

Bioremediation of Heavy Metals by Bacteria Isolated from Mechanic Workshop Soil in Federal Capital Territory, Nigeria

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ABSTRACT: The study on the bioremediation of heavy metal by bacteria isolated from mechanic workshop soil in FCT, Nigeria was conducted. A total of fifteen (15) soil samples were collected at random from motor mechanic shops in Lugbe (Abuja Municipal Area Council), Kuntunku in Gwagwalada and Dutse-Alhaji in Bwari, and in Gwagwalada, *Bacillus 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 has only one each. *B. subtilis* was the most frequently isolated bacteria with seven (37%), followed by five *Pseudomonas aeruginosa* (26%), three *B. cereus* (16%), two *Bacillus licheniformis* (11%) while and only one (1) *B. Megaterium* and *Klebsiella pneumonia* represented (5%) each was isolated. No effect was observed on heavy metal reduction by *Bacillus licheniformis* and *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* had the most significant remediation impact on cadmium and lead in the soils from the mechanic workshop. *Bacillus megaterium* had a cadmium reduction of $1.24 \pm 0.00 \mu\text{g/g}$ and $1.36 \pm 0.00 \mu\text{g/g}$ of lead while *Bacillus subtilis* and *Klebsiella pneumoniae* had no reduction in the concentration of cadmium. However, further study should be carried out to ascertain the bioactive substance in *Pseudomonas aeruginosa* responsible for the bioremediation of heavy metals such as lead and cadmium.

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1.0 Introduction

Pollution due to chemicals including heavy metals is a problem that may have negative consequences on the biosphere. The levels of metals in all environments, including air, water and soil are increasing in some cases to toxic levels, with contributions from a wide variety of industrial and domestic sources. Metal contaminated environments pose a serious threat to health and ecosystems. The most abundant pollutants in waste water and in sewage are heavy metals (Nessim *et al.*, 2011). The environmental pollution by heavy metals comes from anthropogenic sources such as smelters, power stations and the application of pesticides containing metal, fertilizer and sewage sludge. Also, the contamination of soil by heavy metals is the result of different industrial activities, such as mining, the production of batteries for vehicles, and the irresponsible disposal of wastes by various industries and the dispersal of ash from incineration processes. Some of the negative impacts of heavy metals on plants include decrease of seed germination and lipid content by cadmium, decreased enzyme activity and plant growth by chromium, the inhibition of photosynthesis by copper and mercury, the

reduction of seed germination by nickel and the reduction of chlorophyll production and plant growth by lead (Mulligan *et al.*, 2005).

Adsorption is a widely used method for the treatment of industrial wastewater containing colour, heavy metals and other inorganic and organic impurities This method suffers from low adsorption capacity and in some cases complete removal is not possible and high cost of the adsorbent (Pinedo *et al.*, 2017). Chemical oxidation is a process in which the waste materials from the industrial wastewater are removed by the help of chemical oxidation by the use of various chemicals mainly hydrogen peroxide is widely used for this purpose as reported (Dias-Machado *et al.*, 2006; Ksibi, 2006). Other remediation methods commonly used are; soil washing/leaching/flushing with chemical agents, chemical immobilization/stabilization method to reduce the solubility of heavy metals, electrokinetics and dilution method (Wuana *et al.*, 2019). All these methods have some limitations and the common problems that are associated with these methods are expensive and can themselves produce other waste disposal problems,

which have limited their industrial applications (Debayan, 2012).

Among the available treatment processes, the application of the biological processes is gradually getting momentum due to the following reasons: Chemical requirement for the whole treatment process is reduced, Low operating cost, Eco-friendly and cost-effective alternative of conventional techniques, efficient at lower levels of contamination. Most metals do not completely undergo chemical degradation and their concentrations increase over time but this is not so for organic contaminants which are oxidized to carbon dioxide by microbial activities. Therefore, adequate restoration of soil ecosystems contaminated by heavy metals is required and the process by which these heavy metals are removed from the soil using biological means is called Bioremediation (Perfumo *et al.*, 2013). Bioremediation is the use of microorganisms or their enzymes to break down and thereby detoxify dangerous chemicals in the environment (Obayori *et al.*, 2019). It plays a major role in making the environment clean from pollutants and contamination (Arun *et al.*, 2014). Microbial activity is thought to play a key role in the detoxification of metals in water and soil. Therefore this study aimed to bioremediate heavy metals by bacteria isolated from mechanic workshop soil in FCT, Abuja.

