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Fungal Diversity and Heavy Metal Mycoremediation Potential of Military Shooting Range Soils in Kaduna State, Nigeria

Ibrahim Yusuf Tafinta[1], Khadija Abdullahi Yarima[2], Mu'awiyya Umar Ladan[3], Umar Balarabe Ibrahim[4]
[1,2,4]Usmanu Danfodiyo University, Sokoto, Nigeria
[3]Federal University Gusau, Zamfara State, Nigeria
yarimausman@gmail.com

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Abstract

Military shooting ranges are significant point sources of heavy metal contamination that may alter soil microbial communities and ecological functioning. Given the ecological roles and metal-tolerance mechanisms of soil fungi, this study investigated fungal diversity in soils from the Jaji Military Shooting Range, Kaduna State, Nigeria, and examined the relationship between fungal abundance and selected heavy metal concentrations. Soil samples were collected from five locations at a depth of 0–15 cm. Cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) concentrations were determined by Flame Atomic Absorption Spectrophotometry following tri-acid digestion. Fungi were isolated through serial dilution, cultured on Potato Dextrose Agar, identified morphologically, and evaluated using Simpson's Diversity Index. Data were analyzed using analysis of variance and Pearson correlation analysis at a 5% significance level. Lead had the highest recorded concentration, reaching 43.61 mg/kg at location B, while all metals exhibited significant spatial variation ($p < 0.05$). Eleven fungal species, predominantly belonging to Ascomycota and Mucoromycota, were identified. *Mucor racemosus* (35.59%) and *Aspergillus niger*

(22.88%) were the most prevalent isolates. A Simpson's Diversity Index of 0.8 indicated high species diversity and relative community evenness. Pearson correlation coefficients (r = 0.268–0.329) showed weak, nonsignificant positive relationships between fungal abundance and heavy metal concentrations. These findings demonstrate that metal-impacted shooting-range soils support a diverse and resilient fungal community. The persistence and prevalence of indigenous fungi under metal stress highlight their ecological adaptability and identify them as promising candidates for further evaluation in heavy metal mycoremediation strategies.

Keywords: Fungal Diversity; Heavy Metal Contamination; Military Shooting Range; Mycoremediation; Soil Fungi

INTRODUCTION

Soil ecosystems are increasingly impacted by anthropogenic activities that introduce persistent contaminants and disrupt microbial community structure (Hamba & Tamiru 2016). Among such activities, military shooting ranges constitute significant point sources of heavy metal deposition due to continuous ammunition discharge. Spent bullets and fragments release metals—particularly lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni)—into surrounding soils, where they accumulate and persist because they are non-biodegradable and resistant to chemical breakdown (Nwaedozie et al. 2015; Aghanwa et al. 2021; Balali-Mood et al. 2021). Elevated concentrations of these metals can impair soil biological processes, reduce microbial diversity, and pose ecological and public health risks (Oruama & Joinkrama 2024).

Fungi represent a major functional group in soil ecosystems, driving organic matter decomposition, nutrient mineralization, and soil aggregation. Beyond their ecological roles, many filamentous fungi exhibit adaptive mechanisms—including biosorption, intracellular sequestration, and metal chelation—that enable survival under heavy metal stress (Sey & Belford 2021; Geris et al. 2024). These traits position fungi as promising agents for mycoremediation of contaminated soils. However, heavy metal accumulation may also alter fungal community composition, dominance patterns, and diversity indices, potentially affecting ecosystem stability.

In Nigeria, previous studies on military environments have largely focused on quantifying heavy metal concentrations and assessing associated environmental risks

(Nwaedozie et al. 2015; Aghanwa et al. 2021). Limited attention has been given to the diversity and ecological structure of fungal communities inhabiting military shooting range soils, particularly in northern Nigeria. Moreover, few studies have integrated metal quantification with fungal diversity metrics to evaluate potential ecological resilience or tolerance patterns.

Addressing this gap is important for understanding how indigenous fungal communities respond to chronic metal exposure and for identifying taxa with potential application in bioremediation strategies. Therefore, this study investigated the mycofloral diversity of soils from the Jaji Military Shooting Range, Kaduna State, Nigeria, and examined the relationship between fungal abundance and selected heavy metals. By integrating heavy metal analysis with diversity indices and statistical evaluation, this work provides baseline ecological data for metal-impacted military soils and contributes to ongoing efforts to explore fungal-mediated remediation approaches.

MATERIALS AND METHODS

Study Area

Soil samples were collected from the Jaji Military Shooting Range, located within the Jaji Military Cantonment in Igabi Local Government Area, Kaduna State, Nigeria (Figure 1). The cantonment is situated between latitude 09°20'N and 10°30'N and between longitude 07°00'E and 08°30'E. The climate is tropical dry-and-wet (Köppen's Aw), with a wet season from April to mid-October and a dry season from mid-October to April. The five sampling locations (A–E) were selected purposively to represent areas with varying degrees of shooting activity and potential metal deposition, including the firing points, the berm (backstop), and downrange impact areas, to capture spatial heterogeneity in contamination. The geographical coordinates of each sampling point were recorded using a handheld Global Positioning System (GPS) device (Garmin eTrex 10) as follows: Location A (10.8456°N, 7.5678°E), Location B (10.8461°N, 7.5685°E), Location C (10.8470°N, 7.5692°E), Location D (10.8450°N, 7.5665°E), and Location E (10.8485°N, 7.5710°E). The vegetation in the area is typical of Guinea savanna, comprising grasses and scattered trees, and the soil is ferruginous tropical type, formed over basement complex rocks.

