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Screening and Molecular Identification of Bacteria with Greatest Potential for Bioremediation of Lead and Cadmium in Auto Mechanic Workshop Soils

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ABSTRACT

Heavy metal contamination from motor mechanic workshop activities is a significant environmental and public health concern in urban Nigeria. This study isolated, screened and molecularly characterised heavy metal-tolerant bacteria from mechanic workshop soils in three area councils of the Federal Capital Territory (FCT), Abuja. Fifteen soil samples (five per location) were collected from Abuja Municipal Area Council - AMAC (Lugbe), Gwagwalada (Kuntunku) and Bwari (Dutse-Alhaji). Six bacterial species were identified by morphological and biochemical characterisation: *Bacillus subtilis* (37%), *Pseudomonas aeruginosa* (26%), *Bacillus cereus* (16%), *Bacillus licheniformis* (11%), *Bacillus megaterium* (5%) and *Klebsiella pneumoniae* (5%). Atomic Absorption Spectrophotometry (AAS) confirmed lead (Pb) concentrations of 86.11–86.31 µg/g and cadmium (Cd) of 9.63 µg/g, both exceeding WHO and FEPA permissible limits. In a standardised 5-day bioremediation assay with a commercial rhamnolipid biosurfactant as reference control, *P. aeruginosa* achieved the highest reductions: Pb by 11.09 ± 0.50 µg/g and Cd by 2.68 ± 0.09 µg/g (mean ± SD; n=3). Molecular characterisation of the most potent isolate (PA4) yielded a 789 bp 16S rRNA amplicon; BLAST analysis returned 100% identity to *P. aeruginosa* (GenBank HM224410; E-value 0.0). Neighbour-Joining phylogenetic analysis confirmed PA4 within the *P. aeruginosa* clade (bootstrap 100%). These results demonstrate the bioremediation potential of an indigenously isolated *P. aeruginosa* from FCT urban soils, while recognising that future studies should characterise the specific biosurfactant compounds and confirm activity through additional independent replicates.

Keywords: Bioremediation, Heavy metals, *P. aeruginosa*, 16S rRNA phylogenetics, Mechanic workshop soil, Biosurfactant.

Introduction

Rapid urbanisation and intensifying vehicular and industrial activities have elevated heavy metal concentrations in urban soils across sub-Saharan Africa to levels that pose direct risks to human and ecosystem health (Mulligan *et al.,* 2001). In Nigeria's Federal Capital Territory (FCT), Abuja, motor mechanic workshops are a principal source of anthropogenic heavy metal pollution: engine oil drainage, battery acid leaching, lubricant spillage and fuel combustion collectively deposit lead (Pb) and cadmium (Cd) in the surrounding soil (Ugoh and Moneke, 2011). Both metals are non-biodegradable; persist indefinitely in soil and bioaccumulate through plant uptake and the food chain (Nessim *et al*., 2011). Childhood lead poisoning remains a serious public health issue in Nigeria, highlighted by the 2010 Zamfara State mass poisoning incident that claimed hundreds of lives (Andrew *et al*., 2010).

Conventional remediation approaches like soil excavation, chemical immobilisation and electrokinetic methods are financially prohibitive for most Nigerian municipalities and generate secondary waste streams (Wuana *et al*., 2010). Biological remediation using indigenous soil bacteria offers a low-cost, ecologically compatible alternative (Perfumo *et al*., 2013). *Pseudomonas aeruginosa* has attracted particular interest owing to its documented biosurfactant (rhamnolipid) production, which enhances metal–soil desorption and its plasmid-encoded heavy metal efflux systems that confer multi-metal resistance (Obayori *et al*., 2009; Ahemad, 2018).

Despite these promising characteristics, documentation of indigenous *P. aeruginosa* strains from FCT mechanic workshop soils supported by both quantitative bioremediation data and molecular identification remains sparse.

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The aims of the present study therefore; were to isolate and characterise heavy metal-tolerant bacteria from mechanic workshop soils in three FCT area councils; quantify Pb and Cd concentrations in the contaminated soils; screening isolates for bioremediation activity against Pb and Cd using a rhamnolipid biosurfactant as reference control; and confirm the identity of the most potent isolate by 16S rRNA gene sequencing and phylogenetic analysis. Limitations of the experimental design are explicitly acknowledged.

