Isolation and Characterization of Bacteria Associated With Heavy Metal Polluted Soil in Federal Capital Territory, Nigeria

Oyetunji,i Funmitan Nike

Department of Life Science, National Space Research and Development Agency
Email: Ofunmitan@gmail.com

Abstract: This study investigated the isolation and characterization of bacteria associated with heavy metal–polluted soils in the Federal Capital Territory (FCT), Nigeria. A total of fifteen soil samples were collected from Lugbe, Gwagwalada, and Dutse-Alhaji and analyzed for physicochemical properties and microbial content. Standard microbiological techniques, including serial dilution, culture on selective media, and biochemical tests, were employed for the isolation and identification of bacterial species. The results revealed that soil pH ranged from 5.1 to 5.6 while the moisture of the soil ranged from 2.12 to 2.81. The organic matter content and the water holding capacity content also ranged from 1.03 to 2.03 and 3.35 to 3.43 respectively. A total of nineteen bacterial isolates were identified, including *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus megaterium*, and *Klebsiella pneumoniae*. In Gwagwalada, *B. cereus* (3) was the most occurring bacteria, followed by *Bacillus licheniformis* (2) and only one (1) *B. subtilis*, and *Pseudomonas aeruginosa* each, were isolated. In Dutse, *Bacillus subtilis* (3) and *Pseudomonas aeruginosa* (2) were the only bacteria present in the soil samples. Meanwhile, *B. subtilis* (3) was the most prevalent in Lugbe soil, followed by *Pseudomonas aeruginosa* (2), *B. Megaterium* and *Klebsiella pneumonia* present recorded only one each. The bacterial associated with the heavy metal polluted soil at different locations in FCT, Abuja was not significantly different ($P \leq 0.05$). Among these, *Bacillus subtilis* (37%) was the most frequently occurring species, followed by *Pseudomonas aeruginosa* (26%), three *B. cereus* (16%), and two *Bacillus licheniformis* (11%) while only one (1) *B. Megaterium* and *Klebsiella pneumonia* represented 5% each were isolated. The findings highlight the presence of indigenous bacteria with significant potential for bioremediation of heavy metal–polluted soil. The study therefore recommends further exploration of these bacterial isolates for sustainable and eco-friendly soil remediation strategies.

[Oyetunji,i Funmitan Nike. **Isolation and Characterization of Bacteria Associated With Heavy Metal Polluted Soil in Federal Capital Territory, Nigeria.** *Researcher* 2026;18(6):7-13]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). http://www.sciencepub.net/researcher. 02. doi:10.7537/marsrsj180626.02

Keywords: Bacterial isolates; Heavy metal; polluted soil.

1.0 Introduction

Heavy metals occur naturally in the environment and are found in rocks, soil, plants and animals. Metals occur in different forms as dissolved ions in water or vapour or minerals in rocks, sand and soil. These materials can also bond to organic and inorganic molecules or adhere to particles in the air. Both natural and anthropogenic processes emit metals into the air and water (Mulligan *et al.*, 2001).

One of the most important environmental problems throughout the world today is the contamination of soil by heavy metals as the result of increase in industrial activities (Nouri *et al.*, 2019). In developing countries such as Nigeria, activities such as; welding, vulcanizing, auto-electrical works, battery charging and motor transportation have introduced heavy metals into the air which are subsequently deposited in soils. The problem caused by heavy metals has been a subject of concern especially after the Zamfara State (Nigeria) lead poisoning in 2020 that claimed the lives of so many people especially children. The method used in mitigating this problem was through excavation of the soil to landfill and covering the original polluted soil surface with clean soils (Andrew *et al.*, 2020). This method of mitigation (Landfill) is limited due to lack of available land and landfill sites, other remediation methods commonly used are; soil washing/leaching/flushing with chemical agents (Wuana *et al.*, 2020).

