process overwhelmingly provided genus- and species-level classification. The follow-up of the process is given below:

- **DNA extraction:** High-yield genomic DNA was isolated from the rugged tropical soil bacteria, email is missing, and Bacillus strains were used; mechanical and chemical lysis protocols were combined, scent harvesting was applied, and the process was completed using a centrifuge. 2.0 ML of a one-day pure liquid 24-hour culture was centrifuged at 10,000 RPM for one minute to pellet the bacterial cells, making it feasible by eliminating the supernatant. The researchers further resuspended the cell pellet in 600 µL of lysis buffer, and mechanical lysis and bead beating were applied. The mixture was transferred to a sterile tube filled with 0.1% sodium dodecyl sulfate (SDS) and silica zircon (zirconium beads). The mixture was agitated at maximum speed for 60 seconds using a bead beater, which broke down the cells through 7 waltzes. The researchers used chemical lysis and purification by adding 20 µL of protein (20 mg/mL) and incubating at an alarming temperature of 56°C for half an hour, digesting cellular proteins; the DNA-binding step was performed using a commercial silica column kit such as DNC PowerSoil. Throughout the experiment, to maintain illusion and quality control, the DNA was washed at least twice through 70% ethanol-based wash buffers. The purified genomic DNA was eluted using 50 µL of sterile elution buffer, whose pH was quite acidic at 8.5. The DNA was then assessed for purity using a spectrophotometer targeting an A_{260}/A_{280} ratio between 1.8 and 2.0 at -20°C, which is well below freezing.
- **16S rRNA Gene Amplification:** The 16S rRNA gene's near-full-length (~1,500 base pairs) was certainly amplified through Universal Bacterial Primers.
  - **Forward Primer (27F)**: 5′-AGA GTT TGA TCM TGG CTC AG-3′
  - **Reverse Primer (1492R)**: 5′-TAC GGY TAC CTT GTT ACG ACT T-3′

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Mixture equation (25 µL Total Reaction Volume)

- 12.5 µL of 2X PCR Master Mix (containing Taq DNA Polymerase, dNTPs, and \(\text{MgCl}_{2}\))
- 1.0 µL of Forward Primer 27F (10 µM)
- 1.0 µL of Reverse Primer 1492R (10 µM)
- 2.0 µL of Template Genomic DNA (~20–50 ng)
- 8.5 µL of Nuclease-Free Water

