

# Bacteriological and physicochemical changes of automobile workshop contaminated soil amended with fish pond sludge: A bioremediation study from Lokoja, Nigeria

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**ABSTRACT:** The bacteriological and physicochemical changes of automobile workshop polluted soil in Lokoja, Kogi State, Nigeria, amended with varying concentrations of fish pond sludge (10%, 20% and 40%) was studied for six months. The bacteria population was assessed using serial dilution and plate counts after 24 hours of incubation on nutrient agar. Pure isolates were Gram-stained and subjected to molecular analysis. Physicochemical properties of the soil, such as pH, moisture content, organic carbon, organic matter content, nitrate and phosphate, were analysed using standard procedures. Molecular analysis of the isolates showed the presence of *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium xerosis*, *Lysinibacillus fusiformis*, *Micrococcus luteus*, *Paenibacillus polymyxa* and *Rhodococcus erythropolis*. The physicochemical analysis revealed the range of  $5.78 \pm 0.40$ -  $7.20 \pm 0.20$  for pH,  $6.47 \pm 1.03$  to  $12.72 \pm 1.91$  % for moisture content,  $0.24 \pm 0.04$  to  $0.67 \pm 0.17$  mg/kg for nitrate and  $13.25 \pm 2.50$  to  $20.22 \pm 3.17$  mg/kg for phosphate. There were no significant differences in the phosphate level of the soil undergoing bioremediation at 0.05 probability limits. However, significant differences were observed in the pH, moisture content, organic carbon, organic matter content and nitrate concentration of the automobile workshop polluted soil fused with fish pond sludge at 95% confidence interval. The findings from this study revealed that automobile workshop soil treated with 40% sludge (M+40%) had higher bacterial population and rate of utilisation of organic carbon, organic matter content, nitrate and phosphate compared to other treatments. This study recommends the use of organic waste in the bioremediation of automobile workshop-polluted soil.

**Keywords:** Bioremediation, fish pond sludge, automobile workshop soil, organic waste.

## INTRODUCTION

Automobile workshop soils are known to contain different hydrocarbon derivatives (Amupitan *et al.*, 2026). These derivatives include petrol, diesel, spent engine oil, greases and other lubricants spilt on the soil over a long period of time, thereby enhancing the black colour linked to the automobile workshop environ (Fredrick *et al.*, 2024). These hydrocarbon derivatives are rich in trace metals, alkanes, alkenes, branched-chain hydrocarbons and aromatic compounds (Stephen *et al.*, 2025).

Automobile workshop soils are often contaminated due to constant spillage of used hydrocarbon derivatives, which are not subjected to treatment or recycling. These

oils impact the microbial and also the physicochemical properties of the soil (Okwute and Ijah, 2014). Soil properties, including bacterial population and types, pH, moisture retention capacity, organic matter content and electrical conductivity, are all influenced by harmful substances present in petroleum waste products and solvents often used in automobile workshop soil (Fredrick *et al.*, 2024). Soil loses its fertility and aesthetic value when negatively impacted by hydrocarbons over a long period of time (Amupitan *et al.*, 2026).

Biofertilizers originating from organic materials such as animal manure, plant residues and compost have been

utilised in bio-stimulation and bioaugmentation of soil contaminated with hydrocarbon derivatives (Akeem *et al.*, 2024). Recently, the potential of fish pond sludge in the bioremediation of hydrocarbon-polluted soil research has gained prominence because of its high nutrient content and beneficial microbial population (Stephen *et al.*, 2025). Lanzén *et al.* (2020), reported that fish pond sludge is rich in nutrients and bacteria capable of fixing nitrogen as well as phosphate-solubilising bacteria, capable of improving soil quality and plant growth. Some studies have investigated the use of organic manures such as cow droppings, poultry litter, and pigeon droppings for soil bioremediation (Okwute and Ijah, 2014; Stephen and Temola, 2014; Fadina *et al.*, 2019). Despite these literatures, there remains a vast knowledge gap regarding the utilisation of fish pond sludge in bio-remediation of automobile workshop polluted soil.