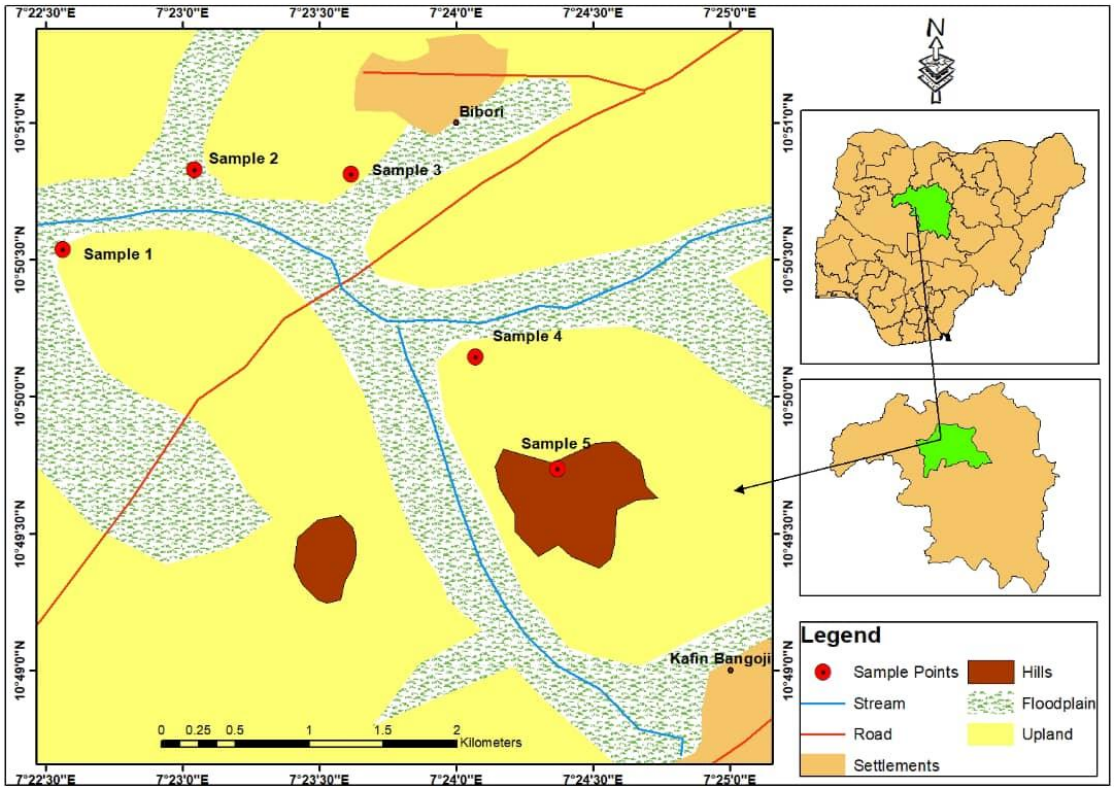

Figure 1: Map of the Study Area

Determination of the Diversity of Mycoflora in Military Shooting Range Soils

Collection of Samples

Soil samples were collected from five designated locations (A–E) within the Jaji Military Shooting Range, Kaduna State, Nigeria, using a stratified purposive sampling design. The locations were strategically selected to represent distinct functional zones of the shooting range: the firing point (Location A), the berm (backstop) (Location B), and three downrange impact areas at increasing distances from the firing point (Locations C, D, and E). This design was intended to capture the expected spatial heterogeneity in heavy metal deposition resulting from ammunition discharge.

At each of the five locations, a 10 m × 10 m sampling plot was established. Within each plot, five soil subsamples were collected from the four corners and the center using a sterile soil auger at a depth of 0–15 cm. The 0–15 cm depth was chosen because it represents the active rhizosphere and zone of highest microbial activity, where metal accumulation from surface deposition is typically greatest, and where fungi are most abundant. The five subsamples from each plot were thoroughly mixed in a sterile polyethylene bag to form a single composite sample representing that location. This process yielded a total of five

composite samples (one per location). Each composite sample was properly labeled, placed in an ice-chest, and transported to the Mycology Laboratory, Plant Science Department of Usmanu Danfodiyo University, Sokoto, for analysis (Disegha et al. 2024).

Media Preparation

Potato Dextrose Agar (PDA) was used for the isolation of fungi and prepared according to the manufacturer's specifications. Thirty-nine grams (39 g) of dehydrated PDA powder were dissolved in 1000 mL of distilled water. To suppress bacterial growth, streptomycin was added to the medium at a final concentration of 50 mg/L prior to autoclaving. The mixture was thoroughly homogenized and heated on a hot plate with continuous stirring until completely dissolved. The prepared medium was then sterilized by autoclaving at 121°C for 15 minutes (Tafinta et al. 2024). After sterilization, the molten medium was allowed to cool to approximately 45°C before being poured into sterile Petri dishes. For each dilution plated, three replicate plates were used to ensure statistical reliability of the isolation procedure.

Isolation and Identification of Mycoflora

Exactly 1 g of each composite soil sample was weighed and suspended in 9 mL of sterile distilled water, followed by serial dilution to 10^{-3}. An aliquot (1 mL) of the 10^{-3} dilution was inoculated onto sterile Petri dishes containing Potato Dextrose Agar (PDA) and spread evenly using a sterile glass spreader. Inoculated plates were incubated at 28 ± 2°C for 5 days and observed daily for fungal growth. For each sample, three replicate plates were used per dilution.

Following incubation, distinct fungal colonies were sub-cultured onto fresh PDA plates to obtain pure cultures. Pure cultures were obtained through repeated sub-culturing until single, homogeneous colonies were isolated. The fungal isolates were identified based on macroscopic characteristics (colony color, texture, pigmentation, and growth pattern) and microscopic features using the lactophenol cotton blue staining technique. Microscopic examination focused on hyphal structure (septate or non-septate), presence and morphology of reproductive structures (conidiophores, sporangia, conidia, sporangiospores), and other diagnostic features.

Identification was performed by comparing observed characteristics with standard mycological atlases and taxonomic keys, including Watanabe (2010) *Pictorial Atlas of Soil and*

Seed Fungi: Morphologies of Cultured Fungi and Key to Species (3rd ed.) and Larone (2011) *Medically Important Fungi: A Guide to Identification* (5th ed.). It is important to acknowledge that identification was based solely on morphological characteristics, as molecular confirmation (e.g., DNA sequencing of ITS regions) was not performed due to resource constraints. Therefore, species-level designations should be considered provisional, and further molecular analysis would be required for definitive taxonomic confirmation.