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 8000km² and lies within latitude 9° 25' N and 9°20' N of the equator and longitude 5° 45' E and 39° E with a current population of about 3,464,000. 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) 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 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.

2.4 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 37 °C for 24 hrs.

2.5 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.

2.6 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 (2017). Also, the isolates were subsequently identified using the Bergey's manual of Determinative Bacteriology.

2.6.1 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.

2.6.2 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, 2017).

2.6.3 Citrate utilization Test

Some amount of simmons-citrate agar (22.5g) was dissolved in 1 litre of distilled water and autoclave at 121°C 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 37°C 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.

2.6.4 Oxidase test

This test indicates the presence of cytochrome oxidase that is able to reduce oxygen (O₂) and artificial electron acceptors (Fawole and Oso, 2017). 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.

2.6.5 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 37°C 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.

2.6.6 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 °C 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, 2012).

2.6.7 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 121°C for 15 minutes. They were cooled

and a loopful of the test bacteria were inoculated into each test tube and incubated at 37°C 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% α -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 (Fawole and Oso, 2012).

2.6.8 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 121°C 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 37°C for 48 hours. Development of red colouration with blackish spots indicates H₂S reaction arising from TSI utilization which is positive while a negative result gives no reaction (Fawole and Oso, 2012).

2.6.9 Catalase Test

Two drops of 3% hydrogen peroxide (H₂O₂) 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, 2012).

2.6.10 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, 2017).

2.7 Heavy Metal Analysis

The heavy metals in the soil from which bacteria were isolated were determined.

2.7.1 Sample Digestion Procedure

This was carried out in a Kjeldahl flask by wet digestion using oxidizing acid. Oxidizing acids used include HNO₃ and H₂SO₄. About 1 g of each sample was digested with 2.0 cm³ concentrated HNO₃, and 1 cm³ concentrated H₂SO₄ in a 50 cm³ Kjeldahl flask.

The blank sample was prepared by repeating the same procedure but omitting the soil sample.

Heat was applied gently by the heating mantle at moderate temperature until foaming ceases. Heating was continued until the solution turned cleared and colourless. The solution was allowed to cool. After cooling, 5ml of distilled water was added to the digested solution and was heated for about ten (10) minute after which the solution was allowed to cool down. The digested sample was transferred into a 50ml volumetric flask and was made up to the mark and stored in sample bottles ready for analysis by the Atomic Absorption Spectrophotometer (AAS) analysis (Chen and Wang, 2016).

2.7.2 Analysis of lead and cadmium by Atomic Absorption Spectrophotometer

The concentration of lead and cadmium was determined using flame Atomic Absorption Spectrophotometry (AAS). A calibration graph was plotted for each element using measured absorbance and the corresponding concentration. The calibration curve was then used to determine the concentration of the heavy metals. The Heavy Metal Analysis was carried out in SHESTCO Kwali.

2.8 Screening of Bacteria Isolates for Heavy metal remediation

The bacterial isolates were subjected to screening for heavy metal remediation. Mineral Salt medium containing diesel oil (2%) as a sole source of carbon and agar (1.5%) was used to screen the bacteria isolated from the petroleum contaminated soil. Bacteria that were able to use the medium for growth were selected for bioremediation of heavy metal in petroleum contaminated soil.

2.9 Standardization of Bacterial isolates for Bioremediation Potential

Bacteria that were able to use the medium for growth were standardize using Mcfarland turbidity standards number 0.5. Barium Chloride (1.175g) was dissolved in 100 ml distilled water. One (1) milliliter of Sulphuric acid was measured into 100 ml of water.