All these methods have been considered to be destructive, not eco-friendly, expensive and do not provide a permanent or lasting solution to the problems (Mejare and Bulow, 2022). There is therefore, the need for adequate protection and restoration of the soil ecosystems, these can be achieved through other remediation process, that make use of inexpensive, environmentally friendly materials in the detoxification and removal of heavy metals from of the contaminated soil (Manjunathan *et al.*, 2021). Hence, this research work is to use bacteria to bind metals and remove them from heavy metal contaminated soil (Kord *et al.*, 2020). Therefore this

study aim to isolate and characterized bacteria associated with heavy metal polluted soil in FCT, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study Area

The Federal Capital Territory (FCT) lies between latitudes 8°25'N and 9°20'N and longitude 6°39' and 7°45' East of the Greenwich meridian (NPC, 2006). The FCT has a land mass of about 8000km^2 and lies within latitude 9^0 25^1 N and $9^0 20^1$ N of the equator and longitude 5^0 45^1E and 39^1E (Badaru*et al*., 2014), with a current population of about 3,464,000 (NPC, 2006). The FCT is divided into six area councils namely, Abaji, Abuja Municipal, Bwari, Gwagwalada, Kuje, and Kwali. Of this six, three (Bwari, Gwagwalada and AMAC) were selected for the purpose of this study.

2.2 Collection of Soil Samples

A total of fifteen (15) heavy metals-polluted soil samples were collected at random from motor mechanic shops in Lugbe (Abuja Municipal Area Council), Kuntunku in Gwagwalada and Dutse-Alhaji in Bwari. Five (5) samples were collected from each location and about 50g of each sample were collected in sterile container and then transported to the laboratory for analysis.

2.3 Determination of physico-chemical parameters of soil samples

2.3.1 Soil pH Determination

About 20g of air-dried sieved soil into a 100ml beaker and 20ml of sterile distilled water were added. The suspensions were left to stand for 30 minutes with occasional stirring to enhance equilibrium reaction. The pH of the suspension was taken by inserting the glass hydrogen (H^+) electrode of the pH meter (Pye Unicam, model 292mk2 pH meter) into the partly settled suspension.

2.3.2 Soil Water Holding Capacity Determination

Soil sample which has been dried in the oven at 105 ^{0}C for 24 hours were used for the water holding capacity determination according to the method of Pramer and Schmidt (1964) thus; about 5 gm of the dried soil was placed on Whitman filter paper which was placed on a 250 Erlenmeyer flask and 20 ml of water was added, then the time taken for all the water to move down to the flask was taken and used to determine the water holding capacity.

2.3.3 Soil Moisture Content Determination

The method used for this determination was that of Pramer and Schmidt (1964). About one gram of each sample was put into a washed and dried crucible dish and placed in a Phoenix oven at a temperature of 70-80 ^{0}C for 2 hours and at 100-105 ^{0}C until the weight is constant. The samples were cooled in a desiccator and weighed. The weight loss was obtained as the moisture content and calculated as:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W1 = Initial weight of empty crucible, W_2 = Weight of crucible + sample before drying, W_3 = Final weight of crucible + sample after drying.

2.3.4 Determination of organic matter

About one gram (1g) of oven dried soil sample (at 105 ^{0}C) was placed in a constant mass silica crucible and heated in a muffle furnace at 550 ^{0}C for 2 h. The crucible was allowed to cool down in a dessicator and again weighed. The organic matter of the soil was calculated thus;

$$\text{Organic matter (\%)} = \frac{\text{Initial mass - Final mass}}{\text{Initial mass}} \times 100$$

2.4 Preparation and Sterilisation of Media

All media were prepared and sterilized according to their manufacturer's specifications. The media used include Nutrient agar, Cetrimide agar, Tryptone Soya agar as well as basal medium.

