UMYU Scientifica, Vol. 4 NO. 2, June 2025, Pp 448 – 456
https://doi.org/10.56919/usci.2542.047
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Degradation of Used Engine Oil by Bacteria Isolated from the Rhizosphere of Neem Plant

*[1]Shamsudeen, S.; [1]Mudassiru, S; [2]Aisha, A.H; [3]Lawal, N And [4]Balumi Z.M.
[1]Department of Microbiology, Bayero University, Kano, Nigeria
[2]Department of Microbiology, Sokoto State University, Sokoto, Nigeria
[3]Department of Biology, Ahmadu Bello University, Zaria, Kaduna, Nigeria
[4]Department of Science Laboratory Technology, Federal College of Science Agricultural Technology, Kano, Nigeria

ABSTRACT
This study examined the degradation of used engine oil by bacteria isolated from the rhizosphere of the neem tree within Sokoto metropolis. The study aimed to determine the physico-chemical properties of the spent engine oil contaminated soil, isolate bacteria from the neem plant rhizosphere, and test their degrading capability on soil contaminated with spent engine oil. Nine soil samples were collected from the rhizosphere of a neem plant contaminated by spent engine oil. The samples were examined and characterised first before testing their degrading ability. Microscopic and Biochemical identification of isolates shows that nine (9) bacteria species were isolated and identified from the samples. The isolated species includes *Bacillus* sp., *Proteus* sp., *Klebsiella* sp., *Citrobacter* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Yersinia* sp., *Staphylococcus* sp., *Salmonella* sp., and *Serratia* sp. The soil samples collected from B1 had the highest temperature of 36°C, and the lowest temperature recorded was 33 °C from A3, C2, and C3 soil samples. The pH of the soil samples from A, A2, B1, B2, B3, and C2 was observed to be slightly basic in the range of 7.1-7.5, while the soil samples of C1 and C3 were found to be acidic. Soil samples B1 and B2 were discovered to have the highest percentage of Organic Carbon (1.05%) and Sodium (Na) content (1.80% and 1.85%) respectively, while sample C3 had the lowest percentage of Organic Carbon (0.56). The percentage biodegradability potential of the isolated bacteria are; *Citrobacter* sp (55.95%), *Proteus* sp (23.19%), *Klebsiella* sp (29.09%), *Klebsiella* sp (48.28%), *Salmonella* sp (25.89%), *Serratia* sp (15.26%), *Bacillus* sp (19.09%) and *Yersina* sp (37.04%). From the results of the present study, it can be concluded that bacteria can be present in soil contaminated with used petroleum products, and these bacteria play important roles in the biodegradation of petroleum substances from oil-contaminated soil by utilising the hydrocarbon substrate as their sole source of carbon. It is recommended that the use of native bacteria or fungal strains with petroleum hydrocarbon-utilising capabilities as a weapon for petroleum degradation could prove a more environmentally friendly approach to the soil than the use of chemicals.

ARTICLE HISTORY
Received November 24, 2024
Accepted June 28, 2025
Published June 30, 2025

KEYWORDS
Biodegradation, engine oil, Bacteria, Contamination, soil

INTRODUCTION
Environmental pollution through petroleum and petrochemical products has attracted much attention in recent decades. Used petroleum hydrocarbons are an environmental and human health hazard if not disposed of correctly. Lubrication oil is a complex mixture of hydrocarbons, additives, and contaminants. (Meena *et al.*, 2021 and Sun *et al.*, 2021). It is used to lubricate and protect machinery and other equipment. However, the indiscriminate disposal of used engine oil not only contaminates the environment but also introduces highly recalcitrant as well as toxic PAH compounds that may cause serious health implications to both humans and animals (Meena *et al.*, 2021 and Sun *et al.*, 2021). The recalcitrant nature of PAHs is due to their hydrophobic, hydrophilic and less soluble nature, which might affect the complete removal of used engine oil from the environment (Umar *et al.*, 2018). Thus, effective remediation methods need to be investigated. There are a number of physicochemical techniques for the remediation of engine oil and other petroleum products, such as diesel and kerosene; conversely, all methods employed have been identified with certain advantages and disadvantages (Fu *et al.*, 2022).

Automobile workshops generate millions of gallons of used engine oil containing toxic compounds, which are released untreated into the environment (Goveas *et al.*, 2020). The release of such toxic substances into the

Correspondence: Shamsudeen, Shuaibu. Department of Microbiology, Faculty of Life Science, Bayero University, Kano, Nigeria. ✉ shamsudubai25@gmail.com.
How to cite: Shamsudeen, S., Mudassiru, S., Aisha, A. H., Lawal, N., & Balumi, Z. M. (2025). Degradation of Used Engine Oil by Bacteria Isolated from the Rhizosphere of Neem Plana. *UMYU Scientifica*, 4(2), 448 – 456. https://doi.org/10.56919/usci.2542.047

environment has been identified as a major threat to the drinking water and soil ecosystem (Meena et al., 2021). In modern days, the use of engine oil among vehicle and machine users is continuously increasing (Goveas *et al.*, 2020).

