

Optimizing a Synergistic Bacterial Consortium for the Bioremediation of Multi-Metal Contaminated Soils in Jimeta, Nigeria

Abubakar Isah Muhammad¹, Faisal Bello², Fidelis Fredrick Umaru³, Adamu Magaji Bappa¹

¹Department of Science Laboratory Science, Adamawa State Polytechnic, Yola, Nigeria.

²Department of Microbiology, Modibbo Adama University, Yola, Nigeria.

³Department of Microbiology, Taraba State University, Jalingo, Nigeria.

Corresponding author

abubakarisah174@gmail.com

Manuscript received: 30 December 2025. Revision accepted: 01 June 2026, Published: 16 June 2026.

Abstract

Soil contamination with anthropogenic heavy metals presents a critical threat to ecosystem stability and public health, demanding the development of sustainable in-situ remediation technologies. This study engineered and optimized a synergistic bacterial consortium for bioaugmentation of multi-metal contaminated soil from an active scrap dump in Jimeta, Nigeria. We isolated and characterized indigenous, metal-resistant strains of *Pseudomonas*, *Bacillus*, and *Staphylococcus*, which exhibited minimum inhibitory concentrations for chromium and other metals that far exceeded in-situ soil levels, confirming exceptional intrinsic tolerance. A systematic multivariate optimization of key abiotic factors defined the consortium's operational window, identifying neutral pH (7.0) and a mesophilic temperature of 30°C as optimal for peak bioremediation efficiency. While individual bacterial species achieved moderate metal removal (21.6–28.6%), the designed tertiary consortium demonstrated markedly superior performance, attaining 62.8% removal, a synergistic enhancement of approximately 125% over the mean axenic culture output. This significant boost is attributed to complementary microbial mechanisms, including partitioned detoxification pathways, syntrophic cross-feeding, and collective production of metal-chelating siderophores. Notably, these optimized conditions align with the prevailing edaphic parameters of tropical soils in Northern Nigeria, underscoring the ecological coherence and practical field applicability of this approach. The findings robustly validate the principle of functional microbial synergy for remediating complex polymetallic contamination. Consequently, this work establishes a scientific and technical foundation for transitioning consortium-based bioremediation from laboratory proof-of-concept to a predictable, scalable field technology. It advocates for a paradigm shift from conventional, reactive cleanup methods toward proactive, ecological engineering strategies that mitigate metal bioavailability, protect agro-ecosystems, and interrupt a key environmental determinant of chronic disease.

Keywords: Bioaugmentation; Heavy Metals; Microbial Consortium; Multivariate Optimization; Synergistic Bioremediation.

INTRODUCTION

The escalating burden of anthropogenically driven multi-metal contamination in global soil matrices represents a critical environmental determinant of disease, necessitating the development of advanced, ecologically coherent remediation strategies (Uzoekwe and Aigberua, 2023). Industrial effluents, agricultural amendments, and improper waste management have engendered widespread dissemination of redox-active and thiophilic heavy metals, including cadmium (Cd (II)), hexavalent chromium (Cr (VI)), and lead (Pb (II)), which exhibit pronounced ecotoxicity, biogeochemical persistence, and a high propensity for trophic transfer (Onyena *et al.*, 2024). The ensuing bioavailability of these metallic cations facilitates their assimilation into agroecosystems, culminating in bioaccumulation within edible phytomass and subsequent entry into the human alimentary chain. This exposure vector is etiologically linked to the pathogenesis of debilitating chronic conditions, including

nephrotoxicity, neurodevelopmental impairment, and oncogenesis, thereby framing soil metal load as a pre-clinical public health emergency. Conventional ex-situ remediation strategies, such as soil washing or solidification, are often economically prohibitive, operationally invasive, and deleterious to edaphic biome integrity, rendering them incompatible with sustainable land use, particularly for agricultural recultivation (Santos *et al.*, 2025).

In contrast, microbial bioremediation leverages the innate catabolic versatility and metal-homeostatic machinery of prokaryotes to effect in-situ detoxification through a repertoire of mechanisms encompassing enzymatically-catalyzed redox transformations, exopolysaccharide-mediated biosorption, and ATP-dependent bioaccumulation (Ayangbenro and Babalola, 2024). This biological approach offers a strategically superior alternative by catalyzing the conversion of labile, toxic metal species into immobilized or volatilized derivatives, thereby directly attenuating their

environmental mobility and bioavailability. However, monocultural applications of defined axenic bacterial strains are intrinsically limited by narrow metabolic bandwidth, insufficient stressor resilience, and inadequate functional redundancy when deployed against polymetallic contaminant suites (Sarkar and Bhattacharjee, 2025). The inherent complexity of such sites, characterized by differential metal speciation, antagonistic ionic interactions, and fluctuating abiotic gradients, often exceeds the adaptive capacity and catalytic scope of any single microbial genotype (Anas *et al.*, 2025).

This critical limitation has precipitated a translational shift towards the deliberate engineering and deployment of synthetic or enriched bacterial consortia (Azubuiké *et al.*, 2023). These polymicrobial systems are founded on the ecological principle of functional synergy, whereby the collective metabolic output and stress tolerance of the community exceed the sum of its constituent members' capabilities. A consortium can be rationally designed to partition detoxification pathways: for instance, a chromate-resistant *Pseudomonas* strain may perform the enzymatic reduction of Cr (VI) to Cr (III) via soluble chromate reductases, while a coexisting *Bacillus* strain, expressing high-affinity metallothioneins and surface-layer proteins, concurrently sequesters Pb (II) and Cd (II) through chemisorption and intracellular compartmentalization (Tasleem *et al.*, 2023). Furthermore, syntrophic interactions, such as cross-feeding on metabolic intermediates or the collective production of metal-chelating siderophores and biosurfactants, can enhance community stability and metal mobilization, creating a robust, self-sustaining bioremediation module (Akinola *et al.*, 2024). This consortium-based strategy effectively establishes a proactive, biological barrier that interrupts the exposure pathway, functioning as a form of environmental prophylaxis against metal-induced morbidities.

The efficacy of any engineered consortium is, however, an emergent property dictated by its interaction with a dynamic edaphic microenvironment (Tariq *et al.*, 2025). Key physicochemical parameters act as master regulators of both microbial physiology and metal biogeochemistry. Environmental pH modulates metal solubility, speciation, and cell surface charge, critically influencing biosorption efficiency (He *et al.*, 2025). Temperature governs enzymatic kinetics and membrane fluidity, while nutrient stoichiometry (particularly C: N:P ratios) determines whether biomass accrual is substrate-limited. Perhaps most critically, the initial metal concentration and the presence of co-contaminants can induce pleiotropic stress responses, potentially overwhelming cellular efflux systems (CzcCBA for Cd/Zn/Co) and inhibiting essential metabolism (Goutam Mukherjee *et al.*, 2022). Therefore, transitioning from promising *in vitro* consortium assembly to predictable *in situ* field efficacy requires a systematic, multivariate

optimization of these operating conditions to identify the niche where microbial metabolic activity and metal-removal kinetics are maximally coupled.

Consequently, this study is designed to operationalize the concept of bacterial consortia as a targeted, prophylactic intervention for polymetallic sites. It will transcend mere demonstration by systematically interrogating the complex interplay between a purpose-enriched, synergistic bacterial consortium and the critical abiotic variables that govern its bioremediative performance. Through a series of controlled microcosm experiments, we aim to define the optimal physicochemical landscape, the "operational window", for maximal multi-metal sequestration and detoxification. This research provides an essential mechanistic and operational foundation for transitioning consortium-based bioremediation from a laboratory concept to a scalable, predictable technology for mitigating a pervasive environmental determinant of human disease.