Hence, this study sets out to utilise an affordable biodegradable waste, such as fish pond sludge, in enhancing the metabolism of hydrocarbon contaminants from the environment. This study aims to address this gap by assessing the potential of fish pond sludge in the bioremediation of automobile workshop-polluted soil. Fish pond sludge may act as a bio-stimulating (nutrients) as well as bio-augmentation (bacteria and fungi are present in the sludge) product, thus becoming an economically viable tool which developing countries can adopt in reclaiming low-level contaminants present in automobile workshop polluted soil.

## MATERIALS AND METHODS

### Study area

This study was carried out in the Department of Biological Sciences, Faculty of Basic Allied Sciences of Salem University, Lokoja, Nigeria. Lokoja is the capital of Kogi State and the confluence of Rivers Benue and Niger.

### Fish pond sludge

Fish pond sludge was collected as described by Stephen *et al.* (2025). The residue was spread on an elevated concrete platform to air dry for a period spanning four weeks to ensure proper drying.

### Experimental design

This study involved pot experiment. The base and sides of the pots were perforated and filled with automobile workshop soil seeded with varying amounts of dry fish pond sludge (10%, 20% and 40%). Five pots were used for the automobile workshop soil as well as the pristine soil. Two of the plastic pots contain 6Kg of pristine soil (positive

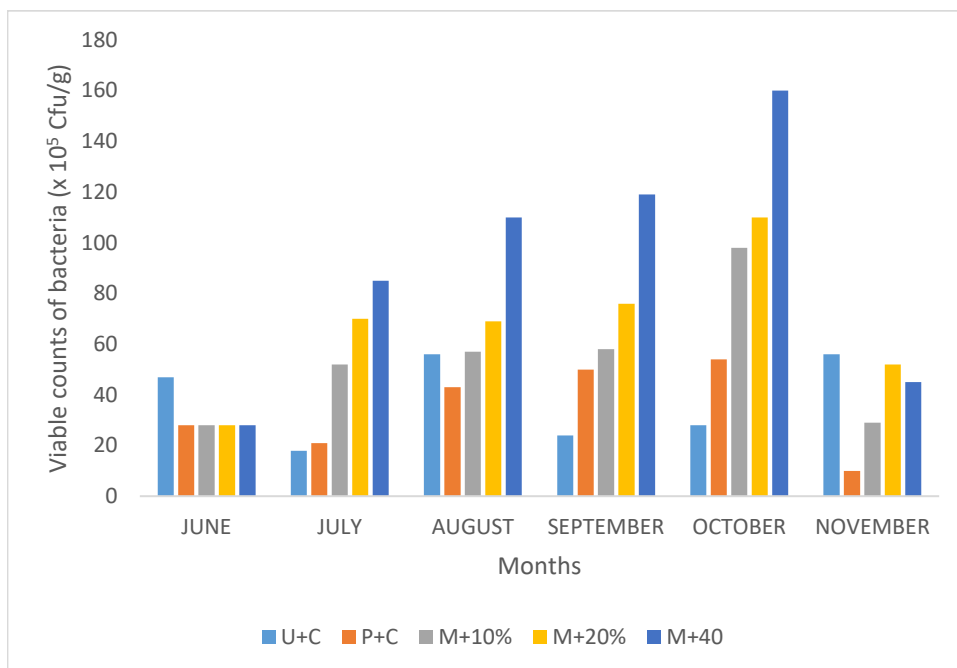
control) and automobile workshop soil (negative control), while the remaining pots containing automobile workshop soil were incorporated with 10% (M+10%), 20% (M+20%) and 40% (M+40%) of dried fish pond sludge.

### Sample analysis

Sampling was carried out monthly for a period of six months to assess the bacteriological and physicochemical properties of the automobile workshop-polluted soil undergoing bioremediation.