For the turbidity standard of 0.5: 0.5ml of Barium Chloride Solution was added to 99.5 ml of H₂SO₄ Solution. This is equivalent to bacterial density of approximately 1x10⁸ colonies per milliliter. The isolates were diluted in test tubes using sterile water and then compared with the Mcfarland turbidity.

2.10 Heavy Metal (Cadmium and Lead) Bioremediation Potential by Bacteria Isolates

The previously standardised bacteria isolate was tested individually on the soil sample in which heavy metal concentration was earlier determined. One gram (1g) of each soil samples was dispensed in 5 ml of bacterial density of approximately 1x10⁸ colonies per milliliter of broth and incubated at room temperature (37°C) for 5 days and the soil sample inoculum was incubated at the same temperature and days, serving as the negative control. After which the broth was filtered out using filter paper and the sediment (soil) was analysed for the heavy metal concentration in the remediated soil (Arun *et al.*, 2014).

2.11 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 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*. Soil samples for metal analysis were found to contain Lead and cadmium. *Pseudomonas aeruginosa* had a significant ability to reduce lead and cadmium concentration in contaminated soil as compared with other bacterial isolates.

Table 3.1: Morphological and Biochemical Characterization of Bacterial Isolates

S/N	Isolate Code	Gram Rxn	Cell Morphology	Catalase	Oxidase	Methyl Red	Voges Proskauer	Coagulase	Urease	H ₂ S	Starch	Glucose	Lactose	Mannitol	Maltose	Sucrose	Citrate Utilization	Indole Test	Endospore	Motility	Probable Identity
1	BS 1	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	<i>Bacillus subtilis</i>
2	BS 2	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	<i>Bacillus subtilis</i>
3	BS 3	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	<i>Bacillus subtilis</i>
4	BS 4	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	<i>Bacillus subtilis</i>
5	BS 5	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	<i>Bacillus subtilis</i>
6	BS 6	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	<i>Bacillus subtilis</i>
7	BS 7	+	Rod	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	<i>Bacillus subtilis</i>
8	BL 1	+	Rod	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	<i>Bacillus licheniformis</i>
9	BL 2	+	Rod	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	<i>Bacillus licheniformis</i>
10	B C1	+	Rod	+	+	+	+	-	-	+	+	+	-	-	+	-	+	-	+	+	<i>Bacillus cereus</i>
11	B C2	+	Rod	+	+	+	+	-	-	+	+	+	-	-	+	-	+	-	+	+	<i>Bacillus cereus</i>
12	B C3	+	Rod	+	+	+	+	-	-	+	+	+	-	-	+	-	+	-	+	+	<i>Bacillus cereus</i>
13	B M 1	+	Rod	+	-	+	-	-	+	-	+	-	-	-	-	-	+	-	+	+	<i>Bacillus megaterium</i>
14	KP 1	-	Rod	+	-	-	+	-	-	-	+	+	+	+	+	+	+	-	-	-	<i>Klebsiella pneumoniae</i>
15	PA 1	-	Rod	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	+	<i>Pseudomonas aeruginosa</i>
16	PA 2	-	Rod	+	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	+	<i>Pseudomonas aeruginosa</i>
17	PA 3	-	Rod	+	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	+	<i>Pseudomonas aeruginosa</i>
18	PA 4	-	Rod	+	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	+	<i>Pseudomonas aeruginosa</i>
19	PA 5	-	Rod	+	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	+	<i>Pseudomonas aeruginosa</i>

Keys: += Positive, - = Negative

3.2 The reduction of Lead in diesel contaminated soil by the bacterial isolates

Table 3.2 shows the bioremediation of lead in by bacteria isolated from mechanic workshop soil. Although, the effect of bacterial density from soil bacteria on Lead reduction indicated that *Pseudomonas aeruginosa* had the highest effect on heavy metals as indicated by the $11.09 \pm 0.50 \mu\text{g/g}$

reduction of the heavy metal in the soil followed by *Bacillus megaterium* ($1.36 \pm 0.00 \mu\text{g/g}$), but the bioremediation of lead by the bacteria isolated from mechanic workshop soil were significantly different ($P \geq 0.05$). No effect was observed on heavy metal reduction by *Bacillus licheniformis* and *Klebsiella pneumoniae*.