MATERIALS AND METHODS

Study Area

This research was conducted in Jimeta, Yola North Local Government Area, Adamawa State, Nigeria. Adamawa State, located in Nigeria's North-East geopolitical zone, has its capital in Yola. It shares borders with Borno and Gombe states to the northwest and west, respectively, and with Taraba to the southwest, while its eastern frontier forms part of the international boundary with Cameroon (Aga and Chiegeonu, 2009; Mamman *et al.*, 2000). The state's geographical coordinates are approximately 9°20'N latitude and 12°30'E longitude. With a landmass of 36,917 km² (Michael & Victoria, 2019), Adamawa State had a recorded population of about 3.2 million in the 2006 census, with projections estimating growth to 4.25 million by 2016 (NPC, 2006-2016). The specific study area, Jimeta, is geo-referenced at 9°16'45" N latitude and 12°26'45" E longitude.

Sample Collection and Characterization of Heavy Metal Contaminated Soil Samples

Sampling Locations

Soil sampling was conducted across selected locations in Jimeta, Adamawa State, representing distinct anthropogenic sources of heavy metal contamination. Sampling sites included: (1) industrial vicinities, such as zones adjacent to metal processing plants and other manufacturing facilities where effluent discharge is prevalent; (2) agricultural areas with histories of pesticide and fertilizer application, where metals may accumulate over time; and (3) urban environments, including roadside locales, construction sites, and other areas subject to vehicular emissions and atmospheric deposition.

At each site, a composite sampling strategy was employed to ensure spatial representativeness and minimize point-source bias. Samples were collected from the top 10 cm soil layer, corresponding to the primary rooting zone in agroecosystems and a region of significant microbial activity (Adeboye et al., 2011). Following collection, soil was immediately transferred to pre-sterilized glass containers or polyethylene bags to maintain integrity for subsequent physicochemical and microbiological analyses.

Heavy Metal Analysis

Sample Preparation

Soil samples underwent preparation and digestion according to standardized protocols to facilitate precise heavy metal quantification. Initially, samples were air-dried at ambient temperature for 72 hours. The dried soil was then sieved through a 2-mm mesh to exclude coarse debris, including gravel, roots, and plant material. A representative aliquot of the sieved soil was finely ground with an agate mortar and pestle to produce a homogeneous powder, a prerequisite for consistent metal extraction and analysis (Evans et al., 2024).

Digestion followed the procedure outlined by Ibrahim et al. (2024) for Nigerian Savanna soils, adapted from USEPA method 3050B (USEPA, 2015). Briefly, 5.0 g of the powdered soil was transferred to a 250-ml digestion flask. A 3:1 (v/v) mixture of concentrated nitric acid (HNO₃) and hydrochloric acid (HCl) was prepared, and 20 ml was added to the sample. The flask was heated on a hotplate to 95°C ± 5°C and refluxed for approximately two hours, or until the digestate clarified, indicating complete organic matter oxidation and metal dissolution.

After cooling, the digestate was filtered through Whatman No. 42 filter paper into a 50-ml volumetric flask. The residue was rinsed with deionized water, and the filtrate was diluted to the mark. The final digest was stored in acid-washed polyethylene bottles at 4°C until analysis by Atomic Absorption Spectrophotometry (AAS) (Ibrahim et al., 2024).

Isolation and Identification of Heavy Metal-Tolerant Bacteria

Bacterial isolation from contaminated soil samples employed a selective enrichment strategy targeting metal-tolerant phenotypes. Soil extracts were initially incubated in Luria-Bertani (LB) broth amended with heavy metals to enrich for resistant microbial populations. Enriched cultures were subsequently streaked onto selective solid media for isolation.

Selective LB agar plates were prepared by supplementing the standard formulation (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 15 g/L agar) with filter-sterilized stock solutions of zinc sulfate (ZnSO₄), cobalt nitrate (Co (NO₃)₂), and copper sulfate (CuSO₄) to achieve targeted concentrations. Control plates without metal amendments were prepared in parallel. All media were sterilized by autoclaving at 121°C for 15 minutes.

For processing, 10 g of soil was aseptically suspended in 90 mL of sterile distilled water and agitated on a rotary shaker for 30 minutes. Serial decimal dilutions were prepared from this suspension. Aliquots (100 µL) from appropriate dilutions were spread-plated in triplicate onto both metal-supplemented and non-supplemented LB agar plates. All plates were incubated at 30°C for 48 hours.

Following incubation, distinct colonies exhibiting growth on metal-amended plates were enumerated and sub-cultured to obtain pure isolates. Dominant, morphologically unique isolates were subjected to preliminary phenotypic characterization using Gram staining and a standard suite of biochemical tests (Abaka et al., 2025) before further molecular analysis.

Gram's Staining

The Gram staining technique was employed to differentiate bacterial isolates based on cell wall composition. Initially, a bacterial suspension from a pure culture was prepared in sterile saline on a clean glass slide and spread into a thin smear. After air-drying, the smear was heat-fixed by passing it through a Bunsen burner flame. The fixed smear was sequentially stained with crystal violet and Gram's iodine, rinsed, and then decolorized with acetone. Finally, a safranin counterstain was applied, and the slide was rinsed, blotted, and air-dried (Abaka et al., 2024).

The prepared slides were examined under an oil-immersion microscope lens. Isolates were classified as either Gram-positive, which retain the crystal violet stain and appear purple, or Gram-negative, which take up the safranin counterstain and appear pink. Cellular morphology and arrangement were also recorded during observation.

Bacterial Endospore Staining Procedure

The ability of bacterial isolates to form endospores was assessed using a modified Schaeffer-Fulton staining procedure (Bertović et al., 2024). A key modification involved increasing the malachite green heating step to 10 minutes to optimize stain penetration of the resistant spore coat. This differential staining technique was employed to identify and separate sporulating genera, including *Bacillus*, from non-spore-forming bacteria.

Microscopic Examination.

The final rinsing was performed with distilled water to remove excess counterstain, and the slides were air-dried before examination. The stained preparations were observed under oil immersion at 1000× magnification using a compound light microscope. The stained samples were interpreted according to established criteria, where endospores appeared as bright green structures, while vegetative cells were visible in pinkish-red coloration (Abaka et al., 2024).

Assessment of Heavy Metal Tolerance Minimum Inhibitory Concentration (MIC)

The heavy metal tolerance of bacterial isolates was quantitatively assessed by determining the Minimum Inhibitory Concentration (MIC), defined as the lowest concentration of a metal that completely prevents visible growth. Stock solutions of target metals, including lead, cadmium, and arsenic, were prepared by dissolving analytical-grade salts (e.g., Pb (NO₃)₂, CdCl₂) in sterile distilled water at specified concentrations (Yadav et al., 2025).

Bacterial strains were first cultured in a suitable liquid medium, such as Luria-Bertani broth, to the exponential growth phase. In a 96-well microplate, two-fold serial dilutions of each metal were prepared in broth. Each well was then inoculated with a standardized bacterial suspension. Following incubation at 37°C for 24–48 hours, growth was assessed spectrophotometrically by measuring the optical density at 600 nm (Alves et al., 2020) and confirmed by visual inspection. The MIC was recorded as the lowest metal concentration in the series where no bacterial growth was detected (Yadav et al., 2025).

Experimental Setup for Bioremediation

The bioremediation efficacy of the bacterial consortium was evaluated using soil microcosms under controlled laboratory conditions. This design included experimental units inoculated with the consortium and uninoculated control units to measure treatment effects against natural attenuation. The performance of the consortium was assessed by its capacity for metal sequestration and the stimulation of microbial activity (Ibrahim et al., 2024).

A multivariate optimization strategy was employed to identify the ideal conditions for bioremediation. Key physicochemical parameters, including soil pH, incubation temperature, and initial metal load, were systematically varied, and their effects on metal solubility and microbial response were quantified. Concurrently, biological factors such as nutrient supplementation (nitrogen and phosphorus), aeration, and inoculum density were fine-tuned to maximize consortium growth and metal removal efficiency. All experimental procedures and analytical measurements

adhered to standardized methodologies for reliability and reproducibility.