Engine oil could simply be defined as a thick mineral liquid applied to a machine or engine so as to reduce friction between the moving parts of the machine (Shahida *et al.*, 2015). Used engine oil represents oil that has undergone destructive changes in its properties when subjected to oxygen, combustion gases, and high temperature. The said oil also undergoes viscosity changes as well as additive depletion and oxidation (Mark *et al.*, 2018). The disposal of spent engine oil (SEO) into gutters, water drains, open plots and farms is a common practice in Nigeria, especially by motor mechanics.

In a study, the Biodegradation of Used Engine Oil by *Pseudomonas* sp. Isolated from an Automobile Workshop's Soil by Abubakar and Faggo, 2022, the results revealed that, out of the 5 isolates, *Pseudomonas* sp., *Bacillu*s sp. and *Corynebacterium* sp. were the best degraders, with *Pseudomonas* sp. outperforming the other isolates and was chosen for further studies. The results revealed that *Pseudomonas* sp. was able to degrade 60-82% of the used engine oil within 5 days of incubation. The superiority of *Pseudomonas* species over *Bacillus* species in hydrocarbon degradation has been well established by many researchers (Adamu *et al.*, 2022; Faggo *et al.*, 2020; Tirmizhi *et al.*, 2022).

Several researchers have isolated *Pseudomonas* sp. (Salam, 2016 and Abayoru, 2014) *Bacillus* sp. (Larik *et al.*, 2019), *Citrobacter freundii* and *Ochrobactrum anthropic* (Ibrahim, 2016) with great success for the degradation of used engine oil. *Pseudomonas* and *bacillus* species were not only efficient engine oil degraders, but also have been used to degrade crude oil and various petroleum fractions such as diesel (Faggo *et al.*, 2020; Tirmizhi *et al.*, 2022; Adeleye *et al.*, 2022).

Notably, a wide variety of plants have been explored for the biodegradation of engine oil, demonstrating the versatility of botanical resources in microbial degradation. Examples include: *Aloe vera, Carica papaya, Nelumbo nucifera, Allium sativum, Moringa oleifera, and many others, such as Garcinia mangostana, Vitex negundo, Acalypha indica, Eucalyptus globules, Dodonaea viscosa, Aframomum melegueta, Anacardium occidentale, Psidium guajava* (Elegbede and Latteef, 2019; Okeiyeto *et al.*, 2019; Kamal *et al.*, 2020). Each contributes uniquely to the eco-friendly creation of microbial degradation. This method supports the natural power of plant extracts and highlights the potential of using renewable resources in advanced material sciences, paving the way for more sustainable practices in biodegradation.

Despite the availability of numerous reports on microbial degradation, there remains a significant gap in the literature concerning the efficiency in degrading spent diesel oil. This deficiency shows the need for focused research in this area. This study seeks to meticulously evaluate the microbial degradation potential processes applied to engine oil-contaminated soil, utilising advanced microbial degradation strategies. This research aims to bridge the knowledge gap and enhance the effectiveness of microbial degradation potentials in environmental cleanup efforts.

METHODOLOGY

Study Area

The soil sample was collected from three (3) automobile workshops situated at different locations in Sokoto. Sokoto State is located in the extreme northwestern part of Nigeria, between longitudes 4^0E and $6^0$54E and latitudes 12^0N and $13^0$58N. It shares common borders with Niger to the North, Kebbi state to the southwest and Zamfara to the East. The total land area is about 32,000km2. (Mamman *et al.*, 2000). Triplicate samples were collected at each location, and the samples were brought to the laboratory, Faculty of Life Sciences, Department of Microbiology, Sokoto State University, for bacteriological and biochemical analysis.

Physicochemical Analysis of the Soil Samples

Determination of Soil pH

Exactly 10ml of distilled water was added to 10g of soil in a 100ml beaker. The solution was stirred thoroughly with a glass rod and allowed to stand for 30 minutes. The electrode was calibrated at 15 pH 7 and then immersed in the suspension, and the pH value was read and recorded (Baldwin and Marshal, 2009).

Determination of Temperature

This was determined at the point of sample collection by dipping the bulb of a mercury-in-glass thermometer into the soil suspension and recording the temperature reading (AOAC, 2000).

Determination of Phosphorus (P)

About 2.85g of soil sample was put into an extraction bottle, and 20ml of the extracting solution (NHexane) was added. The bottle was shaken for 1 minute, and the contents filtered immediately through filter paper. 20ml of aliquot extract was pipette into a 50ml volumetric flask, 2ml of ammonium molybdate solution was added and mixed. Water was added to bring the volume to about 48ml, and 1ml of freshly prepared stannous chloride solution was added. After 5 minutes, the colour intensity was measured on the calorimeter at 660nm wavelength. Phosphorus content in soil was calculated (Uriyo and Singh, 2001). Using formula

$$P = \frac{\text{Absorbance} \times \text{CF} \times \text{DF}}{\textit{Atomic weight of phosphorus}}$$

