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ORIGINAL RESEARCH ARTICLE

Characterization of Heavy Metal-Tolerant Bacteria from Dumpsites in Katsina Metropolis and their Bioremediation Potential

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ABSTRACT

Heavy metals have harmful effects on living organisms, microorganisms, and the environment as a whole. There is a lack of studies on heavy metal-tolerant bacteria from dumpsites in Katsina Metropolis, despite their potential for application in heavy metal bio-removal. Therefore, this study was conducted to characterize heavy metal-tolerant bacteria from refuse dump sites in Katsina Metropolis, with the goal of assessing their potential for bioremediation of heavy metal contamination. Soil samples were collected from four dumpsites: A, B, C, and D. Bacteria were isolated using serial dilutions and plating techniques. These isolates were then Gram-stained and underwent various biochemical tests, including indole, catalase, citrate, motility, starch hydrolysis, strings (exopolysaccharide), urease, and oxidase tests. Heavy metal tolerance was assessed by culturing the bacteria in minimal salt medium supplemented with various metal types at different concentrations: zinc, cobalt, nickel, lead, and chromium. The metal salts used were $ZnSO_4$, $NiSO_4$, $Pb(NO_3)_2$, $CoCl_2$, and $K_2Cr_2O_7$. Molecular identification of metal-tolerant isolates was identified through 16S rRNA gene sequencing. The concentration of heavy metal salts in the soil samples ranged from 0.058 to 0.42 (chromium), 0.341 to 0.952 (zinc), 0.17 to 1.54 (lead), 0.001 to 0.04 (cobalt), and 0.037 to 0.103 (nickel) ppm. The highest bacterial count was 9.8×10^{13} CFU/g in sample A, which also had the highest zinc concentration (0.952 ppm). Isolates A1 and B1 showed high chromium tolerance at lower concentrations. Molecular identification revealed *Bacillus sonorensis*, *Achromobacter mucicolens*, *Bacillus licheniformis*, and *Lysinibacillus composti* as the dominant metal-tolerant species. This study provides the first characterization of heavy metal-tolerant bacteria from dumpsites in Katsina Metropolis, identifying novel strains with bioremediation potential.

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INTRODUCTION

Urban dump sites, particularly in fast-growing cities like Katsina Metropolis, are a huge environmental problem that results from the indiscriminate dumping of heterogeneous waste materials. The dump sites typically comprise municipal, agricultural, industrial, and domestic waste, like plastics, organic waste, and toxic substances like heavy metals (Akinyemi *et al.*, 2021). The lack of proper waste management systems in most developing nations has caused the expansion of such dumpsites, where uncontrolled dumping of metallic materials, used containers, and industrial waste is the cause of soil and water contamination and long-term danger to ecosystems and human health (Ali *et al.*, 2022; Adewole *et al.*, 2021; Chukwuma *et al.*, 2022). Heavy metals like lead, cobalt, nickel, cadmium, chromium, and mercury are of greatest concern because of their toxicity, inability to biodegrade, and persistence in the environment. They are most easily admitted into the environment through industrial effluent, runoff from mining, and breakdown of improperly disposed waste (Jadaa *et al.*, 2023). When they are released, they can percolate into neighboring soils and aquifers, bioaccumulate in organisms, and remain in food chains, hence having severe ecological and human health effects (Kalu *et al.*, 2020). Traditional heavy metal remediation methods are available; they are energy-intensive, expensive, and environmentally invasive. Conversely, bioremediation, utilizing the natural decontaminating ability of microbes, offers a greener and more cost-effective option (Adebayo *et al.*, 2021). Specific bacteria, in return, possess complex mechanisms of resistance biosorption, bioaccumulation, enzymatic transformation, and efflux systems that allow them to survive and convert toxic metals into less toxic metals (Kalu *et al.*, 2020; Olawale *et al.*, 2022).

Despite the global recognition of heavy metal pollution in dumpsites, there is a dearth of information on the microbial communities and their bioremediation potential in Katsina Metropolis, Nigeria, which is experiencing rapid urbanization and waste management challenges.

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This study addresses this gap by isolating and characterizing heavy metal-tolerant bacteria from Katsina dumpsites, with a focus on their potential for bioremediation applications in the region.

Katsina's dumpsites represent special microhabitats formed by the elevated organic load, heterogeneous metal composition, and anaerobic environment, which are proven to favor the development of metal-resistant microbial populations. Studies of the indigenous microbes provide valuable information regarding the adaptation strategies of bacteria in polluted environments and offer an opportunity for locally adapted bioremediation.

This study aimed to isolate, characterize, and identify bacteria resistant to multiple heavy metals from four primary dumpsites of Katsina Metropolis. Using a combination of culture-based techniques, metal tolerance assays, and 16S rRNA gene sequencing, the study evaluated their potentiality for utilization in bioremediation of metal-contaminated soil.

MATERIALS AND METHODS

Research Site

The survey was conducted in Katsina Metropolis, the capital of Katsina State, Nigeria. The City lies approximately at 12.9908° N and 7.6014° E. In this area, wastes are highly accumulated mainly around its numerous disposal sites. Different disposal sites, based on the degree of pollution, were selected for the determination of an environment which may suit microbial populations that could be adapted to the tolerance of heavy metals with ease. Poor waste management and rapid urbanization have turned Katsina Metropolis into a very fertile ground for the study of microbial diversity and possible bioremediation strategies against polluted areas.

Sample Collection

The soil samples used for the research work were aseptically collected from four different dumpsites within the Katsina municipality, labeled as dumpsites Kofar Kaura, Kofar Guga, Kofar Marusa, and Kofar Kwaya. The selection of these sites was based on their proximity to the refuse dump site. Kofar Kaura is a medium to large roadside dump site where waste is primarily domestic and agricultural waste, such as metallic cans, plastic bottles, batteries, and vegetable waste. The waste is quite recent (less than 10 months), but the fact that there are abandoned electronic components and batteries indicates signs of potential initial heavy metal leaching into the ground.

The Kofar Guga dumpsite is a huge uncontrolled dumpsite with humongous domestic, industrial, and street wastes consisting of metal scraps, rusty wires, used batteries, plastics, and clothes. The dumpsite has been operational for more than one year, and the high compactness of waste points towards long-term metal accumulation and long-term environmental stress, conditions that would support the presence of metal-resistant bacteria. Kofar Marusa dumpsite is medium in capacity and accepts mixed household and commercial rubbish, including discarded electronics, packaging materials, food waste, and aluminum foils. The age of the waste is 6-10 months, and mid-stage metal pollution is likely to occur in the dumpsite, based on the nature of the refuse present on site. Kofar Kwaya is an open dump of medium size with ongoing dumping of organic refuse, domestic refuse, plastic bags, and rusty metallic items. A combination of fresh and old waste with poor draining makes metal ion accumulation and mobility in the soil more probable. The method adopted for sample collection was aseptic sampling, where composite sampling of the soil samples was collected 10m apart in three (3) different places in each sampling site and mixed together to make a single sample. To prevent contamination and ensure the integrity of the samples, collection was made from the selected dumpsites by scraping the surface debris with a sterilized trowel and extracting subsurface to a depth of 10 cm using another sterilized trowel. The collected waste samples were transferred into a pre-labeled polyethylene bag and taken for microbiological analysis at the Microbiology Laboratory Unit, Umaru Musa Yar'adua University, Katsina.