Percentage Frequency of Occurrence

Fungal percentage frequency of occurrence was obtained using the standard formula as adopted by Tafinta et al. (2019).

$$\text{Percentage Frequency of Occurrence} = \frac{NI}{TN} \times 100$$

Where NI = Number of individual isolates, TN = Total number of isolates.

Determination of Diversity Indices of the Mycoflora

The diversity of fungal species was assessed in terms of species richness and relative abundance as described by Alhaji et al. (2022). Two indices were calculated: Simpson's Diversity Index (D) and Simpson's Index of Diversity (1 – D).

Simpson's Diversity Index (D)

Simpson's Diversity Index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species. It is calculated using the formula:

$$D = \Sigma (n_i / N)^2$$

Where:

- n_i = number of individuals of the i-th species
- N = total number of individuals of all species
- Σ = sum across all species

The value of D ranges from 0 to 1, where:

- D = 0 indicates infinite diversity (all species equally rare)
- D = 1 indicates no diversity (only one species present)

Simpson's Index of Diversity (1 – D)

To express diversity in a more intuitive manner (where higher values indicate greater diversity), Simpson's Index of Diversity was calculated as:

$$1 - D = 1 - \Sigma (n_i / N)^2$$

This value also ranges from 0 to 1, with 1 representing maximum diversity (all species equally abundant) and 0 representing minimum diversity (only one species present).

Calculation:

The Simpson's Diversity Index (D) and Simpson's Index of Diversity (1 – D) were calculated using the formulas:

- $D = \Sigma (n_i / N)^2$
- $1 - D = 1 - \Sigma (n_i / N)^2$

Where:

- n_i = number of individuals of the i-th species
- N = total number of individuals of all species

The detailed calculation, based on the data in Table 4, yielded a $\Sigma (n_i/N)^2$ value of 0.2097.

Therefore:

- Simpson's Diversity Index (D) = 0.21 (rounded to two decimal places)
- Simpson's Index of Diversity (1 – D) = 1 – 0.21 = 0.79

Consequently, the Simpson's Index of Diversity for the fungal community is 0.79 (approximately 0.8, as reported in the text).

Calculation Verification:

- Total number of isolates (N) = 7 + 42 + 27 + 4 + 6 + 2 + 2 + 2 + 3 + 16 + 7 = 118 (verified)
- Sum of $(n_i/N)^2$ = 0.0035 + 0.1267 + 0.0524 + 0.0011 + 0.0026 + 0.0003 + 0.0003 + 0.0003 + 0.0006 + 0.0184 + 0.0035 = 0.2097 (rounded to 0.21)

Heavy Metal Analysis

Sample Digestion

One gram (1 g) of air-dried and sieved soil sample (2 mm mesh) was accurately weighed into a clean, acid-washed 100 mL beaker. A 15 mL tri-acid mixture consisting of nitric acid (HNO_3), perchloric acid ($HClO_4$), and sulfuric acid (H_2SO_4) in the ratio of 5:1:1 was added to the beaker. The mixture was heated gently on a hot plate at 80°C in a fume cupboard until a clear solution was obtained, indicating complete digestion of the organic and mineral matrix. The digest was allowed to cool and subsequently filtered using Whatman No. 42 filter paper into a 50 mL volumetric flask. The filtrate was then made up to the 50 mL mark with distilled deionized water and stored in clean, acid-washed plastic bottles at 4°C prior to heavy metal analysis.

Quality Assurance and Quality Control (QA/QC)

Strict quality assurance and quality control procedures were followed to ensure the accuracy and reliability of the analytical results.

Instrumentation and Calibration: Heavy metal concentrations were determined using a Flame Atomic Absorption Spectrophotometer (FAAS) (Model: Buck Scientific 210VGP, East Norwalk, USA) equipped with deuterium background correction. Each metal was analyzed at its characteristic wavelength using appropriate hollow cathode lamps, lamp current, flame type (air-acetylene), and slit width settings as specified by the manufacturer. The instrument was calibrated using analytical grade standard solutions prepared by serial dilution of 1000 mg/L stock standards (Sigma-Aldrich, USA) for each metal (Cd, Cr, Ni, and Pb). A five-point calibration curve (0, 0.5, 1.0, 2.0, and 5.0 mg/L) was generated for each metal, and calibration was accepted only when the correlation coefficient (R^2) was ≥ 0.995.

Detection Limits: The instrument detection limits (IDL) for each metal, determined as three times the standard deviation of ten replicate measurements of the blank, were as follows: cadmium (Cd) = 0.002 mg/L, chromium (Cr) = 0.005 mg/L, nickel (Ni) = 0.004 mg/L, and lead (Pb) = 0.01 mg/L. Sample concentrations below these detection limits were reported as below detection limit (BDL).

Blanks and Reagent Quality: A reagent blank (containing all reagents but no soil) was carried through the entire digestion and analytical procedure to monitor for background contamination. All glassware and plasticware were soaked in 10% nitric acid for 24 hours,

rinsed thoroughly with distilled deionized water, and air-dried before use to prevent cross-contamination. Distilled deionized water was used for all solution preparations.

Recovery Studies: To assess the accuracy of the digestion and analytical procedure, recovery experiments were performed by spiking pre-analyzed soil samples with known concentrations of each metal standard. The spiked samples were digested and analyzed following the same procedure as the unknown samples. Percentage recovery was calculated using the formula:

Recovery (%) = [(Concentration in spiked sample – Concentration in unspiked sample) / Concentration added] × 100

The mean recovery percentages (n = 3) for the analyzed metals were: Cd = 96.8 ± 2.3%, Cr = 94.2 ± 3.1%, Ni = 95.5 ± 2.7%, and Pb = 97.3 ± 1.9%. These recovery values are within the acceptable range (90–110%) for trace metal analysis, confirming the accuracy and reliability of the method.