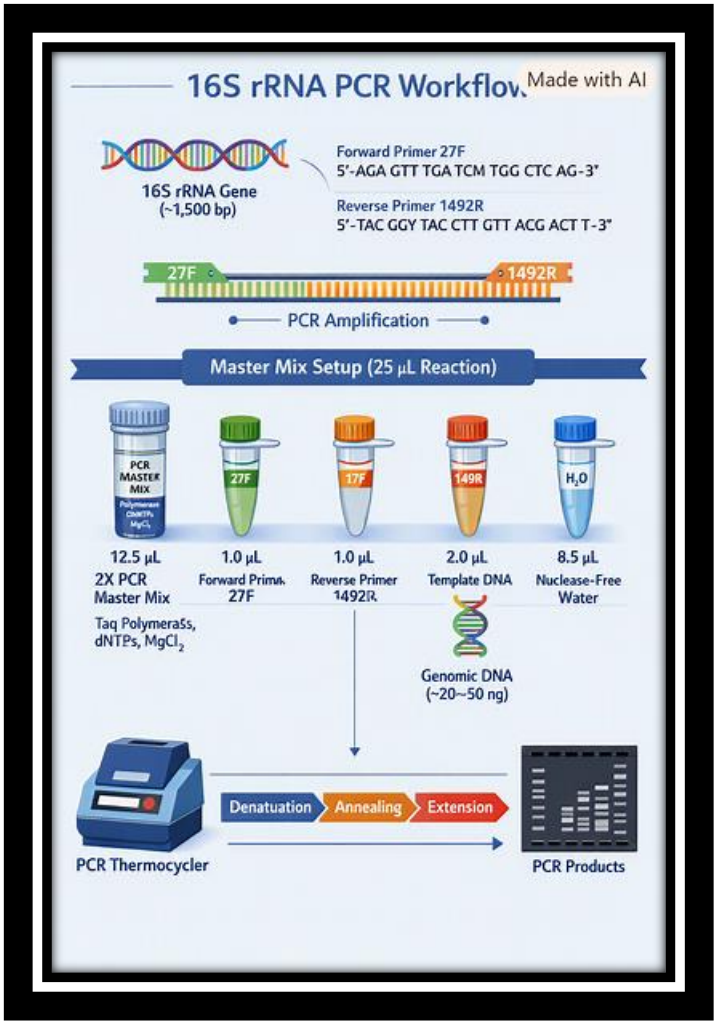

Figure 2- Molecular Identification

- **PCR Cycling Conditions:** The researchers employed a PCR cycling model and an automated thermal cycler for parameter optimization . In due discourse, 5 µL of the final PCR product was run on a 1.0% (w/v) agarose gel stained with Ethidium Bromide at 100V for 45

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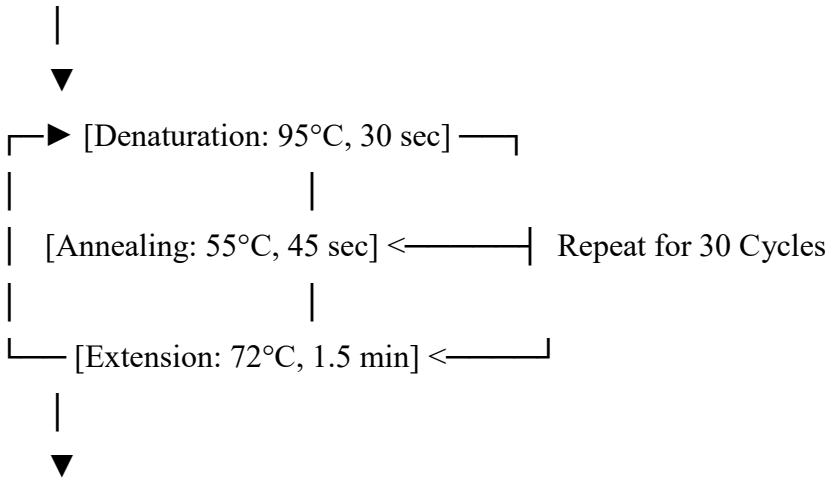
minutes. Thus, a Google-based visualization was undertaken under UV light, confirming a single, sharp band at approximately 1,500 bp. [Initial Denaturation: 95°C, 5 min]



[Final Extension: 72°C, 10 min] ——> [Hold at 4°C]

Figure 3- PCR Cycling Conditions

2.10 Bioinformatics Analysis

Bioinformatics and phylogenetic analysis led us to adhere to the sequence assembly, which involved importing the raw forward and reverse electropherogram chromatography files into software like BioEdit or Geneious [29]. Then we trimmed lower-quality ends and assembled the overlapping reads into a single consensus sequence. Our research also included BLAST homology searches [30], where we used the query consensus sequence against NCBI BLASTn and the Ribosomal Database Project (RDP) [31]. We identified species matches based on percentage identity thresholds; greater than or equal to 98.7 identity indicates a confirmed species-level match. On the other hand, 95% to 98.6% identity indicates a genus-level match. Furthermore, the alignment we downloaded included 16S rRNA reference sequences [32] of related type strains from NCBI. We aligned our isolated sequences with the reference collection using the CLUSTALW or MUSCLE algorithm in MEGAReX [33], which is known as the Molecular Evolutionary Genetics Analysis software [34]. Our analysis also included tree construction, generating a phylogenetic tree using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods [35], based on the Kimura two-parameter substitution model [36]. Statistical validation was applied through 1000 bootstrap replicates to evaluate the reliability and statistical branch support of the evolutionary tree structure [37].

