World Journal of
Environmental Research
ISSN 2301-2641

Volume 15, Issue 1, (2025) 19-30

www.wjer.eu

Physicochemical characterization of lead-resistant soil bacteria from Maimalari Military Cantonment, Maiduguri

Esewi Agho [a], Ferdowsi University of Mashhad, Mashhad, Iran. University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
Ibrahim Alkali Allamin [b1], University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria, ibnallamin@gmail.com
Rahim Karami [c], University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
Kaumi Ali Misherima [d], University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
Idris Umar Hambali [e], University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.
Hussaini Shettima [f], University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.

Suggested Citation:
Agho, E., Allamin, I. A., Misherima, K. A., Hambali, I. U., & Shettima, H. (2025). Physicochemical characterization of lead-resistant soil bacteria from Maimalari Military Cantonment, Maiduguri. *World Journal of Environmental Research*, *15*(1), 19-30. https://doi.org/10.18844/wjer.v15i1.9518

Received from July 16, 2024; revised from January 22, 2025; accepted from April 23, 2025.
Selection and peer review under the responsibility of Prof. Dr. Haluk Soran, Near East University, Cyprus..

Abstract

Lead contamination in soils poses significant environmental and public health risks due to its toxicity and persistence. Shooting ranges are recognized as hotspots for lead pollution, primarily resulting from the accumulation of lead dust generated during firing. Although various remediation strategies exist, bioremediation has emerged as a sustainable and ecologically viable approach that utilizes the natural metabolic processes of microorganisms to detoxify or immobilize pollutants. This study investigates the lead concentration in soils and the tolerance capacity of bacterial isolates, alongside the physico-chemical characteristics of soils from a firing range. Soil samples were collected from five randomly selected sites, both at the surface and at a depth of fifteen centimeters. Lead presence was confirmed using spectrophotometric analysis, and thirteen bacterial strains were successfully isolated. Among these, Proteus mirabilis, Providencia species, and Acinetobacter species exhibited notable tolerance to elevated lead concentrations. The findings suggest that these bacteria possess potential for application in bioremediation strategies aimed at mitigating heavy metal contamination in firing range environments and other similarly impacted ecosystems.

Keywords: Acinetobacter; bioremediation; heavy metals; lead pollution; Proteus mirabilis.

* ADDRESS FOR CORRESPONDENCE: Ibrahim Alkali Allamin, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria. *E-mail address*: ibnallamin@gmail.com

Agho, E., Allamin, I. A., Misherima, K. A., Hambali, I. U., & Shettima, H. (2025). Physicochemical characterization of lead-resistant soil bacteria from Maimalari Military Cantonment, Maiduguri. *World Journal of Environmental Research*, *15*(1), 19-30. https://doi.org/10.18844/wjer.v15i1.9518

1. INTRODUCTION

Lead is a toxic element associated with severe adverse health effects. During firing activities in shooting ranges, lead dust is released due to the presence of lead in ammunition components (Diaz et al., 2012). Standard bullets are composed of a copper jacket enclosing a lead core, with the primer also containing lead (Baum et al., 2022). Upon impact by the firing pin, ignition of the primer initiates combustion of the gunpowder, resulting in the volatilization of lead. As the empty cartridge is expelled through the ejection port, lead particulates are simultaneously discharged into the surrounding environment. These particulates tend to settle in proximal soil and air rather than dispersing over long distances.

Lead dust frequently accumulates on surfaces such as clothing and skin, and in the surrounding soil and airspace, facilitating its potential transport via soil water to adjacent agricultural areas near military installations. This scenario constitutes a significant occupational hazard for military personnel in training, instructors, and local populations residing near military bases. Empirical evidence has demonstrated that personnel engaged in automatic weapon training at outdoor firing ranges can be exposed to elevated levels of airborne lead (Laidlaw et al., 2017). Wang and Bezerra (2017) emphasized the necessity of educating military officers on associated risks, given that firing ranges are intended for skill development and weapons proficiency demonstrations.

Safety protocols at such facilities often prioritize protection from ballistic injuries, whereas environmental hazards related to lead exposure receive insufficient attention (Fayiga and Saha, 2016). Elevated blood lead levels among shooters represent a serious health risk, with toxicity dependent on dose thresholds rather than mere presence (Appenroth et al., 2010). Specialized training programs, including those for special forces, tactical units, and elite combat teams, involve frequent and prolonged exposure to firing ranges, exacerbating the potential for lead poisoning and associated environmental hazards (Arnemo et al., 2016). Soil contamination from lead at firing ranges has been documented, with concentrations frequently surpassing 10,000 milligrams per kilogram, representing significant ecological and health concerns (Rodríguez-Seijo et al., 2017).

Military personnel often lack access to or do not utilize specialized protective equipment commonly employed by civilian recreational shooters, such as safety clothing, ear protection, and goggles. In addition to bullets, explosives detonated at firing ranges release various toxic metals, including lead, antimony, copper, nickel, and zinc (Nwaedozie et al., 2013). Excessive soil lead concentrations have been reported globally at numerous shooting range locations. The Maimalari Military Cantonment in Maiduguri, Borno State, Nigeria, functions as a primary operational base in the long-standing conflict against insurgency. This installation witnesses intensive and regular shooting exercises, resulting in persistent environmental lead discharge.