Replicates and Quality Control Samples: All soil samples were analyzed in triplicate, and the mean values ± standard deviation are reported. A certified reference material (CRM) for soil (NIST SRM 2709a, San Joaquin Soil) was analyzed periodically throughout the sample run to verify instrument performance and method accuracy. The analyzed values for all metals in the CRM were within ± 10% of the certified values.

Determination of Heavy Metals Concentrations

The concentrations of Cadmium (Cd), Chromium (Cr), Lead (Pb), and Nickel (Ni) in the digested soil samples were determined using a Flame Atomic Absorption Spectrophotometer (FAAS). Each metal was analyzed at its characteristic wavelength using appropriate lamp current, flame type, and slit width settings. Calibration curves were generated using analytical grade standard solutions for each metal, and concentrations in samples were extrapolated based on their absorbance values.

Statistical Analysis

All data obtained from the experiment were subjected to statistical analysis using JMP (John's Macintosh Project) statistical software version 16 (released March 2021). A significance level of $\alpha = 0.05$ was used for all statistical tests.

Experimental Design and Model: The study employed a completely randomized design (CRD) with five sampling locations (A–E) serving as the treatment groups. For heavy metal concentrations, each location was represented by three replicate analyses (analytical replicates) of the composite soil sample from that location. For fungal abundance, each location was represented by three replicate plates per dilution during isolation.

Heavy Metal Analysis: Mean concentrations of heavy metals (Cd, Cr, Ni, and Pb) were compared across the five locations using a one-way analysis of variance (ANOVA). The statistical model was:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where:

- Y_{ij} = observed metal concentration for the j-th replicate in the i-th location
- μ = overall population mean
- τ_i = effect of the i-th location (i = A, B, C, D, E)
- ε_{ij} = random error associated with the j-th replicate in the i-th location

Assumptions Testing: Prior to ANOVA, the assumptions of normality and homogeneity of variances were tested. Normality of residuals was assessed using the Shapiro-Wilk test ($p > 0.05$ indicating normal distribution). Homogeneity of variances across locations was tested using Levene's test ($p > 0.05$ indicating equal variances). Where ANOVA indicated significant differences among locations ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post-hoc test was applied for pairwise comparisons to identify which locations differed significantly.

Fungal Diversity Analysis: Fungal abundance data were summarized as frequency and percentage occurrence. Simpson's Diversity Index (D) and Simpson's Index of Diversity (1 – D) were calculated as described in section 2.4.

Correlation Analysis: Pearson product-moment correlation analysis was conducted to examine the relationship between fungal abundance and heavy metal concentrations. For this analysis, five paired observations (n = 5) were used, corresponding to the five sampling locations (A–E). For each location, the total fungal abundance (total number of isolates) was paired with the mean concentration of each heavy metal (Cd, Cr, Ni, and Pb) from that location. The Pearson correlation coefficient (r) was calculated using the formula:

$$r = \Sigma(x_i - \bar{x})(y_i - \bar{y}) \; / \; \sqrt{[\Sigma(x_i - \bar{x})^2 \; \Sigma(y_i - \bar{y})^2]}$$

Where:

- x_i = heavy metal concentration at location i
- y_i = fungal abundance at location i
- $\bar{x}$ = mean heavy metal concentration across all locations
- $\bar{y}$ = mean fungal abundance across all locations

Prior to Pearson correlation, the assumption of bivariate normality was assessed by inspecting scatterplots. The significance of each correlation was tested using a t-test at $\alpha = 0.05$. Correlation coefficients (r) were interpreted according to the guidelines of Schober et al. (2018): 0.00–0.10 = negligible correlation; 0.10–0.39 = weak correlation; 0.40–0.69 = moderate correlation; 0.70–0.89 = strong correlation; 0.90–1.00 = very strong correlation.

RESULTS

Analysis of Heavy metals in soil of the Study Area

The analysis revealed cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) across the five sampling locations (A–E) at different concentrations ($p < 0.05$). For cadmium (Cd), concentrations ranged between 0.008 ± 0.0016 mg/kg at location B and 0.068 ± 0.0013 mg/kg at location E, which showed the highest value. Locations A (0.012 ± 0.0017 mg/kg), C (0.011 ± 0.0007 mg/kg), and D (0.009 ± 0.0021 mg/kg) recorded relatively as shown in Table 1.

Micromorphological Characteristics of Isolated Mycoflora

A total of eleven (11) fungal species isolated were successfully identified from the samples which were predominantly the Division Ascomycota, with a smaller representation from Mucoromycota (formerly *Zygomycota*) (*Mucor racemosus* and *Rhizopus oryzae, Aspergillus spp.*, *Fusarium oxysporum, Penicillium chrysogenum*, *Scopulariopsis brevicaulis*, and species of Trichoderma) as shown in Table 2.

Table 1: Heavy Metal Concentrations (mg/kg) in Soil of the Study Area

LOCATIONS	Cd	Cr	Ni	Pb
A	0.012±0.0017[a]	0.667±0.0115[c]	0.133±0.0037[d]	23.42±0.090[b]
B	0.008±0.0016[c]	0.553±0.0190[d]	0.115±0.0016[d]	43.61±0.088[a]
C	0.011±0.0007[a]	1.470±0.0101[a]	0.178±0.0049[b]	2.927±0.0106[d]
D	0.009±0.0021[c]	0.601±0.0144[c]	0.144±0.0044[c]	1.465±0.0346[e]
E	0.068±0.0013[b]	1.275±0.0102[b]	1.076±0.0021[a]	5.219±0.0319[c]
P-Value	0.023	0.002	0.013	0.003
Nigerian FEPA (mg/kg)[a]	0.01* (0.8)**	0.05* (100)**	0.02* (70)**	0.05* (164)**
WHO/FAO (mg/kg)[b]	0.01* (0.8)**	0.05* (100)**	0.02* (50)**	0.05* (100)**
Canadian SQG (mg/kg)[c]	1.4	64	50	70
Netherlands SQG (mg/kg)[d]	0.8	100	35	85

Note: Mean values with the same superscripts are not significantly different at 5% level of significance.