2.5 Isolation of bacteria associated with heavy metal polluted soil

Isolation of bacteria associated with heavy metal polluted soil were carried out using spread plate technique. Serial dilutions of the soil samples were carried out to obtain the proper dilution factors. One (1 g) of the soil samples from mechanic workshop were aseptically transferred into 9 ml of sterile distilled water as the stock culture. Ten folds serial dilutions of the stock culture were made using sterile water as diluents. Then 1.0 ml of the dilution sample was aseptically pipetted into a sterile test tube containing 9.0 ml of sterile distilled water. The contents were mixed thoroughly. Other ten-fold dilutions were similarly made up to 10^{-6}. Some 1 ml of 10^{-6} was inoculated on the Nutrient agar and Tryptone soya agar while 10^{-3} was inoculated on Cetrimide agar using the spread plate method. The plates were allowed to stand undisturbed for about 15 minutes and then incubated at at 37 ^{0}C for 24 hrs.

2.6 Purification of Isolates

Resulting colonies on nutrient agar were counted on colony counter while colonies on Tryptone soya agar and Cetrimide agar were sub-cultured on freshly prepared Tryptone soya agar and Cetrimide agar and then incubated for another 24h in the incubator to obtain pure cultures. The pure cultures were then maintained on nutrient agar slant at 4°C in a refrigerator.

2.7 Identification of Bacterial Isolates

The bacterial cultures were characterised according to their colony's morphological appearance (colony, shape, edge or margin, pigmentation, consistency and optical characteristics) on the plate. In addition to the colonial characterization, cellular morphologies and biochemical characteristics as described in the laboratory manual by Fawole and Oso (2018). Also, the isolates were subsequently identified using the Bergey's manual of Determinative Bacteriology.

2.7.1 Gram staining and microscopy of isolates

A wire loop was flamed to red hot and allowed to cool, the sterile loop was used to pick culture from a discrete colony. Then, a smear of the discrete colony was fixed on a slide which had been air dried by passing it through a flame. The fixed smear was flooded with crystal violet for 60s after which the stain drained off and washed over running water; then, it was flooded with lugol's iodine for 60s and washed gently using running water. This was flooded with 95% alcohol to decolorize for 30s and rinse under running water. The slide was counter-stained using safranin for 60s, after which the slide was washed gently with tap water and left to air-dry. The slide was viewed under the oil immersion objective lens of the microscope.

2.7.2 Motility Test

The method used in determining motility was the hanging drop method. Loop full of sterile distilled water was placed on a cover slip and a small portion of each bacterial isolate from 24hrs old culture was transferred to the drop of water on the cover slip using a sterile inoculating loop. A smooth suspension was made by mixing it thoroughly. Vaseline was applied around the edges of the cover slip so as to disallow air and it was carefully covered with a clean cavity glass slide. Cover slip was pressed down to make an airtight seal and was subsequently observed. The cover slip was inverted upon the cavity slide under the x40 objective lens. Motile bacterial cells were seen moving rapidly in the field (Fawole and Oso, 2018).

2.7.3 Citrate utilization Test

Some amount of simmons-citrate agar (22.5g) was dissolved in 1 litre of distilled water and autoclave at 121^0C for 15 mins, after which it was allowed to cool and about 20ml was poured into sterile petri-dish and left to solidify. The test organisms were streaked on the solid media and incubated in an inverted position at 37ºC for 48 h. A change in the colour from green to blue indicates an alkaline reaction from citrate utilization which is a positive result and a negative result gives no colour change.

2.7.4 Oxidase test

This test indicates the presence of cytochrome oxidase that is able to reduce oxygen (O_2) and artificial electron acceptors (Prescott *et al.*, 2019). A drop of 1% tetramethyl-p-phenylenediamine hydrogen chloride was dropped on a filter paper. Fresh culture of the isolate was then rubbed on the filter paper and observed. A possible result was indicated by a purple colour change within 10 seconds.

2.7.5 Indole test

Some 5 ml of prepared peptone is poured into sterile test tubes. The pure culture of different isolates is then inoculated into the test tubes. The test tubes are corked and incubated at 37ºC for 48 hours. About 0.5 ml of Kovacs reagent was added after incubation. It swirled gently and was allowed to stand. The formation of a deep red ring shows positive results.

2.7.6 Urease production

A dense milky suspension of the test organism was prepared in a small tube containing 0.25ml physiological saline. A urease tablet was added; the tube would then be closed and incubated at 37 ºC for 4 h. The isolate gave a positive reaction within 4 h, showing purple-pink colour for positive urease test and yellow/orange colour for negative urease test (Fawole and Oso, 2021).