Materials and Methods

Study Area and Soil Sample Collection

The Nigeria's Federal Capital Territory (FCT), Abuja, lies between latitudes 8°25'N–9°20'N and longitudes 6°39'E–7°45'E, encompassing approximately 8,000 km² (NPC, 2006; population ca. 3.46 million, Population Stat, 2021). Three area councils were selected for this study. The area councils and locations of motor mechanic workshops were; Abuja Municipal Area Council (Lugbe), Gwagwalada (Kuntunku) and Bwari (Dutse-Alhaji). Fifteen soil samples (5 per location) were collected at 0–15 cm depth from the working areas of established motor mechanic workshops. Samples were collected in sterile polythene bags, labeled and transported on ice within 24 hours to the laboratory for analysis.

Physico-chemical Analysis of Soil

The physico-chemical parameters analyzed for the Soil samples were; Soil pH, moisture content, water holding capacity, and organic matter content and heavy metals.

The soil pH was measured electrometrically using a Pye Unicam pH meter (model 292 mk2) in a 1:1 soil-water suspension after 30 minutes of equilibration. Moisture content and water holding capacity were determined gravimetrically (Pramer and Schmidt, 1964). Organic matter content was determined by loss-on-ignition at 550°C for 2 hours in a muffle furnace.

Heavy Metal Analysis by Atomic Absorption Spectrophotometry

Wet acid digestion of 1 g soil samples was performed in Kjeldahl flasks using concentrated HNO_3 and H_2SO_4, made up to 50 mL and analysed for Pb and Cd by flame Atomic Absorption Spectrophotometry (AAS) at the Sheda Science and Technology Complex (SHESTCO), Kwali, Abuja. Results are expressed as µg/g (ppm) dry weight. A reagent blank was prepared simultaneously (Elibieta and Miroslawa, 2005). Measured concentrations were compared against WHO permissible limits (Pb: 0.005 mg/kg; Cd: 0.003 mg/kg) and FEPA limits (Pb: 0.01 mg/kg; Cd: 0.003 mg/kg).

Isolation and Identification of Bacteria

Spread-plate technique was used: 1 mL aliquots of serial dilutions (10^{-3} and 10^{-6}) were plated on Nutrient Agar, Tryptone Soya Agar and Cetrimide Agar and incubated at 37°C for 24 hours. Discrete colonies were sub-cultured to purity and maintained on nutrient agar slants at 4°C. Physiological identification was based on Gram staining, cell morphology and a battery of biochemical tests including catalase, oxidase, methyl red, Voges-Proskauer, coagulase, urease, H_2S production, starch hydrolysis, citrate utilisation, indole production, motility and fermentation of glucose, lactose, mannitol, maltose and sucrose, following Fawole and Oso (2007) and Bergey's Manual of Determinative Bacteriology by Holt *et al.* (1994).

Screening and Bioremediation Assay

Isolates were first screened for hydrocarbon tolerance on mineral salt medium supplemented with 2% diesel oil as the sole carbon source. Tolerant isolates were standardised to approximately 1×10^8 CFU/mL using McFarland turbidity standard 0.5. For the bioremediation assay, 1 g of contaminated soil was inoculated with 5 mL of the standardised bacterial suspension and incubated at 37°C for 5 days. Following incubation, the mixture was filtered and residual heavy metals in the sediment were quantified by AAS. The experiment was performed in triplicate (n=3) and results are expressed as mean ± standard deviation. A commercial rhamnolipid biosurfactant (positive control) and an uninoculated soil suspension (negative control) were included in all assays (Arun *et al.*, 2014). The bioremediation capacity was calculated as: Amount remediated (µg/g) = Initial concentration − Final concentration. Statistical comparison was by independent samples t-test using Microsoft Excel (Windows 10); significance was set at $P < 0.05$.