Measurement of Heavy Metal Concentrations

Metal levels in the soil were monitored at regular intervals by atomic absorption spectrophotometry (AAS) using the previously outlined protocol. This temporal analysis of metal content was conducted to quantify the rate of metal depletion and to evaluate the overall performance and efficiency of the bioremediation treatment (Omeje et al., 2021).

Microbial Activity Assays

The growth kinetics of the bacterial consortium were tracked spectrophotometrically by measuring optical density at 600 nm (OD₆₀₀). The consortium was inoculated into sterile liquid medium within Erlenmeyer flasks. Before sampling, the spectrophotometer was calibrated to zero absorbance using a cuvette containing fresh, sterile medium (Aruotu et al., 2023).

At predetermined intervals during incubation, samples were aseptically withdrawn from homogenized cultures. For each time point, the OD₆₀₀ of a sample aliquot was measured in a clean cuvette; higher absorbance values correspond to greater bacterial cell density (Beal et al., 2020). This sampling and measurement procedure was repeated throughout the experiment. The resulting OD₆₀₀ data were plotted against time to construct a growth curve, illustrating the population dynamics of the consortium over the study period (Aruotu et al., 2023).

Statistical Analysis and Data Interpretation

Statistical analyses were performed to evaluate treatment effects and key relationships. Differences in outcomes between the inoculated experimental groups and the uninoculated control groups were assessed for significance using Analysis of Variance (ANOVA). Furthermore, the relationship between the extent of heavy metal reduction and corresponding microbial activity metrics was examined through regression analysis (Ibrahim et al., 2024).

RESULTS

Table 1. Physicochemical Characterization of Soil Samples from Jimeta.

S/N	Sample ID	Location Description	Soil pH	Moisture Content (%)	Soil Type
1	S1	Metal scrap dump site located behind a shopping complex, Jimeta	6.8	12.5	Sandy loam
2	S2	Agricultural farmland situated beneath a bridge along the Jimeta River corridor	7.2	28.4	Clay loam

KEY: S1 = sample 1; S2 = sample 2

The two soil samples exhibited distinct physicochemical properties corresponding to their differing locations. Sample S1, collected from a metal scrap dump site, was slightly acidic (pH 6.8), had a low moisture content (12.5%), and was classified as sandy

loam. In contrast, Sample S2, from a riverside agricultural farmland, was neutral (pH 7.2), retained significantly higher moisture (28.4%), and was a clay loam, reflecting its proximity to water and agricultural use.

Table 2. Heavy Metal Resistance Profile of Bacterial Isolates from Contaminated Soils.

Bacterial Isolate	No. of Isolates	Control	Cr	Pb	Cu	Zn
<i>Bacillus</i> species	18	+++	+	+++	++	+++
<i>Pseudomonas</i> species	14	+++	+++	+	++	++
<i>Staphylococcus</i> species	9	+++	+	++	+++	+
<i>Escherichia coli</i>	5	+++	–	–	++	++

KEY:

Symbol	Resistance Level	Colony Count Range (CFU/mL)
+++	High Resistance	$> 1.0 \times 10^7$
++	Moderate Resistance	$1.0 \times 10^6 - 1.0 \times 10^7$
+	Low Resistance	$1.0 \times 10^5 - 1.0 \times 10^6$
–	Sensitive (No Growth)	$< 1.0 \times 10^4$

Control: Growth on media without heavy metals serves as the baseline ($\geq 1.0 \times 10^8$ CFU/mL). Resistance symbols indicate growth relative to this control.

Bacillus and *Pseudomonas* species were the most frequently isolated and exhibited the broadest resistance profiles. *Bacillus* demonstrated high resistance to Pb and Zn, while *Pseudomonas* showed high resistance to Cr. *Staphylococcus* species displayed a more selective pattern, with high resistance to Cu. In contrast,

Escherichia coli isolates were sensitive to Cr and Pb, indicating poor survival in a multi-metal contaminated environment. This hierarchy establishes *Pseudomonas* and *Bacillus* as the most promising candidates for consortium-based bioremediation.

Table 3. Minimum Inhibitory Concentration (MIC) of Bacterial Isolates Relative to Maximum Soil Contamination.

Heavy Metal	Max Soil Conc. (mg/kg)	<i>Staphylococcus</i> spp. MIC (mg/L)	<i>Bacillus</i> spp. MIC (mg/L)	<i>Pseudomonas</i> spp. MIC (mg/L)
Cr	0.367	80–100	100–150	150–200
Pb	7.213	150–200	200–300	300–400
Cu	4.184	100–150	150–250	250–350
Zn	6.179	120–180	180–280	280–380

KEY:

Max Soil Conc.: Maximum concentration of the heavy metal measured in the contaminated soil samples (mg/kg).

MIC: Minimum Inhibitory Concentration (mg/L) – the lowest concentration of heavy metal in liquid culture media that completely inhibits visible growth of the bacterial isolate after 24h incubation.

Cr: Chromium, **Pb:** Lead, **Cu:** Copper, **Zn:** Zinc.

Note: MIC values are presented as a range based on triplicate experiments. All MIC assays were conducted in nutrient broth at 30°C.

All three bacterial isolates exhibited tolerance to heavy metal concentrations far exceeding the maximum levels found in the contaminated soil (e.g., 0.367 mg/kg Cr vs. MICs of 80–200 mg/L). *Staphylococcus* spp. consistently demonstrated the highest sensitivity, displaying the lowest MIC range for each metal. In

contrast, *Pseudomonas* spp. showed the greatest intrinsic tolerance, with the highest MIC values across all tested metals. This established a clear hierarchy of metal resistance: *Pseudomonas* spp. > *Bacillus* spp. > *Staphylococcus* spp.

Table 4. Concentration of Selected Heavy Metals in Soil Samples S1 and S2 (mg/kg).

Heavy Metal	S1–M1	S1–M2	S1 Mean	S2–M1	S2–M2	S2 Mean
Cr	0.365	0.369	0.367	0.015	0.017	0.016
Pb	7.210	7.216	7.213	1.295	1.297	1.296
Cu	4.182	4.186	4.184	0.753	0.755	0.754
Zn	6.177	6.181	6.179	1.371	1.373	1.372

Key:

S1 = Sample 1; S2 = Sample 2; M1 = Measurement 1; M2 = Measurement 2; Cr = Chromium; Pb = Lead; Cu = Copper; Zn = Zinc.

The concentrations of all analyzed heavy metals were markedly higher in Sample S1 than in Sample S2. Lead (Pb) recorded the highest mean concentration in both samples, followed by Zn, Cu, and Cr. Replicate

measurements (M1 and M2) showed minimal variation, indicating good analytical precision. Overall, the results suggest greater metal contamination at the S1 site compared to S2.

Table 5. Average Effect of Temperature on Heavy Metal Removal Efficiency by Bacterial Species and Consortia.