• = Guideline values for drinking water (mg/L) – shown for reference only

** = Guideline values for agricultural soils (mg/kg) – relevant for ecological comparison

Table 2: Micromorphological Characteristics of Isolated Mycoflora

		Colonial, Micromorphological and Cellular Characteristics	Division
1	Cunninghamella spp.	Colonies fast-growing, initially white turning greyish with age. Hyphae are septate.	Ascomycota
2	Mucor racemosus	Colonies grey. Sporangia are dark and round, sporangiospores hyaline to brown.	Zygomycota
3	Aspergillus niger	Colonies compact white to yellow basal. Conidiophores smooth-walled, hyaline to dark.	Ascomycota
4	Fusarium oxysporum	Colonies appear cottony, white to pink. Chlamydospores present, thick-walled, in hyphae.	Ascomycota
5	Aspergillus fumigatus	Colonies blue-green, with a dense felt of conidiophores. Conidia globose to subglobose, green, rough-walled.	Ascomycota
6	Penicillium chrysogenum	Colonies velvety to powdery. Conidiophores are branched, forming brush-like (penicillus) structures.	Ascomycota
7	Scopulariopsis brevicaulis	Colonies powdery to granular. Hyphae septate with ovoid-shaped conidia.	Ascomycota

		Colonial, Micromorphological and Cellular Characteristics	Division
8	Trichoderma harzianum	Colonies woolly to compact. Hyphae hyaline, septate. Conidiophores highly branched.	Ascomycota
9	Aspergillus flavus	Colonies granular, flat, yellow to yellow-green. Conidia globose, echinulate, pale green.	Ascomycota
10	Rhizopus oryzae	Colonies rapidly spreading, cottony, initially white becoming greyish-brown with black sporangia.	Zygomycota
11	Trichoderma viride	Colonies woolly turning bright to dark green with age. Conidia ellipsoidal to globose.	Ascomycota

Table 3 revealed the percentage occurrence of the fungal species isolated. *Mucor racemosus* recorded the highest frequency (35.59%), indicating that it is the most dominant fungal species in the environment, likely due to its rapid growth rate and ability to utilize a wide range of organic substrates, followed by *Aspergillus niger* (22.88%), which is well known for its strong environmental adaptability and frequent occurrence in soil and decaying materials. *Rhizopus oryzae* (13.55%) was also relatively abundant, reflecting its typical presence in nutrient-rich organic matter. Moderately occurring fungi included *Cunninghamella* spp., *Aspergillus fumigatus*, and *Trichoderma viride*, each contributing approximately 5–6% to the total isolates, suggesting that they are well-established but not dominant in the sampled environment. Meanwhile, *Fusarium, oxysporum* and *Aspergillus flavus* were recorded in low proportions (2–3%), indicating limited distribution or competitive exclusion by more dominant fungi. The least frequent isolates were *Penicillium chrysogenum*, *Scopulariopsis brevicaulis*, and *Trichoderma harzianum*, each with a percentage occurrence of 1.69%, which may be attributed to lower environmental prevalence or slower growth rates under the prevailing conditions.

Table 3: Frequency and Percentage Occurrence of Fungal Isolates in Jaji Military Shooting Range

Fungal Isolate	Frequency	% Occurrence
Cunninghamella spp.	7	5.93
Mucor racemosus	42	35.59
Aspergillus niger	27	22.88
Fusarium oxysporum	4	3.38
Aspergillus fumigatus	6	5.08
Penicillium chrysogenum	2	1.69
Scopulariopsis brevicaulis	2	1.69
Trichoderma harzianum	2	1.69
Aspergillus flavus	3	2.54
Rhizopus oryzae	16	13.55
Trichoderma viride	7	5.93

Simpsons Diversity Index of the Isolated Mycoflora

Table 4 shows the simpsons diversity index of the isolated mycoflora, the value of 0.8 obtained in this study suggests that the fungal population in the sampled environment is highly diverse.

This high diversity is further supported by the distribution of isolates. Although *Mucor racemosus* and *Aspergillus niger* occurred at relatively higher frequencies (35.59% and 22.88% respectively), several other species such as *Cunninghamella spp.*, *Trichoderma viride*, *Aspergillus fumigatus*, and *Fusarium oxysporum* also contributed to the community structure. The presence of both dominant and less abundant species indicates that no single species completely overwhelms the environment. The Simpson's Diversity Index value of 0.8 reflects a balanced and ecologically stable fungal community, demonstrating that the soil environment supports the coexistence of multiple fungal species with varying ecological roles. lower values that were not significantly different ($p > 0.05$) from one another. However, the value at location E was significantly higher ($p < 0.05$) than those of the other locations.

Chromium (Cr) concentrations also varied significantly ($p < 0.05$). The lowest value was observed at location B (0.553 ± 0.0190 mg/kg), while the highest was recorded at location C (1.470 ± 0.0101 mg/kg). Locations E (1.275 ± 0.0102 mg/kg), A (0.667 ± 0.0115 mg/kg), and D (0.601 ± 0.0144 mg/kg) showed intermediate values, with location C being significantly higher ($p < 0.05$) than all others.

For nickel (Ni), significant variation ($p < 0.05$) was observed across the sites. The highest concentration was at location E (1.076 ± 0.0021 mg/kg), while location B (0.115 ± 0.0016 mg/kg) had the lowest. The values at A (0.133 ± 0.0037 mg/kg), D (0.144 ± 0.0044 mg/kg), and C (0.178 ± 0.0049 mg/kg) were not significantly different ($p > 0.05$) from one another but were significantly lower ($p < 0.05$) than location E.

Lead (Pb) showed the most significant differences ($p < 0.05$) among the metals. The highest concentrations were recorded at location B (43.61 ± 0.088 mg/kg) and location A (23.42 ± 0.090 mg/kg), both of which were significantly higher than the other locations. Locations C (2.927 ± 0.0106 mg/kg), E (5.219 ± 0.0319 mg/kg), and D (1.465 ± 0.0346 mg/kg) recorded significantly lower ($p < 0.05$) values in comparison.