Ammunition used in these exercises contributes to local contamination, with lead dust adhering to clothing and skin and subsequently depositing in the surrounding soil. Contaminated soil may facilitate lead migration via surface runoff to nearby agricultural fields, introducing risks to food safety and public health. Several remediation techniques can be implemented to address soil contamination, including soil washing, thermal desorption, and, most prominently, bioremediation. Bioremediation employs microorganisms such as bacteria and fungi to degrade pollutants and has been recognized as a sustainable and effective strategy for remediating lead-contaminated soils at shooting ranges (Mendes et al., 2023).

This technique is relatively simple to assess using site investigation data and is applicable across diverse contamination contexts. Recent investigations have underscored the efficacy of bioremediation in reducing soil lead concentrations. For instance, combining biochar amendments with phytoremediation has yielded lead concentration reductions exceeding 70 percent (Maceiras et al., 2024). While comprehensive surveys on lead poisoning prevalence among active or former military personnel remain limited, the toxicity of lead is well-documented, and its health implications are severe (Kabir et al., 2015). Cardiovascular health, critical for military readiness and endurance, is particularly vulnerable to lead-induced damage (Smith and Cashman, 2002).

Certain bacterial strains, including *Bacillus cereus* PSB-2, have demonstrated potential in immobilizing lead through interactions with modified biochar, enhancing bioremediation outcomes (Zhang et al., 2025). Additionally, microbial induced carbonate precipitation by urease-producing bacteria has been shown to immobilize up to 97 percent of lead in contaminated environments (Kang et al., 2015). Utilization of agro-industrial byproducts such as bone meal and biochar further contributes to lead immobilization, thereby reducing bioavailability and ecological risk (Rodríguez-Seijo et al., 2021). Continued investigation into microbial agents capable of binding and transforming lead remains critical to environmental protection and the health of military trainees (Mendes et al., 2023).

1.1. Purpose of study

The present investigation evaluates lead concentrations in soils, the tolerance capacity of bacterial isolates, and the physicochemical properties of soils collected from a firing range.

2. MATERIALS AND METHODS

2.1. Study area

The study area was limited to the shooting range of the Maimalari military cantonment in Maiduguri, Borno state, where leaded ammunitions are used for training activities. Maiduguri is located at a longitude of 11.50^o North and a latitude of 13.15^o East, having an elevation of 364m above sea level. The temperature of the metropolis ranges between 35^o- 42^{o}C in the heat season (March to May) and in cold seasons it ranges between 19^o- 25^{o}C. The amount of rainfall varies between 150-600mm during the short rainy season (June to September) each year.

2.2. Sampling technique and lead detection on soil samples

Soil samples were randomly collected from five separate sampling sites within the research area and designated A_1, B_1, C_1, D_1, and E_1 for surface soils and A_2, B_2, C_2, D_2, and E_2 for depth soils. Surface soil samples were collected alongside depth soil with a soil auger at a depth of 15cm below the surface. All 10 samples were preserved in clean, sterile polythene bags, labeled, and kept at room temperature. The soils were air-dried and sieved through a 2mm sieve and weighed for analysis. Lead detection was carried out on all 10 samples using the Smart Spectrophotometer 2000 at a wavelength of 385nm. The test was carried out at the NAFDAC Research Laboratory, Maiduguri. The Smart Spectrophotometer is a portable device that can customize sequences for frequently run tests, having a wide wavelength range of 350 to 1000nm with wavelength accuracy of ±2nm. 5ml of sample solution was placed into the spectrophotometric tube with a syringe, and 5ml of ammonium chloride buffer was added to fill the tube to the 10 mL line. It was stirred to mix. 3 drops of sodium cyanide (10%) were added and stirred to mix. The 0.5ml pipette was used to add the stabilizing reagent cap and mixed. The tube was inserted into the chamber, and the lid closed. The sample was scanned, and the result was recorded in ppm as reading A. The tube was then removed from the spectrophotometer; 3 drops of DDC reagent cap were added and mixed. The tube was inserted into the chamber and scanned, the result was recorded in ppm as reading B. The Final concentration of Lead (ppm) is equal to Reading A minus Reading B.

2.3. Chemical and physical analysis of soil samples

Standard techniques were used to analyze the physicochemical characteristics of representative soil samples. The pH of soil samples was measured using a pH meter at the Department of Microbiology, and the physicochemical analysis was carried out at the Department of Soil Sciences, both at the University of Maiduguri. The percent organic carbon and organic matter were determined using the Walkley and Black process, and total nitrogen was calculated using the Kjeldahi method (Udo & Ogunwale, 1986).

2.3.1. Isolation and enumeration of bacteria from soil

Isolation and Enumeration of Bacteria from the soil samples were carried out at the Department of Microbiology. Bacteria were isolated from soil samples using the pour plate technique on nutritional agar and mineral salt agar supplemented with 0.01g of standard lead to isolate bacteria tolerant to lead and assess their population density in soils. Seven serial dilutions were prepared for each of the 10 samples. In 0.1 ml quantities, serial dilutions of tubes 10^{-5}, $10^{-6}$and 10^{-7} were pour-plated onto duplicate plates of Nutrient agar and Mineral salt agar. The plates were incubated at 28 + 2°C for 48 hours. The Quebec colony counter was used to count developed colonies. Viable colonies were selected from plates based on their various forms and purified using the streak-plate procedure, which involves subculturing onto fresh nutrient agar plates. Isolated colonies formed on the plates were transferred to nutrient agar slants and kept as stock cultures for future testing. The mineral salt agar was composed of 4.0g of Sodium chloride (NaCl), 0.2g of Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$), 0.02g Calcium chloride ($CaCl_2$), 0.1g Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), 1.0 Ammonium nitrate (NH_4NO_3), 1.73g Dipotassium hydrogen phosphate (K_2HPO_4), and 0.68g of Potassium di-hydrogen sulphate (KH_2PO_4) at a pH of 7.0.