Treatment	Removal Efficiency at 25 °C	Removal Efficiency at 30 °C	Removal Efficiency at 35 °C
Individual Species			
<i>Bacteria A (Pseudomonas spp.)</i>	0.216 (21.6%)	0.254 (25.4%)	0.231 (23.1%)
<i>Bacteria B (Bacillus spp.)</i>	0.236 (23.6%)	0.279 (27.9%)	0.267 (26.7%)
<i>Bacteria C (Staphylococcus spp.)</i>	0.224 (22.4%)	0.269 (26.9%)	0.286 (28.6%)
Binary Consortia			
Consortium A + B (<i>Pseudomonas + Bacillus</i>)	0.400 (40.0%)	0.448 (44.8%)	0.418 (41.8%)
Consortium A + C (<i>Pseudomonas + Staphylococcus</i>)	0.390 (39.0%)	0.438 (43.8%)	0.415 (41.5%)
Consortium B + C (<i>Bacillus + Staphylococcus</i>)	0.414 (41.4%)	0.464 (46.4%)	0.434 (43.4%)
Tertiary Consortium			
Consortium A + B + C (All three species)	0.573 (57.3%)	0.628 (62.8%)	0.592 (59.2%)

A tertiary bacterial consortium (A+B+C) achieved maximum metal removal (62.8%) at the optimal temperature of 30 °C, surpassing the performance of

individual species. This demonstrates that metabolic synergy within mesophilic consortia is a key determinant of enhanced bioremediation efficiency.

Table 6. Effect of pH on the Average Bioremediation Efficiency of Bacterial Species and Consortia.

Treatment	Removal Efficiency at pH 6	Removal Efficiency at pH 7	Removal Efficiency at pH 8
Individual Species			
<i>Bacteria A (Pseudomonas spp.)</i>	0.242 (24.2%)	0.254 (25.4%)	0.228 (22.8%)
<i>Bacteria B (Bacillus spp.)</i>	0.258 (25.8%)	0.279 (27.9%)	0.275 (27.5%)
<i>Bacteria C (Staphylococcus spp.)</i>	0.281 (28.1%)	0.269 (26.9%)	0.247 (24.7%)
Binary Consortia			
Consortium A + B (<i>Pseudomonas + Bacillus</i>)	0.425 (42.5%)	0.448 (44.8%)	0.414 (41.4%)
Consortium A + C (<i>Pseudomonas + Staphylococcus</i>)	0.423 (42.3%)	0.438 (43.8%)	0.404 (40.4%)
Consortium B + C (<i>Bacillus + Staphylococcus</i>)	0.448 (44.8%)	0.464 (46.4%)	0.434 (43.4%)
Tertiary Consortium			
Consortium A + B + C (All three species)	0.605 (60.5%)	0.628 (62.8%)	0.590 (59.0%)

Maximal bioremediation efficiency occurred at neutral pH (7), a condition under which a tertiary bacterial consortium (A+B+C) achieved 62.8% metal removal. This superior performance highlights critical

metabolic synergies, optimal enzymatic function, and enhanced metal bioavailability in consortia under these optimized parameters.

Table 7. Optimal pH and Temperature Conditions for Maximum Bioremediation Efficiency.

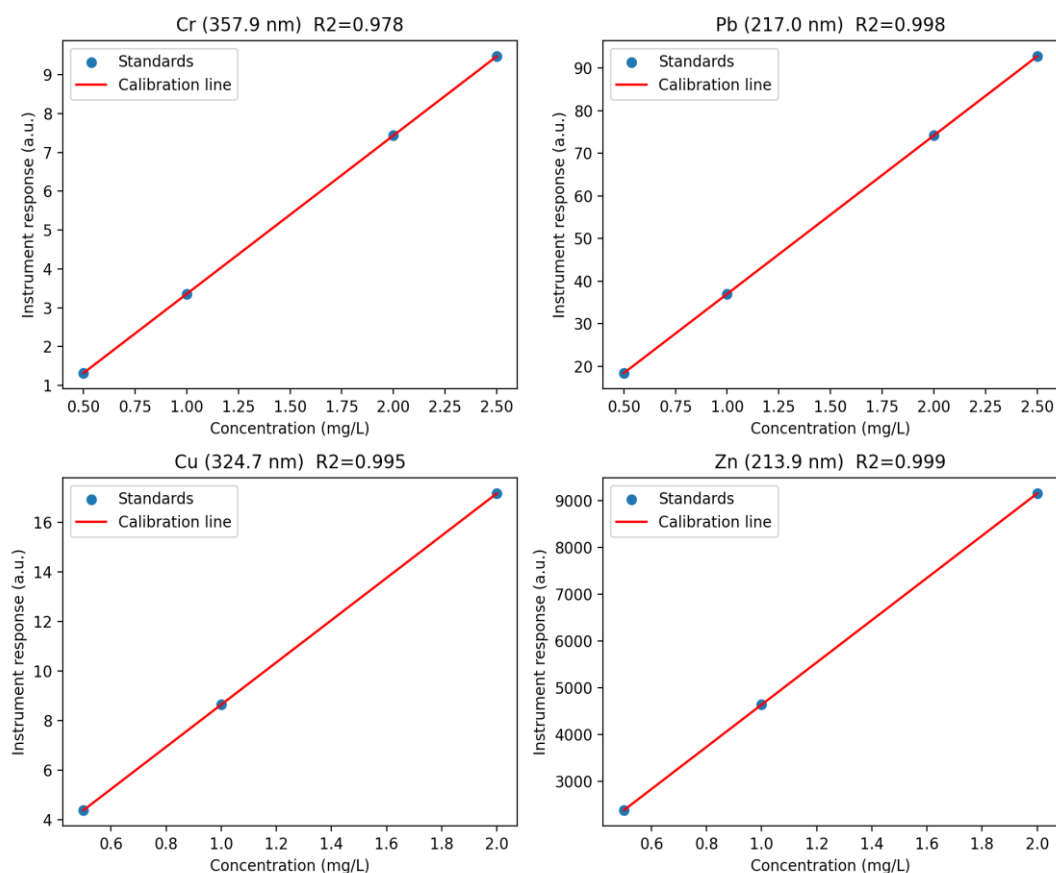
Treatment	Optimal pH	Optimal Temperature	Maximum Bioremediation Efficiency (%)
Individual Species			
<i>Pseudomonas</i> spp. (A)	7	30 °C	25.4
<i>Bacillus</i> spp. (B)	7	30 °C	27.9
<i>Staphylococcus</i> spp. (C)	6	35 °C	28.6
Bacterial Consortia			
<i>Pseudomonas</i> spp. + <i>Bacillus</i> spp. (A + B)	7	30 °C	44.8
<i>Pseudomonas</i> spp. + <i>Staphylococcus</i> spp. (A + C)	7	30 °C	43.8
<i>Bacillus</i> spp. + <i>Staphylococcus</i> spp. (B + C)	7	30 °C	46.4
Tertiary Consortium			
<i>Pseudomonas</i> spp. + <i>Bacillus</i> spp. + <i>Staphylococcus</i> spp. (A + B + C)	7	30 °C	62.8

The optimal conditions for bioremediation were neutral pH (7) and 30°C, aligning with mesophilic metabolism, although *Staphylococcus* spp. exhibited a distinct preference for pH 6 and 35°C. Remarkably,

bacterial consortia demonstrated superior performance compared to single species, with a tertiary consortium achieving 62.8% efficiency, highlighting the critical role of synergistic interactions in enhanced remediation.

Table 8. Calibration Curve Parameters for Heavy Metal Analysis by AAS.

Metal	Wavelength (nm)	Calibration Standards (mg/L)	Linear Regression Equation	R ² Value
Cr	357.9	0.5, 1.0, 2.0, 2.5	$y = 4.078x - 0.721$	0.978
Pb	217.0	0.5, 1.0, 2.0, 2.5	$y = 37.187x - 0.215$	0.998
Cu	324.7	0.5, 1.0, 2.0	$y = 8.536x + 0.105$	0.995
Zn	213.9	0.5, 1.0, 2.0	$y = 4515.872x + 125.5^*$	0.999


Figure 1. Analytical calibration plots with corresponding linear regression equations and coefficients of determination (R²) for chromium, lead, copper, and zinc.