Assessment of Heavy Metal Concentration in Waste Sample

One gram of the resulting ground sample was measured to an accuracy of measurement by a top-loading balance and transferred to a 250 mL beaker pre-cleaned with nitric acid, followed by washing with distilled water (Thompson and Lee 2018). To the sample were added sequentially, pipetting: 5 mL of nitric acid (HNO_3), 3 mL of concentrated sulfuric acid H_2SO_4, and 2 mL of perchloric acid $HClO_4$. This solution was transferred to a fume hood where it was heated until it had produced dense white fumes (ISO, 2020; Thompson and Lee, 2018). During this process, the digestion time used was 15 minutes, and then cooled. This was further diluted with distilled water, filtered through acid-washed Whatman No. 44 filter paper, and finally transferred into a 50 mL volumetric flask, making up to the mark with further dilution. The aspirated sample solution that was prepared was introduced in regular intervals into the Atomic Absorption Spectrometer for analysis (ISO, 2020; Thompson and Lee, 2018).

Determination of metal tolerance in bacterial isolates

Preparation of Heavy Metal Solutions and Minimal Salt Medium Agar Plates

Five heavy metals, namely Zn, Ni, Co, Pb, and Cr, were prepared at concentrations of 25 mg/L, 50 mg/L, and 100 mg/L by adding their respective metal salts to sterile deionized water. The metal salts used included ZnSO4, NiSO4, PbNO3, CoCl2, and K2Cr2O7. All solutions were filtered through a 0.22 µm membrane filter to ensure sterility (Ayangbenro and Babalola, 2017). Next, the MSM agar was prepared using standard methods and

supplemented with heavy metal ions at appropriate concentrations. Various volumes of the metal solutions were added into MSM agar to achieve the final concentrations of 25 mg/L, 50 mg/L, and 100 mg/L. The plates were allowed to solidify under a laminar flow hood to maintain sterility. The concentration of heavy metals in the prepared solutions was expressed in mg/L, as they were used to assess bacterial tolerance. However, for soil sample analyses, the concentration of heavy metals was measured in ppm, which is a standard unit for environmental contamination studies.

Inoculation and incubation of Bacterial isolates in MSM

The isolates A1–A3, B1–B3, C1–C3, and D1–D3 obtained from heavy metal-contaminated sites were pre-cultivated in nutrient broth at 37°C for 24 hours to achieve log-phase growth. This is indeed the most suitable phase of growth for active biodegradation (Ahmad *et al.*, 2022; El-Sersy *et al.*, 2020). Each of the bacterial isolates A1–A3, B1–B3, C1–C3, and D1–D3 was inoculated on MSM agar plates containing different concentrations of heavy metal salts, namely: $ZnSO_4$, $NiSO_4$, $PbNO_3$, $CoCl_2$, and $K_2Cr_2O_7$. Triplicate sets for each strain were prepared at each concentration to ensure accuracy in the results obtained. Control plates without metal ions were also prepared, and the results were compared (El-Naggar *et al.*, 2018). The inoculation plates were then incubated at 30°C for 5 days.

Assessment of heavy metal tolerance in Bacteria

Daily observations were made on the growth of bacteria in the presence of heavy metals. The bacteria's ability to tolerate heavy metals was assessed by quantifying colony growth on MSM plates supplemented with these metals. The colony-forming units (CFU/g) were manually counted from MSM plates incubated for 5 days to quantify bacterial growth with every metal concentration. The growth patterns on the plates exhibited characteristics of bacterial tolerance and potential for biodegradation, as noted by Ayangbenro and Babalola (2017). Color changes around the colonies visually indicated bacterial activity, which may be related to interactions with heavy metals (Ali *et al.*, 2021; Ahmad *et al.*, 2022).

Molecular characterization of the Heavy Metal-Tolerant isolates

Isolates that showed high tolerance to the heavy metals tested were purified and identified using 16S rRNA gene sequencing

DNA Deoxyribonucleic Acid Extraction

The genomic DNA extraction was conducted using the traditional phenol-chloroform method (Prabha *et al.*, 2017). Initially, the sample suspension was made in sterile distilled water, and the resulting material was mixed with 200 µL of an extraction buffer and 20 µL of proteinase K. Then, it was left to incubate at 55°C overnight to facilitate cell lysis.

Subsequently, the phenol-chloroform extraction process was initiated by adding 200 µL of phenol:chloroform: isoamyl alcohol (25:24:1) solution to the sample. The mixture was centrifuged at 12,000 g for 5 minutes, resulting in the separation of aqueous and organic phases. The upper aqueous phase, which contained DNA, was carefully collected with a pipette and transferred to a new 1.5 µL microcentrifuge tube (Prabha *et al.*, 2017).

The DNA was then precipitated by adding 200 µL of cold 70% ethanol, followed by centrifugation at 12,000 g for 5 minutes, resulting in the formation of a DNA pellet. This pellet was washed with ethanol to eliminate impurities (Prabha *et al.*, 2017).

After air-drying to remove any remaining ethanol, the DNA pellet was dissolved in distilled water. Finally, the DNA was stored at -20°C for preservation.

Polymerase Chain Reaction (PCR) Amplification

The bacterial 16S rRNA gene was amplified using the bacterial universal primers 27F and 1492R (27F: 5'-TAGAGTTTGATCMTGGCTCAG-3', 1492R: 5'-GGTTACCTTGTTACGACTT-3'), which are designed to target the conserved region of the bacterial 16S gene. The PCR reactions were set up to a total volume of 50 µL, comprising 1 µL of template DNA, 10 µL of PCR buffer, 0.5 µL of Taq polymerase, 1 µL of each primer (10 µM), 1 µL of dNTP mix, and 36.5 µL of nuclease-free water. The conditions for PCR were as follows: pre-denaturation at 94°C for 3 min; amplification for 35 cycles, each consisting of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 7 min (Zhao *et al.*, 2023). PCR reactions were conducted with positive and negative controls. Positive control consisted of a known bacterial DNA to monitor the efficiency of the reaction, while no-template control (NTC) was added to screen out any possible contamination.

Sequencing and Bioinformatics Analysis

The purified 16S amplicons were sent for Sanger sequencing at Inqaba Biotec in Ibadan. The obtained sequences were then analyzed and matched with reference sequences in the GenBank database using the Basic Local Alignment Search Tool. Identification was considered confirmed if there was at least 98% similarity to recognized bacterial species in the database (Park *et al.*, 2023).

Agarose Gel Electrophoresis

The PCR products were then examined on a 1.5% agarose gel impregnated with ethidium bromide. Following 30 min of electrophoresis at 100V, bands were examined under a UV light. A molecular marker utilizing a 100bp DNA ladder was utilized to verify the expected amplicon size for the 16S region (Sun *et al.*, 2022).

Purification of PCR Products

The respective manufacturers' commercial kit for the purification of the PCR products was performed. After that, the quantification and quality checking of the purified DNA were made using a NanoDrop spectrophotometer before being washed in 30 µL of nuclease-free water for sequencing (Chen *et al.*, 2022).

Data Analysis

Statistical analysis was carried out using SPSS version 25.0 (IBM Corp., USA). One-way Analysis of Variance (ANOVA) was employed to measure extreme differences in concentration of heavy metal and bacterial density between sampling points at $p < 0.05$. Pearson and Spearman correlation tests were also conducted to measure the relationship between heavy metal concentration and total bacterial abundance, to determine possible inhibitory effects of individual metals.

RESULTS

Total Bacteria Count

Table 1 presents the data of Bacterial microbial count, indicating microbial population of the soil sample for samples A, B, C, and D. The highest microbial population in both units was enlisted for Sample A, which recorded 9.8×10^{13} CFU/g ±1.0. This means that sample A provides more favorable conditions for microbial growth, potentially due to higher nutrient availability or other environmental conditions in the soil sample. Sample B shows counts of 8.35×10^{13} CFU/g ±1.5, relatively a good environment, though not as optimum as sample A for microbial growth.