2.7.7 Methyl-red and Voges Proskauer test

Glucose phosphate broth was prepared and dispensed into 2ml portions in sterile test tubes labelled A and B, sterilized at 121ºC for 15 minutes. They were cooled and a loopful of the test bacteria were inoculated into each test tube and incubated at 37ºC for 48 hours, after which 3-4 drops of the test reagent (methyl red) is added to test tube A. Appearance of a red colour shows a positive result while a negative result gives a yellow-orange colouration. To the test tube B, 1ml of 5% á-naphthol solution was added followed by 1ml of 40% potassium hydroxide (KOH) solution. The mixture was shaken and was allowed to stand for some minutes and observed. A red colour within 5 minutes is indicative of a positive reaction while no colour change indicates negative reaction.

2.7.8 Triple sugar iron test

About 6.5 g of triple sugar iron (TSI) agar was dissolved in 100 ml of distilled water and mixed. It is then autoclaved at 121ºC for 15 minutes. 5ml of prepared TSI agar is dispensed into sterile test tubes and placed in a slanted position to solidify. After which bacterial isolates are then incubated at 37ºC for 48 hours. Development of red colouration with blackish spots indicates H_2S reaction arising from TSI utilization which is positive while a negative result gives no reaction (Fawole and Oso, 2018).

2.7.9 Catalase Test

Two drops of 3% hydrogen peroxide (H_2O_2) were placed on each end of a clean grease free slide and labelled A and B. The test organism is inoculated into drop A and was observed immediately for gas bubbling (effervescence) while drop B serves as

control. Result was recorded based on the evolution of gas or bubbles formed (Fawole and Oso, 2018).

2.7.10 Starch Hydrolysis

The ability of some bacteria to hydrolyze starch is detected by the presence of enzyme amylase. Soluble starch was added to already prepared nutrient agar in the ratio 2g soluble starch to 1 liter of nutrient agar and it was sterilized in the autoclave. The medium was thereafter poured into Petri dishes, and was inoculated and then incubated at 37°C for 48hours. After incubation, the plates were flooded with Gram's iodine solution and observed. A clear zone around a distinct colony indicates hydrolysis of starch (a positive result) while a blue-black colouration gives a negative result (Fawole and Oso, 2018).

2.8 Data Analysis

Data obtained from this study were analysed statistically using Ms Excel Statistics (Window 10 version) and the test applied was t-test statistics at P< 0.05.

3.0 RESULTS

3.1 Physico-chemical Parameter of Soil

Figure 3.1 shows the physico-chemical parameter of heavy metals polluted soil samples. The pH of the soil ranges from 5.1 to 5.6 while the moisture of the soil ranged from 2.12 to 2.81. The organic matter content and the water holding capacity content also ranged from 1.03 to 2.03 and 3.35 to 3.43 respectively.