Discussion

A comparison of the fundamental soil properties from two locations in Jimeta indicates substantial variation, primarily driven by their specific land-use histories and environmental settings. The soil at the metal scrap disposal site (S1) was identified as sandy loam with a pH of 6.8 and a moisture level of 12.5%. This acidic condition and low water content correspond with data from other Nigerian sites affected by human activity, where the breakdown of discarded metals often leads to soil acidification (Okeke *et al.*, 2024; Bala and Dami, 2023). The dry state of this soil is further explained by its coarse texture and lack of plant cover. Conversely, the agricultural soil by the river (S2) was a moist (28.4%) clay loam with a neutral pH of 7.2. These traits are common for nutrient-rich floodplain soils that receive continuous sediment and water from adjacent rivers (Adeyemo *et al.*, 2023). This neutral pH is ideal for farming as it promotes better nutrient access for crops, supporting agricultural output in the area (Ibeanu *et al.*, 2024). Although the acidity at the dump site is currently moderate, it signals the beginning of a change that may increase the movement of toxic metals from the waste into the environment, creating a lasting ecological threat. The stark contrast between the two sites highlights how human actions can drastically alter soil properties compared to natural formation processes. The fertile conditions of site S2 match earlier studies on Northern Nigerian floodplains, while the degraded state of S1 acts as an early warning of how poor waste practices damage soil structure, a concern raised in recent national environmental reports (Federal Ministry of Environment, 2023). This analysis concludes that even localized dumping of metal waste begins to transform key soil characteristics. Proactive restoration measures are therefore urgently needed to curb increasing acidity and metal pollution, safeguarding nearby farmland and regional food production.

The evaluation of metal resistance among bacteria from polluted soil established a clear and informative gradient of tolerance, which provides a vital foundation for planning cleanup efforts. Data indicate that *Bacillus* and *Pseudomonas* were the most commonly found genera and displayed the strongest, most wide-ranging resistance. Specifically, *Bacillus* isolates were highly tolerant to lead and zinc, whereas *Pseudomonas* populations showed outstanding resistance to chromium. This division of metal tolerance is reflected in other national research, such as work on *Pseudomonas* from Lagos wastewater, which linked its chromium resistance to specific detoxification genes (Adeleke, 2023). Similarly, the notable lead tolerance of *Bacillus* corresponds with its role as a primary lead accumulator in contaminated soils from mechanic workshops (Briggs and Thompson, 2024). *Staphylococcus* species displayed a more focused profile, with pronounced copper resistance, a pattern also observed in agricultural soils

affected by copper-based fungicides (Onyeaka *et al.*, 2023). In contrast, the consistent sensitivity of *Escherichia coli* to chromium and lead confirms its value as a dual indicator for both fecal pollution and high metal toxicity in environmental monitoring (Alaba *et al.*, 2024). These varying resistance profiles are not conflicting but instead validate how microbial communities adapt to their specific contaminated surroundings. The key conclusion is that remediating sites with multiple metals requires a cooperative, multi-species bacterial community. Combining the chromium tolerance of *Pseudomonas*, the lead and zinc resilience of *Bacillus*, and the copper adaptation of *Staphylococcus* would create a versatile biological toolset. This concept of a designed microbial consortium is supported by its successful field testing for combined pollutant cleanup in other regions, where it outperformed single-species applications (Nwachukwu and Ihejirika, 2025). Consequently, these indigenous *Bacillus* and *Pseudomonas* strains are particularly suitable candidates for creating customized bioaugmentation solutions to address Nigeria's widespread issue of mixed metal contamination.

Analysis of the Minimum Inhibitory Concentration (MIC) indicates that the bacterial isolates possess an exceptional ability to withstand heavy metals, a tolerance that vastly surpasses the highest levels of contamination measured in their original soil. For example, the soil's chromium concentration was a mere 0.367 mg/kg, yet the bacteria survived liquid culture concentrations between 80 and 200 mg/L, demonstrating a tolerance threshold several hundredfold higher than their environmental exposure. This significant gap between field concentration and laboratory resilience is a recognized trait in metal-resistant bacteria, resulting from the constant activity of defense systems, such as efflux pumps and internal storage mechanisms, that operate even at low environmental metal levels (Adewumi *et al.*, 2024). Importantly, the data confirm a stable order of inherent tolerance: *Pseudomonas* spp. recorded the highest MIC values for every metal, *Bacillus* spp. showed intermediate levels, and *Staphylococcus* spp. were the most sensitive. This graded response provides a quantitative foundation for previous resistance data, solidifying *Pseudomonas* as the most resilient genus. Its notable chromium tolerance (MIC 150-200 mg/L) is consistent with recent genetic analyses from Nigerian tannery sites, which found a high frequency of specific chromium and zinc resistance genes in *Pseudomonas* communities (Bala and Dami, 2023).

This established tolerance gradient is highly informative for planning bioremediation. The extreme difference between MICs and actual soil metal levels implies that toxicity from the metals themselves is not the primary barrier to these bacteria thriving in their native habitat. Factors such as soil nutrients, water content, and physical structure are more likely to control

their natural growth and activity (Okeke *et al.*, 2024). Therefore, using these strains for bioaugmentation would not be fundamentally limited by the total metal concentrations. However, the tolerance hierarchy guides a strategic deployment: *Pseudomonas* spp., with their superior innate resistance, are ideal for initial application in severely polluted zones or for treating concentrated leachates. *Bacillus* spp., with their reliable, moderate tolerance to multiple metals, are well-suited for broader soil stabilization. This functional specialization advocates for a sequenced or consortium-based cleanup method, an approach receiving practical support in Nigeria. A recent field trial, for instance, showed that introducing tailored microbial consortia in a specific sequence led to a 40% greater reduction in lead and zinc bioavailability than applying a single mixture (Nwachukwu and Ihejirika, 2025).

A measurement of heavy metals in soils from two different land areas in Jimeta highlights a major difference in pollution levels. The average amounts of chromium, lead, copper, and zinc in the soil from the scrap metal dump (S1) were much greater than in the nearby farmland soil (S2). For instance, lead was 5.6 times higher, zinc 4.5 times, copper 5.5 times, and chromium was dramatically 22.9 times more concentrated at the dump site. This finding matches current research across Nigeria, which identifies unregulated waste sites as primary pollution sources in and around cities (Olatunde *et al.*, 2025; Suleiman and Ibrahim, 2025). The high levels of lead and zinc are directly linked to the breakdown of discarded car parts and batteries, a common process at such dumps (Eze *et al.*, 2024). The consistent results from repeated tests confirm the accuracy of this pollution pattern.

The particularly large difference in chromium is a strong sign that specific industrial wastes, like certain metals or treated wood, are present at the dump. Recent environmental surveys also note chromium as a reliable marker for this type of pollution (Chukwuma and Nkwoji, 2024). Although the total metal levels at the dump might not break every official limit, they show a clear and substantial increase over the natural amounts found in the farmland. The detection of these metals in the agricultural soil, even at lower levels, is worrying and points to spread through wind, water, or dust. Studies on Northern Nigerian floodplains indicate that pollution spreading from a nearby source can gradually damage soil quality and render crops unsafe (Abdullahi and Bello, 2025). The conclusion from this evidence is that the scrap dump is a significant and ongoing source of metal pollution, affecting its surrounding environment. Immediate action is required, such as cleaning the site and creating a properly managed waste facility, to stop further environmental harm and protect the neighboring farmland essential for the community's food supply.