2.3.2. Lead tolerance screening of the isolates

Screening of the isolates was done in two phases, the primary screening in which the isolates were cultured in the mineral salt agar supplemented with variable concentration of lead (0.01-0.05g).

2.3.3. Characterization and identification of bacterial isolates

Characterization and Identification of Bacterial Isolates from the soil samples were carried out at the Department of Microbiology. Gram stain reaction and Biochemical tests, such as catalase test, methyl red test, motility test, indole test, oxidative fermentative utilization of glucose, H_2S production, and other biochemical tests, were performed. The colony morphology, cell micromorphology, and biochemical tests on pure cultures of the bacterial isolates were tabulated and compared with Bergey's manual of determinative bacteriology, 8th edition (Buchanan & Gibbons, 1995) as well as the handbook for identifying bacteria (Cowan & Steel, 1981).

3. RESULTS

Based on the data presented, Table 1 highlights the physicochemical variability of the soil samples, with lead concentrations ranging from 0.11 ppm (B1) to 0.72 ppm (D1). The soils also varied in pH (5.63 to 6.44), organic matter content, and nitrogen levels, suggesting diverse microenvironments potentially influencing microbial diversity and metal tolerance. Figure 1 illustrates the population distribution of total heterotrophic bacteria (THB) and lead-tolerating bacteria (LTB) across these samples, with some sites showing complex growth (CG) or counts that were either too few (TFTC) or too numerous (TNTC) to quantify. Table 2 provides a preliminary screening of bacterial isolates, where several, including A1a, B2, C1, C2b, and D1b, demonstrated resistance to increasing lead concentrations (up to 0.05 mg/l). These were further biochemically characterized and identified in Table 3, revealing a variety of genera such as Proteus, Bacillus, Micrococcus, and Pseudomonas. Finally, Table 4 presents a secondary screening of the most tolerant strains, with Proteus mirabilis showing the highest resistance, tolerating up to 0.1 mg/l of lead, suggesting its potential utility in bioremediation of contaminated soils.

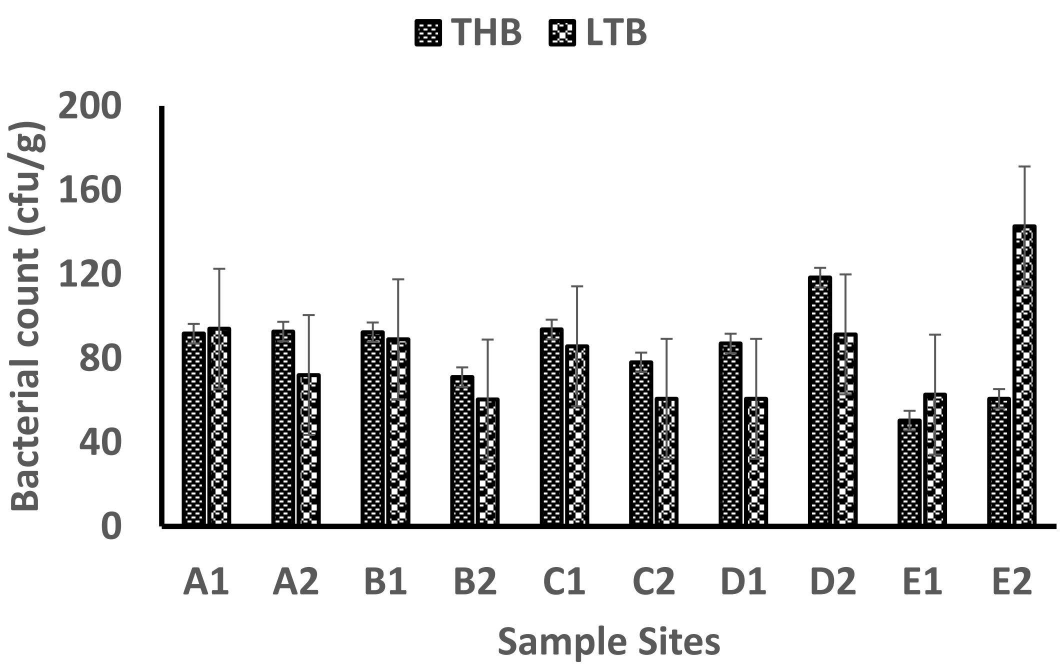
Table 1

Physicochemical parameters of soil

Samples	Lead Conc. (ppm)	pH	Electrical Conductivity	Total Organic Matter %	Organic Carbon %	Soil Water Content	Total Nitrogen
A1	0.14	6.44	49.5	0.20	0.12	0.78	0.31
A2	0.18	5.86	451	0.39	0.23	0.79	1.30
B1	0.11	5.64	45.4	0.19	0.11	0.77	0.30
B2	0.16	5.75	31.9	0.79	0.46	0.78	0.24
C1	0.68	5.63	60.3	0.39	0.30	1.37	0.40
C2	0.53	5.73	54.6	1.00	0.60	0.80	0.42
D1	0.72	6.15	58.0	0.40	0.23	0.78	0.37
D2	0.48	6.25	44.4	0.40	0.23	0.19	0.25
E1	0.18	5.93	51.1	0.78	0.50	0.58	0.19
E2	0.27	5.73	58.4	0.19	0.11	0.59	0.21