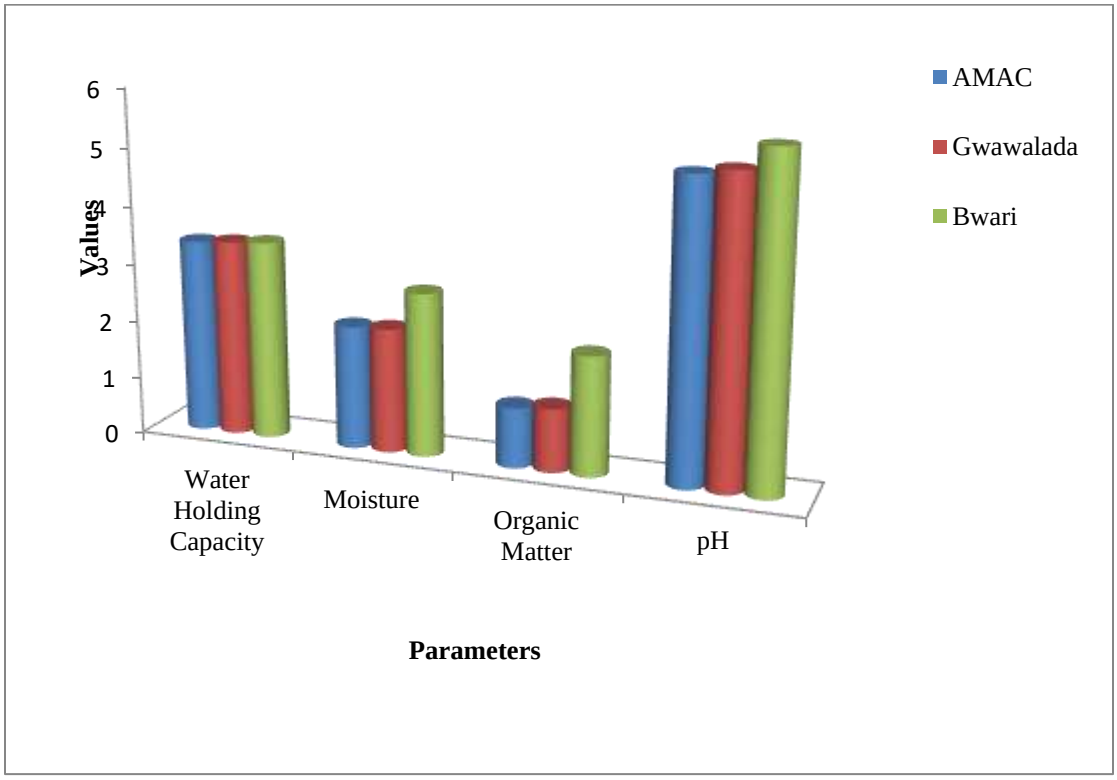

Figure 3.1: Physico-chemical Parameters of Soil

3.2 Biochemical Characterization of Isolated Bacteria

The results of the research show the biochemical characteristics of bacteria isolated from the contaminated soil are as indicated in Table 3.1. Based on the biochemical characteristics of the isolated bacteria, the bacteria were identified to include *Bacillus subtilis, Klebsiella pneumonia, Bacillus cereus, Bacillus licheniformis, Pseudomonas aeruginosa* and *Bacillus megaterium*.

Table 3.1: Morphological and Biochemical Characterization of Bacterial Isolates

S/N	Isolate Code	Gram Rxn	Cell Morphology	Catalase	Oxidase	Methyl Red	Voges Proskaeur	Coagulase	Urease	H_2S	Starch	Glucose	Lactose	Mannitol	Maltose	Sucrose	Citrate Utilization	Indole Test	Endospore	Motility	Probable Identity
1	BS1	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	*Bacillus subtilis*
2	BS2	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	*Bacillus subtilis*
3	BS3	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	*Bacillus subtilis*
4	BS4	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	*Bacillus subtilis*
5	BS5	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	*Bacillus subtilis*
6	BS6	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	*Bacillus subtilis*
7	BS7	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	*Bacillus subtilis*
8	BL1	+	Rod	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	*Bacillus licheniformis*
9	BL2	+	Rod	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	*Bacillus licheniformis*
10	BC1	+	Rod	+	+	+	+	-	-	+	+	+	-	-	+	-	+	-	+	+	*Bacillus cereus*
11	BC2	+	Rod	+	+	+	+	-	-	+	+	+	-	-	+	-	+	-	+	+	*Bacillus cereus*
12	BC3	+	Rod	+	+	+	+	-	-	+	+	+	-	-	+	-	+	-	+	+	*Bacillus cereus*
13	BM1	+	Rod	+	-	+	-	-	+	-	+	-	-	-	-	-	+	-	+	+	*Bacillus megaterium*
14	KP1	-	Rod	+	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	-	*Klebsiella pneumoniae*
15	PA1	-	Rod	+	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-	+	*Pseudomonas aeruginosa*
16	PA2	-	Rod	+	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-	+	*Pseudomonas aeruginosa*
17	PA3	-	Rod	+	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-	+	*Pseudomonas aeruginosa*
18	PA4	-	Rod	+	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-	+	*Pseudomonas aeruginosa*
19	PA5	-	Rod	+	+	-	-	-	+	+	-	-	-	+	-	-	+	-	-	+	*Pseudomonas aeruginosa*