In contrast, Sample D shows the minimum Bacterial count in the particular class of 5.2×10^{13} CFU/g category which may indicate harmful conditions such as nutrient deficiencies and its availability or higher amount of microbial inhibitors in the soil sample. Sample C had recorded lower values than the samples A and B with 8.2×10^{13} CFU/g ±2.0, respectively.

Table 1: Total Bacterial Count

Samples ID	(X10^{13}) CFU/g
A	9.8±1.0
B	8.35±1.5
C	8.2±2.0
D	5.2±1.0

Key: Mean± Standard deviation

Heavy metal Concentration obtained from the Soil samples

Concentrations of Chromium, Zinc, Lead, Cobalt, and Nickel were analyzed in four different soil samples: A, B, C, and D. The results obtained (Table 2) reveal significant differences. Chromium varied from 0.058±0.00 PPM in Sample D to 0.42±0.001 PPM in Sample B, where the high level can be indicative of localized contamination. Zinc levels were highest in Sample A at 0.952 ± 0.006 PPM and lowest in Sample B at 0.341 ± 0.0029 PPM, thus showing significant variation and are hence environmentally influenced by factors such as industrial waste discharge. The lead concentration was highest in Sample D at 1.54 ± 0.0033 PPM, with appreciable levels also present in Samples C and D; hence, it could be contaminated from sources like industrial wastes. In general, the cobalt concentrations are low for all samples, with Sample A the highest at 0.04 ± 0.003 PPM and Sample B having the least at 0.001 ± 0.0008 PPM, which indicates trace contamination with cobalt. These results reflect a high amount of lead and chromium contamination, while the large variations of zinc indicate possible industrial and agricultural activities. The Asterisk * denotes that it is statistically significant, and it means there is a significant difference between sample sites.

Table 2: Heavy metals concentrations obtained from the soil

Soil Samples ID	Chromium(PPM)	Zinc (PPM)	Lead (PPM)	Cobalt (PPM)	Nickel
A	0.214±0.0014	0.952±0.006	0.17±0.0042*	0.04±0.003	0.037±0.0013
B	0.42±0.001	0.341±0.0029	0.75±0.0132	0.001±0.0008*	0.009±0.0034
C	0.238±0.0006*	0.699±0.002	1.18±0.0110	0.005±0.0016*	0.010±0.0013
D	0.058±0.00	0.894±0.0006*	1.54±0.0033*	0.011±0.0012	0.103±0.0012

Key: Mean ± Standard deviation**, (*)** Showing significance difference

Tolerance of Bacteria Isolated from Refuse Dump Sites to Heavy Metals

The bar graphs (Figures 2, 3, 4, 5 and 6) represent the mean count of bacteria, reflecting chromium tolerance in different soil samples treated with three different concentrations of chromium (25 mg, 50 mg, and 100 mg), compared with the control. In control, the count of bacteria is highest among all soil samples, which means the absence of chromium favors bacterial growth. With increased chromium content, the average bacterial count decreases across samples, pointing out the ill effect of chromium on bacterial growth. Some samples, A1 and B1, for instance, at lower concentrations showed a bigger bacterial count than at 100 mg, indicating an increase in tolerance. In the control group, B1 had the highest average bacterial count of 278 CFU. At 25 mg, this count decreased to 169.5 CFU, and at 50 mg, it further decreased to 88.5 CFU, with a significant drop to 35.5 CFU at 100 mg. Sample A3 showed the lowest bacterial count at the higher tested doses, specifically 30 CFU at 50 mg and 8.5 CFU at 100 mg. This shows that this bacterium was much more sensitive to chromium.

Molecular Characterisation of Soil-Isolated bacteria

Molecular characterization of the heavy metal-tolerant bacteria from the 12 isolates (A1–A3, B1–B3, C1–C3, and D1–D3) revealed significant growth in all tested concentrations of the five heavy metals, as shown in

Figures 2, 3, 4, 5 and 6. Hence, these isolates were subjected to PCR amplification targeting the 16S rRNA gene using 27F and 1492R bacterial universal primers and subsequent species identification by comparing their partial 16S rRNA gene sequences with those in NCBI GeneBank.

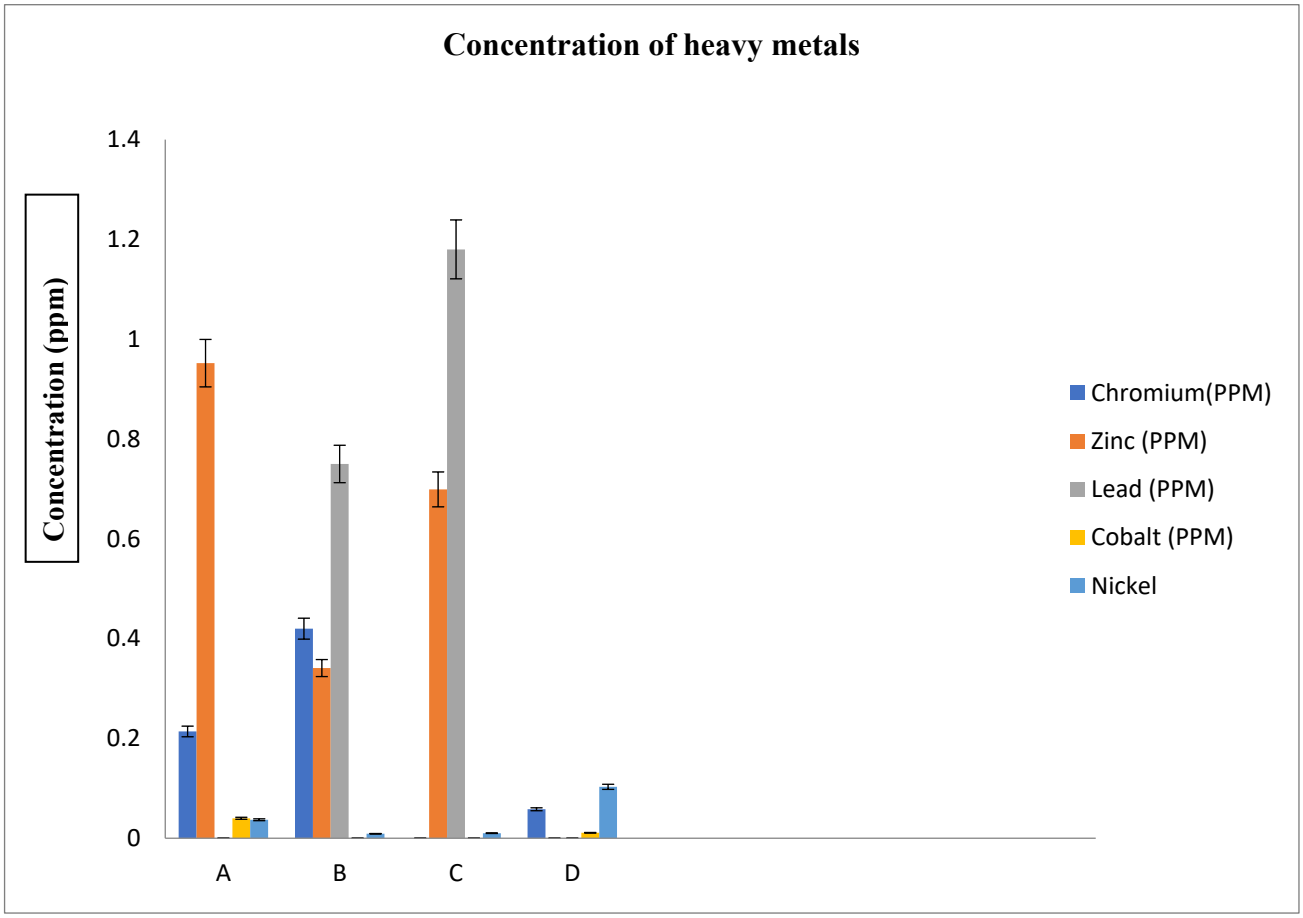

Figure 1: Concentrations of Heavy metals obtained from the dumpsites
Legend: Error bars indicate % error