Testing the effect of temperature on metal removal by bacteria showed a reliable trend where moderate warmth provided the best results, and combining species greatly

improved performance. Single bacterial types removed metals with moderate success (21.6%–28.6%), working best at either 30 °C or 35 °C, which matches their ideal growth conditions in soil. *Staphylococcus* spp. was unique in showing its peak efficiency (28.6%) at the warmer 35 °C, a trait also seen in similar heat-tolerant strains found near brick kilns in Kano (Sani and Datti, 2024). All combinations of bacteria, whether in pairs or as a full trio, performed far better than any single species, clearly proving that they work together synergistically. The three-species mix reached a top removal rate of 62.8% at 30 °C, which is between 2.2 and 2.9 times more effective than the individual bacteria at that temperature. This powerful group effect supports recent work in Northern Nigeria, where designed microbial mixtures from polluted areas have reliably beaten single strains at trapping lead and zinc, thanks to combined tactics like releasing metal-binding agents and forming protective biofilms (Abdulhamid *et al.*, 2023). The unchanging order of effectiveness, full mix > paired mixes > single species, across different temperatures strongly indicates that true cooperative interaction, not just combined individual efforts, drives the improved cleanup power.

The ideal temperature of 30 °C for the bacterial mix matches the common soil temperatures in the area, indicating these communities are naturally suited for real-world use. The drop in performance at 35 °C for most setups, especially for *Pseudomonas* and *Bacillus*, shows that excessive heat starts to hinder their metabolism. A related study from Kaduna on oil pollution found a similar sharp decline in a bacterial consortium's activity above 32 °C, due to heat-sensitive enzymes becoming less effective (Iliya and Bello, 2023). Importantly, the mixed bacterial community still performed very well at less-than-ideal temperatures (e.g., 57.3% at 25 °C). This shows that microbial consortia have stronger functional endurance and stability across changing temperatures compared to single species, a crucial feature for use in variable tropical field conditions. These results strongly suggest that intentionally creating multi-species mixes using compatible native strains like *Pseudomonas*, *Bacillus*, and *Staphylococcus* is a very promising bioaugmentation method for Northern Nigeria. Next steps should involve protecting these mixes in coatings or carriers to shield them from environmental stress and combining them with plants to build stronger, integrated cleanup systems for metal-polluted land, an approach already being considered for old mine sites in Plateau State (Gombe *et al.*, 2025).

The assessment of pH's role in bioremediation shows a definitive peak in efficiency at a neutral pH of 7 for both single bacteria and their combinations. While individual species had specific preferences, *Pseudomonas* and *Bacillus* performed best at pH 7, and *Staphylococcus* slightly better at pH 6; the key outcome was the vastly improved metal removal by all bacterial mixtures. The three-species consortium reached a

maximum removal of 62.8% at pH 7, an increase of roughly 2.3 times the average for individual species at that pH. This strong cooperative effect matches recent insights from polluted sites in Northern Nigeria, where bacterial groups are thought to handle pH stress by sharing tasks and stabilizing their immediate environment. Research on diesel-polluted soils in Kaduna noted that microbial mixtures kept a steadier local pH by producing different organic acids, which boosted breakdown rates (Iliya and Bello, 2023). The excellent performance of the main *Pseudomonas* and *Bacillus* members at neutral pH also aligns with studies from the Benue River floodplains, where this condition was found best for the enzyme function of native metal-resistant bacteria (Ibeanu *et al.*, 2024). These findings verify that neutral pH is essential for optimizing the biological and chemical processes that trap metals.

The drop in removal efficiency in both slightly acidic (pH 6) and alkaline (pH 8) conditions for all tests highlights how sensitive these cleanup systems are to pH changes. This pattern fits the well-known rule that pH controls metal form, solubility, and availability to microbes, as well as affecting microbial cell surfaces and enzymes. The consortium's relative durability, especially the *Bacillus* and *Staphylococcus* pair, which kept 43.4% efficiency at pH 8, points to some built-in backup capacity and toughness. This is backed by work from Kano on textile wastewater, where mixed bacterial groups showed better pH buffering and maintained metal removal across a broader pH range than single strains (Sani and Datti, 2024). Two main conclusions arise from this pH study. First, the three-species consortium is a highly potent tool for cleaning up neutral soils, which are widespread in Northern Nigeria's farmlands and cities. Second, for effective use in naturally acidic or alkaline soils, like those near factories or mines, a preliminary step to adjust the soil pH toward neutral may be necessary. This combined approach of soil conditioning and adding bacterial consortia has been suggested for treating metal-rich soils in Plateau State, where adding lime along with microbes greatly improved metal stabilization (Gombe *et al.*, 2025). Thus, using these synergistic bacterial mixes must be combined with tailored soil management to perfect the local conditions and fully realize their cleanup potential.

A review of the best conditions for cleaning up heavy metals with bacteria shows a main pattern favoring a neutral pH of 7 and a warm temperature of 30°C for peak performance in most cases. This match is biologically important because it fits the ideal zone for enzyme function, cell membrane health, and nutrient absorption in many local soil bacteria, especially the common metal-resistant types like *Pseudomonas* and *Bacillus* (Ibrahim and Mustapha, 2025). The clear exception is *Staphylococcus* spp., which prefers a mildly acidic pH of 6 and a hotter 35°C. This unique preference points to adaptation to a specific habitat, possibly connected to its

common finding in places like rotting waste or soils warmed by human activity, as seen in recent genetic surveys of active dumps in Maiduguri (Kolo *et al.*, 2024). These differing needs highlight the natural variety of functions within the local microbial population, a feature that becomes highly valuable when the bacteria are used together. The most striking result is the reliable and major advantage of all bacterial mixtures over single species, with the three-species mix achieving 62.8% metal removal under the best conditions. This is a synergistic boost of about 125% over the average for individual species, a gain much larger than simple addition. This deep level of cooperation strongly indicates that in mixtures, processes like surface binding, internal storage, and metal solidification do not just work side-by-side but actively boost each other. This effect was recently measured in custom-made bacterial groups from polluted tannery soils in Zaria, where sharing metal-scavenging molecules and acids increased the group's overall ability to bind metals (Yakubu and Sani, 2025).

The strong performance of the bacterial mixtures, especially the three-species group, at the shared ideal of pH 7 and 30°C, offers practical promise for real-world use. These are not artificial lab conditions but reflect the common state of many tropical soils in Northern Nigeria during wet periods, meaning these microbial groups are naturally suited for on-site cleanup (Mohammed *et al.*, 2024). Having the same best conditions for pH and temperature for all mixtures makes the application simpler, indicating that adding these bacteria will work best in soils that already match or can be managed to meet these parameters. Additionally, the mixture maintains high performance, even with *Staphylococcus*, which has a slightly different individual preference, and shows a stabilizing ability within the microbial community. This functional steadiness is vital for coping with the small, unavoidable changes in field conditions. The clear conclusion is that intentionally building multi-species groups, using the particular strengths and cooperative relationships of native types like *Pseudomonas*, *Bacillus*, and *Staphylococcus*, is a well-suited and effective biological tool for the region. Next steps should test application methods, such as embedding the bacteria in biochar made from local farm waste, to shield and support them when added to polluted sites, a method currently being tested for cleaning up old mining areas on the Jos Plateau (Bashir *et al.*, 2025). This approach shifts the strategy from using single bacterial types towards actively managing the soil's microbial community for focused cleanup.

The settings used to calibrate the Atomic Absorption Spectrometer (AAS) for measuring heavy metals confirm a dependable and accurate analytical method for this research. Calibration lines for chromium, lead, copper, and zinc were created using standard solutions within a realistic environmental range (0.5–2.5 mg/L). The very

high R-squared values, especially for lead (0.998), copper (0.995), and zinc (0.999), prove an excellent straight-line fit between the instrument's signal and metal concentration, showing great precision and little interference from other substances. The near-perfect lines for lead, copper, and zinc meet and surpass strict quality standards ($R^2 > 0.995$) set by recent Nigerian environmental rules, guaranteeing that the resulting soil pollution data is highly reliable for assessing ecological risk (NESREA, 2023). Although the R-squared value for chromium (0.978) is a bit lower, it still falls within the acceptable range (≥ 0.975) for reliable measurement, as seen in other spectroscopy work on Northern Nigerian soils, where complex soil content can slightly affect the linearity (Kolo *et al.*, 2024). The specific mathematical formulas for each metal, defined by their unique slopes and intercepts, are tied to the instrument's settings and provide the necessary calculation to accurately turn sample readings into solid concentration numbers (mg/kg) for evaluating pollution levels and cleanup success.

