DOI: https://doi.org/10.57233/ijsgs.v11i3.943 ISSNp: 2488-9229; ISSNe: *3027-1118*

IJSGS FUGUSAU VOL.11(3) WEBSITE: https://fugus-

INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL USTAINABILITY
(A PUBLICATION OF FACULTY OF SCIENCE, FEDERAL UNIVERSITY GUSAU, NIGERIA)

Isolation and molecular Identification of Lead Tolerance Bacteria from Contaminated Soil of Different Mining Site in Minna, Niger State, Nigeria

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Received on: August, 2025 **Revised and Accepted on:** September, 2025 **Published on:** October, 2025

ABSTRACT

Lead contamination resulting from mining activities pose a significant environmental and health risk. this study focused on the isolation and molecular identification of bacteria exhibiting high tolerance to lead from contaminated soil samples collected from various mining sites in Minna, Niger State. The serial dilution and plating technique was employed to isolate lead tolerance bacteria on nutrient agar. The isolates were screened on nutrient agar supplemented with varying concentration of lead acetate (0.2 -1.0mg/ml) for lead tolerant determination. The isolates lead removal potential was determined using mineral salt medium (MSM) and incubated for 8 days at room temperature. The isolates were characterized based on their morphological, biochemical properties and molecular identification using 16S RNA gene sequence analysis. Four bacterial strains HM1, HM2, HM3 and HM4 were isolated. Bacteria HM3 effectively tolerated lead at various concentrations ranging from 0.2mg/ml - 1.0mg/ml. lead removal potential results showed the potency of HM3 in the reduction of lead on the 8th day with absorbance value of 0.002. Results from the biochemical test, blasting, and phylogenetic analysis showed that bacterial HM3 belongs to *bacillus subtilis* with 99% maximum identity to this specie. These isolates, particularly HM3 exhibit high tolerance and removal potential, represent potential bioremediation agents for mitigating lead pollution in contaminated soils, offering an eco-friendly approach to environmental clean-up.

Keywords: Lead, Lead tolerance, Bacteria isolation, Mining sites, Bioremediation.

1.0 INTRODUCTION

Environmental pollutants are compounds that impair human health and enter the environment as a result of human activity (Shilpa *et al.*, 2023). Mining is a human activity that involves extracting mineral resources from the surface or beneath the earth's surface. It is carried out where minerals are abundant and commercially viable. Mining activities release lead into the environment via polluting water, air, and soil. The three main ways lead enters the human body are through inhalation, oral absorption, and ingestion (Shilpa *et al*., 2023).

Lead is a metal that has long-term effects on the neurological, gastrointestinal, cardiovascular, hematological, and renal systems. Lead's neurological impact is especially harmful in children, and even low levels of exposure can cause severe and even irreversible brain damage. In early 2015, a lead poisoning epidemic killed 28 young infants in Niger State, Nigeria. The contamination was connected to unlawful mining by artisanal miners. A similar instance was recorded in 2010 in Zamfara State, Nigeria, when 400 children died and another 2000 were impacted by lead poisoning (Public Health, 2024).

Lead in polluted soil may interact with soil bacteria, either directly or indirectly, and be converted into non-toxic forms. According to Datta *et al.* (2012), bioremediation is a method that employs living organisms to remove or convert polluted or hazardous chemicals into harmless compounds. Microorganisms are beneficial to the environment, do not affect the ecosystem, and are extremely effective at cleaning up contaminants (Shams, 2012). As a result, it is obvious that bioremediation has significant promise for addressing particular types of site pollution. Lead is bio-remediated by bacteria such as Bacillus species and P.aeruginose. Numerous studies have shown that native bacteria isolated from metal-contaminated sites may readily interact with hazardous metals via both direct and indirect methods.

A large number of lead tolerant bacteria have been identified previously all around the world. Because lead is hazardous to all living beings, identifying and characterizing indigenous bacteria capable of tolerating lead is of significant interest. In the present investigation, bacteria capable of using lead as a carbon and energy source were isolated from lead-contaminated soil at a mining site in Minna, Niger State. This organism has the potential to act as a bioremediation agent in the environment.

2.0 MATERIAL AND METHOD

2.1 Sample Collection

The lead-contaminated soil sample was collected at a depth of 15 cm^3 from four different mining sites in Minna, Niger State, Nigeria. The samples were allowed

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to air dry at room temperature then sieved with a sieve mesh.

2.2 Growth Media Preparation

28g of nutrient agar was dissolved in 1000ml of distilled water and autoclaved for 15 minutes at 15 psi (121 ^{0}C). The medium was poured into petri dishes and allowed to solidify.