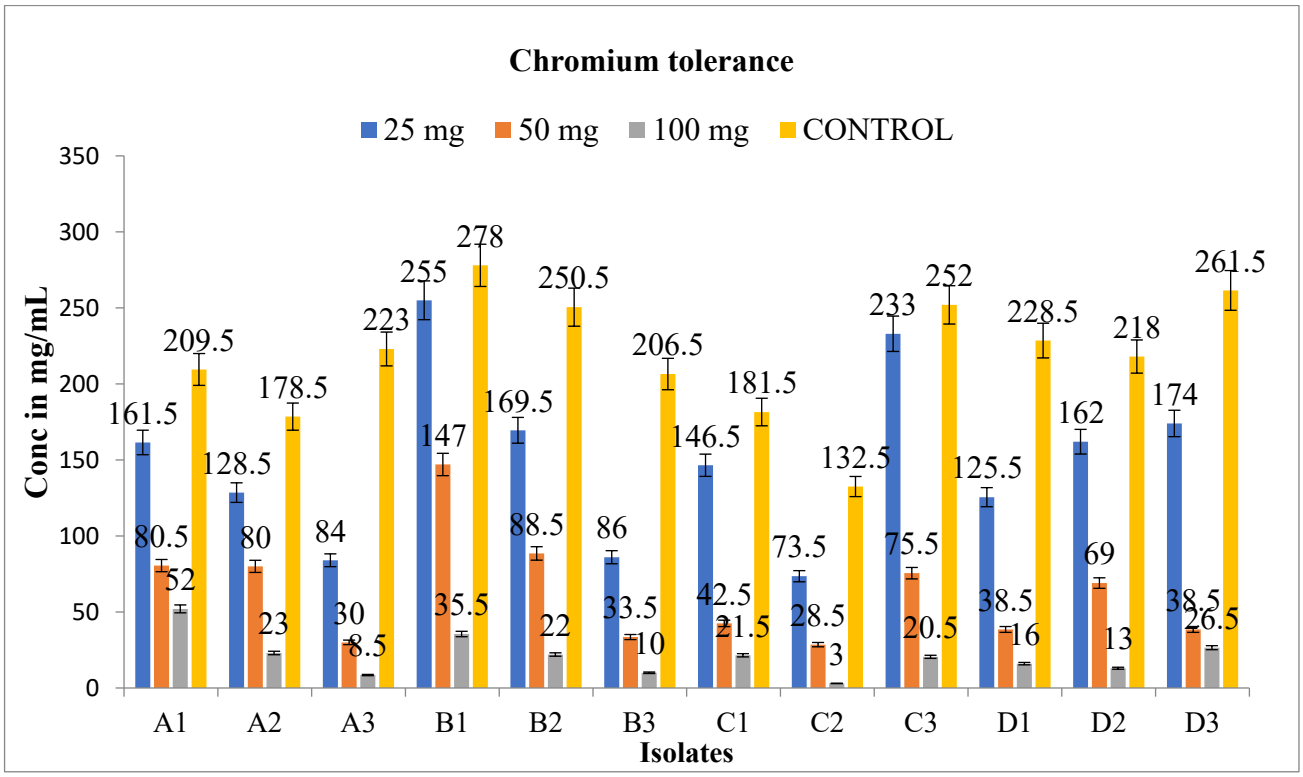

Figure 2: Mean count of chromium tolerant Bacteria isolated from the soil
Legend: Error bars indicate % error

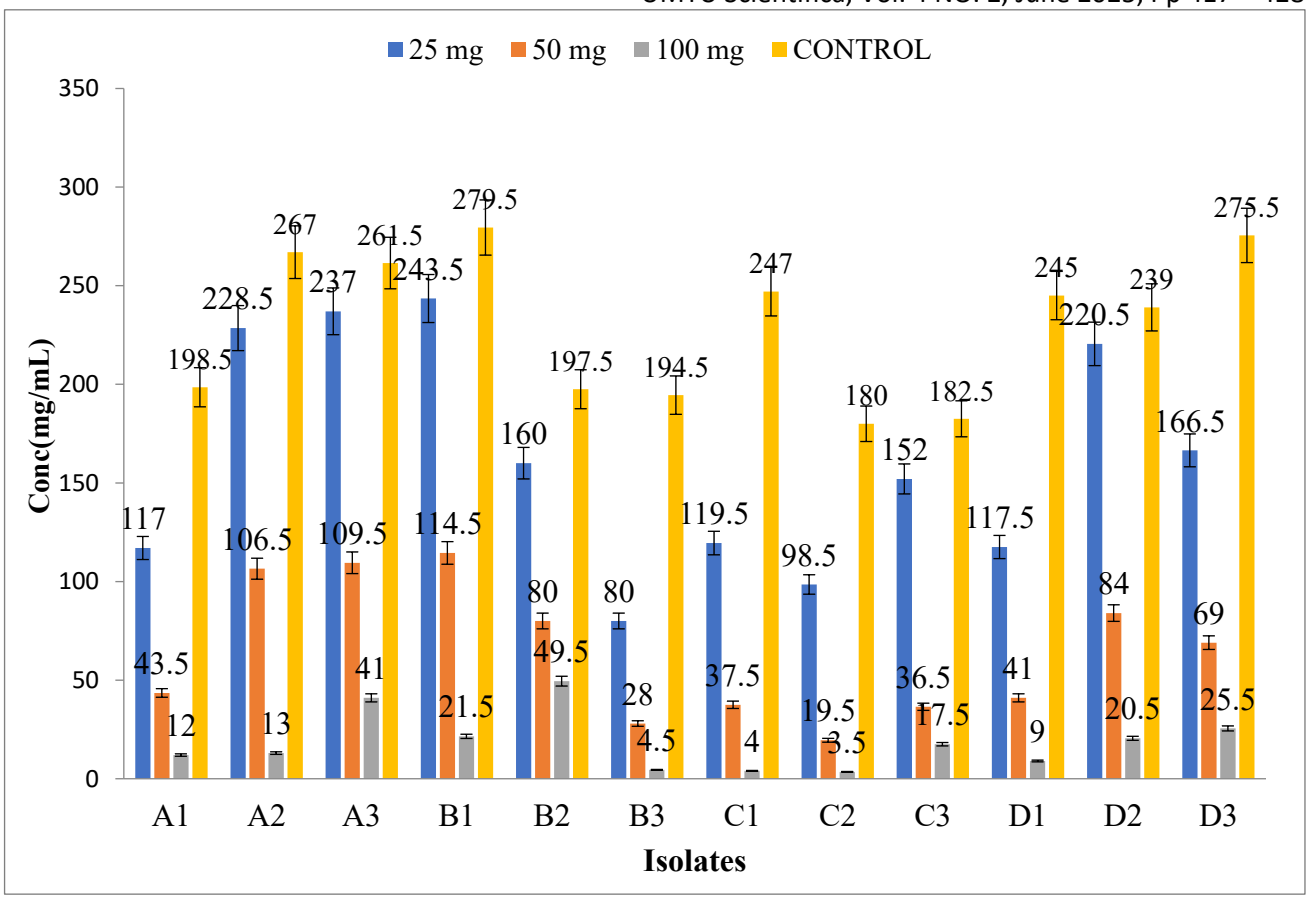

Figure 3: Mean count of lead tolerant bacteria isolated from the soil
Legend: Error bars indicate % error