Keys: += Positive, - = Negative

3.3 Occurrence of bacteria isolated from Heavy Metal Polluted Soil

The frequency of occurrence of bacteria isolated from Heavy metals polluted soil of different locations is indicated in Table 3.2. In Gwagwalada, *B. cereus* (3) was the most occurring bacteria, followed by *Bacillus licheniformis* (2) and only one (1) *B. subtilis*, and *Pseudomonas aeruginosa* each, were isolated. In Dutse, *Bacillus subtilis* (3) and *Pseudomonas aeruginosa* (2) were the only bacteria present in the soil samples. Meanwhile, *B. subtilis* (3) was the most prevalent in Lugbe soil, followed by *Pseudomonas aeruginosa* (2), *B. Megaterium* and *Klebsiella pneumonia* present recorded only one each. The bacterial associated with the heavy metals polluted soil at different locations in FCT, Abuja was not significantly different (P≤ 0.05).

Also, the same Table 3.2 showed the overall percentages of bacteria isolated in this study. *Bacillus subtilis* was the most frequently isolated bacteria with 7 (37%), followed by five *Pseudomonas aeruginosa* (26%), three *B. cereus* (16%), and two *Bacillus licheniformis* (11%) while only one (1) *B. Megaterium* and *Klebsiella pneumonia* represented 5% each were isolated. The frequencies of occurrence of bacteria isolated from heavy metals polluted soil at different locations in FCT, Abuja were not significantly different (P≤ 0.05).

Table 3.3: Overall percentages of bacteria isolates from Heavy Metals Polluted Soil

Isolates	AMAC	Gwagwalada	Bwari	Total N (%)
Bacillus subtilis	3	1	3	7 (37)
Bacillus cereus	0	3	0	3 (16)
Bacillus licheniformis	0	2	0	2 (11)
Bacillus megaterium	1	0	0	1 (5)
Pseudomonas aeruginosa	2	1	2	5 (26)
Klebsiella pneumoniae	1	0	0	1 (5)
Total	**7**	**7**	**5**	**19 (100)**

4.0 Discussion

The findings from this study revealed that heavy metal–polluted soils from selected motor mechanic workshops in the Federal Capital Territory (FCT), Nigeria, harbor diverse bacterial populations with potential bioremediation capabilities. The slightly acidic pH range (5.1–5.6) observed in the soil samples is consistent with conditions commonly reported for contaminated soils, where metal solubility tends to increase under acidic conditions. This acidic environment may enhance the mobility and bioavailability of heavy metals such as lead and cadmium, thereby influencing microbial adaptation and survival. The relatively low moisture content (2.12–2.81%) and organic matter (1.03–2.03%) recorded in this study suggest limited nutrient availability, which may impose selective pressure on microbial communities. However, the presence of measurable water holding capacity indicates that the soil still retains sufficient moisture to support microbial metabolic activities. These physico-chemical conditions likely contributed to the selection of hardy and metabolically versatile bacterial species capable of tolerating environmental stress and heavy metal toxicity.

The bacterial isolates identified in this study include: *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* are widely reported in literature as common inhabitants of contaminated environments (Ugoh and Moneke, 2021; Kord *et al*., 2020). This result is similar to the report of Ugoh and Moneke (2021), in the aspect of high incidence of *Bacillus* spp. and *Pseudomonas aeruginosa.* The dominance of *Bacillus subtilis* (37%) may be attributed to its ability to form endospores, allowing it to survive harsh environmental conditions such as heavy metal stress, desiccation, and nutrient limitation. Similarly, the occurrence of *Pseudomonas aeruginosa* (26%) highlights its ecological adaptability and metabolic diversity, which enable it to thrive in polluted environments. The presence of *Bacillus cereus* and *Bacillus licheniformis* further supports the potential for bioremediation, as these organisms are known to produce metal-binding proteins and enzymes that facilitate detoxification