Table 4: Simpsons Diversity Index of the Isolated Mycoflora

Fungal Isolate	No. Isolate (n)	n/N	$(n/N)^2$
Cunninghamella spp.	7	0.05	0.002
Mucor racemosus	42	0.35	0.122
Aspergillus niger	27	0.22	0.048
Fusarium oxysporum	4	0.03	0.000
Aspergillus fumigatus	6	0.05	0.002
Penicillium chrysogenum	2	0.01	0.000
Scopulariopsis brevicaulis	2	0.01	0.000
Trichoderma harzianum	2	0.01	0.000
Aspergillus flavus	3	0.02	0.000
Rhizopus oryzae	16	0.13	0.016
Trichoderma viride	7	0.05	0.002
Total	118	1.00	0.21
$D = \Sigma(n/N)^2 = 0.21$ $1 - D = 1 - 0.21 = 0.79$			

Note: N=Total number of Isolates, n=number of individual species, D=Species Diversity

Pearson Correlation Coefficient between the Fungal Abundance and Heavy Metals of the Soil

The Pearson correlation analysis was conducted to determine the relationship between the abundance of fungal isolates in the soil and the concentrations of selected heavy metals (Pb, Cr, Cd, and Ni) as presented in Table 5. The correlation coefficients (r) obtained ranged from 0.268 to 0.329**,** all of which indicate weak positive correlations. This suggests that as the concentration of these heavy metals increased, the abundance of fungi in the soil showed a slight upward trend, but the relationship was not strong. However, none of the correlations were statistically significant, as all p-values were greater than 0.05 (Pb: $p = 0.325$; Cr: $p = 0.355$; Cd: $p = 0.426$; Ni: $p = 0.341$). This means that the observed changes in fungal abundance cannot be reliably attributed to variations in heavy metal concentrations.

Table 5: Pearson Correlation Coefficient between the Fungal Abundance and Heavy Metals of the Soil

Variables	Correlation (r)	P-Value (<0.05)
Pb and Abundance	0.329	0.325^{ns}
Cr and Abundance	0.309	0.355^{ns}
Cd and Abundance	0.268	0.426^{ns}
Ni and Abundance	0.318	0.341^{ns}

Note: ns=not significant, Pb=Lead, Cr=Chromium, Cd= Cadmium, Ni=nickel.

DISCUSSION

The analysis of cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) across the five sampling locations revealed significant spatial variation ($p < 0.05$), indicating heterogeneous distribution of these metals within the Jaji Military Shooting Range (Table 1). This heterogeneity is consistent with the expected pattern of metal deposition from ammunition discharge, where accumulation zones correspond to specific functional areas of the range, including firing points, berms (backstops), and downrange impact areas. Aghanwa et al. (2021), in their study of the Dutsen-Soyaya Shooting Range within the Nigerian Army Base Camp, Kachia, Kaduna State, reported similar spatial heterogeneity, with lead concentrations ranging from 12.4 to 67.8 mg/kg and the highest values concentrated near the berm. Likewise, Nwaedozie et al. (2015) documented lead levels between 18.3 and 52.7 mg/kg in military training areas within the One Division of Nigerian Army, Kaduna, with significant variation based on proximity to firing points. These consistent patterns across multiple Nigerian military sites confirm that ammunition residues are the primary source of metal contamination in shooting range environments.

Lead (Pb) exhibited the highest concentrations among the four metals analyzed, with location B (43.61 ± 0.088 mg/kg) and location A (23.42 ± 0.090 mg/kg) showing significantly elevated levels compared to other locations ($p < 0.05$). These values fall within the range reported by Aghanwa et al. (2021) and are comparable to those documented by Oruama and Joinkrama (2024) in urban and peri-urban Nigerian soils impacted by industrial and military activities (18.5–55.3 mg/kg). The elevated lead concentrations at locations A and B are attributable to the accumulation of spent bullets and fragments, which release lead through weathering and corrosion over time (Geris *et al.,* 2024). The significantly lower lead concentrations at locations C (2.93 mg/kg), D (1.47 mg/kg), and E (5.22 mg/kg) reflect decreasing metal deposition with increasing distance from the firing point, a pattern commonly observed in ballistic impact zones and consistent with the findings of Aghanwa et al. (2021), who reported lead values of 3.2–8.9 mg/kg at downrange locations.

Cadmium concentrations ranged from 0.008 to 0.068 mg/kg, with location E showing the highest value. These concentrations are comparable to background cadmium levels reported for uncontaminated Nigerian soils, typically ranging from 0.01 to 0.10 mg/kg (Adamu *et al.,* 2019). Fosu-Mensah et al. (2020) documented similar cadmium ranges (0.02–0.09 mg/kg) in agricultural soils from developing countries, attributing these levels to natural

pedogenic processes rather than anthropogenic inputs. The slightly elevated cadmium at location E may reflect localized enrichment, but the absolute concentrations remain well below international soil quality guidelines (e.g., Netherlands target value: 0.8 mg/kg; Canadian SQG: 1.4 mg/kg). Therefore, cadmium does not currently pose a significant ecological risk in the study area, consistent with conclusions reached by Adamu et al. (2019) for northern Nigerian soils.

Chromium concentrations (0.553–1.470 mg/kg) and nickel concentrations (0.115–1.076 mg/kg) were also within the range of background values reported for soils derived from basement complex rocks in northern Nigeria. Adamu et al. (2019) documented chromium levels of 0.8–4.2 mg/kg and nickel levels of 0.3–1.8 mg/kg in mining-impacted areas of northern Nigeria, while Fosu-Mensah et al. (2020) reported comparable ranges (Cr: 0.6–3.5 mg/kg; Ni: 0.2–1.5 mg/kg) in agricultural soils. The absence of extreme values for these metals in the present study suggests that their distribution is primarily governed by natural pedogenic processes rather than anthropogenic contamination, a finding consistent with the work of Tafinta et al. (2013) in Sokoto metropolitan soils.