### Bacteria

The total viable bacteria counts were determined using serial dilution on nutrient agar via pour plate methods. The plates were incubated at 37°C for 24 hours. The isolates that grew within 24 hours were enumerated and Gram-stained. Pure isolates of the Gram-stained bacteria were further identified by molecular techniques as described by Onyemaobi *et al.* (2022) and Okwute *et al.* (2025). DNA extraction from the isolates was resuscitated by sub-culturing and inoculated into nutrient broth for DNA extraction. DNA from bacteria cells were extracted using the Zymo QuickDNA Fungal/Bacterial Miniprep Kit in line with the manufacturer's instructions. Approximately 70 mg (wet weight) bacteria cells were re-suspended in 200 µl of sterile distilled water and lysed in 750 µl Lysis Solution for about 30 minutes using the ZR Bashing Bead™ Lysis Tube. They were then centrifuged at 10,000 x g for 1 minute. After which about 400 µl supernatant was collected in the ZymoSpin™ IV spin filter (Orange Top) in a collection tube and centrifuged at 7,000 x g for 1 minute. 1,200 µl of genomic lysis buffer was added to the filtrate in the collection tube, of which 800 µl of the mixture was transferred into a Zymo-Spin™ IIC column in another collection tube and then centrifuged at 10,000 x g for 1 minute. The flow from the Collection Tube was discarded, and the step was repeated and flowed through discarded. 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at 10,000 x g for 1 minute, after which another 500 µl g-DNA Wash Buffer was added to the ZymoSpin™ IIC Column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IIC Column was then transferred into a clean 1.5 ml microcentrifuge tube, and 100 µl DNA Elution Buffer was added directly to the column matrix. This was centrifuged at 10,000 x g for 30 seconds to elute the DNA. The extracted DNA samples were measured with the NanoDrop™ One/One C (Thermo Fisher Scientific). All DNA samples were stored at -20°C until further use. The 16S rRNA sequences obtained for the isolates using the universal primer were compared with those of the National Centre for Biotechnology Information (NCBI) genebank database by performing the Basic Local Alignment Search Tool (BLAST) search programme.



**Figure 1.** Viable counts from Lokoja automobile workshop soil undergoing bioremediation. **Keys:** U+C: Pristine soil, P+C: Automobile workshop unamended soil, M+10% : Automobile workshop polluted soil amended with 10% fish pond sludge, M+20%: Automobile workshop polluted soil amended with 20% fish pond sludge, M+40% : Automobile workshop polluted soil amended with 10% fish pond sludge.

### Physicochemical analysis

pH and Phosphorus were determined as described by Onyeonwu (2000). Organic carbon, organic matter content, nitrate and moisture were determined as outlined by Ibitoye (2006). Nitrate was determined using the micro Kjeldahl method as described by the Association of Official Analytical Chemists, AOAC (2005) and Ibitoye (2006). The moisture content of the soil was determined using the dry weight method as described by Ibitoye (2006).

### Statistical analysis

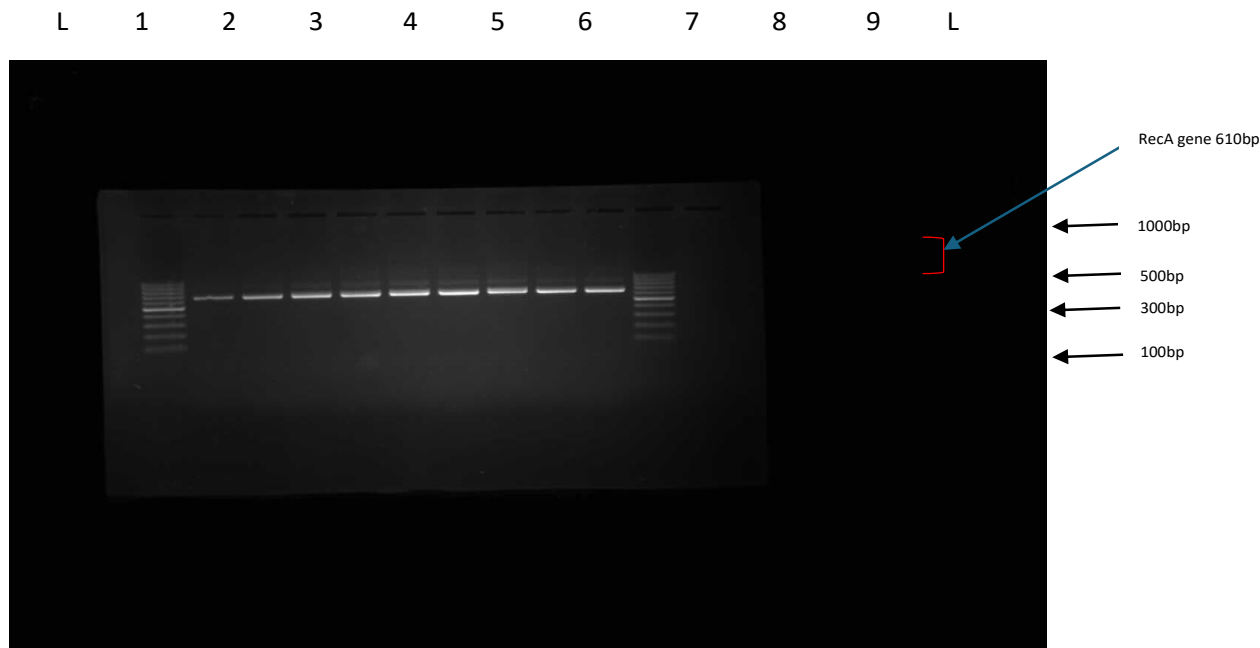
The data obtained were analysed using the Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistics was performed to determine the mean and standard error of variables, while a one-way Analysis of Variance (one-way ANOVA) was carried out to determine the association between variables.