Figure 1

Population of total heterotrophic bacteria and lead-tolerant bacteria in soil samples



Keys: THB= total heterotrophic bacteria, LTB= lead tolerating bacteria, TNTC= too numerous to count, TFTC= too few to count, CG= complex growth

Table 2

Primary screening of bacterial isolates from the soil

Bacterial Isolates Code	Different concentrations of lead mg/l					Remarks
	0.01	0.02	0.03	0.04	0.05	
A1a	+	+	+	+	+	*
A1b	+	+	+	+	-	
A2	+	+	+	+	-	
B1	+	+	+	+	-	
B2	+	+	+	+	+	*
C1	+	+	+	+	+	*
C2a	+	+	-	-	-	
C2b	+	+	+	+	+	*
D1a	+	+	-	-	-	
D1b	+	+	+	+	+	*
D2	+	+	+	+	-	
E1	+	+	-	-	-	
E2	+	+	+	-	-	

Key: * tolerant species at different concentrations

Table 3

Biochemical characterization and identification of the bacterial isolates

Isolate code	Staining properties			Biochemical Characteristics													Identified bacteria
	GR	Shape	Spore	CAT	OXI	MR	VP	UR	IND	CIT	TSI						
											GLU	LAC	SUC	H_2S	GAS	MOT	
A1a	-	SR	-	+	-	-	+	+	-	+	+	-	-	-	-	+	*Proteus mirabilis*
A2	+	LR	+	-	-	-	-	+	-	-	+	+	+	-	-	-	*Lactobacillus* sp.
B1	+	LR	+	-	+	-	+	+	+	-	+	-	-	-	-	+	*Bacillus subtilis*
B2	-	SR	-	+	-	+	-	+	-	-	+	-	-	-	-	+	*Providencia* sp.
C1	+	CP	-	+	-	-	+	+	+	-	-	-	-	-	-	-	*Micrococcus* sp.
C2	+	LR	+	+	+	-	+	+	-	+	+	-	-	-	-	+	*Bacillus cereus*
C2a	-	SR	-	+	-	-	+	+	-	+	-	-	-	+	-	-	*Acinetobacter* sp.
C2b	+	CP	-	+	+	-	+	+	-	+	-	-	-	-	-	-	*Arthrobacter* sp.
D1a	-	LR	-	+	+	-	+	+	-	+	+	-	-	-	-	+	*Agrobacterium* sp.
D1b	-	SR	-	+	-	-	+	+	-	+	+	+	+	-	-	+	*Serratia* sp.
D2	+	LR	+	+	-	-	+	+	-	+	+	-	-	-	-	+	*Bacillus subtilis*
E1	+	LR	+	+	-	-	+	+	-	+	+	-	-	+	-	+	*Azotobacter*
E2	-	SR	-	+	-	-	+	+	-	+	+	-	-	+	-	+	*Pseudomonas* sp.

Keys: GR- Gram Reaction, COA- Coagulase, CAT- Catalase, OXI- Oxidase, MR- Methyl Red, VP- Voges-Proskauer, IND- Indole, CIT- Citrate, TSI- Triple Sugar Iron, GLU- Glucose, LAC- Lactose, SUC- Sucrose, H_2S- Hydrogen Sulphide, GAS- Gas Production, MOT- Motility, SR- Short Rod, LR (shape)- Long Rod, CC- Cocci Clusters, CP- Cocci Pairs.

Table 4

Secondary screening of lead-tolerant bacterial isolates

Bacteria	Concentrations of lead mg/l					Remarks
	0.06	0.07	0.08	0.09	0.1	
Proteus mirabilis	+	+	+	+	+	*
Providencia sp	+	+	+	-	-	
Acinetobacter sp	+	-	-	-	-	
Arthrobacter sp	+	-	-	-	-	
Serratia sp	+	-	-	-	-	