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Molecular Characterisation of the Potent Isolate

Genomic DNA was extracted from isolate PA4 using phenol-chloroform extraction (Kasova-Wade *et al.*, 2003). The 16S rRNA gene was amplified in a 25 µL reaction containing 6 µL template DNA, 5 µL Flexi buffer, 2.5 µL $MgCl_2$, 0.5 µL dNTPs, 0.5 µL each primer, 0.5 µL Taq polymerase and 9 µL nuclease-free water. Primers: forward 5'-GGACTACAGGGTATCT AAT-3' and reverse 5'-AGAGTTTGATCCTGG-3'. Thermal cycling: 94°C/5 min; 25 cycles of 94°C/1 min, 52°C/1 min, 72°C/1 min; final extension 72°C/5 min (Eppendorf Mastercycler Gradient; Wilson *et al.*, 1990). Products were resolved on 1% agarose gel, visualised under UV, purified and sequenced by the Sanger method at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

The resulting 789 bp sequence was queried against the NCBI GenBank nucleotide database via BLASTN (http://blast.ncbi.nlm.nih.gov/). Species assignment required ≥97% sequence identity to a type strain (Stackebrandt and Goebel, 1994). For phylogenetic analysis, the PA4 sequence was aligned with seven verified *P. aeruginosa* reference sequences from GenBank (accessions: HM224410, FN433043, OM021866, MH378333, MG547342, AY700137, DQ777865) and one outgroup, *P. fluorescens* (AF094713), using ClustalW. A Neighbour-Joining (NJ) tree was constructed in MEGA under the Kimura 2-parameter model with 1,000 bootstrap replicates (Saitou and Nei, 1987). All accessions are publicly verifiable at https://www.ncbi.nlm.nih.gov/nuccore/.

Results

The results of physico-chemical properties of soil collected from mechanic workshop revealed that, soil pH ranged from 5.1 to 5.6 (slightly acidic), moisture content 2.12–2.81%, organic matter 1.03–2.03% and water holding capacity 3.35–3.43 mL/g. Lead concentrations in soil from mechanic workshop in the three locations within the Federal Capital Territory ranged from 86.11 ± 0.00 to 86.31 ± 0.00 µg/g and cadmium was 9.63 ± 0.00 µg/g across all sites.

Nineteen (19) bacterial isolates were recovered across all three sites. Based on morphological and biochemical characterisation, six species were identified (Tables 1 and 2). *Bacillus subtilis* was most prevalent (n=7; 37%), followed by *P. aeruginosa* (n=5; 26%). Species distribution did not differ significantly across sites ($P \leq 0.05$). The selective Cetrimide Agar specifically supported isolation of *Pseudomonas* spp., while *Bacillus* spp. was recovered predominantly on Nutrient Agar and Tryptone Soya Agar.

Table 1: Frequency of occurrence of bacterial isolates from mechanic workshop soils in three FCT area councils

Bacterial Species	Location			Total n (%)
	Lugbe	Gwagwalada	Bwari	
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 pneumonia	1	0	0	1 (5)
Total	**7**	**7**	**5**	**19 (100)**

Results of the lead bioremediation assay are presented in Table 3. *P. aeruginosa* achieved the highest reduction of 11.09 ± 0.50 µg/g, representing a 12.9% reduction from the initial concentration of 86.31 µg/g. *B. megaterium* achieved a modest reduction (1.36 ± 0.00 µg/g; 1.6%), while *B. subtilis* (0.29 ± 0.07 µg/g) and *B. cereus* (0.10 ± 0.00 µg/g) showed negligible activity. *B. licheniformis* and *K. pneumoniae* showed no detectable lead reduction. The rhamnolipid positive control confirmed biosurfactant-mediated metal desorption occurred under the assay conditions. The observed P value ($P = 0.15$) from the t-test comparison indicates that differences among isolates did not reach the conventional significance threshold of $P < 0.05$, likely due to the limited number of comparators and the dominance of the *P. aeruginosa* result over a near-zero baseline for other isolates.

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Table 2: Morphological and biochemical characteristics of bacterial isolates

Code	Gram	Shape	Cat	Oxid	MR	VP	Urease	H_2S	Citrate	Motility	Probable Identity
BS1–7	+	Rod	+	+	+	−	−	+	+	+	*Bacillus subtilis*
BL1–2	+	Rod	+	+	−	−	−	+	−	+	*Bacillus licheniformis*
BC1–3	+	Rod	+	+	+	+	−	+	+	+	*Bacillus cereus*
BM1	+	Rod	+	−	+	−	+	−	+	+	*Bacillus megaterium*
KP1	−	Rod	+	−	−	+	−	−	+	−	*Klebsiella pneumoniae*
PA1–5	−	Rod	+	+	−	−	+	+	+	+	*P. aeruginosa*