processes. Additionally, the detection of *Klebsiella pneumoniae*, although in low frequency (5%), suggests its possible involvement in metal resistance, as some strains have been reported to possess plasmid-mediated resistance to heavy metals. The distribution of bacterial isolates across the three study locations (AMAC, Gwagwalada, and Bwari) showed no significant difference ($P \leq 0.05$), indicating a relatively uniform pattern of microbial adaptation to heavy metal contamination within the study area. This uniformity may be attributed to similar anthropogenic activities such as automobile repair, battery charging, and oil spillage occurring across the sites, leading to comparable contamination profiles. This result varies from the result of Udeani *et al*. (2019) who reported the presence of *B. Stearothermophilus* and *Cyanobacteria* in mechanic workshop soil. The difference in the bacteria present in heavy metals polluted soils from mechanic workshops as observed in the current study and in the report of Udeani *et al*. (2019) may be as a result of different environmental factor predisposing the survival of bacteria in the different location and the fact that Udeani *et al*. (2019) study location may have a different geological area being that the study was carried out in Enugu metropolis and the current study was carried out in Abuja, the Federal Capital Territory. Overall, the results of this study demonstrate that heavy metal–polluted soils in the FCT harbor indigenous bacterial species with significant bioremediation potential.

4.1 Conclusion

Based on the result of this study, it can be concluded that; of the bacteria isolated, *Bacillus subtilis* is the highest frequently isolated bacterial from mechanic workshop soil, followed by *Pseudomonas aeruginosa Bacillus cereus, Bacillus licheniformis, Bacillus megaterium* and *Klebsiella pneumonia* respectively.

4.2 Recommendation

Based on the result of this study, it is therefore recommended that further exploration of these bacterial isolates for sustainable and eco-friendly soil remediation strategies should be carry out.

REFERENCES

Andrew, M, J., Dan, B. A., David, H., Bert, E.. andrea, T., Richard, F. and Jack. (2020). *World's Worst Pollution Problems Report*, 3(4): 1-73.

Fawole, M.O and Oso, B.A. (2018). Laboratory manual of Microbiology. 5th Edition, Spectrum Books Limited, Ibadan. Pp. 22-23

Kord, B., Mataji, A. and Babaie, S. (2020). Pine needles as indicator for heavy metals pollution. *International Journal of Environmental Science and Technology*, 7(1): 79- 84.

Manjunathan, J.M., Ayyasamy, V. and Kaviyarasan. (2021). Effect of microbially treated industry effluent on the growth of green gram negative bacteria *International Journal of Pharmaceutical and Biological Sciences*, 2(1).

Mejare, M. and Bulow, L. (2022). Metal binding proteins and peptides in bioremediation and phytoremediation of heavy metals. *Trends Biotechnology*, 19:67–73.

Mulligan, C.N., Yong, R.N. and Gibbs, B.F. (2018). Remediation technologies for metal contaminated soils and groundwater: an evaluation. *Engineering Geology*, 60:193.

Nouri, J., Mahvi, A. H., Jahed, G.R. and Babaei, A. A. (2019). Regional Distribution pattern of groundwater fluoride in the Shush aquifer of Khuzestan Country. *Current Research in Microbiology and Biotechnology*, 9 (4): 321-325.

Udeani, T. K. C., Obroh, A .A., Okwuosa, C .N., Achukwu, P. U. and Azubike, N. (2019). Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil. *African Journal of Biotechnology*, 8 (22), 6301-6303.

Ugoh, S.C. and Moneke, L.U. (2021). Isolation of Bacteria from Engine Oil Contaminated Soils in Auto mechanic workshops in Gwagwalada, Abuja, FCT-Nigeria. *Academia Arena*, 3(5):28-33

Wuana, R. A., Okeieimen, F. E. and Imborvungu, J. A. (2020). Removal of heavy metals from a contaminated soil using chelating acids. *International Journal of Environmental Science and Technology*, 7(3): 484-496.