4. DISCUSSIONS

The soil comprises the foremost element in fulfilling an individual's necessities. In agriculture, soil finds extensive employment (Wang et al., 2023). The agricultural sector occupies a significant status globally in the cultivation of wheat, rice, jawar, pulses, sugarcane, vegetables, and fruits, among others, owing to the indispensable role of the physical and chemical characteristics of the soil in the application of other management practices (Eliazer Nelson et al., 2015). Since both physical and chemical properties influence soil productivity, the assessment of soil physico-chemical attributes in a region is crucial. These include pH, electrical conductivity, texture, moisture, temperature, soil organic matter, and the amount of available nitrogen, phosphorus, and potassium. The physico-chemical analysis of soil is based on these parameters (Kekane et al., 2015). The physicochemical analysis conducted on the soil employed for this experiment reveals that the pH value was found to be pH 6.0 on average. It is noteworthy that the pH of the soil may be slightly acidic or slightly neutral. The significance of the pH level of the soil cannot be overstated, as it has a considerable impact on all other soil parameters. Consequently, while analyzing the soil, due consideration was given to the pH. It is pertinent to mention that a soil is characterized as acidic when its pH is less than 6, normal when its pH falls within the range of 6 to 8, and alkaline when its pH exceeds 8.5 (Jensen & Thomas 2010). The sample of the study has been shown to contain a considerable amount of lead. Lead in soil decreases the soil pH, making it acidic, however, results show that the pH of the soils is between 5.63 (lowest) and 6.44 (highest), showing that the pH is within the normal range that can support the growth of plants. This also goes to confirm the activities of microorganisms that can tolerate high concentrations of lead. These microorganisms act to biosorb lead in the soil and maintain pH levels to support agricultural activities.

Soil's electrical conductivity is an essential characteristic that is utilized to evaluate its quality. This parameter quantifies the number of ions present in a solution, wherein an increase in ion concentration leads to a corresponding rise in the electrical conductivity of the soil solution. Consequently, this parameter offers a rapid, facile, and cost-effective method to assess the soil's health, making it an indispensable tool for soil quality evaluation. Higher EC-value nonsaline soils have more readily available nutrients than lower EC-value nonsaline soils. In comparison to soils with a higher concentration of larger silt and sand particles, soils with a higher proportion of tiny soil particles (higher content of clay) transmit more electrical current (lower content of clay). Higher EC values are frequently found in soils with a layer of restriction, like a claypan. Because the restrictive layer restricts water movement, the salts cannot be leached from the root zone and instead build up on the soil's surface. Using the prescribed methods, the physicochemical characteristics of the test soil were ascertained. Conductivity meters and pH meters, respectively, were used to determine pH and electric conductivity, and results show that both pH and electrical conductivity were within normal ranges suitable for plant growth.

The composition of sand, silt, and clay in a soil is commonly referred to as its texture, which is a critical attribute that has a significant impact on soil fertility. Natural soils are made up of particles of varying sizes. Texture influences the soil's water absorption capacity, water storage capacity, ease of tillage, aeration, and other factors that contribute to soil fertility (Patel et al., 2021). A coarse sandy soil, for example, is easy to cultivate and till, has sufficient airflow for healthy root development, and is easy to irrigate. However, it also dries out rapidly and can lose plant nutrients through leaching. In contrast, soils with high clay content (> 35% clay) have tiny, densely packed particles that leave little room for pore spaces and water percolation into the soil (Broadbent, 2008). Due to this particular matter, it presents a considerable challenge to appropriately saturate, drain, and cultivate the soil. The allocation of soil texture names is determined by the respective ratios of the three primary soil constituents: sand, silt, and clay. In particular, soil with a significant presence of clay is designated as clay (textural class), while soil rich in silt is referred to as loam (textural class), and soil displaying a high concentration of sand is identified as sand (textural class). The three fundamental categories of soil texture classes are recognized as sands, loams, and clays. Utilizing the hand feel method, the soils located within the study area have been classified as silty clay soils, which are generally arid and refined under high temperatures that characterize the local climatic conditions (Al-Kaisi & Licht 2005).

During the transportation and storage of soil samples, their water content tends to fluctuate, which can potentially impact the resultant data. The mathematical expression for water content in a given soil mass is expressed as the ratio of the weight of water to that of soil. As plants absorb water, nutrients from the soil are absorbed into the roots of the plants. Water is retained in a multitude of minuscule pores (micropores) and infiltrates the soil via larger pores (macropores). In porous soils, there exists a delicate balance between the large and small pores (Fenton et al., 2008). Plants have a greater requirement for Nitrogen (N) than for any other nutrient. However, the availability of soil nitrogen to plants is restricted, as a mere 2% of nitrogen in soil is derived from mineral sources. A majority of 98% of soil nitrogen is present in organic forms, which cannot be easily absorbed by plants except for a few diminutive organic molecules, as stated by Angus (2001. Nitrate and ammonia, two types of nitrogen found in minerals, can be easily absorbed by plants. Soil microbes break down organic materials and new plant remnants, transforming organic forms of nitrogen into mineral forms. This process is known as mineralization. Angus and Peoples have defined five descriptive categories that range from "Very Low" to "Very High" for the values of soil nitrogen supply (Angus & Peoples 2012). More organic nitrogen will likely be converted into mineral nitrogen for plant uptake in a soil if the value for soil nitrogen supply is higher. However, it is more probable that nitrate will undergo leaching down the soil profile, rendering it inaccessible to plant roots and potentially entering waterways in coarse-textured soils that exhibit heightened soil nitrogen supply values (Murphy et al., 2009).