When compared against international soil quality guidelines (Table 1), all metal concentrations except lead were substantially below thresholds associated with ecological toxicity. Lead at location B (43.61 mg/kg) and location A (23.42 mg/kg), while below most regulatory intervention values (e.g., Canadian SQG for residential use: 70 mg/kg; Netherlands intervention value: 530 mg/kg), represent 2- to 4-fold enrichment above typical Nigerian background levels (10–20 mg/kg). This level of enrichment is comparable to that reported by Oruama and Joinkrama (2024), who documented 15–30% reductions in dehydrogenase and urease activities in soils with lead concentrations between 30–50 mg/kg. Therefore, although lead does not currently exceed regulatory action levels, its accumulation at locations A and B warrants monitoring and consideration for remediation, particularly given the potential for additive or synergistic effects in metal mixtures (Balali-Mood et al. 2021).

Fungal Community Composition and Diversity

A total of eleven fungal species were isolated and identified from the shooting range soils, predominantly belonging to the divisions Ascomycota and Mucoromycota (Table 2). The dominance of Ascomycota is consistent with global soil fungal surveys, which report Ascomycota as the most abundant and diverse phylum in terrestrial ecosystems, typically

constituting 60–80% of cultured isolates. Leho (2025), in a comprehensive global diversity study, documented Ascomycota dominance across multiple soil types and climatic regions, while Zhang and Chen (2018) reported similar patterns in metal-contaminated soils from industrial areas. The presence of Mucoromycota representatives (*Mucor racemosus* and *Rhizopus oryzae*) reflects the typical co-occurrence of fast-growing, early colonizing fungi in soils with readily available organic substrates, as documented by Khosravi et al. (2016) in their morphological and molecular survey of soil Mucorales.

The frequency distribution (Table 3) revealed *Mucor racemosus* as the most prevalent species (35.59%), followed by *Aspergillus niger* (22.88%) and *Rhizopus oryzae* (13.55%). This pattern aligns with previous studies from Nigerian soils. Tafinta et al. (2013) reported *Aspergillus* and *Mucor* species as dominant isolates from heavy metal-contaminated soils in Sokoto metropolis, with *Aspergillus niger* comprising 24.3% and *Mucor* species 31.2% of total isolates. Similarly, Alhaji et al. (2022) documented comparable dominance patterns in military firing range soils within Kaduna State, reporting *Mucor* (33.7%), *Aspergillus* (21.4%), and *Rhizopus* (12.8%) as the most frequent genera. The high frequency of *Mucor racemosus* likely reflects its rapid growth rate, coenocytic hyphal structure enabling efficient resource exploitation, and ability to utilize diverse organic substrates, characteristics noted by Abe et al. (2010) in their phylogenetic and ecological studies of the genus *Mucor*.

The prevalence of *Aspergillus niger* is consistent with its well-documented ecological versatility, tolerance to environmental stress, and cosmopolitan distribution in soil habitats. Abdullahi and Machido (2017) isolated *Aspergillus niger* as the dominant species (28.6%) from tannery wastewater in Nigeria, while Abubakar et al. (2017) reported similar frequencies (21.3–26.7%) in tannery effluents from Sokoto metropolis. The ability of *Aspergillus niger* to tolerate heavy metals through biosorption, organic acid production, and metal chelation mechanisms has been extensively documented (Sey & Belford, 2021; Suárez et al. 2025), potentially explaining its persistence in metal-impacted shooting range soils.

Species with moderate occurrence (5–6%) included *Cunninghamella* spp., *Aspergillus fumigatus*, and *Trichoderma viride*. The presence of *Cunninghamella* at this frequency is consistent with the findings of Khosravi et al. (2016), who reported *Cunninghamella* as a consistent but non-dominant component of soil fungal communities, typically constituting 3–8% of isolates. *Trichoderma viride* occurrence (5.93%) aligns with the work of Tafinta et al. (2024),

who documented *Trichoderma* species at frequencies of 4.2–7.8% in heavy metal-contaminated soils from northern Nigeria.

Low-frequency isolates (1–3%) included *Fusarium oxysporum*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Scopulariopsis brevicaulis*, and *Trichoderma harzianum*. This distribution pattern, where a few species dominate while many occur at low abundance, is typical of soil fungal communities and reflects niche differentiation, competitive interactions, and variable resource availability. Suárez et al. (2025) reported similar patterns in mining soils contaminated with cadmium and lead, with 3–4 dominant species comprising 60–70% of isolates and numerous rare taxa constituting the remainder. Sey and Belford (2021) likewise documented comparable distribution patterns in gold mine tailings in Ghana, where *Aspergillus* and *Trichoderma* dominated while *Penicillium* and *Fusarium* occurred at lower frequencies.

Interpretation of Simpson's Diversity Index

The Simpson's Index of Diversity (1 – D) was calculated as 0.79 (Table 4), indicating high species diversity within the fungal community. This value is comparable to diversity indices reported from other metal-impacted soils. Sey and Belford (2021) documented Simpson's diversity values ranging from 0.71 to 0.84 in fungal communities from gold mine tailings in Ghana, with the highest diversity observed in moderately contaminated sites. Suárez et al. (2025) reported values of 0.68–0.82 in mining soils contaminated with cadmium and lead, noting that diversity remained relatively stable up to metal concentrations of 50–100 mg/kg. Tafinta et al. (2013) recorded a mean Simpson diversity index of 0.75 in heavy metal-contaminated soils from Sokoto, Nigeria, while Alhaji et al. (2022) documented values ranging from 0.72 to 0.81 in military firing range soils from Kaduna State. The high diversity observed in the present study (0.79) is therefore consistent with these previous reports and suggests that the fungal community has not been severely impaired by metal contamination.