Where CF = Conversion Factor, DF = Dilution Factor

Determination of Nitrogen in Soil

2g of soil, 1 tablespoon of catalyst, and 20ml of concentrated sulphuric acid were put in an 800ml conical flask and shaken gently until all the acid was thoroughly mixed. The flasks were placed on the digestion rack until all the organic matter was destroyed. The flask turned to a light grey colour. 50ml of distilled water was added to cool the flask. 10ml of the aliquot was taken from the flask, and the flask was mounted on a distillation rack. 20ml of boric acid mixed indicator was pipette into the flask. It was placed under the condenser of the distillation apparatus just to touch the surface of the solution. Tap water was used to cool the mixture. A 20ml solution of 40% sodium hydroxide was added to the side arm of the flask, and distillation started after thoroughly mixing the contents. 40ml of the distillate was collected and titrated, and the percentage of nitrogen content was calculated (Uriyu and Singh, 2001). Using the formula below:

$$\%Nitrogen = \frac{xml\ HsSO4 \times Normality\ of\ acids \times N}{Weight\ of\ soil\ xml\ of\ aliquots} \text{ x } 100$$

Determination of Organic Carbon

Exactly 10ml of normal potassium dichromate and 10ml of concentrated sulphuric acid were added to 1g of soil in a 100ml flask. The mixture was allowed to swell for 30 minutes. 100ml of water was added to the flask containing the samples. 5ml of Orthophosphoric acid and 3 drops of dimethylamine indicator were also added. It was then titrated with ferrous sulphate solution. The colour changed from purple to blue before the endpoint reached. The titration was completed by addition ferrous sulphate drop by drop (Uriyu and Singh, 2001). The percentage of organic carbon (OC) was calculated using the formula

$$\%\ OC = \frac{me\ K2Cr2O7 - FeO4XF}{Weight\ of\ soil\ xml\ of\ aliquos} \text{ x } 100$$

Determination of Calcium and Magnesium

Two 5ml aliquots of the prepared solution were pipetted into a titration flask, and distilled water was added to get a volume of about 150ml. 15ml of buffer solution and 10 drops each KCN, NH_2O, HCl, $K_4Fe(CN)_6$ and triethanoalime were added. The mixture was allowed to stand for a few minutes. Ten drops of ferrochrome black T were added, and the content was titrated until a permanent blue colour was obtained. Black determination was carried out in the same way with 5ml of distilled water instead of the sample solution, and the net EDTA for the solution was calculated. Two 5ml aliquots of the pre-prepared solution of soil were pipette into two titration flasks and made to 150ml volume of distilled water. Ten drops of NH_2OH, HCl and triethanolamine were added. The pH was raised to about 12 by the addition of 10ml of 10% NaOH solution. Murixed was added, and the mixture was titrated with EDTA; the titration result was recorded. EDTA for mg = net ml for (Ca + Mg) – net ml for Ca along (Uriyu and Singh, 2001). From the net ml of EDTA obtained above, the concentrations of Ca and Mg in the original samples were calculated using the formula below:

$$CA\ or\ Mg = \frac{Tm \times Solution\ Volume\ (m)}{20 \times Sample\ weight.}$$

Determination of Potassium (K)

The flame photometer was switched on and allowed to warm for 30minutes and the potassium lamp was adjusted. The photometer was adjusted to 100% transmittance by feeding a 25ppm solution and then set to 0% with a blank. All the standard solutions were run, and a standard curve was prepared by plotting transmittance against the concentration of the standard K solution. The transmittance value was read from the standard solution and finally calculated using the formula

$$K = \frac{PP \times Volume\ of\ Solution \times 100}{1000\ sample\ atomic\ weight\ of\ K}$$

Where K = Standard

Determination of Sodium (Na)

The Sodium (Na) lamp of the flame photometer was aligned and then set to 0% with a blank appropriately. The photometer was set to 100% transmittance by feeding a 25ppm sodium (Na) solution. Other standard solutions were fed, and a standard curve was prepared. The concentration of Na was read, and the curve and finally calculated by plotting the transmittance against concentration (Uriyu and Singh, 2001). Formula used below

$$Na = \frac{Xppm \times Volume\ of\ Solution \times 1000}{1000\ sample\ atomic\ weight\ of\ Na}$$

Sample Inoculation

One gram of the soil sample was inoculated into the nutrient broth. Three test tubes containing nutrient broth served as the growth medium. All tubes were incubated at a temperature of 37 °C for 24 hours before checking turbidity.

Isolation of the Bacteria

The bacterial isolate was subcultured using a wire loop and inoculated into a nutrient agar, mannitol salt agar, and eosin methyl blue agar using the streak plate method. This was done for a different colony that was identified on the primary culture plates. The plates were incubated at 37 °C for 24 hours.

Isolation of Pure Culture

The collected bacterial isolate was subcultured using a wire loop and inoculated into slant bottles containing nutrient agar and incubated at 37 °C for 24 hours. The slant bottles containing bacteria were kept in the refrigerator after the 24-hour incubation for further analysis.

Biochemical Identification and Characterisation

All isolates were sub-cultured to obtain a pure culture, and a Gram staining technique was carried out.