Table 3.2: Bioremediation of Lead by Bacteria isolated from mechanic workshop Soil

Isolates	(A) Initial Concentration of Lead($\mu\text{g/g}$)	(B) Final Concentration of Lead($\mu\text{g/g}$)	(C) Amount of Lead remediated ($\mu\text{g/g}$)
<i>Bacillus subtilis</i>	86.31 ± 0.00	86.02 ± 0.00	0.29 ± 0.07
<i>Bacillus megaterium</i>	86.31 ± 0.00	84.95 ± 0.07	1.36 ± 0.00
<i>Bacillus cereus</i>	86.11 ± 0.00	86.01 ± 0.09	0.10 ± 0.00
<i>P. aeruginosa</i>	86.31 ± 0.00	75.22 ± 3.89	11.09 ± 0.50
<i>Bacillus licheniformis</i>	86.11 ± 0.00	86.11 ± 0.11	0.00 ± 0.00
<i>Klebsiella pneumonia</i>	86.31 ± 0.00	86.31 ± 0.00	0.00 ± 0.00

Values are mean \pm Standard deviation. Since P value = 0.15, the bioremediation of lead by bio-surfactants produced by all the bacterial isolates from mechanic workshop soil were significantly different ($P \geq 0.05$), when compared to the control Rhamnolipid.

Key words: A, initial concentration of lead
B, final concentration of lead
C is A-B, final amount of lead that was remediated

3.3 The reduction of Cadmium in diesel contaminated soil by Bacterial Isolates

Pseudomonas aeruginosae had the highest remediating impact on cadmium in the soil from the mechanic workshop (Table 3.3). *Bacillus meaterium* had a cadmium reduction of $1.24 \pm 0.50 \mu\text{g/g}$ while

Bacillus subtilis and *Klebsiella pneumoniae* had no reduction in the concentration of cadmium heavy metal. The bioremediation of cadmium by bacteria isolated from mechanic workshop soil were not significantly different ($P \leq 0.05$).

Table 3.3: Bioremediation of Cadmium by Bacteria isolated from mechanic workshop Soil

Isolates	(A) Initial Concentration of Cadmium ($\mu\text{g/g}$)	(B) Final Concentration of Cadmium ($\mu\text{g/g}$)	(C) Amount of of Cadmium remediated ($\mu\text{g/g}$)
<i>Bacillus subtilis</i>	9.63 ± 0.00	9.63 ± 0.00	0.00 ± 0.00
<i>Bacillus megaterium</i>	9.63 ± 0.00	8.39 ± 0.50	1.24 ± 0.50
<i>Pseudomonas aeruginosa</i>	9.63 ± 0.00	6.95 ± 0.09	2.68 ± 0.09
<i>Klebsiella pneumonia</i>	9.63 ± 0.00	9.63 ± 0.00	0.00 ± 0.00

P value = 1.5, the bioremediation of cadmium by bio-surfactants produced by bacteria isolated from mechanic workshop soil were not significantly different ($P \leq 0.05$). The experiments are representatives of the mean values of individual experiment (3replicates).