2.3 Isolation of Lead-Tolerant Bacteria

0.1ml of 10^{-3} and 10^{-4} diluted lead contaminated soil samples from four different mining sites were spread on the solidified nutrient agar. The medium was incubated at 35 ^{0}C for 24 hrs. The prominent bacteria colonies observed were sub cultured. The pure culture obtained was stored in slants bottle.

2.4 Bacteria's Lead Tolerance Determination

Lead acetate (substrate) with the following concentrations 200, 400, 600, 800 and 1000mg/ml was discharged into a petri dish before pouring the nutrient agar and allowed to solidify. The four bacterial isolates were spread by streaking on each petri dish containing the substrate with different concentrations and incubated for 24 hrs at 37 ^{0}C. Bacteria growth was observed (Balogu, 2019).

2.5 Screening of Bacterial Isolates with Lead Removal Potential

Bacteria isolates with potential to remediate soil samples were tested using the method of Ibrahim (2023). 5ml of nutrient broth grown isolates was added to a test tube containing 10ml of mineral salt medium (MSM), lead contaminated soil sample was added to the solution and incubated at room temperature for 8 days. The absorbance at 290nm was measured with a UV spectrophotometer to determine bacterial lead removal potential.

2.6 Biochemical Test

The following biochemical assays were performed on the isolates using Bergy's manual: citrate, catalase, coagulase, urease, methyl red, mannitol, Gram staining, and sugar fermentation.

2.7 DNA Extraction and 16S rRNA Gene Amplification

Genomic DNA was extracted from the bacteria isolates using the Zymo Research DNA prep kit. After the extraction, polymerase chain reaction was done to amplify the bacteria using gene amplification of the 16S gene with RIBOS 1 (for) GGACTACAGGGTATCTAAT and RIBOS 2 (rev) AGAGTTTGATTCCTGG primers. They were amplified using a thermal cycler (Bioer, Japan). Each 20 $\mathcal{M}$ L hotstart PCR reaction mixture volume contained approximately 50ηg purified DNA 0.1 $\mathcal{M}$M primers, 25 $\mathcal{M}$M of each dNTPS (3mM MgCl2, 0.5$\mathcal{M}$ of Taq DNA polymerase, in 10mM Tris-HCl (pH 9) and 50mM KCl. The temperature profile is as follows: 1 minute at 94 ^{0}C, 1 minute at 52 ^{0}C, I minute at 72 ^{0}C and 7 minutes extension at 72 ^{0}C. 1.5% agarose gel electrophoresis was used to analyze amplified products. The 16S gene's final amplicon size was 789 base pairs. Purification and sequencing of the amplicon were done by Inqaba Biotec, Ibadan, Nigeria.

2.7.1 Phylogenetic Analysis

The Blastn analysis was performed at the National Center for Biotechnology Information to establish the genetic relationships between the bacteria and others in the NCBI gene bank. The bacterial sequences were aligned using the cluster W technique in MEGA 11 software. The phylogenetic tree was constructed with cluster W, distance matrix analysis, and the neighbor-joining technique (Saitou & Nei, 1987).

3.0 RESULTS AND DISCUSSION

3.1 Isolation of lead-tolerant bacteria

After 3 days of incubation, bacteria strains with distinct abilities to use lead sole carbon and energy source were isolated from lead contaminated soil of four different mining sites in Minna, Niger State. Four prominent isolates designated as HM1, HM2, HM3, and HM4 were isolated from the contaminated soil (Figure 1).