Gram Staining

The Gram staining was aimed at differentiating gram reactions, sizes, shapes and arrangement of cells of the isolates. For the Gram staining of the various isolates, glass slides were washed and air dried. A drop of normal saline was placed on the slide. Using a flame-inoculation wire loop, a small amount of inoculum was also taken and smeared on the drop of normal saline on the slide. The smear was then allowed to air dry and heat fixed by passing over a flame three times. The fixed smear was flooded with crystal violet for a minute and then rinsed with clean water. Lugol's iodine was added for an additional minute, which served as a mordant. This was 19 later rinsed and cleaned with distilled water. Acetone-alcohol was added as a decolouriser and rinsed immediately with clean water. A counter stain, "safaranin" was then added and allowed to stand for a minute before it was rinsed with clean water. This was allowed to dry before observing under oil immersion with a 100X objective lens microscopically (Cheesbrough, 2006).

IMVIC Tests

IMVIC is an acronym which stands for Indole Test, Methyl Red, Voges Proskauer Test and Citrate Utilisation test.

Indole Test

The test demonstrates the ability of certain bacteria to decompose the amino acid to tryptophan, which then decomposes into indole, accumulating in the medium. The test organism was inoculated in a test tube containing 3ml of sterile water. It was incubated are 35-37 °C for up to 48 hours. 0.5ml of Kovac's reagent was then added to test for indole, and the content was gently shaken. It was examined for a red colour in the surface layer within 10 minutes (Cheesbrough, 2006).

Methyl Red Test

A loopful of pure culture was inoculated into the sterile glucose phosphate peptone water medium and was incubated at 37 °C for 48 hours. Then, 5 drops of the methyl red reagent were added to the culture after incubation. The mixture was read immediately (Chessbrough, 2006).

Voges Proskauer Test

Two millilitres of sterile glucose peptone was inoculated with the test organism, and then incubated at 37 °C for 48 hours. 1ml of 40% potassium hydroxide and 3ml of 5% solution of alpha naphthalene in absolute alcohol were added after incubation. The tube was then shaken at intervals to ensure maximum aeration (Chessbrough, 2006).

Citrate Utilisation Test

Here, Simon's citrate broth medium was used. The sterile medium was inoculated from a saline suspension of the test organism and incubated for 96 hours at 37 °C , and the medium was observed for a colour change (Chessbrough, 2006).

Catalase Test

Three millilitres of the hydrogen peroxide solution were poured into a test tube while a small quantity of the test organism was picked and immersed in the hydrogen peroxide solution. The mixture was then observed for the production of bubbles (Chessbrough, 2006).

Oxidase Test

A piece of filter paper was placed in a clean petri dish, and 2 drops of freshly prepared oxidase reagent were added. A stick or glass rod was used to remove a colony of the test organism and smeared on the filter paper. It was then observed for development of blue-purple colour within a few seconds (Chessbrough, 2006).

Urease Test

The medium Christensen's medium was prepared and autoclaved at 121^0C for 30 minutes. pH 6.8 glucose and urea were sterilised by steaming at 100 °C for 15 minutes. The mixture was then added and mixed, and poured into tubes as deep slopes. The test organism was inoculated into the agar slopes and incubated at 37 °C for 4 hours, followed by overnight incubation. It was observed for colour change (Chessbrough, 2006).

Motility Test

A drop of suspension was placed on a cover glass and inverted over a normal slide supported on a ring of plasticine. The preparation was microscopically examined for motile organisms by using 10X and 40X objective lenses to observe the movement (Chessbrough, 2006).

Sugar Fermentation Test

Glucose, Sucrose, and Lactose were carried out using Triple Sugar Iron (TSI) agar. The TSI agar medium was prepared and sterilised using an autoclave. The medium was then allowed to cool and solidify. A speck of each isolate was then inoculated by stabbing into the medium and incubated at 37 °C for 24 hours (Chessbrough, 2006).

Utilisation of Petroleum Products by Isolates

A loop full of isolates on agar plates was picked and inoculated on mineral salt media. To meet with the nutritional requirements of the bacteria isolates for proper growth, mineral salts media was prepared using the

following salts: KH_2PO_4(2.0g/l), $NaNO_3$ (2.0g/l), NaCl (0.8g/l), KCl (0.8g/l), $Na_2HPO_4.12H_2O$ (2.0g/l), $MgSO_4$ (0.2g/l) and $FeSO_4.7H_2O$ (0.001g/l). All the salts were dissolved in one litre (1L) of water and sterilised in an autoclave at 121^0C for 15 minutes. A quantity of the test substrate (Engine Oil) was measured aseptically using a wire loop using a ratio of 1 on McFarland's scale. The test tubes containing the inoculum and petroleum substrates were inoculated at 35 °C for 48-72 hours, and the presence of turbidity and absorbance was determined using a calorimeter and recorded for day 1 and day 7 (Mandri and Lin, 2017).

Statistical Analysis

The data were analysed by one-way analysis of variance (ANOVA), using Microsoft Office Excel 2007. P value was calculated to see the significant results. Results showing P value less than 0.05 were considered significant ($P<0.05$).

RESULTS

Physicochemical Properties of Soil Samples

The soil samples collected from B1 have the highest temperature of 36^0 °C, while the lowest temperature recorded was 33 °C from A3, C2, and C3 soil samples. The pH of the soil samples from A, A2, B1, B2, B3, and C2 was observed to be slightly basic in the range of 7.1-7.5, while the soil samples of C1 and C3 were found to be acidic. Soil samples B1 and B2 were discovered to have the highest percentage of Organic Carbon (1.05%), and Sodium (Na) content (1.80% and 1.85%), respectively, while sample C3 had the lowest percentage of Organic Carbon (0.56), as in Table 1.