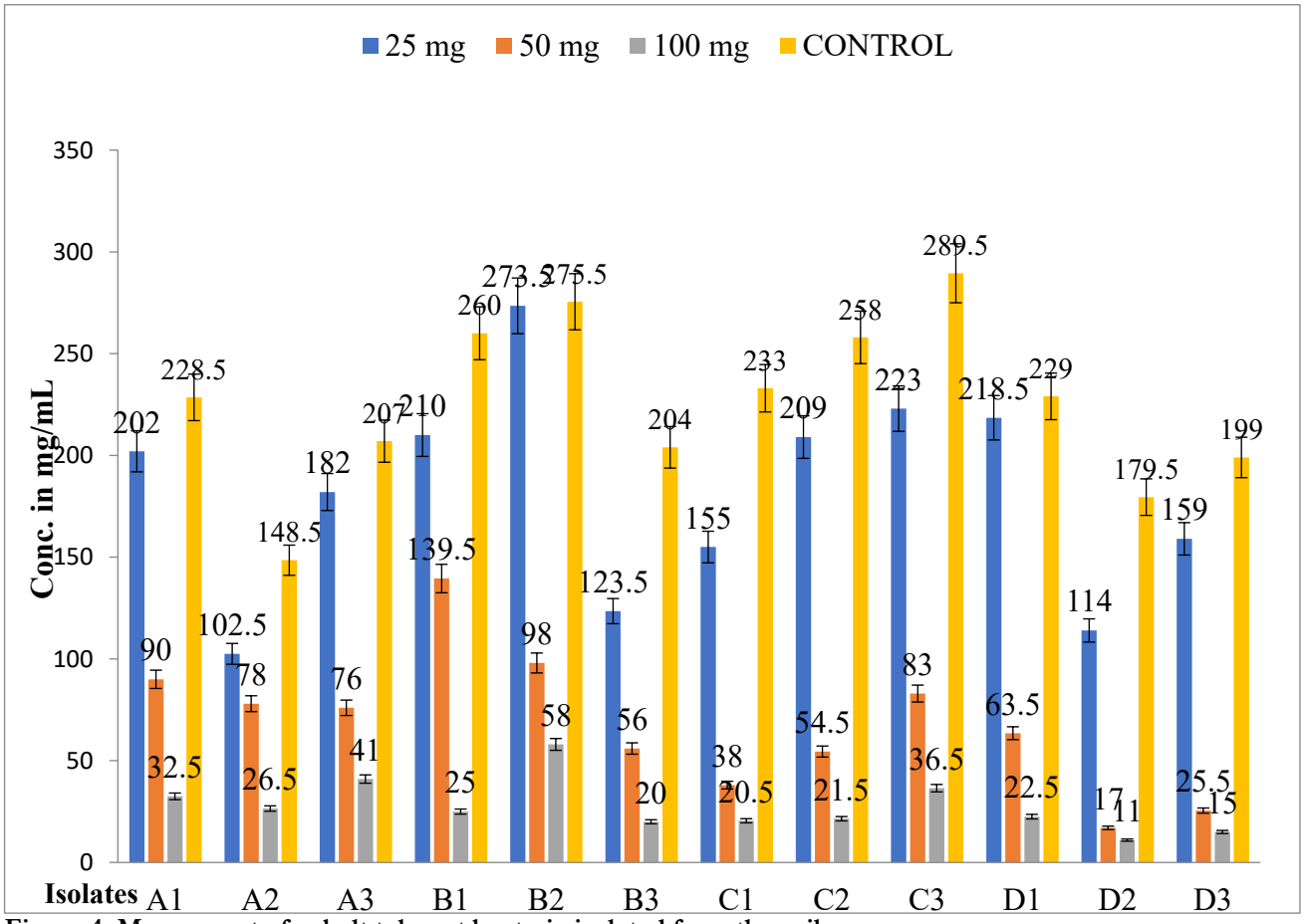

Figure 4: Mean count of cobalt tolerant bacteria isolated from the soil
Legend: Error bars indicate % error

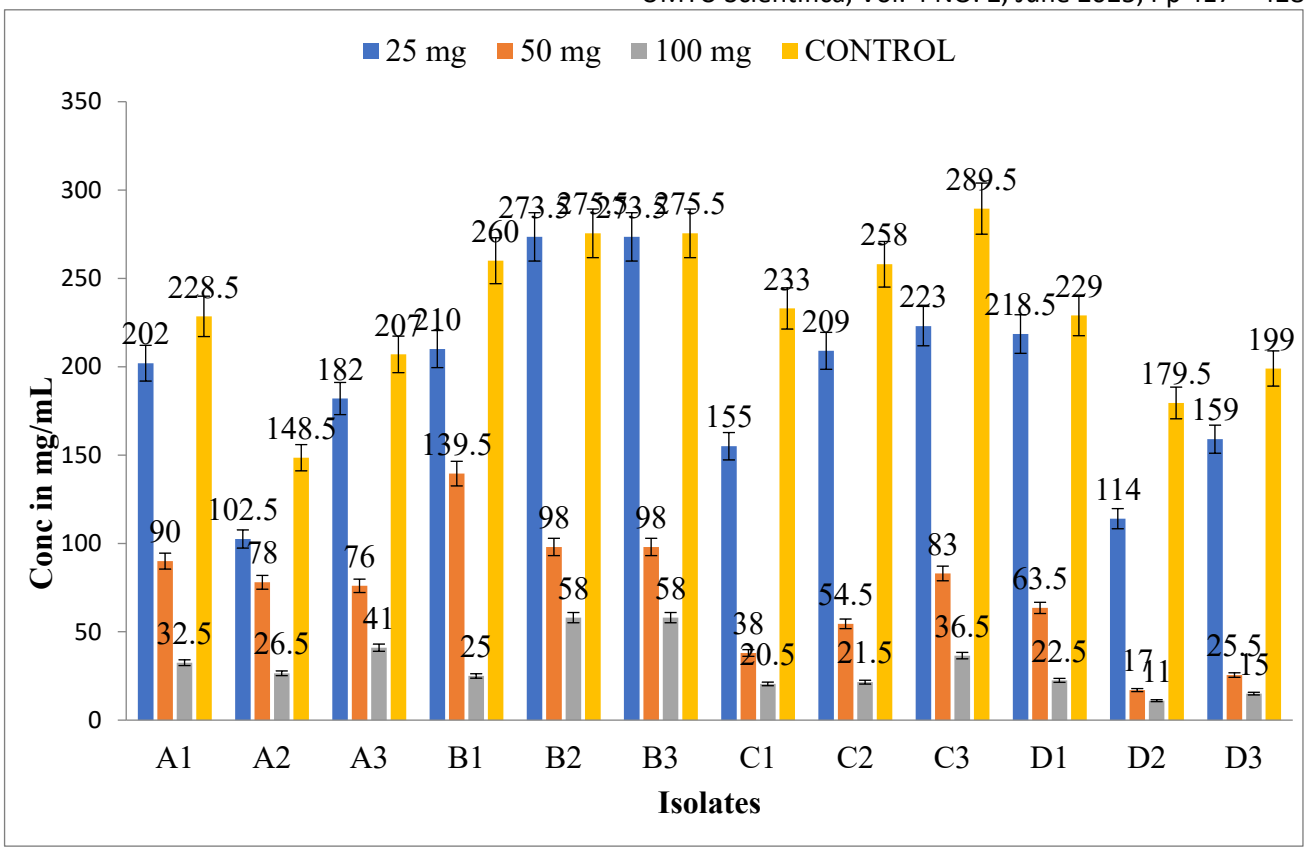

Figure 5: Mean count of zinc tolerant bacteria isolated from the soil
Legend: Error bars indicate % error