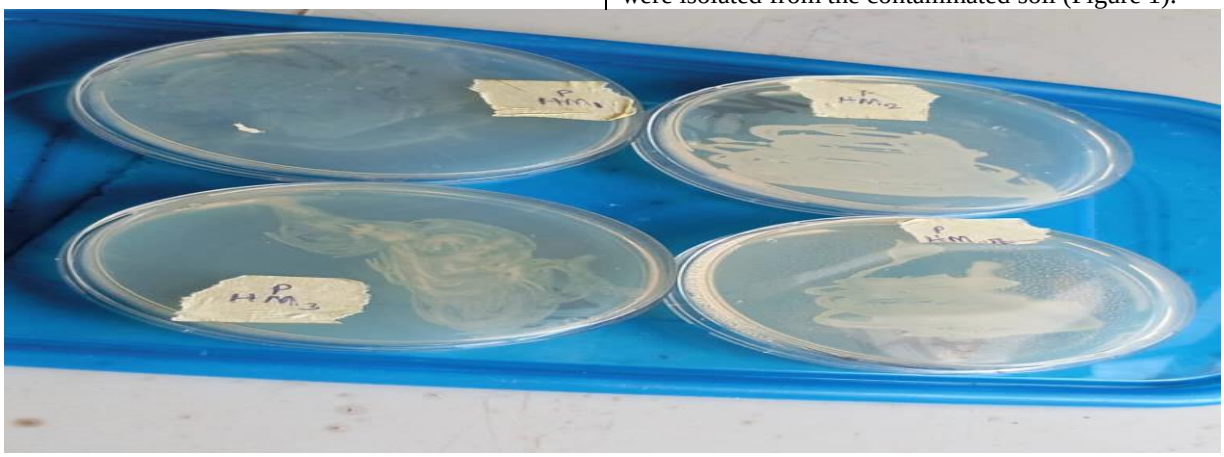

Figure 1: Bacteria Isolated From Contaminated Soil Of Four Different Mining Sites, Minna Niger State.

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3.2 Determination of Lead Tolerant Bacteria

The lead tolerance activities of the four isolates were evaluated on nutrient agar with different concentration of lead acetate (substrate) as a carbon and energy source (Table 1). It revealed that more growth was observed on isolate HM3, with substantial growth at concentrations of 200, 400, 600, 800 and 1000mg/ml. The bacteria growth for the four isolates was consistent with the bacteria growth principle, which states that the greater the growth, the lower the concentration and the lower the concentration, the greater the growth (Table 1). Compared to the potency of isolates to other isolates, HM3 was chosen for subsequent molecular identification.

Table 1: Determination of Lead Tolerant Bacteria

Bacteria Isolates	200mg/ml	400mg/ml	600mg/ml	800mg/ml	1000mg/ml
HM1	+++	+++	++	+	+
HM2	+++	+++	++	+	+
HM3	+++	+++	+++	++	+
HM4	++	++	+	+	-

Keywords: +++ = high growth, ++ = moderate growth, + = low growth, - = no growth.

3.4 Bacteria Isolates with Lead Removal Potential

The lead degrading activities of the four isolates were evaluated on MSM broth with lead contaminated soil sample as carbon and energy source (Figure 1). It revealed that isolate HM2 and HM3 drastically reduce lead on the 8th day of the treatment showing the potency of the bacteria isolates to the contaminated soil (Figure 1). Before incubation indicate the absorbance taken of the isolates on the 1st day of treatment as shown on (figure 2).

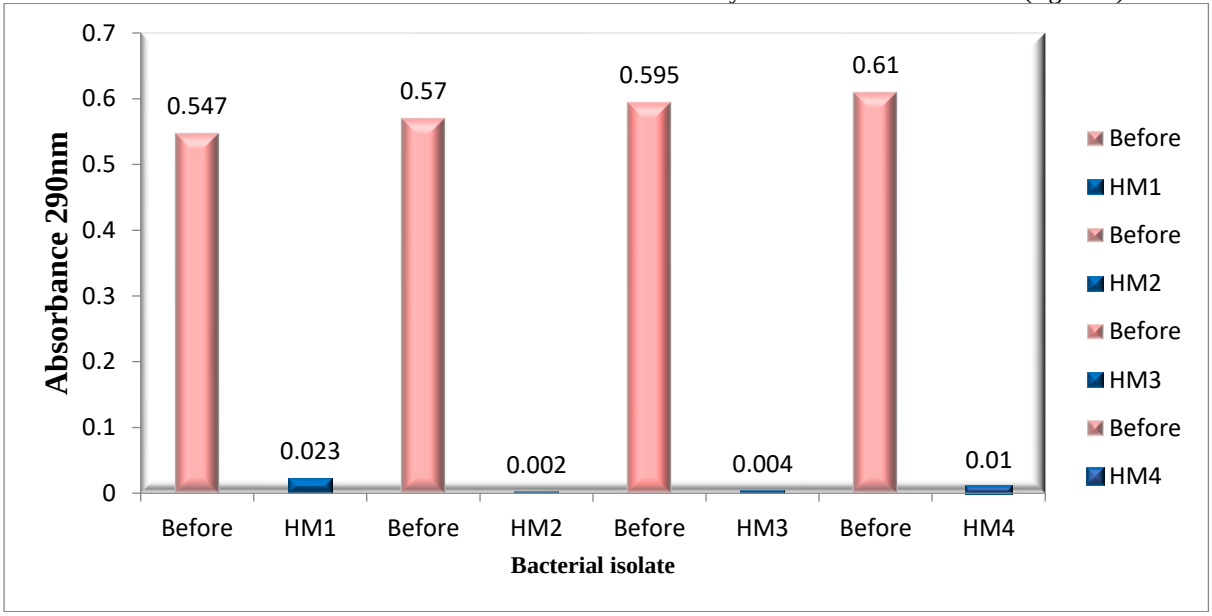

Figure 2: Lead Reduction By Bacteria Isolates Isolated From Contaminated Soil Of Mining Sites.