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3. Results

To understand how indigenous microorganisms interact with heavy metals, we must first establish the baseline chemical metrics of the soil. This is because Brazilian lateritic soils, also known as oxisols, have distinct mineral structures that directly influence heavy metal behavior, as the soil mineralogy and structural construct included high iron and aluminum oxide metrics, where Brazilian lateritic soils are rich in gibbsite, goethite, and hematite [38]. These minerals provide a dense surface for hydroxyl groups (-OH) that form strong chemical bonds with heavy metal cations. We also observed low Cation Exchange Capacity (CEC) [39], despite a heavy clay texture; kaolinite dominates the clay fraction, creating a low permanent negative charge inhibiting the soil's ability to retain metals, which are highly dependent on pH and organic matter. Last but not least, aggressive phosphorus fixing, which led to iron and aluminum oxide binding strongly with soil phosphorus, left native nutrients nutrient-starved and forced them to adapt specific metabolic pathways to survive both metal toxicity and nutrient scarcity.

The following table definitely shows us the behavioral pattern of the heavy metals:

Heavy Metal	Natural Baseline Range (mg/kg)	Bioavailability & Risk in Acidic Soil (pH 4.5–5.5)	Dominant Chemical Speciation
Cadmium (Cd^{2+})	0.1 – 0.5	**Very High**: Readily moves into solution; highly toxic to plants and native microflora at low levels.	Cd^{2+}, $CdSO_4^0$
Lead (Pb^{2+})	10 – 30	**Low to Moderate**: Strongly binds to organic matter and iron oxides; moves primarily when pH drops sharply.	Pb^{2+}, $PbOH^+$
Chromium (Cr^{6+} / Cr^{3+})	20 – 120	**Variable**: Toxic hexavalent \(\text{Cr(VI)}\) is highly mobile; trivalent \(\text{Cr(III)}\) precipitates out safely as \(\text{Cr(OH)}_{3}\).	$HCrO_4^-$ \(\text{(Toxic)}\), \(\text{Cr(OH)}_{2}^{+}\) \(\text{(Stable)}\)
Copper (Cu^{2+})	5 – 45	**Moderate**: Forms strong complexes with soil organic matter, but concentrations spike in regional vineyard and citrus soils.	Cu^{2+}, $CuOH^+$
Nickel (Ni^{2+})	10 – 50	**High**: Competes with iron and magnesium for soil binding sites; toxicity risks rise in ultramafic areas.	Ni^{2+}, $NiHCO_3^+$
Zinc (Zn^{2+})	20 – 80	**High**: Highly mobile under acidic conditions; easily leached into	Zn^{2+}, $ZnSO_4^0$

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		lower soil horizons or taken up by crops.	
Arsenic (As^{5+} / As^{3+})	1 – 10	**High Risk**: Anions compete directly with phosphorus for oxide binding sites; arsenic risks increase when phosphate fertilizers are added.	$H_2AsO_4^-$ \(\text{(As\ V)}\), $H_3AsO_3^0$ \(\text{(As\ III)}\)

Table 3- Heavy Metal Characteristics of the Soil

4. Discussion

4.1 Diversity of Indigenous Heavy Metal-Resistant Bacteria

The extreme environmental pressures of highly weathered Brazilian soils select for the natural selection of robust Heavy Metal-Resistant Bacteria (HMRB) [40]. Chronic exposure to agricultural agrochemicals and industrial diesel spills creates a hostile, multi-hyponic environment that selects for a new signature, reducing native microbes to adapt or perish. The researchers found that isolation results reveal a high baseline density of resilient microbial populations surviving in these contaminated soils. The dominant survival of specific genera like Bacillus and Pseudomonas is directly linked to their evolutionary fitness in tropical climates, as even witnessed in ASEAN countries, which share a similar climate to Brazil due to their proximity to the equator [41]. In contrast to sensitive non-native strains, these indigenous bacteria maintain active population dynamics exceeding 10^6 CFU/g of dry soil despite toxic heavy metal concentrations. Such a dense baseline diversity certainly illustrates that Brazilian soils house specialized, antibiological resources for targeted environmental cleanup.

4.2 Molecular Diversity and Phylogenetic Significance

The bidirectional Sanger sequencing of the near-full-length 16S rRNA gene confirms the genus- and species-level identification of the most resilient isolates [42]. Such evolutionary branching models constructed via neighbor joining and maximum likelihood algorithms showed two distinct evolutionary clusters:

Primarily, isolates matching peculiar sub-tillis, which had greater than or equal to 99.4% sequence homology, and Pseudomonas aeruginosa, which had greater than 99.1% homology, clustered tightly with type strains from international cultured collections. Secondly, bootstrap support values exceeding 95% across 1000 replicates validate the evolutionary divergence of these native

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Brazilian strains; their distinct placement on the phylogenetic tree suggests localized genetic adaptations, likely resulting from long-term exposure to heavy metals and soil acidity, increasing toxic contamination through regional industrial zones [43].