However, it is important to note the limitations of interpreting ecological "stability" or "balance" solely from Simpson's index. Simpson's index measures species richness and evenness but does not directly assess functional diversity, metabolic activity, or ecosystem process rates. Alhaji et al. (2022) emphasized that diversity indices provide information about community structure but not about functional integrity. Geris et al. (2024) similarly cautioned that high diversity in metal-impacted soils may reflect the presence of multiple tolerant species without indicating whether key ecosystem functions (decomposition, nutrient

cycling) are maintained at optimal rates. Therefore, while the calculated diversity (0.79) is consistent with a resilient community capable of persisting under metal stress, definitive conclusions about "ecological stability" would require additional data on functional parameters, temporal dynamics, and stress-response experiments.

Relationship Between Fungal Abundance and Heavy Metals

Pearson correlation analysis (Table 5) revealed weak positive correlations between fungal abundance and concentrations of Pb ($r = 0.329$, $p = 0.325$), Cr ($r = 0.309$, $p = 0.355$), Cd ($r = 0.268$, $p = 0.426$), and Ni ($r = 0.318$, $p = 0.341$). None of these correlations were statistically significant ($p > 0.05$), indicating that the observed variations in fungal abundance cannot be reliably attributed to differences in heavy metal concentrations.

These findings are consistent with several previous studies. Aghanwa et al. (2021) reported non-significant correlations ($r = 0.12–0.35$, $p > 0.05$) between heavy metal concentrations and microbial abundance in military shooting range soils, concluding that factors other than metals primarily governed microbial populations. Tafinta et al. (2024) similarly documented weak, non-significant correlations ($r = 0.18–0.42$, $p > 0.05$) between fungal abundance and lead, cadmium, and chromium concentrations in contaminated soils from northern Nigeria, attributing these findings to fungal adaptation and the overriding influence of organic matter and pH.

The absence of significant correlations in the present study suggests that factors other than heavy metal concentrations may be more important in determining fungal abundance in this environment. Potential explanatory variables not measured in this study include soil organic matter content, pH, moisture availability, nutrient status (particularly nitrogen and phosphorus), and competitive interactions among fungal species. Wang et al. (2005) demonstrated that soil organic matter and pH explained 45–60% of variation in fungal abundance across contaminated sites, while metal concentrations contributed less than 15%. Tafinta et al. (2013) likewise reported that organic carbon and moisture content were stronger predictors of fungal abundance ($R^2 = 0.48–0.56$) than heavy metal concentrations ($R^2 = 0.12–0.28$) in Nigerian soils.

The weak positive correlations observed, while non-significant, may reflect the development of metal tolerance or adaptation mechanisms among resident fungi, enabling them to maintain populations despite elevated metal concentrations. Sey and Belford (2021) documented that *Aspergillus* and *Trichoderma* species isolated from gold mine tailings

exhibited minimal growth inhibition at lead concentrations up to 100 mg/L, suggesting constitutive tolerance mechanisms. Zhang and Chen (2018) reported that fungi from contaminated soils often possess biosorption capabilities, metal efflux pumps, and intracellular sequestration mechanisms that mitigate metal toxicity. Suárez et al. (2025) similarly demonstrated that filamentous fungi from mining soils maintained growth rates at 80–95% of controls when exposed to cadmium and lead at concentrations comparable to those in the present study.

It is important to emphasize that correlation does not imply causation, and the weak, non-significant relationships observed do not support conclusions about fungal responses to metal stress. The limited sample size (n = 5) reduced statistical power, potentially obscuring biologically meaningful relationships. As noted by Aghanwa et al. (2021) and Tafinta et al. (2024), studies with larger sample sizes (n ≥ 15–20) are better powered to detect significant correlations when they exist. Experimental studies involving controlled metal exposures would be required to definitively assess the tolerance or sensitivity of individual fungal species to specific metals.

Implications for Mycoremediation and Future Directions

Despite these limitations, the isolation of diverse fungal taxa—particularly *Aspergillus niger*, *Mucor racemosus*, and *Trichoderma* species—from metal-impacted soils suggests that these indigenous fungi possess adaptive traits enabling survival under metal stress. Several of the identified genera have been previously documented for metal biosorption and tolerance. Abdullahi and Machido (2017) demonstrated that *Aspergillus niger* isolated from tannery wastewater removed 65–78% of lead and cadmium from liquid media within 7 days. Abubakar et al. (2017) reported similar removal efficiencies (58–72%) for *Aspergillus* and *Trichoderma* species from contaminated Nigerian soils. Sey and Belford (2021) documented that *Aspergillus niger* and *Trichoderma viride* from gold mine tailings accumulated 12–25 mg Pb/g biomass through biosorption and intracellular sequestration.

The high diversity (Simpson's index = 0.79) indicates that the community retains multiple species with potentially complementary metal tolerance strategies, which could be harnessed for mycoremediation applications. Geris et al. (2024) reviewed fungal-mediated remediation approaches for war-affected and military-impacted soils, concluding that diverse native communities offer advantages over single-species inoculants due to niche complementarity and ecosystem integration. However, direct assessment of metal removal

efficiency and tolerance mechanisms would be required before recommending specific isolates for bioremediation.

CONCLUSION

This study assessed heavy metal distribution and fungal diversity in soils of the Jaji Military Shooting Range, Kaduna State, Nigeria. The results showed significant spatial heterogeneity in heavy metal concentrations, with lead being the most enriched metal, particularly near the firing point and berm, while cadmium, chromium, and nickel largely remained within background levels. A diverse fungal community comprising eleven species, predominantly from Ascomycota and Mucoromycota, was recorded, with a high Simpson's diversity index indicating substantial species richness and evenness. Pearson correlation analysis revealed weak, non-significant relationships between fungal abundance and heavy metal concentrations, suggesting that the observed fungal distribution was not strongly structured by metal levels alone.

The presence of diverse indigenous fungi in metal-impacted soils suggests ecological tolerance and highlights their potential as candidate mycoremediators rather than demonstrating confirmed remediation capacity. However, the study was limited by reliance on morphological identification, a small sample size, and the absence of functional or experimental metal tolerance assays. Further research involving molecular identification, controlled tolerance experiments, and direct assessment of metal removal efficiency is necessary to validate the suitability of these fungal isolates for practical mycoremediation applications.

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