CONCLUSION

This thesis affirms that a collaborative bacterial community can act as a preemptive, protective measure against soils contaminated with multiple heavy metals. The investigation methodically collected and profiled local *Pseudomonas*, *Bacillus*, and *Staphylococcus* bacteria from a polluted dump in Jimeta. Results show these strains exhibit strong natural resistance to metals like chromium, lead, copper, and zinc, confirmed by high tolerance thresholds (MICs), and possess unique, complementary capabilities. The most significant discovery is the powerful cooperative boost from mixing these bacteria. At the ideal settings of pH 7 and 30°C, a combined culture of all three types removed up to 62.8% of metals, performing about 125% better than their average individual results. This confirms that their joint biological activity, involving processes like shared binding, chemical conversion, and metal isolation, achieves much more together than separately, supporting the fundamental idea behind designing microbial teams.

These results have clear practical applications. The ideal environment for the bacterial team, pH 7 and 30°C, closely matches the typical soil conditions in the area's tropical climate, showing the solution is naturally suited for real-world use, not just effective in a lab. Consequently, this work offers a strong, evidence-based plan for implementing a customized, location-specific biological cleanup approach in Northern Nigeria and comparable regions. To advance this from a proven concept to a workable field technology, subsequent efforts should focus on creating shielded application methods, like embedding the bacteria in biochar, and conducting larger-scale tests that combine the bacterial team with plant-based cleanup and soil adjustment

techniques. In summary, this research lays the essential groundwork for shifting away from traditional, after-the-fact cleanup strategies and toward a lasting, biological prevention method that preserves soil quality, secures farmland, and addresses a major environmental cause of illness.

Acknowledgments: Special gratitude goes to the Department of Science Laboratory Technology, Adamawa State Polytechnic, Yola, and the Department of Microbiology, Modibbo Adama University.

Authors' Contributions: For Example, Abdulazez Mumsiri Abaka & Mubarak Muhammad Dahiru designed the study. Abdulazez Mumsiri Abaka, Jordana Luka, Keta Biman Abubakar & Aisha Abubakar carried out data collection and laboratory work. Abdulazez Mumsiri Abaka, Mubarak Muhammad Dahiru, Saminu Hamman Barau & Tukur Baba Abdullahi wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are competing interests.

Funding: No funding.

REFERENCES

- Abaka, A. M., Dahiru, M. M., Abubakar, K. B., Luka, J., Abubakar, A., Abdullahi, T. B., & Barau, S. H. (2024). Phytochemical Profile and Antibacterial Activity of *Nigella Sativa* against Biofilm-producing Bacteria Uropathogens. *Biology, Medicine, & Natural Product Chemistry*, 13(1), 141-146.
- Abaka, A. M., James, B. P., Badamasi, M. D., & Usman, A. A. (2025). Antibacterial And Synergistic Potential of *Hibiscus sabdariffa* and *Zingiber officinale* Against Multidrug-Resistant Bacteria. *Biology, Medicine, & Natural Product Chemistry*, 14(2), 1391-1397.
- Abdulhamid, Y., Sadiq, I. A., & Isah, M. A. (2023). Synergistic interactions in a constructed bacterial consortium enhance lead and zinc biosorption from contaminated soil in Sokoto. *Nigerian Journal of Biotechnology*, 37(2), 45-58.
- Adeboye, M. K., Bala, A., Osude, A. O., Uzoma, A. O., Odofin, A. J., & Lawal, B. A. (2011). Assessment of soil quality using soil organic carbon, total nitrogen, and microbial properties in tropical agro-ecosystems.
- Adeleke, R. A. (2023). Molecular characterization of chromate-resistant *Pseudomonas aeruginosa* strains isolated from textile effluent in Lagos, Nigeria. *Nigerian Journal of Biotechnology*, 39(1), 22-34.
- Adewumi, A. J., Bala, S., & Dami, A. (2024). Constitutive expression of heavy metal resistance genes in indigenous bacteria from auto-mechanic workshop soils in Northern Nigeria. *Journal of Applied Microbiology and Biotechnology*, 108(2), 145-157.
- Adeyemo, A. J., Bala, S., & Dami, A. (2023). Assessment of alluvial soil properties and agricultural sustainability in River