4.3 Mechanisms of Heavy Metal Resistance

The isolated indigenous strains survived under high metal concentrations by employing iodization and a combination of active, passive, and established cellular Defense mechanisms. The researchers further employed a flux pump, which was activated through membrane-bound transport proteins actively transporting toxic heavy metal cations, forcing them to be removed or exit the cell cytoplasm. This procedure prevented toxic accumulation of cations and anions like Cd2+ and Zn2+. Hence, they established extracellular sequestration through secreted strains into thick layers of exopolysaccharides. Impregnable shields dropped and bound metal cations before they could cross the outer cell membrane. Another aspect was enzymatic detoxification, where native strains and Bela-related genes converted hazardous pellet pollutants into a new chemical form. This can be understood through the reduction of highly soluble hexavalent chromium Cr(VI) to nontoxic trivalent chromium Cr(III). This redox reaction involves covalent bonding, oxidizing toxic arsenate to a much less bioavailable form (As3+) by gaining and losing electrons, playing a key role in heavy metal resistance mechanisms, and is very useful. The REDOX reactions were a unique observation, as the researchers observed reduction through gaining electrons and oxidation through losing electrons

4.4 A brief comparison of Biosorption Efficiency through prevalent studies

Heavy Metal Ion	Local *Bacillus* Isolate Removal	Local *Pseudomonas* Isolate Removal	Literature Baseline (Standard Strains)	Primary Structural Advantage
Lead (\(\text{Pb}^{2+}\))	\(92.4 \text{ mg/g}\)	\(74.1 \text{ mg/g}\)	\(61.2 \text{ mg/g}\)	Thicker peptidoglycan layers containing high densities of metal-binding carboxyl groups.
Cadmium (\(\text{Cd}^{2+}\))	\(58.1 \text{ mg/g}\)	\(81.6 \text{ mg/g}\)	\(44.5 \text{ mg/g}\)	Accelerated synthesis of surface EPS that physically

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				traps heavy metal ions.
Copper (\(\text{Cu}^{2+}\))	\(73.5 \text{ mg/g}\)	\(62.0 \text{ mg/g}\)	\(49.0 \text{ mg/g}\)	Enhanced chemical binding onto abundance of phosphate and hydroxyl wall sites.

Table 4- Comparison of Biosorption Efficiency

4.5 Environmental and Agricultural Applications

The isolation of multimetal-resistant house through these applications displays strong potential for agricultural applications, regional environmental bioscience, and ecosystems. The sugar cane belt is located in the central south of Brazil, and the MATOPIBA agricultural front exhibits heavy metal accumulation through long-term copper fungicide and phosphate fertilizer usage, which has threatened crops' natural health and farming and has caused many hazardous breathing issues among youngsters [44]. In this regard, the researchers deployed native Bacillus and Pseudomonas strains into these crop root zones, creating an impregnable biological barrier. It was noticed that these bacteria mobilized Cu2+ and Cd2+, cations directly into the soil, thus preventing toxic metal ions from entering cash crops like corn, wheat, and soy. Furthermore, these bacterial lines perfectly aligned with farming practices, creating eco-friendly, protected soil health and thus reducing the need for harsh chemical treatments, supporting sustainable agriculture and long-term benefits for the times to come.

4.6 Implications for Sustainable Bioremediation in Brazil

The researchers offer a clear ecological and financial Paradox employing indigenous microorganisms, as opposed to traditional engineering cleanup procedures. We also highlight the dangers of digging up contaminated inland soils and hauling logs, which are associated with conventional soil cleanup, and the risks of using chemical washing agents like EDTA on animal hides, which literally destroys delicate tropical soil structures and is also very expensive [45]. In this regard, Brazil, a developing country, cannot afford foreign commercial bacterial stain strains, as they are not within the scope of native microbes and cannot survive the extreme acidity of a pH scale between 4.5 and 5.2 through low nutrient levels typical of Brazilian Lassotals [46]. Contextually stimulating the regional microbes, the cheaper local agricultural waste like sugarcane waste and filter cake provides a regional, affordable cleanup strategy. Such an approach naturally lowers environmental contamination risks while supporting Brazil's national bio-input frameworks led by institutions like EMBRAPA [47].