Table 1: Physiological Properties of Soil Samples

Samples	Temp (°C)	pH	Org. C (%)	N (%)	P (mg/Kg)	Ca (Cmol/kg)	Mg (Cmol/kg)	K (Cmol/kg)	Na (Cmol/kg)
A1	34	7.2	1.04	0.052	0.93	1.42	0.40	0.60	1.75
A2	35	7.2	1.04	0.054	0.92	1.50	0.45	0.62	1.48
A3	33	7.1	0.85	0.053	0.91	1.40	0.45	0.86	1.75
B1	33	7.1	1.05	0.061	0.88	1.55	0.45	1.80	1.85
B2	35	7.4	1.05	0.060	0.91	1.47	0.40	1.86	1.80
B3	35	7.5	1.06	0.061	0.88	1.58	0.45	2.24	1.60
C1	35	7.2	0.70	0.048	0.94	1.45	0.40	2.12	1.56
C2	33	7.1	0.58	0.051	0.95	1.35	0.15	1.62	1.43
C3	33	7.4	0.56	0.049	0.92	1.35	0.15	0.85	1.42

KEY: Temp.Temperature; Org.C, Organic Carbon; N, Nitrogen; P, Phosphorus; Ca,Calcium; Mg.Magnesium; K, Potassium; Na, Sodium.

Frequency of Occurrence of the Isolates

Staphylococcus sp. had the highest frequency of occurrence (20.00%), while *Proteus*, *Citrobacter*, *Corynebacterium*, *Pseudomonas*, *Yersinia*, and *Salmonella* sp. had the lowest frequency of occurrence (6.68%) (Table 2).

Table 2: Frequency of Occurrence of the Bacteria from Soil Contaminated with used spent engine oil in Sokoto State, Nigeria

Bacteria species	Total (% Occurrence)
Bacillus sp.	2 (13.33)
Proteus sp.	1 (6.68)
Klebsiella sp.	2 (13.33)
Citrobacter sp.	1 (6.68)
Corynebacterium sp.	1 (6.68)
Pseudomonas sp.	1 (6.68)
Yersinia sp.	1 (6.68)
Staphylococcus sp.	3 (20.00)
Salmonella sp.	1 (6.68)
Serratia sp.	2 (13.33)
Total	**15 (100)**

Utilisation of Petroleum Products by Isolates

Using a calibrated calorimeter, the absorbance of each organism was recorded with spent engine oil on the first and seventh days after incubation to observe the bacterial species that has the most biodegradative potential. Table 3 shows absorbance.

Biodegradation of Petroleum Products

The percentage of degradation was measured for each bacteria species by subtracting the final absorbance from the initial absorbance and multiply by 100

The formula is as follows

$$\text{Percentage Degradation} = \frac{Final\ Absorbance - Initial\ Absorbance}{Final\ Absorbance} \times 100\%$$

DISCUSSION

The physicochemical properties of oil-polluted soil collected from Emir Yahaya Mechanic Workshop (Site A), Kantin Daji Mechanic Workshop (Site B) and Down

Town Mechanic Workshop (Site C) as presented in Table 1. The analysis of the soil from the three sites was conducted on the Site B and Site A soils, respectively. It was observed that the lowest concentrations of Mg, Na, K, P, and N were found in oil-polluted Site C soil, while the highest concentrations were obtained in Site A, except for K, which has the highest concentration in Site B soils. The values for the organic carbon ranged from 0.56 for Site C to 1.06 for Site B. The results obtained from the physicochemical properties of the soil showed that sample sites A, A2, A3, C1, C2, C3 have a temperature range of 33-36 °C, while sites B1, B2, B3 showed a relatively higher temperature range of 34-35 °C, which generally favours the growth of microorganisms in the soil. The sample sites also had a pH value range of 7.1-7.5, with sample C2 showing the lowest pH of 7.1, and sample site B showing the highest (7.5), which explains why the spent oil-contaminated soil promoted the growth of certain bacterial species. The organic carbon, nitrogen, and phosphorus results obtained show why some bacteria can still grow in the polluted oil soil. These findings are in agreement with the study of Smith *et al.* (2019). In the study conducted by Smith *et al.* (2019), the physicochemical properties of oil-polluted soil were investigated in a similar context, supporting the findings presented in Table 1 of the current study.