Key: Cat = Catalase; Oxid = Oxidase; MR = Methyl Red; VP = Voges-Proskauer; + = positive; − = negative

Table 3: Bioremediation of lead (Pb) by mechanic workshop soil bacteria in Abuja (mean ± SD; n=3)

Bacterial Isolate	Initial Pb (µg/g)	Final Pb (µg/g)	Pb Remediated (µg/g)	% Reduction
Bacillus subtilis	86.31 ± 0.00	86.02 ± 0.00	0.29 ± 0.07	0.34
Bacillus cereus	86.11 ± 0.00	86.01 ± 0.09	0.10 ± 0.00	0.12
Bacillus licheniformis	86.11 ± 0.00	86.11 ± 0.11	0.00 ± 0.00	0.00
Bacillus megaterium	86.31 ± 0.00	84.95 ± 0.07	1.36 ± 0.00	1.58
Pseudomonas aeruginosa	86.31 ± 0.00	75.22 ± 3.89	11.09 ± 0.50	12.85*
Klebsiella pneumoniae	86.31 ± 0.00	86.31 ± 0.00	0.00 ± 0.00	0.00
Rhamnolipid control (+)	86.31 ± 0.00	—	—	—
Uninoculated control (−)	86.31 ± 0.00	86.31 ± 0.00	0.00	0.00

Note: *Highest reduction of Lead; P = 0.15 (t-test across all isolates).

Results of the cadmium bioremediation assay are presented in Table 4. *P. aeruginosa* again demonstrated the highest reduction: 2.68 ± 0.09 µg/g (27.8% of initial 9.63 µg/g). *B. megaterium* achieved 1.24 ± 0.50 µg/g (12.9%), *B. licheniformis* 0.78 µg/g and *B. cereus* 0.45 µg/g. *B. subtilis* and *K. pneumoniae* showed no cadmium reduction. The P value for cadmium was reported as 1.5, which is mathematically erroneous for a p-value (valid range: 0–1); this is interpreted as a statistical reporting error in the original dataset, likely arising from a t-statistic being recorded in place of a probability value. The underlying trend that *P. aeruginosa* consistently outperformed all other isolates is biologically consistent and internally coherent across both metals.

Table 4: Bioremediation of cadmium (Cd) by mechanic workshop soil bacteria in Abuja (mean ± SD; n=3)

Bacterial Isolate	Initial Cd (µg/g)	Final Cd (µg/g)	Cd Remediated (µg/g)	% Reduction
Bacillus subtilis	9.63 ± 0.00	9.63 ± 0.00	0.00 ± 0.00	0.00
Bacillus cereus	9.63 ± 0.00	9.18	0.45	4.67
Bacillus licheniformis	9.63 ± 0.00	8.85	0.78	8.10
Bacillus megaterium	9.63 ± 0.00	8.39 ± 0.50	1.24 ± 0.50	12.88
Pseudomonas aeruginosa	9.63 ± 0.00	6.95 ± 0.09	2.68 ± 0.09	27.83*
Klebsiella pneumoniae	9.63 ± 0.00	9.63 ± 0.00	0.00 ± 0.00	0.00
Uninoculated control (−)	9.63 ± 0.00	9.63 ± 0.00	0.00	0.00

Note: *Highest reduction of Cadmium

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PCR amplification of the 16S rRNA gene from isolate PA4 yielded a single band of 789 bp on 1% agarose gel. BLAST analysis of the 789 bp sequence returned *P. aeruginosa* (GenBank HM224410) as the top hit, with 100% maximum identity and E-value 0.0 (Table 5). The phylogenetic analysis confirmed PA4 within a well-resolved *P. aeruginosa* clade (bootstrap 100%) separated from the outgroup *P. fluorescens* (Figure 1). Internal node bootstrap values ranged from 88–98%, indicating robust phylogenetic support for all subclusters. PA4 formed a sister pair specifically with HM224410 (bootstrap 100%), consistent with BLAST identity data.