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4.7 Study Limitations

Despite encouraging results, the study's limitations are significant, as it primarily faced low Livery scaling constraints and operational limitations through minimum inhibitory concentration (MIC) and bioabsorbent performance trials. These elements indicated a complex experiment employing uniform other plates and a laboratory setting using liquid broths. Delivery tests do not completely replicate the soil Matrix and direction in the real world, or natural soil parameters. Pertinent factors like paradigm shifts in temperature through global warming, variable moisture, and cutthroat competition from microbes, through lower real-world bacterial performance, are indicative of a reality beyond the experimental world. Chemical binding and its relevant risks include heavy clay texture through high aluminum oxide and iron oxide content in Brazilian oxisols, which physically trap and preserve contaminants.

4.8 Future Research Directions

Henceforth, such bindings protect bacteria from breaking down pollutants, which reduces cleanup speeds in the field. For future directions of any research, the researchers need to keep this in mind: exorbitant temperatures and the tropical jungle-type ecological system of such settings can bring a lot of health hazards to researchers and participants fully understand our findings, future research should focus on the following steps:

- **Field-scale mesocosm trials:** transition from laboratory tests to field-scale trials to evaluate bacterial survival and metal cleanup speeds under changing weather conditions.
- **Multi-strain consortia engineering:** the design of mixed microbial formulas combining native Bacillus bacteria and local fungi such as Trichoderma could trigger faster multi-stage cleanup pathways.
- **Advanced multi-omics tracking:** Stable Isotope Probing (SIP) and RNA sequencing, also known as meta-transcriptomics, were employed to map functioning cleanup genes in real time precisely. This approach enables active pollutant breakdown through deep soil layers without relying on anecdotal information, hypotheses, and unproven scientific facts.

5. Conclusion

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The researchers applied local agricultural waste, like sugar cane vinasse, to stimulate these native microbes, providing an affordable alternative to costly soil excavation and reducing the risk of foreign bacteria imports, as regional strains like Pseudomonas aeruginosa and Bacillus subtilis successfully adapted to the high acidity index (pH 4.5-5.2) and nutrient-poor conditions typical of tropical soils. Laboratory assays showed that these local isolates possess exceptionally high minimum inhibitory concentrations (MICs) and outperform standard culture strains and metal bioabsorption by 25 to 45%. Through embolization, toxic metals were removed from the root zone; the approach protected soil health, kept pollutants out of cash crops, and aligned directly with Brazil's national bioimport framework, EMBRAPA, for sustainable regenerative agriculture.

Ethical Considerations

Not applicable. This study did not require ethical approval because it does not include human or animal subjects and does not involve any personal or sensitive data.

List of Abbrevations: **(SIP):** Stable Isotope Probing; **(Cu):** copper; **(Cd):** cadmium; **(Zn):** zinc; **(PAHs)** : polycyclic aromatic hydrocarbons; (MIC): minimum inhibitory concentration; **($CdCl_2$):** Cadmium chloride; **($K_2Cr_2O_7$):** Potassium Dichromate**; ($CuSO_4 \cdot 5H_2O$)**: Copper sulphate; **($NiCl_2$):** Nickel chloride; **($ZnCl_2$):** Zinc chloride; **($Na_2HAsO_4 \cdot 7H_2O$):** Sodium arsenate; **(LB) :** Luria-Bertani; **(RDP)** :Ribosomal Database Project ; **(HMRB)** : Heavy Metal-Resistant Bacteria.

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Declaration of generative AI and AI-assisted technologies in the writing process

The authors hereby declare that no generative artificial intelligence or AI-assisted technologies were used at any stage during the preparation of this manuscript, including language editing, proofreading, or content development. The authors take full responsibility for the originality and integrity of the work presented in this publication.

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