Table 3: Showing Absorbance of Petroleum Products by Day 1 & 7

Test organisms	Day 1 (Mean Absorbance)	Day 7 (Mean Absorbance)
Control Engine oil	0.044	0.29
Citrobacter sp.	±0.31	±0.14
Proteus sp.	±0.15	±0.29
Klebsiella sp.	±0.10	±0.12
Klebsiella sp.	±0.23	±0.11
Salmonella sp.	±0.15	±0.14
Serratia sp.	±0.43	±0.33
Bacillus sp.	±0.21	±0.12
Yersinia sp.	±0.17	±0.17
Standard deviation	0.257	0.207

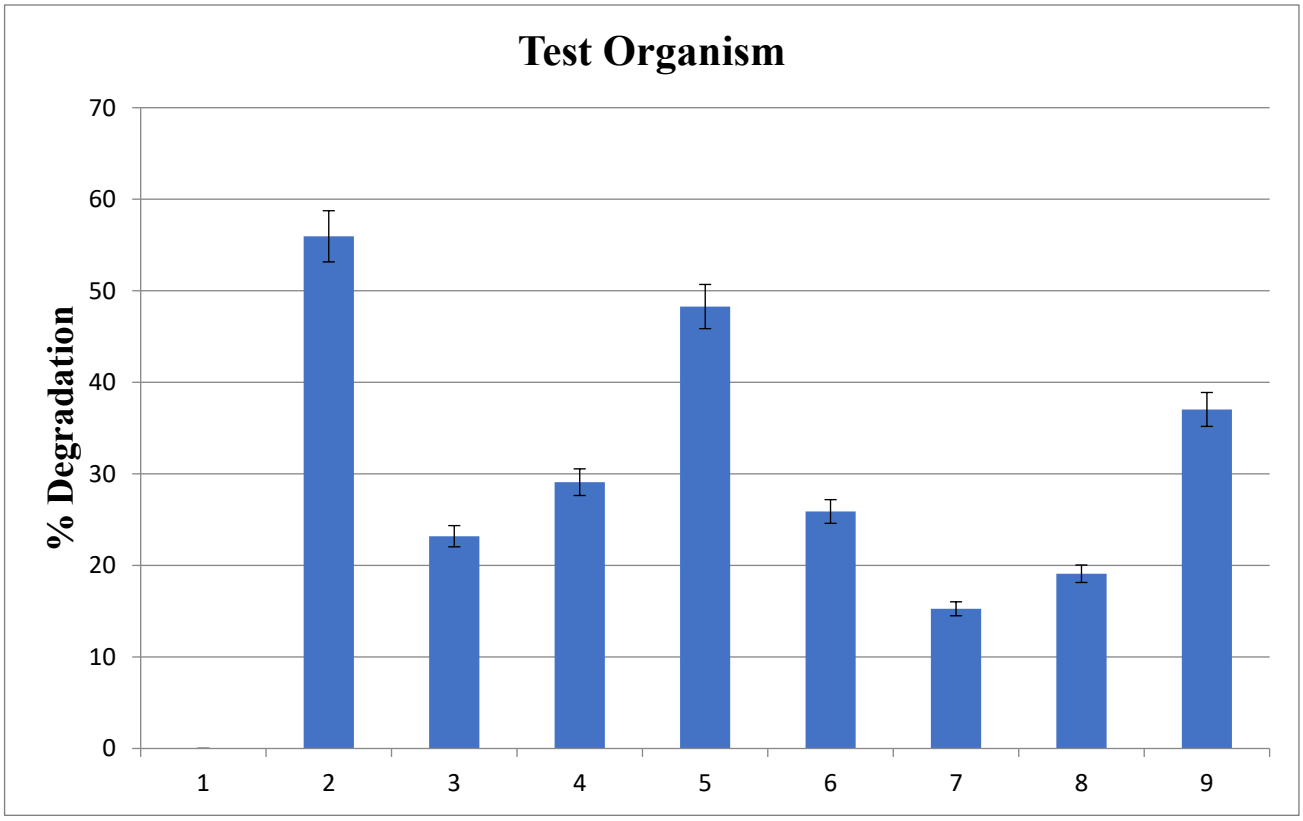

Figure 1: Percentage Absorbance of Bacteria Species to describe their Biodegradability Potential

Smith *et al.* collected soil samples from multiple mechanic workshop sites and analysed the concentrations of various elements. Their results showed a consistent pattern with the present study, indicating variations in Mg, Na, K, P, and N concentrations across different polluted soil sites. Smith et al. reported that, similar to the findings in Site A of the current study, the soil from one of their sites (referred to as Site X) exhibited higher concentrations of

Mg, Na, K, P, and N. Conversely, another site (referred to as Site Y) displayed lower concentrations of these elements, aligning with the lower values observed in Site C of the present study. Furthermore, the temperature and pH ranges observed in the current study are consistent with the results reported by Smith *et al.* (2019). In both studies, the polluted soil sites exhibited temperature ranges conducive to microbial growth. The pH values reported by Smith *et al.* (2019) also fell within the range observed in the current study, supporting the idea that spent oil-contaminated soil can create conditions favourable for bacterial species. This consistency strengthens the generalizability of the results and contributes to a more comprehensive understanding of the impact of oil pollution on soil properties. It has been observed from recent studies that the microorganisms isolated from soil polluted areas have the potential to biodegrade the petroleum-contaminated sites (Chaerun *et al.,* 2004). Since all the bacteria isolates used in this finding were isolated from used petroleum-contaminated soil, the potential of degradation exhibited by these bacteria is in accordance with the reports of Chaerun *et al.* (2004) and Vidalii (2001). *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp., *Klebsiella* sp., *Salmonella* sp., *Yersinia* sp., *Proteus* sp., *Serratia* sp., etc. were isolated from used petroleum contaminated with soil, with *Staphylococcus sp.* showing the highest frequency of occurrence. The bacteria species identified in this study were: *Salmonella enterica, Staphylococcus aureus, Bacillus subtilis, Klebsiella oxytoca, Bacillus megaterium, and Pseudomonas aeruginosa.* These are naturally occurring populations that participate in soil processes and have an inherent ability to degrade petroleum hydrocarbons (Larsen et *al.,* 2009).