## RESULTS AND DISCUSSION

Figure 1 shows the viable bacteria population in the automobile workshop polluted soil obtained from Lokoja, undergoing bioremediation. The viable counts ranged from  $18.0 \times 10^5$  -  $56.0 \times 10^5$  CFU/g in pristine soil (U+C),  $10.0 \times$

$10^5$  -  $55.0 \times 10^5$  CFU/g in un-amended automobile workshop polluted soil (P + C),  $28.0 \times 10^5$  –  $98.0 \times 10^5$  CFU/g in M+10% fish pond sludge,  $24.0 \times 10^5$  –  $77.0 \times 10^5$  CFU/g in M+20% fish pond sludge,  $28.0 \times 10^5$  –  $125.0 \times 10^5$  CFU/g in M+40% fish pond sludge. There was no significant difference in the viable counts obtained from the Lokoja automobile workshop soil undergoing bioremediation at 0.05 probability limits. The highest bacterial population in the automobile workshop polluted soil undergoing bioremediation obtained from Lokoja was observed in M+40% amended soil, followed by M+20% all through the months of sampling. This is an indication that higher sludge amendment provides more nutrients required for the metabolism of the hydrocarbon present in the automobile workshop-polluted soil. These results agree with earlier studies by Fadina *et al.* (2019) and Stephen *et al.* (2025). These authors reported an elevated population in sludge and organic manure amended soil compared to un-amended hydrocarbon polluted soil due to its rich organic matter content. The result also showed that the rainy months of August and September had a higher bacterial population compared to October and November. This could be due to a reduction in the moisture content of the soil as the rainy season began to wind down, as well as dwindling nutrients in the soil (Fredrick *et al.*, 2024).

The bacteria isolated from the automobile workshop soil from Lokoja, undergoing bioremediation is shown in Table 1. The organisms include *Bacillus cereus*, *Bacillus subtilis*,



**Plate 1.** DNA agarose gel electrophoresis of the isolates. **Lane L:** DNA ladder 100bp plus, **Lane 1:** *Bacillus subtilis*, **L2:** *Bacillus cereus*, **L3:** *Lysinbacillus fusiformis*, **L4:** *Paenibacillus polymyxa*, **L5:** *Rhodococcus erythropolis*, **L6:** *Micrococcus luteus*, **L7:** *Corynebacterium xerosis*.