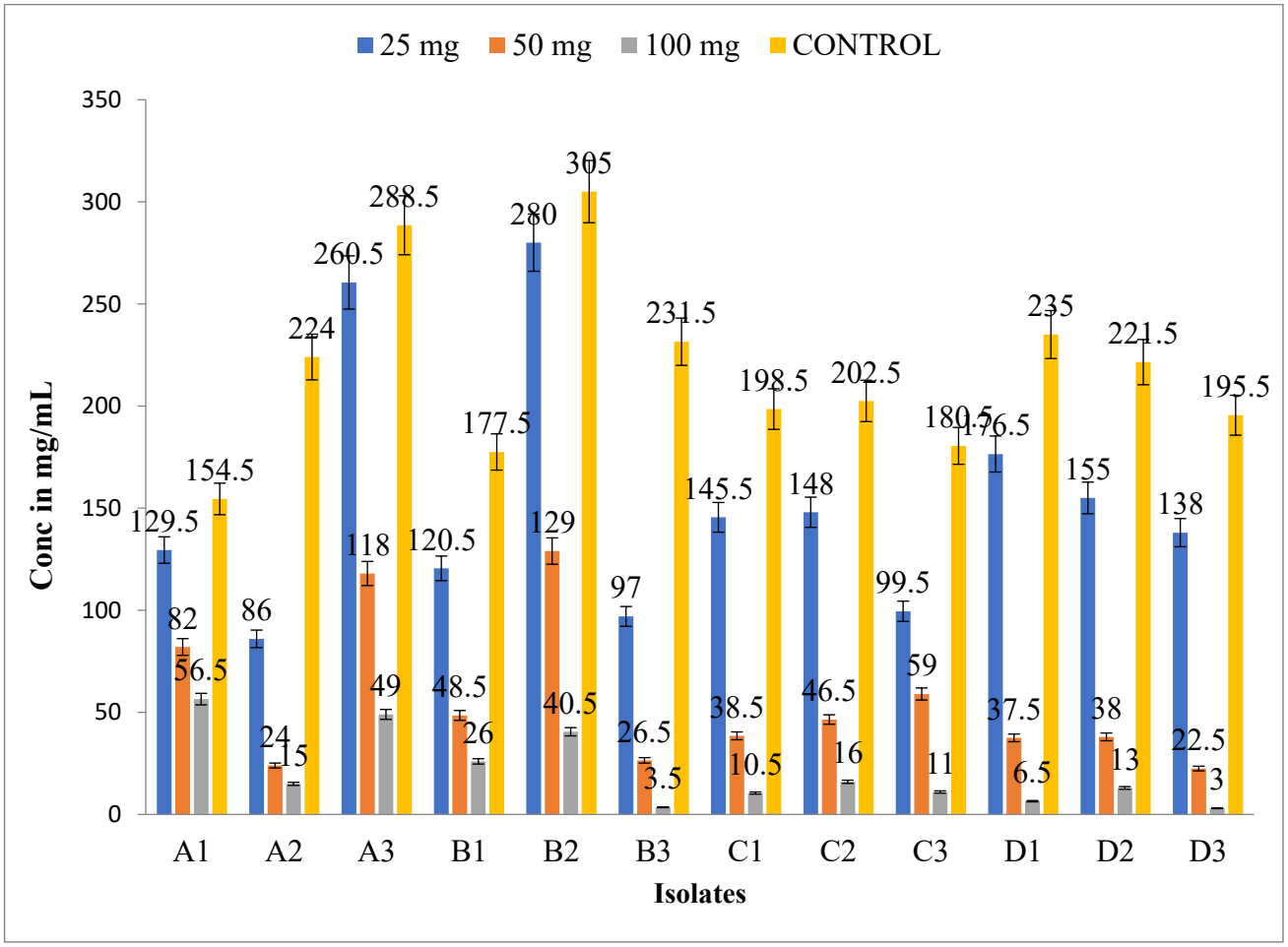

Figure 6: Mean count of Nickel tolerant bacteria isolated from the soil
Legend: Error bars indicate % error

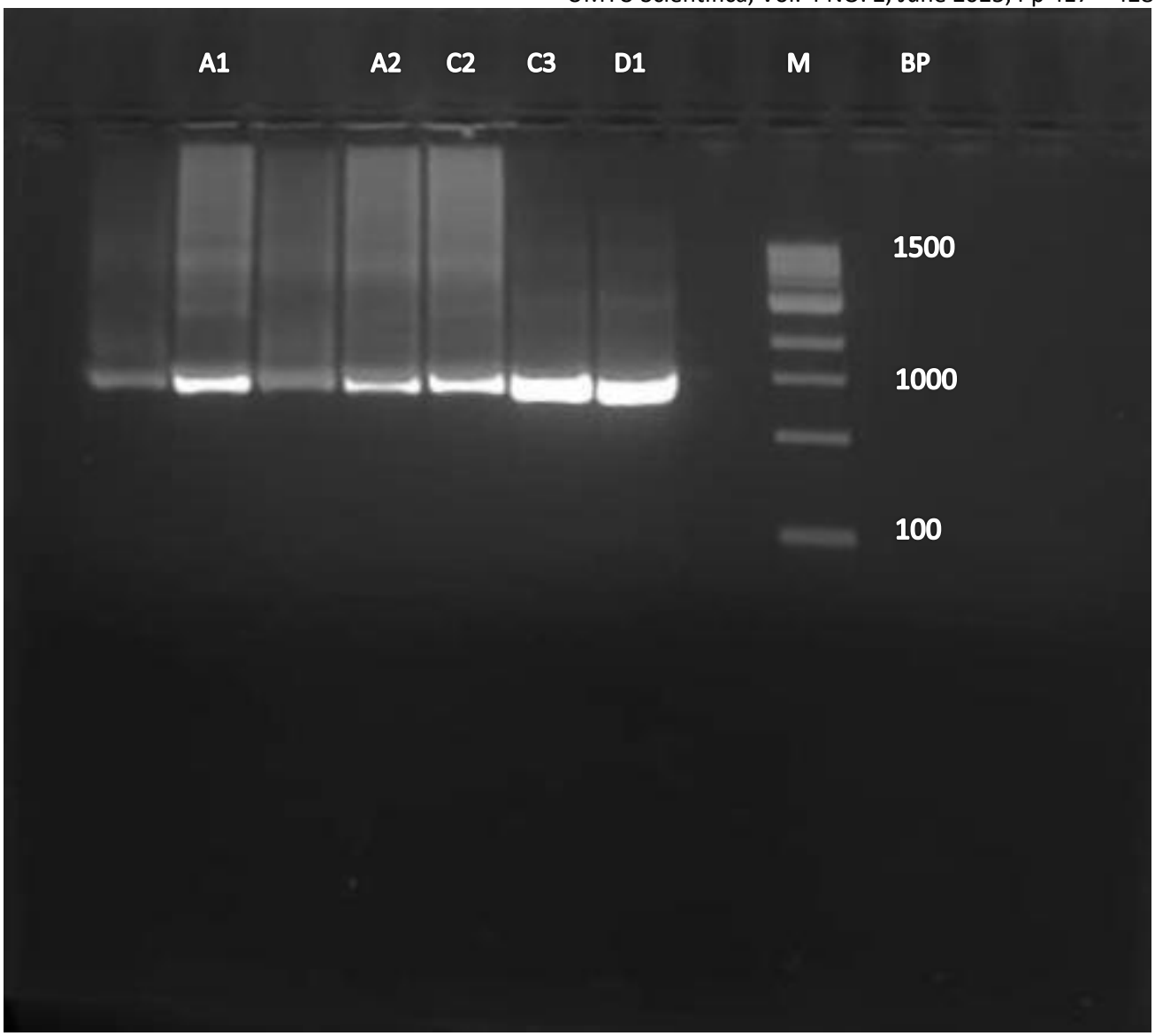

Figure 7: Gel Electrophoresis showing DNA Amplification of Bacterial Isolates
Key: Lane 2: A1, Lane 3: A2, Lane 4: C2, Lane 5: C3, Lane 6: D1, Lane M: Molecular marker, Lane BP: Base pair