An optimal balance between an increase in nitrogen availability for plant uptake and a reduction in the risk of nitrate leaching is attained at intermediate levels of soil nitrogen delivery. The quantity of soil nitrogen that achieves the best equilibrium between benefits and hazards is dependent on the clay content of the soil (Ye et al., 2022). In sand soils, the most favorable equilibrium is realized with "Moderate" soil nitrogen delivery (25–50 mg-N/N/kg soil). In contrast, "High" soil nitrogen supply is most effective for loam and clay soils (50 to 75 and 75 to 125 Mg-N/kg soils, respectively). Results from the sample site show a significantly low level of soil nitrogen, organic carbon, and water content; the temperature and soil texture may be responsible. Patel demonstrated how soil water content and other factors responsible for soil fertility can be affected by soil texture (Patel et al., 2021). Bacterial enumeration is the process of determining the number of bacterial cells in a sample. A method for counting live, culturable heterotrophs in a sample is called the heterotrophic plate count (HPC), formerly known as the standard plate count. A broad heterogeneous collection of organisms known as heterotrophs, which depend on organic carbon for growth, includes bacteria, yeasts, and molds (Bartram et al., 2003). Robert Koch first described the heterotrophic plate count method, a conventional microbiological approach, over 150 years ago, and it has been part of Standard Methods since its first edition. In this study, the total heterotrophic bacteria (THB) were counted alongside the lead-tolerating bacteria (LTB).

Results from the isolation and enumeration of bacterial isolates indicated the presence of extensive colonies of heterotrophic and lead-tolerant bacteria, particularly evident at the 10^5 dilution level. Observed colonies displayed both complex growth patterns and instances of overgrowth too numerous to quantify. Subsequent dilutions (10^6 and 10^{-7}) were utilized to reduce colony numbers and enhance counting precision. For accuracy, it was deemed essential that each colony originate from a single cell, necessitating the dissociation of cell chains and aggregates. However, certain bacterial taxa inherently grow in pairs, chains, or clusters, or possess adhesive extracellular materials such as capsules or slime layers, which impede effective separation into individual cells, thus limiting accurate quantification of the original cell population (Joan and Susan, 2021). As a result, the total viable count was expressed as colony-forming units (cfu), reflecting the number of culturable cells.

High cfu values indicated substantial microbial activity, suggesting survival and proliferation in lead-contaminated environments, and confirming microbial tolerance to elevated lead concentrations. Isolation and enumeration procedures employed two distinct media types: nutrient agar for total heterotrophic bacterial enumeration, and mineral salt agar supplemented with 0.01 g of lead for the selective growth of lead-tolerant bacteria. The microbial activity observed on nutrient agar closely mirrored that on the lead-supplemented medium, indicating a predominance of lead-tolerant bacteria in the sampled soil.

A total of thirteen bacterial isolates were obtained and subsequently cultured in mineral salt agar containing varying concentrations of lead (0.01 g to 0.05 g). Five isolates demonstrating growth at 0.05 g were further subjected to secondary screening across lead concentrations ranging from 0.06 g to 0.1 g. Only isolate A1a exhibited growth at the highest concentration of 0.1 g, indicating high lead tolerance.

Characterization and identification of all thirteen isolates subjected to primary screening were conducted using biochemical assays by Bergey's Manual of Determinative Bacteriology. Biochemical testing provided critical insights into the metabolic profiles necessary for taxonomic classification, based on enzymatic activity. Bacteria produce distinct enzymes that facilitate identification via established biochemical pathways (Sandle, 2016). Enzyme-based tests, such as those detecting catalase, gelatinase, oxidase, and urease, were employed to differentiate bacterial genera. The presence and activity levels of these enzymes served as diagnostic indicators of species identity (Janda and Abbott, 2002).

All thirteen isolates demonstrated the capacity to grow in lead-supplemented media, indicating adaptation and potential involvement in lead biosorption. These isolates included *Proteus mirabilis*, *Lactobacillus* sp., *Bacillus subtilis*, *Providencia* sp., *Micrococcus* sp., *Bacillus cereus*, *Acinetobacter* sp., *Arthrobacter* sp., *Agrobacterium* sp., *Serratia* sp., *Bacillus subtilis*, *Azotobacter*, and *Pseudomonas* sp. These microorganisms may contribute to the transformation and detoxification of lead, supporting survival through metal sequestration mechanisms.

5. CONCLUSION

The shooting range in Maimalari Military Cantonment contains a considerable amount of lead, as shown by this study. However, some bacteria have developed the ability to biosorb this metal and convert it to useful compounds, thereby maintaining a stable soil pH and suitable electrical conductivity for plant growth. About four of these bacteria were identified to be tolerant to high levels of lead concentrations. *Proteus mirabilis*, out of the four, proved to be the most efficient in tolerating elevated lead concentrations. Other bacteria demonstrating similar tolerance include *Providencia sp.* and *Acinetobacter sp.* These various bacteria can be enhanced to provide better bioremedial activities in the study area of interest and even be applied to other areas polluted with heavy metals.

Further analysis of these isolates suggests that their metal tolerance is likely due to specific physiological and biochemical adaptations, such as the production of extracellular polymeric substances, efflux systems, and enzymatic transformation of toxic ions. These mechanisms not only allow the bacteria to survive in contaminated environments but also contribute to immobilizing or detoxifying lead in the soil matrix. The presence of such microbes indicates a natural resilience of the soil microbiome and offers a promising, low-cost, and eco-friendly solution to soil remediation. Future work could focus on optimizing environmental conditions to enhance the activity of these native bacterial strains or developing bioaugmentation strategies using these isolates to accelerate lead detoxification across wider contaminated zones.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: The study adheres to the ethical guidelines for conducting research.

Funding: This research received no external funding.