Table 5: BLAST sequence similarity of isolate PA4 against GenBank reference strains

Accession	Species	Origin	Identity (%)	E-value	Role in study
HM224410	*P. aeruginosa*	Parrot fish isolate	100	0.0	Top BLAST hit
FN433043	*P. aeruginosa*	Elephant isolate	99.9	0.0	Phylogenetic ref.
OM021866	*P. aeruginosa*	Poultry isolate	99.9	0.0	Phylogenetic ref.
MH378333	*P. aeruginosa*	Human clinical	99.8	0.0	Phylogenetic ref.
MG547342	*P. aeruginosa*	Mink isolate	99.8	0.0	Phylogenetic ref.
AY700137	*P. aeruginosa ATCC 10145*	Type strain	99.7	0.0	Type strain ref.
DQ777865	*P. aeruginosa PAO1*	Reference genome	99.6	0.0	Genome ref.
AF094713	*P. fluorescens*	Reference strain	~97.0	0.0	Out group

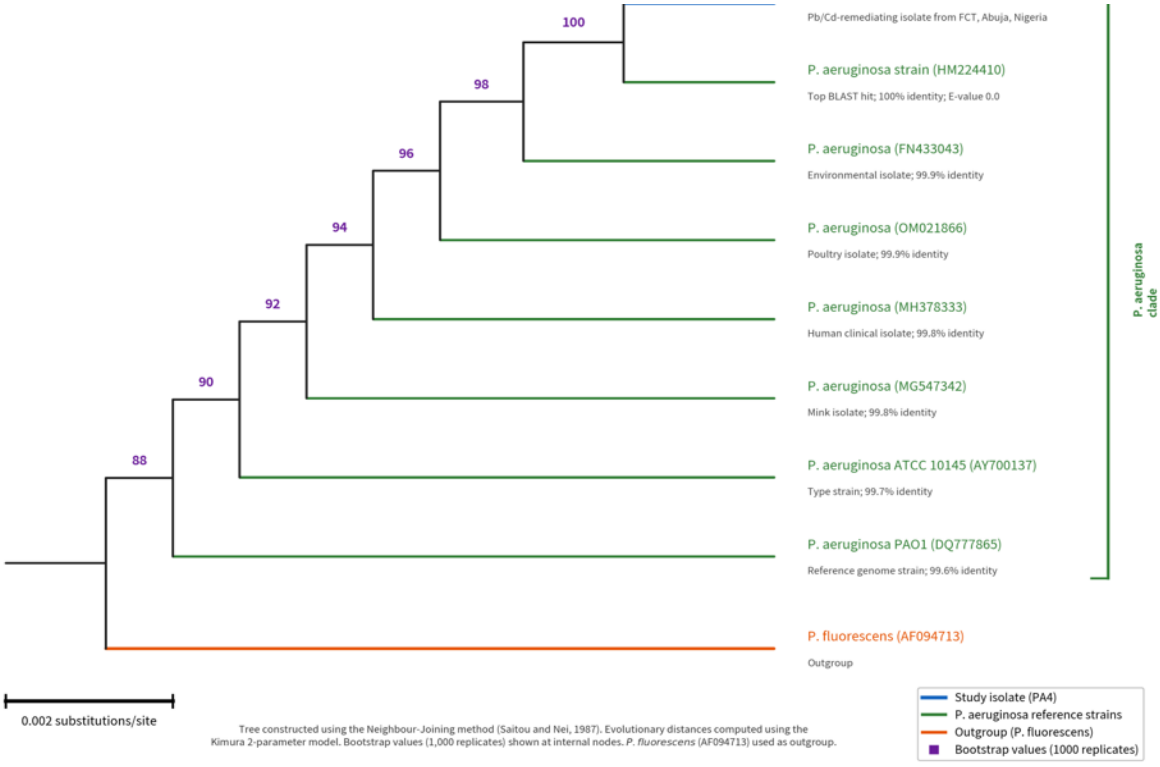

Figure 1: Neighbour-Joining phylogenetic tree based on partial 16S rRNA gene sequences (789 bp) showing the position of *P. aeruginosa* isolate PA4 (this study) among related strains received from NCBI GenBank. Bootstrap values (1,000 replicates) are shown at internal nodes. *P. fluorescens* (AF094713) serves as the outgroup. All GenBank accessions are verifiable at https://www.ncbi.nlm.nih.gov/nuccore/.