Table 3: Identification of the isolates using NCBI BLASTnSearch

Isolate No	Identified Species Name of isolates	Accession Number	% Similarity
A1-907R	*Bacillus sonorensis* strain APBSDSB21 16S ribosomal RNA gene, partial sequence	PV803116	74.12
A2_907R	*Achromobacter mucicolens* strain c 16S ribosomal RNA gene, partial sequence	PV803120	97.37
C2_907R	*Bacillus* sp. (in: Bacteria) strain PPE173 16S ribosomal RNA gene, partial sequence	PV803119	91.46
C3_907R	*Bacillus licheniformis* strain SM19 16S ribosomal RNA gene, partial sequence	PV803118	98.41
D1_907R	*Lysinibacillus composti* strain NCCP-36 16S ribosomal RNA, partial sequence	PV803117	99.72

Statistical Analysis of Bacterial Count and Heavy Metal Concentrations

The electrophoresis image shows the pattern of amplification of the 16S rRNA gene of the five isolates shown in Figure 7. On the other hand, Table 3 shows the BLASTn search result used to identify the five isolates. Isolate A1 was identified as *Bacillus sonorensis* because its genome is most similar to that of *Bacillus sonorensis* strain APBSDSB21 in the NCBI GenBank, showing a homology of 74.12%. It is followed by that of Bacillus *paralicheniformis* RSC-3 DNA, with 74.09%. Other bacteria, such as *Bacillus licheniformis* and uncultured *Bacillus*

clones, share similarity within the range of 73.85 to 73.99%. Similar criteria were used to identify the other four isolates (A2, C2, C3, and D1) as Achromobacter mucicolens, showing a homology of 97.37%, *Bacillus* sp. Strain PPE 16S ribosomal 91.45%, *Bacillus licheniformis* strain SM19 16S ribosomal 98.41%, and *Lysinibacillus composti* strain NCCP-36 16S ribosomal 99.72%, respectively.

Table 4: Pearson and Spearman Correlation Coefficient between Bacterial Count and Heavy Metal Concentrations across the Dumpsite Samples

S/N	Heavy Metal	Pearson r	Pearson p-value	Spearman ϱ	Spearman p-value
1	**Chromium**	0.607	0.393	0.400	0.600
2	**Zinc**	–0.129	0.871	0.200	0.800
3	**Lead**	–0.907	0.093	–1.000	0.000
4	**Cobalt**	0.479	0.521	0.200	0.800
5	**Nickel**	–0.778	0.222	–0.400	0.600

A correlation was identified between the concentration of heavy metals and the density of bacteria in the four dumpsite samples, aiming to establish the relationship between the two. There was an extremely negative correlation between the bacterial count and the concentration of lead. Pearson r = –0.91; Spearman ϱ = –1.00, indicating that an improvement in the concentrations of lead could significantly repress microbial growth. There was a moderate negative correlation with nickel (Pearson r = –0.78), while the other metals presented weak or unsystematic associations.

DISCUSSION

The study focuses on characterizing heavy metal-tolerant bacteria from waste disposal sites within the Katsina metropolitan area. Dump sites are colonized by various types of microbes that exhibit resistance to heavy metal stress.

Heavy metals are persistent environmental pollutants, not easily degraded; hence, they can accumulate in the soil over time and lead to long-term contamination. As shown in Table 2, the concentrations of chromium, zinc, lead, and cobalt varied significantly among the four samples collected from the refuse dumpsites. Sample B has the highest concentration of chromium at 0.42±0.001 ppm, which most likely indicates localized contamination due to some form of industrial activity or dumping of waste. Chromium is classified as a toxic metal and may pose environmental and health risks, as Wang et al. (2022) noted, citing chromium pollution as a common phenomenon in areas surrounding industrial regions due to the dumping of waste products containing chromium.

Similarly, the concentration of zinc present in Sample A was highest, at 0.952 ± 0.006 ppm, possibly resulting from such contamination of the environment via sources that could include agricultural runoff and industrial waste discharge. The essential micronutrient required by plants and microorganisms, on becoming non-toxic to the endotherms when present at a high dosage, brings about substitution for other related metals, subsequently reducing their activity (Bhardwaj *et al.*, 2020). Moreover, Lead, a highly toxic metal, was found in very high concentration in Sample D with a value of 1.54 ± 0.0033 ppm, which is highly contaminated, possibly due to industrial emissions or automobile exhaust. The high levels of lead in some of the samples further confirm the potentiality of industrial contamination, and this finding is in agreement with the study conducted by Ogbodo *et al.* (2018), who observed similar lead contamination in refuse dumpsite areas in urban settings.

Although cobalt concentrations were low across all samples, they still indicate trace contamination, highlighting the importance of even low metal pollutant concentrations in environmental health. Cobalt is a trace element necessary for organisms, though it turns toxic when the level becomes so high that it starts interfering with bacterial metabolism (Mahmoud *et al.*, 2021). These findings illustrate that refuse dumpsites are obviously the hotspots for heavy metal contamination, which in turn causes damage to both the environment and microflora in the habitats.

The data for microbial populations presented in Table 1 show clear variation in the number of bacteria in the four soil samples due to variations in environmental conditions and soil properties at each location. Sample A had the highest microbial population of $9.8 \times 10^{13} \pm 1.0$ CFU/g, reflecting the best conditions for microbial growth. This is likely due to higher nutrient availability, the right moisture content, and favorable soil physicochemical properties, all of which are well documented to favor microbial growth and activity (Siciliano *et al.*, 2017). All these favor the sustenance of a rich, diverse microbial community.

Furthermore, Sample D contained the smallest number of bacteria ($5.2 \times 10^{13} \pm 1.0$ CFU/g), indicating unfavourable or potentially toxic growth conditions for microorganisms. Such low counts can be due to a lack of nutrients or the presence of microbial inhibitors like heavy metals or other contaminants that inhibit microbial growth and diversity (Imran *et al.*, 2018). These unfavorable conditions lower microbial activity in soil, which can affect soil health and nutrient cycling. Samples B and C obtained mid-microbial values of $8.35 \times 10^{13} \pm 1.5$ CFU/g and $8.2 \times 10^{13} \pm 2.0$ CFU/g, respectively. For relatively favorable microbial growth conditions, small differences might account for differences in the content of organic matter, pH, water-holding capacity, or degree of contamination (Jia *et al.*, 2019). The relatively higher Sample B compared to Sample C could represent improved nutritional quality or a reduced number of inhibitory substances at the latter site. Generally, these results point toward the heterogeneity of microbial soil

communities, most probably caused by intrinsic properties of soil and external factors like pollution or dumping of refuse. Quantification of such differences is key to soil ecosystem function and communicating remediation or management interventions to optimize restoration of soil health in affected environments (Khan *et al*., 2020).