REFERENCES

Al-Kaisi, M., & Licht, M. (2005). Evaluating soil moisture before field preparation and planting.

Angus, J. F. (2001). Nitrogen supply and demand in Australian agriculture. *Australian Journal of Experimental Agriculture*, *41*(3), 277-288. https://www.publish.csiro.au/an/EA00141

Angus, J. F., & Peoples, M. B. (2012). Nitrogen from Australian dryland pastures. *Crop and Pasture Science*, *63*(9), 746-758. https://www.publish.csiro.au/CP/CP12161

Appenroth, K. J., Sherameti, I. and Varma, A. (2010). Soil Heavy metals. Soil Biology (Eds), 2010; 19. *Springer-Verlag,* Berlin Heidelberg.

Arnemo, J. M., Andersen, O., Stokke, S., Thomas, V. G., Krone, O., Pain, D. J., & Mateo, R. (2016). Health and environmental risks from lead-based ammunition: science versus socio-politics. *EcoHealth*, *13*, 618-622. https://link.springer.com/article/10.1007/s10393-016-1177-x

Bartram, J., Cotruvo, J. A., Exner, M., Fricker, C., & Glasmacher, A. (Eds.). (2003). *Heterotrophic plate counts and drinking-water safety*. IWA publishing. https://books.google.com/books?hl=en&lr=&id=HQx8a_KtBbAC&oi=fnd&pg=PR7&dq=Bartram,+J.,+Cotruvo,+J.,+Exner+M.,+Fricker,+C.+and+Glasmacher,+A.+(2003).+Heterotrophic+plate+counts+and+drinking-water+safety,+World+Health+Organization.+London+(UK):+IWA+Publishing%3B+Google+Scholar+2003&ots=QQg-Kz4MDy&sig=SMZpUcZEKMl-ceRyYu6IaTKTZhU

Baum, G. R., Baum, J. T., Hayward, D., & MacKay, B. J. (2022). Gunshot wounds: ballistics, pathology, and treatment recommendations, with a focus on retained bullets. *Orthopedic research and reviews*, *14*, 293. https://pmc.ncbi.nlm.nih.gov/articles/PMC9462949/

Broadbent, F. E. (2008). *Soil Nitrogen*. Department of Agronomy, Cornell University, Ithaca, New York.

Buchanan, R. E. and Gibbons, N. E. (1995). *Bergey's Mannual of Determinative Bacteriology*. (8th ed). Williams, Wilkens Co., Baltimore, 1268.

Cowan, S. T. and Steel, K. J. (1981). *Manual for identification of Medical Bacteria*. 2nd Ed. Cambridge University Press, London, 217.

Agho, E., Allamin, I. A., Misherima, K. A., Hambali, I. U., & Shettima, H. (2025). Physicochemical characterization of lead-resistant soil bacteria from Maimalari Military Cantonment, Maiduguri. *World Journal of Environmental Research*, *15*(1), 19-30. https://doi.org/10.18844/wjer.v15i1.9518

Diaz, E., Sarkis, J. E. S., Viebig, S., & Saldiva, P. (2012). Measurement of airborne gunshot particles in a ballistics laboratory by sector field inductively coupled plasma mass spectrometry. *Forensic science international*, *214*(1-3), 44-47. https://www.sciencedirect.com/science/article/pii/S0379073811003318

Eliazer Nelson, A. R. L., Ravichandran, K., & Antony, U. (2019). The impact of the Green Revolution on indigenous crops of India. *Journal of Ethnic Foods*, *6*(1), 1-10. https://link.springer.com/article/10.1186/s42779-019-0011-9

Fayiga, A. O., & Saha, U. K. (2016). Soil pollution at outdoor shooting ranges: Health effects, bioavailability and best management practices. *Environmental pollution*, *216*, 135-145. https://www.sciencedirect.com/science/article/pii/S026974911630450X

Fenton, M., Alber, C., & Ketterings, Q. (2008). Soil organic matter. Agronomy Fact Sheet. Fact Sheet 41.

Janda, J. M., & Abbott, S. L. (2002). Bacterial identification for publication: when is enough enough?. *Journal of clinical microbiology*, *40*(6), 1887-1891. https://journals.asm.org/doi/full/10.1128/jcm.40.6.1887-1891.2002

Jensen, D. and Thomas, L. (2010). Soil pH and the Availability of Plant Nutrients*, IPNI Plant Nutrition TODAY*, Fall, 2.

Joan, P., and Susan, M. (2021). Introduction to enumeration of bacteria; 5.1.

Kabir, E. R., Rahman, M. S., & Rahman, I. (2015). A review on endocrine disruptors and their possible impacts on human health. *Environmental toxicology and pharmacology*, *40*(1), 241-258. https://www.sciencedirect.com/science/article/pii/S1382668915300120

Kang, C. H., Kwon, Y. J., & So, J. S. (2015). Bioremediation of heavy metals by using bacterial mixtures. *Ecological Engineering*, *74*, 302–305. https://doi.org/10.1016/j.ecoleng.2014.10.019

Kekane, S. S., Chavan, R. P., Shinde, D. N., Patil, C. L., & Sagar, S. S. (2015). A review on physico-chemical properties of soil. *International Journal of Chemical Studies*, *3*(4), 29-32. https://www.researchgate.net/profile/Assitant-Professorshrikant-Kekane/publication/333976045_A_review_on_physico-chemical_properties_of_soil/links/5d10be5c458515c11cf30e24/A-review-on-physico-chemical-properties-of-soil.pdf