*Corynebacterium xerosis*, *Lysinbacillus fusiformis*, *Micrococcus luteus*, *Paenibacillus polymyxa* and *Rhodococcus erythropolis*. These organisms are often encountered and isolated from hydrocarbon-enriched environments (Okwute and Ijah, 2014; Fadina *et al.*, 2019; Fashola *et al.*, 2024; Fredrick *et al.*, 2024; Stephen *et al.*, 2025). The DNA agarose gel electrophoresis is shown in Plate 1. All the organisms fell within 610kbp, suggesting a similar molecular weight

Table 2 shows the physicochemical properties of the automobile workshop-polluted soil from Lokoja. The pH ranged from  $5.78 \pm 0.40$  to  $7.20 \pm 0.20$ . The lowest pH was observed in the pristine soil, while the highest pH was observed in the automobile workshop-polluted soil amended with 20% fish pond sludge (M+20%). There were significant differences in the pH of the soil at 0.05 probability limits (Table 2). The pH of the soils was weakly acidic at the end of the study. The range of pH from Lokoja aligns with reports of Fadina *et al.* (2019), Fredrick *et al.* (2024) and Amupitan *et al.* (2026). These researchers observed that soil seeded with organic manure tends to tilt towards weak acidity during the bioremediation process. However, the highest pH observed in M+20% amendment was lower than that of mixed cow dung and poultry litter reported by Fadina *et al.* (2019). This could stem from the fact that mixed manure (cow dung and poultry litter) confers more biostimulatory effect on soil microbes responsible for the metabolism of the hydrocarbon compared to fish pond sludge alone.

The moisture contents ranged from  $6.47 \pm 1.03$  to

$12.72 \pm 1.91$ . The highest moisture content of the soils was observed in the automobile workshop polluted soil amended with M+40%, followed by M+20%, while the least moisture content was observed in U+C. Significant differences were observed in the moisture contents across the soil treatments at 95% confidence limit. This trend has been reported in the literature several times (Okwute and Ijah, 2014; Fadina *et al.*, 2019; Fredrick *et al.*, 2024). According to Stephen *et al.* (2025), the water-holding capacity of soil increases in the presence of organic manure. This may account for the lower moisture content observed in soils (U+C and P+C) without manure during the study.

The organic carbon of the soil from Lokoja ranged from  $0.85 \pm 0.15$  to  $3.13 \pm 0.90\%$ . M+40% had the highest organic carbon, followed by M+20% and M+10%, while the least organic carbon was recorded in U+C. There were significant differences in the organic carbon contents of the soils undergoing bioremediation at 0.05 probability limits. The finding from the Lokoja soil is in agreement with the report of Fadina *et al.* (2019). They reported higher organic carbon in soil containing a higher amount of organic manure during a biodegradation study.

The organic matter content of the soils ranged from  $1.47 \pm 0.25$  -  $3.80 \pm 1.02\%$ . M+20% had the highest organic matter content ( $3.80 \pm 1.02\%$ ), followed by M+40% ( $3.60 \pm 1.05\%$ ), while the least value was observed in the pristine soil, U+C ( $1.47 \pm 0.25\%$ ). Significant differences were observed across the automobile workshop polluted soil treatments undergoing bioremediation. However, the

**Table 1.** Basic Local Alignment Search Tool (BLAST) showing value and percentage similarity of sequences for Bacteria isolated from automobile workshop polluted soil in Lokoja undergoing bioremediation with those in the geneBank.

Isolates	Gram's rxn	Max score	Total score	Query cover (100%)	E-value	Per. Iden	Acc. Len	Accession number
<i>Bacillus cereus</i>	+ve	1140	1140	100	0	100	724	MN938236.1
<i>Bacillus subtilis</i>	+ve	1146	1146	100	0	100	4056765	CP107539.1
<i>Corynebacterium xerosis</i>	+ve	1127	1127	100	0	100	958	PP977494.1
<i>Lysinibacillus fusiformis</i>	+ve	1146	1146	100	0	100	808	KY623090.1
<i>Micrococcus luteus</i>	+ve	1125	1125	100	0	100	801	PP380079.1
<i>Paenibacillus polymyxa</i>	+ve	1094	1094	100	0	100	886	PP621225.1
<i>Rhodococcus erythropolis</i>	+ve	1107	1107	100	0	100	1449	OK136203.1

rxn: reaction, +ve: positive, Per. Iden: percentage identification.

**Table 2.** Physicochemical properties of automobile workshop polluted soil from Lokoja undergoing bioremediation (M+SE).