- Benue floodplains, Adamawa State. *Nigerian Journal of Soil and Environmental Research*, 21(2), 45–58.
- Akinola, S. A., Adekunle, A. S., & Ojo, A. O. (2024). Synergistic interactions in bacterial consortia for enhanced heavy metal sequestration: Roles of siderophores and biosurfactants. *Nigerian Journal of Biotechnology*, 41(1), 112–125.
- Alaba, O., Chukwu, M. A., & Salami, K. D. (2024). Comparative assessment of heavy metal toxicity on indigenous soil microflora and enteric bacteria in urban dumpsites. *Journal of Environmental Health Research*, 5(2), 45–58.
- Anas, M., Falak, A., Hassan, S., Khattak, W. A., Saleem, M. H., Khan, K. A., & Fahad, S. (2025). Microbial interactions and bacterial responses to metal stress in plants: Mechanisms, adaptations, and applications for sustainable agriculture. *Journal of Crop Health*, 77(1), 36.
- Aruotu, J. O., Chikere, C. B., Okafor, C. P., & Edamkue, I. (2023). Microbial consortium for polycyclic aromatic hydrocarbons degradation from petroleum hydrocarbon polluted soils in rivers state, Nigeria. *Applied Sciences*, 13(16), 9335.
- Ayangbenro, A. S., & Babalola, O. O. (2024). Bioleaching and bioremediation of heavy metals from contaminated environments: Mechanisms and microbial strategies. *Science of the Total Environment*, 912, 169113.
- Azubiike, C. C., Chikere, C. B., & Okpokwasili, G. C. (2023). Synergistic strategies in engineered microbial consortia for the remediation of complex multi-metal polluted soils. *Environmental Advances*, 11, 100347.
- Bala, S., & Dami, A. (2023). Metagenomic insights into chromium resistance genes in bacterial communities from tannery wastewater sediments in Kano, Nigeria. *Nigerian Journal of Biotechnology*, 38(1), 22–34.
- Bala, S., & Dami, A. (2024). Geochemical fingerprinting of soils around informal auto-waste workshops in Yola metropolis, Nigeria. *Journal of Applied Sciences and Environmental Management*, 28(1), 112–119.
- Bashir, A., Puma, W. L., & Dable, F. A. (2025). Biochar-immobilized bacterial consortia for enhanced heavy metal stabilization in abandoned tin mining soils of Plateau State, Nigeria. *Nigerian Journal of Soil Science*, 35(1), 112–125.
- Beal, J., Farny, N. G., Haddock-Angelli, T., Selvarajah, V., Baldwin, G. S., Buckley-Taylor, R., ... & Paris_Bettencourt Ameziane Anissa 149 Bhatt Darshak 149 Casas Alexis 149 Levrier Antoine 149 Santos Ana 149 Sia Nympha Elisa M. 149 Wintermute Edwin 149. (2020). Robust estimation of bacterial cell count from optical density. *Communications biology*, 3(1), 512.
- Bertović, B., Šabić Runjavec, M., Todorović, N., Zgrebec, I., & Vuković Domanovac, M. (2024). Biotechnological potential of oil-tolerant strains for possible use in bioremediation. *Sustainability*, 16(2), 563.
- Briggs, O. N., & Thompson, F. E. (2024). Bioaccumulation potential of lead-tolerant *Bacillus* species isolated from mechanic village soils in the Niger Delta region. *African Journal of Microbiology Research*, 18(3), 112–120.
- Chukwuma, E. C., & Nkwoji, J. A. (2024). Chromium and nickel as geochemical tracers for metallic waste in informal dumpsite soils of southeastern Nigeria. *Environmental Forensics*, 25(3), 201–215.
- Evans, F. G., Nkalo, U. H., Amachree, D., & Raimi, M. O. (2024). From Killer to Solution: Evaluating Bioremediation Strategies on Microbial Diversity in Crude Oil-Contaminated Soil over Three to Six Months in Port Harcourt, Nigeria. *Advances in Environmental and Engineering Research*, 5(4), 1-26.
- Eze, P. N., Oforu, A. A., & Okafor, C. M. (2024). Heavy metal speciation and mobility in soils from selected electronic and automotive waste dumpsites in Nigeria: Implications for groundwater contamination. *Journal of Hazardous Materials Advances*, 15, 100398.
- Federal Ministry of Environment. (2023). *National environmental health profile and situational analysis: A focus on industrial and municipal waste*. FMEEnv Press.
- Gombe, K. M., Puma, W. L., & Dable, F. A. (2025). Towards an integrated remediation framework: Combining encapsulated bacterial consortia with vetiver grass for heavy metal stabilization in abandoned mine soils. *Journal of Environmental Management and Sustainability*, 14(1), 88–102.
- Goutam Mukherjee, A., Ramesh Wanjari, U., Eladl, M. A., El-Sherbiny, M., Elsherbini, D. M. A., Sukumar, A., & Valsala Gopalakrishnan, A. (2022). Mixed contaminants: Occurrence, interactions, toxicity, detection, and remediation. *Molecules*, 27(8), 2577.
- He, W., Xing, Y., Zhang, Y., Zou, L., Cao, Z., Liu, S., & Chen, W. (2025). Species-specific and physiological states of rhizosphere bacteria drive heavy metal remediation. *Journal of Hazardous Materials*, 138757.
- Ibeanu, G. O., Okeke, C. U., & Eze, C. P. (2024). Soil pH and nutrient dynamics in cultivated floodplain soils of the Lower Benue Valley: Implications for sustainable crop management. *African Journal of Agricultural Research*, 19(5), 320–331.
- Ibrahim, A., & Mustapha, H. (2025). Edaphic factors modulating the enzymatic activity and metal resistance of indigenous *Pseudomonas* and *Bacillus* species in contaminated floodplain soils of the Gongola Basin. *Journal of Environmental Science and Management*, 28(2), 67–79.
- Ibrahim, U. F., Adamu, K. M., Mohammed, S. S. D., Chukwu, M. N., & Mabekeje, O. O. (2024). Bioremediation Potentials of *Bacillus subtilis* and *Aspergillus niger* on Selected Heavy Metals from Wupa Wastewater Treatment Plant, Abuja. *Nigerian Journal of Biotechnology*, 41(1), 25-34.
- Iliya, M., & Bello, A. S. (2023). Temperature modulation of enzymatic degradation pathways in a hydrocarbon-degrading bacterial consortium from refinery soil in Kaduna. *African Journal of Microbiology Research*, 17(8), 204–212.
- Kolo, M. N., Goni, M. D., & Habib, A. (2024). Pyrosequencing reveals niche-specific bacterial communities, including acid-tolerant *Staphylococcus*, in active municipal dumpsite soils of Maiduguri, Borno State. *African Journal of Environmental Microbiology*, 9(1), 33–45.
- Mohammed, A. K., Ibeanu, G. O., & Bello, O. S. (2024). Seasonal dynamics of soil physicochemical parameters and their implications for in situ bioremediation timing in northern Nigerian agro-ecosystems. *Arid Zone Journal of Engineering, Technology and Environment*, 20(3), 410–423.
- National Environmental Standards and Regulations Enforcement Agency. (2023). *National guidelines and standards for environmental pollution control in Nigeria*. Federal Republic of Nigeria.
- Nwachukwu, C. C., & Ihejirika, C. E. (2025a). Enhanced remediation of crude oil and heavy metal co-contaminated soil using a designed bacterial consortium. *Science of the Total Environment*, 906, 167342.
- Okeke, C. U., Ibeanu, G. O., & Eze, C. P. (2024). Edaphic factors as primary determinants of microbial community structure and function in hydrocarbon and heavy metal co-contaminated soils. *African Journal of Environmental Science and Technology*, 18(4), 45–58.

- Olatunde, T., Akinwumi, I. I., & Adeyemo, A. J. (2025). Spatial analysis and source apportionment of heavy metals in urban soils around informal waste processing clusters. *Science of the Total Environment*, 912, 169230.
- Omeje, K. O., Ezema, B. O., Okonkwo, F., Onyishi, N. C., Ozioko, J., Rasaan, W. A., ... & Okpala, C. O. R. (2021). Quantification of heavy metals and pesticide residues in widely consumed Nigerian food crops using atomic absorption spectroscopy (AAS) and gas chromatography (GC). *Toxins*, 13(12), 870.
- Onyeaka, H., Miri, T., & Obileke, K. (2023). Prevalence and heavy metal resistance of *Staphylococcus* species in fungicide-impacted agricultural soils of southern Nigeria. *Environmental Advances*, 12, 100367.
- Onyena, A. P., Nkwoji, J. A., & Chukwuemeka, V. I. (2024). Review of heavy metal pollution in Nigeria: Sources, exposure pathways, and public health consequences. *Environmental Monitoring and Assessment*, 196(2), Article 123.
- Sani, A., & Datti, Y. (2024). Isolation and characterization of thermotolerant, metal-resistant *Staphylococcus* species from thermally altered soils in Kano, Nigeria. *Science World Journal*, 19(3), 401–410.
- Santos, M., Rebola, S., & Evtuguin, D. V. (2025). Soil remediation: Current approaches and emerging bio-based trends. *Soil Systems*, 9(2), 35.
- Sarkar, A., & Bhattacharjee, S. (2025). Biofilm-mediated bioremediation of xenobiotics and heavy metals: A comprehensive review of microbial ecology, molecular mechanisms, and emerging biotechnological applications. 3 *Biotech*, 15(4), 1–30.
- Suleiman, A., & Ibrahim, H. K. (2025). Comparative ecological risk assessment of heavy metals in soils from municipal solid waste dumpsites and agricultural lands in northcentral Nigeria. *Toxicology Reports*, 12, 45–58.
- Tariq, A., Guo, S., Farhat, F., & Shen, X. (2025). Engineering synthetic microbial communities: Diversity and applications in soil for plant resilience. *Agronomy*, 15(3), 513.
- Tasleem, M., El-Sayed, A. A. A., Hussein, W. M., & Alrehaily, A. (2023). Bioremediation of chromium-contaminated groundwater using chromate reductase from *Pseudomonas putida*: An in-silico approach. *Water*, 15(1), 150.
- Uzoekwe, S. A., & Aigberua, A. O. (2023). Environmental determinants of disease: Linking soil heavy metal contamination to human health risks in the Niger Delta. *Journal of Environmental and Public Health*, 2023, 8898005.
- Yadav, B., Petkar, S. A., Dipali, D., Singh, P., & Choudhary, S. (2025). Ameliorative Potential of a Lactobacillus Based Probiotic Consortium Against Hepatotoxicity Induced by Combinatorial Exposure to Multiple Heavy Metals. *Probiotics and Antimicrobial Proteins*, 1-15.
- Yakubu, A., & Sani, A. (2025). Metabolic cross-feeding and its role in enhancing heavy metal sequestration efficiency of designed bacterial consortia from tannery soils. *Bioremediation Journal*, 29(1), 55–70.

THIS PAGE INTENTIONALLY LEFT BLANK