3.5 Biochemical/ Morphological characterization of lead tolerance bacteria

Table 2 displays the biochemical and morphological characterization of four bacterial colonies that were isolated from lead contaminated soil of mining sites. The isolates were classified to the species level based on morphological and biochemical parameters. Bactria HM1, HM2, HM3, and HM4 are *Bacillus subtilis, Bacillus megaterium, Bacillus subtilis*, and *Bacillus cereus* respectively.

Table 2: Biochemical/ Morphological Characterization

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Isolates	Colour	Shape	Gram staining	Catalase	Methyl red	V/p	Mannitol	Citrate	Coagulase	Urease	Glucose	Fructose	Organism interpreta
HM1	Off-white	Rod shape	+	+	+	+	+	+	-	-	+		*Bacillus subtilis*
HM2	Milk-white	Rod shape	+	+	+	-	+	+	-	+	+		*Bacillus megaterium*
HM3	Off-white	Rod shape	+	+	+	+	+	+	-	-	+		*Bacillus subtilis*
HM4	Gray-white	Oval shape	+	+	+	-	+	+	+	+	+		*Bacillus cereus*

+ = Present, - = absent

3.6 Identification of Bacterial Isolates by DNA Analysis

The 16S rRNA gene sequence of bacterium HM3 was obtained from the PCR product (Figure 3) which showed 789 base pairs. The sequence was blasted in NCBI to compare it with strains in the Gen bank data base (Table 3). From the blasting result, bacterium HM3 is *Bacillus subtilis* with 99% identity to this species. The biochemical tests (Table 2) and MEGA 11 phylogenetic analyses verify this result.

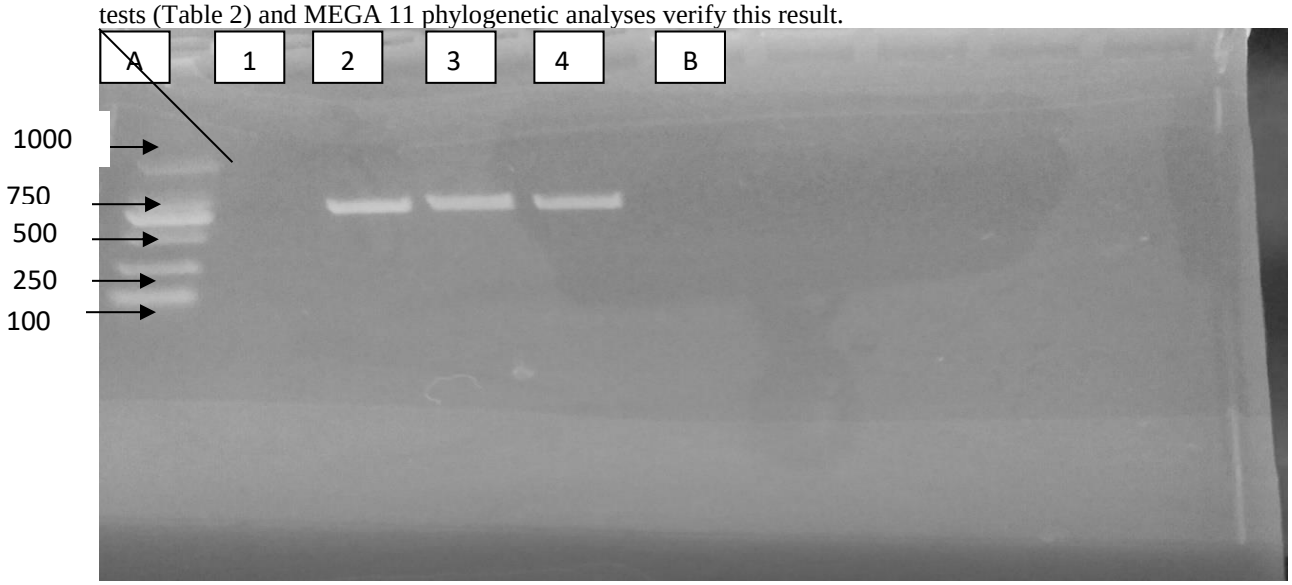

Figure 3: PCR Amplicons Of Rrna Genes Of Four Bacterial Isolates From Lead-Contaminated Soil Of Mining Site, Lane A; Ladder, Lane 1; HM1, Lane 2; HM3, Lane 4; HM4, Lane B; Control

3.6.1 Phylogenetic Analysis

In this study, the bacterium isolate HM3 Neighbor-joining phylogeny tree was constructed with sequences from the NCBI BLAST result. Mega 11 software was used and about 5 sequences were selected together with the isolate sequence (Figure 4).