Exposure to chromium with various concentrations demonstrated dose-dependent inhibition of bacterial survivability. Isolates such as A1 and B1 were tolerant at 25 mg/L with the viable growth rate, while isolates such as A3 demonstrated compromised survivability in high concentrations, 100 mg/L (Khan *et al*., 2019). The high tolerance of *Bacillus licheniformis* to lead suggests its use in the bioremediation of lead-contaminated sites. Such variations reflect strain-specific capacities for tolerance, an important aspect in the selection of effective microbial agents in bioremediation. These findings are consistent with previous work demonstrating that metal resistance in bacteria can occur through processes such as efflux pumping, metal-binding proteins, enzymatic conversion, and biofilm (Nwachukwu *et al*., 2021; Khan *et al*., 2019). The isolates described in this study belong to the environmentally and industrially relevant genera such as *Bacillus*, *Lysinibacillus*, and *Achromobacter*. Microorganisms belonging to these genera have been extremely widely documented to decontaminate heavy metals by biosorption, bioaccumulation, and enzymatic reduction (Das *et al*., 2021). *Bacillus licheniformis*, for instance, was documented to adsorb lead and cadmium and supply chelating agents (Das *et al*., 2021). Similarly, *Lysinibacillus composti* was documented to involve chromium and arsenic metal degradation and immobilization (Mojiri *et al*., 2021). The clinical more common reporting of *Achromobacter mucicolens* is a hallmark of its ecological adaptability and suggests a hitherto untapped function in the decontamination of the environment.

This is the first report of *Achromobacter mucicolens* and *Lysinibacillus composti* from dumpsites in Nigeria, showing their potential for chromium and lead bioremediation. The report closes an important gap in indigenous microbial ecology and bioremediation data. Isolation of such taxa is an indication of the indigenous microbial resources that may be exploited for cost-effective and localized bioremediation methods in northern Nigeria (Mojiri *et al*., 2021). The notable contributions of the work are the first-ever report on *Achromobacter mucicolens*, *Bacillus haynesii*, *Bacillus licheniformis*, and *Lysinibacillus composti* from Katsina Metropolis dumpsites with experimentally confirmed heavy metal resistance (Mojiri *et al*., 2021). This report addresses an important lacuna of knowledge in microbial ecology and bioremediation research in Nigeria. Isolation of these isolates is proof of indigenous microbial resources, which can be redesigned to maximize localized low-cost bioremediation objectives in northern Nigeria.

Phylogenetic comparison among isolates through 16S rRNA gene sequencing revealed the above-mentioned isolates to be congeneric with identified strains. For example, Bacillus haynesii revealed high genetic similarity with *B. paralicheniformis* and *B. sonorensis* which proved convergent evolutionary adaptation under environmental stress. Achromobacter mucicolens also revealed close similarity with the remaining *Achromobacter* species, which meant that it had adapted to environments with high levels of heavy metals. Specifically, *Achromobacter* mucicolens highlights its putative antimicrobial resistance potential, an area that is yet to be thoroughly explored (Smith *et al*., 2021; Green *et al*., 2020).

The potential resistance mechanisms in this study, efflux pumps, enzymatic detoxification, and ion sequestration, are identical to those described in the literature for these same genera (Bhattacharya & Gupta, 2020). Functional diversity and redundancy, as observed in Bacillus and Lysinibacillus spp., are likely to enhance ecological resilience and improve bioremediation of metal pollutant mixtures. (Ilangovan *et al*., 2023; Rajkumar *et al*., 2022).

The decrease in the number of bacteria in lead-polluted soils and heavy metals was found to be consistent with existing contaminated soil studies. This could also be seen through correlation analysis, whereby there was a strong negative relationship between the composition of lead and the number of bacteria (Pearson $r = -0.91$; Spearman $\rho = -1.00$). These reversal trends indicate that lead has potent inhibitory actions against microbial populations because of potential interference with cellular enzymic activities and membrane integrity. Moderately negative correlation was also found with nickel, but the other metals (i.e., chromium, cobalt, zinc) had weaker or significant correlations, perhaps because of varying levels of bioavailability and microbial tolerance. These findings justify the case for emphasizing lead-resistant bacterial strains in bioremediation processes for lead-contaminated urban soils.

Although the results of this research are encouraging, certain limitations should be acknowledged. Application of 16S rRNA sequencing, standard procedure limits resolution to the species identification and provides no indication of functional genes or single operons of resistance (Abellan-Schneyder *et al*., 2021). In addition, the in vitro environments of metal tolerance assays might not always reflect the intricate interactions and stressful environments common in real contaminated environments. Further studies should utilize whole-genome sequencing, functional genomics, and field testing to thoroughly investigate the metabolic pathways, gene expression patterns, and field performance of such isolates under bioremediation conditions (Abellan-Schneyder *et al*., 2021). While the phenol-chloroform extraction protocol was applied successfully in this study, it would be desirable for future work to employ commercially available DNA extraction kits for improved reproducibility and purity of DNA.

Dumpsite isolates from Katsina Metropolis vary with respect to heavy metal resistance, particularly chromium and lead. Their taxonomic characterization, genetic identities, and physiological properties have vast potential for use in bioremediation. Discoveries of novel environmental roles of such microbes as Achromobacter mucicolens add another in microbial adaptation to

polluted environments. Such data provide a fundamental foundation for the establishment of future research and innovation for sustainable, microbe-based remediation technologies to address northern Nigeria's environmental issues.

CONCLUSION

In this study, heavy metal-tolerant bacteria isolated and characterized from refuse dumpsites in Katsina Metropolis include *Achromobacter mucicolens, Bacillus sp., Lysinibacillus composti, Bacillus sonorensis* APBSDSB21, and *Bacillus licheniformis*. These isolates showed high tolerance to lead and chromium, two of the most common toxic metals used in the study area, which are promising candidates for bioremediation application in the region.

Bacterial abundance varied by location and inversely with levels of lead, chromium, nickel, and zinc, implying heavy metal pollution drives microbial community structure. Even in conditions like these, strong species prevalence indicates native microorganisms' ability to adapt in polluted environments. Its findings justify the development of cost-effective and environmentally friendly treatment technologies for waste by incorporating indigenous bacteria's natural detoxification processes. These uses are significant in soil quality development, prevention of landfill contamination, and promotion of environmental well-being in urban areas such as Katsina.

This study is the first to report the characterization of heavy metal-tolerant bacteria from dumpsites in Katsina, identifying novel strains with bioremediation potential.

RECOMMENDATIONS

The following recommendations were made based on the findings of this study:

1. The metal-tolerant bacteria identified in this study, such as *Bacillus sonorensis* and *Lysinibacillus* sp., should be considered for use in bioremediation efforts aimed at reducing soil toxicity in contaminated environments. Their ability to withstand heavy metal stress makes them promising candidates for addressing pollution in affected areas.

2. Regular Monitoring of Heavy Metals is crucial to conduct regular assessments of heavy metal concentrations, particularly in highly polluted sites, to track contamination trends over time. Such monitoring will aid in improving waste management strategies and enable targeted interventions to mitigate pollution.

3. The application of organic amendments and improved aeration should be explored as methods to enhance soil quality and foster microbial growth. These practices can support natural remediation processes, especially in areas with low bacterial populations, and contribute to more effective heavy metal removal.

4. The implementation of proper waste management practices, including the segregation and safe disposal of refuse, is essential to preventing further contamination of soil with heavy metals. Effective waste management will reduce the long-term environmental impact of refuse dumps and support sustainable pollution control.

5. Additional genomic and functional studies are recommended to further explore the mechanisms of heavy metal resistance in the isolated bacteria. Such research will contribute to the development of more efficient bioremediation techniques and broaden the potential industrial applications of these bacteria in environmental cleanup efforts.

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