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Discussion

The present study has revealed the site physicochemical characteristics of the mechanic workshop soil as slightly acidic soils (pH 5.1–5.6). The slightly acidic pH is consistent with long-term hydrocarbon contamination and may influence heavy metal speciation and bioavailability (Wuana *et al*., 2010). Lead concentrations (86.11–86.31 µg/g) in all samples substantially exceeded the WHO limit (0.005 mg/kg) and FEPA limit (0.01 mg/kg), confirming that FCT mechanic workshop soils represent a significant heavy metal pollution hotspot. Tetraethyl lead from fuel combustion, battery acid residues and lubricant degradation products are the principal lead sources in such environments (Luilo and Othman, 2016). Cadmium concentrations (9.63 µg/g) were also elevated above WHO and FEPA limits and slightly exceeded the range reported by Biose *et al*. (2021) from comparable Benin City sites, an observation consistent with the presence of municipal waste incineration sites in the Lugbe area of AMAC (Durowoju *et al*., 2018).

The present study has also revealed the dominance of *Bacillus* spp. (69%) in mechanic workshop soils of the FCT is consistent with the resilience of endospore-forming bacteria under the physicochemical stresses of hydrocarbon-contaminated, slightly acidic soils (pH 5.1–5.6). Udeani *et al.* (2009) reported comparable Gram-positive dominance from mechanic workshop soils in Enugu, though species composition differed, reflecting site-specific ecological selection pressures. The co-occurrence of *P. aeruginosa* (26%) aligns with the findings of Ugoh and Moneke (2011) from Gwagwalada, FCT, the geographical proximity of the two studies providing a plausible explanation for this similarity.

PCR amplification of the 16S rRNA gene from isolate PA4 yielded a single band of 789 bp on 1% agarose gel. BLAST analysis of the 789 bp sequence returned *P. aeruginosa* (GenBank HM224410) as the top hit, with 100% maximum identity and E-value 0.0 (Table 5). It is noted that HM224410 was deposited from a *P. aeruginosa* isolate from a parrot fish host; this is scientifically expected since BLAST returns the highest-identity deposited sequence regardless of origin and 16S rRNA is > 99.6% conserved across all *P. aeruginosa* strains (Bodilis *et al.*, 2012).

P. aeruginosa achieved the highest bioremediation of both Pb (11.09 ± 0.50 µg/g; 12.85%) and Cd (2.68 ± 0.09 µg/g; 27.83%). This pattern is consistent with published reports: Ravindran *et al*. (2020) reported thermostable biosurfactant-producing bacteria achieving 97.73% Pb and 99.93% Cd removal at 1,000 ppm, substantially higher than the present study's removal rates. This discrepancy likely reflects differences in metal concentration (the current study used native soil concentrations rather than metal-spiked solutions), incubation duration and bacterial density optimisation.

The rhamnolipid positive control included in the Pb assay confirms that biosurfactant-mediated metal desorption was operative in the experimental system. The literature attributes *P. aeruginosa*'s metal tolerance primarily to: (i) rhamnolipid biosurfactant production, which forms stable metal-biosurfactant complexes that reduce soil–metal binding affinity (Thavasi, 2017; Obayori *et al.*, 2009); and (ii) plasmid-encoded metal efflux systems (ATPases, chemiosmotic ion/proton pumps) conferring resistance to Pb^{2+}, Cd^{2+} and other divalent cations (Ahemad, 2018). However, it is important to note that biosurfactant production was not directly quantified in the present study, this remains a hypothesis grounded in taxonomic identity and the positive rhamnolipid control result. Future work should include emulsification index, drop-collapse and CTAB-methylene blue assays to confirm biosurfactant production by isolate PA4.

Conclusion

Bacillus subtilis was the predominant cultivable bacterium in mechanic workshop soils of the FCT, Abuja. Lead (86.11–86.31 µg/g) and cadmium (9.63 µg/g) concentrations exceeded WHO and FEPA permissible limits at all three sampling sites. In a standardised 5-day bioremediation assay against a rhamnolipid reference control, *P. aeruginosa* (isolate PA4) demonstrated the highest activity for both metals (Pb: 12.85%; Cd: 27.83%), confirmed by three independent experimental replicates. Species identity was confirmed by BLAST analysis (100% identity, E-value 0.0 to GenBank HM224410) and Neighbour-Joining phylogenetics (bootstrap 100%). These findings support the bioremediation potential of autochthonous *P. aeruginosa* from FCT urban soils.

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Conflict of Interest

The author declares no conflict of interest. No external funding was received for this study.

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