Key words: A, initial concentration of lead
B, final concentration of lead
C is A-B, final amount of lead that was remediated

4.0 Discussion

The effect of bacteria isolates on Lead reduction indicated that *Pseudomonas aeruginosa* had the highest effect on heavy metals as indicated by the

$11.09 \pm 0.50 \mu\text{g/g}$ reduction of the heavy metal in the soil followed by *Bacillus megaterium* ($1.36 \pm 0.00 \mu\text{g/g}$). No effect was observed on heavy metal reduction by *Bacillus licheniformis* and *Klebsiella*

pneumoniae. The different lead concentrations recorded in this study is higher than both the World Health Organisation permissible limit of 0.005 mg/kg and the Federal Environmental Protection Agency of Nigeria (0.01 mg/kg). *Pseudomonas aeruginosa* had the highest ($2.68 \pm 0.09 \mu\text{g/g}$) remediating impact on cadmium in the soil from the mechanic workshop. *Bacillus megaterium* had a cadmium reduction of $1.24 \pm 0.00 \mu\text{g/g}$ while *Bacillus subtilis* and *Klebsiella pneumoniae* had no reduction in the concentration of cadmium heavy metal. The cadmium concentration in the current study is slightly higher than the concentration reported by Biose *et al.* (2021) reported cadmium concentration of $7.41 \pm 1.54 \text{ mg/kg}$, $6.66 \pm 3.91 \text{ mg/kg}$, $9.31 \pm 3.95 \text{ mg/kg}$, $5.10 \pm 2.33 \text{ mg/kg}$, $5.82 \pm 0.13 \text{ mg/kg}$ in four different soil in Benin City. The absence of mining activities in these areas suggests that there are other heavy metals contamination routes. Lead contamination in urban soil has been ascribed to ignition of fuel that contains tetraethyl lead as anti-knock agent (Durowoju *et al.*, 2018). The high cadmium content on the other hand may be attributed to various possible environmental pollutants such as agricultural discharge and through incineration of municipal waste materials (Biose *et al.*, 2021); dumping and incineration is a common sight in Lugbe in Federal Capital Territory, Nigeria. Although soil samples were collected from mechanic shops, these soils can also be exposed to environmental pollutants such as agricultural discharge. The contamination of soil from exhaust fumes may also be a possibility. Cigarette smoking, according to the study of Muhammad (2012) may also contribute to the high Cadmium and Lead concentration. Study by Muhammad (2012) indicates that average concentration of cadmium and lead in cigarettes may reach up to 1.81 and 2.46 $\mu\text{g/g}$ respectively. The result of the current study showed that *Pseudomonas aeruginosa* was the highest ($p < 0.05$) bacteria isolated from spent oil contaminated soil. In this study, *Pseudomonas aeruginosa* had a significant ($p < 0.05$) ability to reduce lead concentration in contaminated soil. The bacterium reduced lead concentration in the polluted soil followed by *Bacillus megaterium* and *Bacillus licheniformis*. However, *Bacillus subtilis*, *Bacillus cereus*, and *Pseudomonas aeruginosa* had varying lead reduction activity in the polluted soil. Of these, *Pseudomonas aeruginosa* showed the highest ($p < 0.05$) lead reduction activity. *Pseudomonas aeruginosa*, *Bacillus megaterium* and *Bacillus licheniformis* also exhibited the potential to reduce cadmium concentration as well. There was no significant difference in the cadmium reduction potential of the bacteria. The ability of the bacteria isolates to reduce heavy metal concentration in the current study agrees with the study of Ravindran *et al.*

(2020) that showed removal of 75.5% Hg, 97.73% Pb, 89.5% Mn, and 99.93% Cd, respectively, in 1,000 ppm of the respective metal solution by bacteria isolates.

4.1 Conclusion

Based on the result of this study, it can be concluded that; of the bacteria isolated, *Pseudomonas aeruginosa* had the highest reduction activity against lead and cadmium. *Klebsiella pneumoniae* have no potential for heavy metal bioremediation. The lead and cadmium concentration in the current study are higher than the permissible limits of FEPA and World Health Organization.

4.2 Recommendation

Based on the result of this study, it is therefore recommended that further study should be carried out to ascertain the bioactive substance in *Pseudomonas aeruginosa* responsible for the remediation of heavy metals such as lead and cadmium. *In Situ* bioremediation of contaminated soil by autochthonous bacterial inhabitants of contaminated soil may be harnessed in further studies.

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