Laidlaw, M. A. S., Filippelli, G. M., Mielke, H. W., Gulson, B., & Ball, A. S. (2017). Lead exposure at firing ranges—a review. *Environmental Health*, *16*(1), 34. https://doi.org/10.1186/s12940-017-0246-0

Maceiras, R., Pérez, M., & López, J. (2024). Biochar amendments and phytoremediation: A combined approach for effective lead removal in shooting range soils. *Toxics*, *12*(3), 520. https://doi.org/10.3390/toxics12030520

Mendes, A. M. S., Oliveira, R. S., & Freitas, H. (2023). Remediation of lead-contaminated shooting range soil: Biodegradable chelating agents and plant-assisted strategies. *Journal of Environmental Management*, 325, 116362. https://doi.org/10.1016/j.jenvman.2022.116362

Mendes, A. M. S., Oliveira, R. S., & Freitas, H. (2023). Remediation of lead-contaminated shooting range soil: Biodegradable chelating agents and plant-assisted strategies. *Journal of Environmental Management*, 325, 116362. https://doi.org/10.1016/j.jenvman.2022.116362

Murphy, D. V., Osman, M., Russell, C. A., Darmawanto, S., & Hoyle, F. C. (2009). Potentially mineralisable nitrogen: relationship to crop production and spatial mapping using infrared reflectance spectroscopy. *Soil Research*, *47*(7), 737-741. https://www.publish.csiro.au/SR/SR08096

Nwaedozie, G., Mohammed, Y., Faruruwa, D. M., & Nwaedozie, J. M. (2013). Environmental impact of toxic metal load in some military training areas within the one division of Nigerian Army, Kaduna, Nigeria. *International Journal of Academic Research in Business and Social Sciences*, *3*(3), 180. https://search.proquest.com/openview/8d375d2af5b1d098e1a774629f8a9388/1.pdf?pq-origsite=gscholar&cbl=696344

Patel, K. F., Fansler, S. J., Campbell, T. P., Bond-Lamberty, B., Smith, A. P., RoyChowdhury, T., ... & Bailey, V. L. (2021). Soil texture and environmental conditions influence the biogeochemical responses of soils to drought and

Agho, E., Allamin, I. A., Misherima, K. A., Hambali, I. U., & Shettima, H. (2025). Physicochemical characterization of lead-resistant soil bacteria from Maimalari Military Cantonment, Maiduguri. *World Journal of Environmental Research*, *15*(1), 19-30. https://doi.org/10.18844/wjer.v15i1.9518

flooding. *Communications Earth & Environment*, *2*(1), 127. https://www.nature.com/articles/s43247-021-00198-4

Rodríguez-Seijo, A., Pereira, R., & Cachada, A. (2017). Lead (Pb) in shooting range soil: A systematic literature review. *Environmental Science and Pollution Research*, *24*(2), 1152–1165. https://doi.org/10.1007/s11356-016-7943-0

Rodríguez-Seijo, A., Pereira, R., & Cachada, A. (2021). Recent trends in sustainable remediation of Pb-contaminated shooting range soils: Rethinking waste management within a circular economy. *Processes*, 9(4), 572. https://doi.org/10.3390/pr9040572

Sandle, T. (2016). Microbial Identification *Pharmaceutical Microbiology*; 103-113. https://www.sciencedirect.com/science/article/pii/B9780081000229000098.

Smith, T. A., & Cashman, T. M. (2002). The incidence of injury in light infantry soldiers. *Military medicine*, *167*(2), 104-108. https://academic.oup.com/milmed/article-abstract/167/2/104/4819665

Udo, E. J. and Ogunwale, J. A. (1986). *Laboratory Manual for the Analysis of Soil, Plant and Water Samples*. 2nd Edition, Imperial Binding Service, Ibadan, 174.

Wang, J., Li, H., & Bezerra, M. L. (2017). Assessment of shooter's task-based exposure to airborne lead and acidic gas at indoor and outdoor ranges. *Journal of Chemical Health & Safety*, *24*(4), 14-21. https://pubs.acs.org/doi/abs/10.1016/j.jchas.2016.11.003

Wang, S., Liao, P., Cen, L., Cheng, H., & Liu, Q. (2023). Biochar promotes arsenopyrite weathering in simulated alkaline soils: electrochemical mechanism and environmental implications. *Environmental Science & Technology*, *57*(22), 8373-8384. https://pubs.acs.org/doi/abs/10.1021/acs.est.2c09874

Ye, J. Y., Tian, W. H., & Jin, C. W. (2022). Nitrogen in plants: From nutrition to the modulation of abiotic stress adaptation. *Stress Biology*, *2*(1), 4. https://link.springer.com/article/10.1007/s44154-021-00030-1

Zhang, Y., Peng, J., Wang, Z., Zhou, F., Yu, J., Chi, R., & Xiao, C. (2025). Metagenomic analysis revealed the bioremediation mechanism of lead and cadmium contamination by modified biochar synergized with Bacillus cereus PSB-2 in phosphate mining wasteland. *Frontiers in Microbiology*, 16, 1529784. https://doi.org/10.3389/fmicb.2025.1529784