The bacterial species of the following genera: *Yersinia* sp., *Bacillus* sp., *Salmonella* sp., *Klebsiella* sp., and *Proteus* sp. were analysed for their hydrocarbon-utilising potential. The results obtained in this study revealed that the five bacterial genera used in this work exhibited different levels of hydrocarbon degradation (Figure 1) showing the utilisation potentials of *Yersinia* sp., *Bacillus* sp., *Salmonella* sp., *Serratia* sp., *Citrobacter* sp., *Klebsiella sp.,* and *Proteus* sp., respectively, on spent Engine Oil under aerobic conditions. The spectrometric absorbance and turbidity determined the growth dynamics of the organisms. All bacterial cultures tested were able to grow on the spent Engine Oil and Petrol as the sole source of carbon and energy when screened for hydrocarbon utilisation. The growth profile of the selected bacterial cultures in test tubes with different petroleum substrate concentrations containing mineral salt medium was obtained through observation of the growth medium turbid metrically using a calibrated calorimeter in accordance with findings by (Kumar *et. al.,* 2006; Lin *et. al.,* 2005; Ziad *et. al.,* 2005), as compared with an uninoculated control by optical density measurement. The results in Figure 1 show the percentage Degradation of the Bacteria based on their absorbance value measured in (nm).

In another research, four (4) bacteria isolated from a soil sample were gram-positive *Bacillus*, *Micrococcus*, *Serratia* and gram-negative *Pseudomonas* species. The results indicated a higher activity of bacterial species from the depth soil than those from the top. The study also revealed that the colony count from soil samples S1Top, S1Depth, S2Top and S2Depth were (20, 26, 18, and 28) cfu/ml and expressed in percentage as 21.74%, 28.26%, 19.56% and 30.44% respectively. The bacterial species were examined for the utility of fresh engine oil, and the result revealed that Okija soil harbours hydrocarbon degraders, which may have occurred as a result of indiscriminate exposure to used engine oil collected from motor vehicles, motor bikes, and other machinery in Okija village (Abioye *et al.*, 2012). An increase in oil degradation corresponded to an increase in cell number during the degradation processes, demonstrating their ability to utilise engine oil as an energy source. The result is in correlation with the work reported by Abioye et al. (2012), who isolated *Pseudomonas, Bacillus, Micrococcus*, and other bacterial strains from engine oil-contaminated soil. *Pseudomonas, Bacillus,* and *Rhodococcus* were also isolated from engine oil-contaminated soil (Osuji *et al.*, 2023).

From the results obtained in the utilisation of petroleum products, it was observed that *Klebsiella* sp. has the highest absorbance after the incubation period. The result of the absorbance using a spectrophotometer from day one to day seven shows an increase (Table 3), indicating the biodegradation potential of the engine oil by the bacteria isolated from soil contaminated with spent petroleum products of mechanic workshops in Sokoto metropolis. The result of the present research is in agreement with the findings of Idemudia *et. al.* (2014), who worked on "Comparative Assessment of Degradation Potentials of Bacteria and Actinomycetes in soil contaminated with Motorcycle Spent Oil".

CONCLUSION

From the results of this study, it can be concluded that bacteria can be present in soil contaminated with used petroleum products. These bacteria play important roles in the biodegradation of petroleum substances from oil-contaminated soil by utilising the hydrocarbon substrate as their sole source of carbon.

RECOMMENDATIONS

It is recommended that;

1. The use of native bacteria or fungal strains with petroleum hydrocarbon utilising capabilities as a weapon for petroleum degradation could prove a more environmentally friendly approach to the soil than the use of chemicals.

2. Mixing of indigenous bacteria consortia found in contaminated soil should be tested in carrying out the biodegradation and bioremediation process in order to yield a higher percentage of degradation.

3. Further research should be conducted to identify other strains of bacteria and fungi that can utilise petroleum products in order to reduce oil pollution, especially in soil,

as this leads to soil deterioration and death of organic and inorganic matter.

REFERENCES

Abioye, O. P., Agamuthu, P., & Abdulaziz, A. R. (2012). Biodegradation of used motor oil in soil using organic waste. *Biotechnology Research International, 2012*, 587041. [Crossref]

Abubkar, Muhammad., & Faggo, F.A. (2022). Biodegradation of Used Engine Oil by Pseudomonas sp. Isolated from an Automobile Workshop's Soil. Journal of Environmental Microbiology and Toxicology, 10(2), 59-62. [Crossref]

Adeleye, A. O., Yerima, M. B., Nkereuwem, M. E., Onasanya, G. O., Onokebhagbe, V. O., & Bate, G. B. (2022). Biochemical and PCR-based identification of hydrocarbonoclastic bacteria isolated from spent engine oil polluted soil. *Sule Lamido University Journal of Science and Technology, 3*(1 & 2), 23–34.