Parameters	Pristine(U+C)	Polluted (P+C)	M + 10%	M + 20%	M + 40%
pH	5.78 ± 0.40 <sup>b</sup>	6.60 ± 0.32 <sup>a</sup>	6.97 ± 0.25 <sup>a</sup>	7.20 ± 0.20 <sup>a</sup>	7.11 ± 0.13 <sup>a</sup>
Moisture (%)	8.50 ± 1.24 <sup>a,b</sup>	6.47 ± 1.03 <sup>b</sup>	9.46 ± 1.24 <sup>a,b</sup>	11.32 ± 1.62 <sup>a</sup>	12.72 ± 1.91 <sup>a</sup>
Organic carbon (%)	0.85 ± 0.15 <sup>b</sup>	1.64 ± 0.20 <sup>a,b</sup>	2.05 ± 0.12 <sup>a,b</sup>	2.20 ± 0.60 <sup>a,b</sup>	3.13 ± 0.90 <sup>a</sup>
O.M.C (%)	1.47 ± 0.25 <sup>b</sup>	2.08 ± 0.24 <sup>a,b</sup>	2.93 ± 0.50 <sup>a,b</sup>	3.80 ± 1.02 <sup>a</sup>	3.60 ± 1.05 <sup>a,b</sup>
Nitrate (Mg/kg)	0.24 ± 0.04 <sup>b</sup>	0.48 ± 0.05 <sup>a,b</sup>	0.56 ± 0.03 <sup>a,b</sup>	0.65 ± 0.17 <sup>a</sup>	0.67 ± 0.17 <sup>a</sup>
Phosphate (Mg/kg)	13.25 ± 2.50 <sup>a</sup>	15.82 ± 2.00 <sup>a</sup>	19.00 ± 3.43 <sup>a</sup>	20.22 ± 3.17 <sup>a</sup>	16.66 ± 3.50 <sup>a</sup>

<sup>a,b,c</sup>: means of six replicates denoted by different superscripts along the same row are significantly ( $p < 0.05$ ) different, M+10%: Automobile workshop polluted soil amended with 10% fish pond sludge. M+20%: Automobile workshop polluted soil amended with 20% fish pond sludge. M+40%: Automobile workshop polluted soil amended with 40% fish pond sludge.

organic matter content of the soil in Lokoja revealed a higher concentration in the intermediate amendment (M+20%), followed by M+40%. These patterns contradict the report by Fadina *et al.* (2019). This may stem from the nature of the soil and level of hydrocarbon derivatives in the automobile workshop polluted soil in Lokoja. The observed values recorded for the treated soils may have a beneficial impact on the physicochemical properties of the soil by influencing the metabolism of the hydrocarbon by soil microbiota (Mbah *et al.*, 2009).

The highest nitrate concentration was recorded

in M+40%, followed by M+ 20%, while the lowest concentration was recorded in the pristine soil (U+C). The nitrate concentration ranged from 0.24±0.04 to 0.67±0.17mg/kg. Significant differences were observed in the nitrate levels of the soil undergoing bioremediation at 95% confidence interval. This result aligns with the reports of Johnson *et al.* (2018). Fish pond sludge is rich in nutrients, hence the higher nitrate contents observed in the amended soils may be attributed to the rich nitrate component of the fish pond sludge used in stimulating the bioremediation process (Zhang *et al.*, 2019).

The phosphate levels of all treatments were higher than the nitrate levels. It ranged from 13.25±2.50 to 20.22±3.17mg/kg. The highest phosphate values were observed in M+20%, followed M+10%, while the lowest value was observed in the pristine soil (U+C). Significant differences were observed in the phosphate levels of the soil undergoing bioremediation at 0.05 probability limits (Table 2). The values of phosphate concentration recorded in this study were higher than those in a similar study by Stephen *et al.* (2025). These authors attributed lower levels of phosphate in fish pond sludge-amended soil to phosphate utilisation by soil microbes.

## Conclusion

The findings from this study showed that automobile workshop polluted soil amended with 40% fish pond sludge (M+40%) had the highest bacterial population and rate of utilization of organic carbon, organic matter content, nitrate and phosphate by soil organisms compared to 10% (M+10%), 20% (M+20%) treatments compared to pristine and unamended polluted soil after six months. Hence, this study recommends the use of higher concentrations of biodegradable organic wastes such as fish pond sludge in the bioremediation of hydrocarbon-polluted soil.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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