Table 3: NCBI Database Blastn Result for Bacterium HM3

Accession number	Description	Maximum identity (%)

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PQ813807.1	*Bacillus velenzensis strain Bac73 16S ribosomal RNA gene*	99%
PQ464786.1	*Bacillus subtilis strain L-20 16S ribosomal RNA gene*	99%
PV972097.1	*Bacillus subtilis strain MP9 16S ribosomal RNA gene*	99%
PQ813753.1	*Bacillus velenzensis strain Bac19 16S ribosomal RNA gene*	99%
OP218662.1	*Bacillus sp. (in: Bacteria) strains SWUSTb-106 16S ribosomal RNA gene*	99%

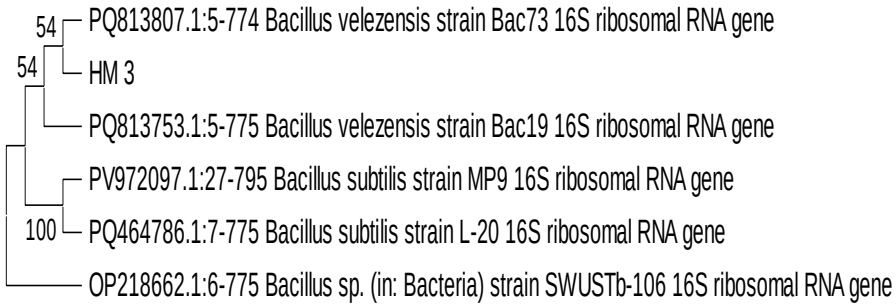

Figure 4: Phylogenetic Tree Displaying Evolutionary Relationship of Bacteria HM3 with Other Bacteria in the Gene Bank.

4.0 DISCUSSION

Four bacteria strains with different abilities to utilize lead as a carbon and energy source were identified from lead-contaminated soil at mining sites. The main isolates were named HM1, HM2, HM3, and HM4.

The lead tolerance activities of the four isolates were assessed on nutrient agar using varying concentrations of lead acetate (substrate) as a carbon and energy source (Table 1). It reveals that HM3 has a high tolerance for lead acetate, with significant growth at concentrations of 200, 400, 600, 800, and 1000 mg/ml. This result is in line with the findings of Fatima *et al*. (2023), who discovered that *Bacillus sp*. grows optimally at 1000mg/L of $Pb(NO_3)_2$ after 24 hours of incubation at 37 ^{0}C. The isolate's growth rises linearly at lead nitrate concentrations of 500mg/L, peaking at 1000mg/L ($Pb(NO_3)_2$) and decreasing considerably above 1500mg/L.

Research on lead-tolerant microorganisms has been conducted over the years, and the most tolerated concentration so far has been reported by Sepale *et al*., (2015). According to Sepale *et al*., (2015), a *Proteus sp*. isolated from sewage in India can tolerate 1600mg/L of lead. The concentration of lead has a direct effect on microbial tolerance. Microbial tolerance to lead concentration varies: the higher the concentration, the less tolerance; the lower the concentration, the greater the tolerance.

The four isolates' lead removal potential was assessed on MSM broth containing lead-contaminated soil as a carbon and energy source (Figure 1). It indicated that HM2 and HM3 could remove lead more than the other isolates (Figure 1). The four Bacteria isolates from polluted mining soil survive in the presence of lead; however the pace of deterioration varies. On the 8th day of incubation, the bacteria isolates undergo significant breakdown and use lead as a carbon and energy source for development.

The 16S rRNA gene sequence of bacteria HM3 was recovered from the PCR result (Figure 3), which had 789 base pairs. According to the blasting results, bacteria HM3 belong to *Bacillus subtilis* with 99% identity to the species. This conclusion is supported by biochemical and morphological analyses. *Bacillus subtilis* is a species of *Bacillus* that may be found in a

wide range of environments. *Bacillus subtilis* is the most prevalent *Bacillus species* isolated from the environment; it is a Gram-positive, rod-shaped, spore-forming, facultative anaerobe bacterium (Xianopei Zhang *et al*., 2020).

CONCLUSION

The study successfully isolated and characterized indigenous lead-tolerant bacteria from the mining environments in Minna. These isolates, particularly those exhibiting high tolerance and degradation efficiency, represent potential bioremediation agents for mitigating lead pollution in contaminated soils, offering and eco-friendly approach to environmental clean-up.

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