AOAC (2000) Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16

Baldwin D. R. and Marshall W. J. (1999). Heavy metal poisoning and its laboratory investigation, Annals of Clinical Biochemistry. 36, no. 3, 267-300, 2-s2.0-0033035111. [Crossref]

Chaerun, S. L., Tazaki, K., Asada, R., & Kogure, L. (2004). Lubricating oil degrading bacteria in soils from filling stations and auto mechanic workshops in Buea, Cameroon: Occurrence and characteristics of isolates. *African Journal of Biotechnology, 7*, 1700–1706. [Crossref]

Cheesbrough, M. (2006) District Laboratory Practice in Tropical Countries. Part 2, 2nd Edition, Cambridge University Press Publication, South Africa, 1-434. [Crossref]

Elegbede, J.A and Lateef, A (2019) Green synthesis of silver (Ag), gold (Au) and silver-gold (Ag Au) alloy nanoparticles: a review on recent advances, trends and biomedical applications. In: Verma DK, Goyal MR, Suleria HAR (eds) Nanotechnology and nanomaterial applications in food, health and biomedical sciences. Apple Academic Press Inc./CRC Press, Taylor and Francis Group, Oakville, Ontario, Canada, pp 3-89. ISBN 978-1-77188-764-9. [Crossref]

Fu, X., Wu, T., Li, H., Xue, J., Sun, J., & Li, L. (2022). Study on the preparation conditions and degradation performance of an efficient immobilised microbial agent for marine oil pollution. *Environmental Technology (United Kingdom), 43*(15), 2352–2358. [Crossref]

Goveas, L. C., Menezes, J., Salian, A., Krishna, A., Alva, M., & Basavapattan, B. (2020). Petroleum hydrocarbon degradation in soil augmented with used engine oil by novel *Pantoea wallisii* SS2: Optimisation by response surface methodology. *Biocatalysis and Agricultural Biotechnology, 25*, 101614. [Crossref]

Ibrahim, H. M. M. (2016). Biodegradation of used engine oil by novel strains of *Ochrobactrum anthropi* HM-1 and *Citrobacter freundii* HM-2 isolated from oil-contaminated soil. *3 Biotech, 6*(2), 226. [Crossref]

Idemudia, M. I., Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, PMB 1100, Benin City, Nigeria.

Kumar, A.G., Vijayakumar, L., Joshi, D.G., Peter, G. Dharani, R. Kirubagaran, Biodegradation of complex hydrocarbons in spent engine oil by novel bacterial consortium isolated from deep sea sediment, Bioresource Technology,Volume 170, 2014, Pages 556-564,nISSN 0960-8524. [Crossref]

Larik, I. A., Qazi, M. A., Phulpoto, A. H., Haleem, A., Ahmed, S., & Kanhar, N. A. (2019). *Stenotrophomonas maltophilia* strain 5DMD: An efficient biosurfactant-producing bacterium for biodegradation of diesel oil and used engine oil. *International Journal of Environmental Science and Technology, 16*, 259–268. [Crossref]

Larsen, J., Cornejo, P., & Barea, J. M. (2009). Interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the plant growth-promoting rhizobacteria *Paenibacillus myxa* and *P. macerans* in the mycorrhizosphere of *Cucumis sativus*. *Soil Biology and Biochemistry, 41*(43), 286–292. [Crossref]

Lin, T.A., Young, C.C., Ho, M.J., Yeh, M.S., Chou, C.L., Wei, H and Chang, J.S. Here's a short and persuasive message you can send to a sponsor:

Mandri, T., & Lin, J. (2017). Isolation and characterisation of engine oil degrading indigenous microorganisms in KwaZulu, South Africa. *Journal of Bioremediation, 6*(1), 23–27.

Mark, O, I., Ogbulie, J.N., Nweke, C,O., (2018). Isolation and identification of bacteria from engine oil polluted soil from mechanic workshops in Okija Town. Journal of Microbiology Experiment. 2018;11(5):138–141. [Crossref].

Meena, K. R., Dhiman, R., Singh, K., Kumar, S., Sharma, A., & Kanwar, S. S. (2021). Purification and identification of a surfactin biosurfactant and engine oil degradation by *Bacillus velezensis* KLP2016. *Microbial Cell Factories, 20*(1). [Crossref]

Muhammad, A., & Adamu, F. A. (2022). Biodegradation of used engine oil by *Pseudomonas* sp. isolated from an automobile workshop's soil. *Journal of Environmental Microbiology and Toxicology, 10*(2), 59–62. [Crossref]

Ogunbayo, A. O., Bello, R. A., & Nwagbara, U. (2012). Bioremediation of engine oil contaminated site. *Journal of Emerging Trends in Engineering and Applied Sciences, 3*(3), 483–489.

Osuji, M. I., Ogbulie, J. N., & Nweke, C. O. (2023). Isolation and identification of bacteria from engine oil polluted soil from mechanic workshops in Okija Town. *Journal of Microbiology and Experimentation, 11*(5), 138–141. [Crossref]

Salam, L. B. (2016). Metabolism of waste engine oil by *Pseudomonas* species. *3 Biotech, 6*(1), 98. [Crossref]

Shahida, A. A., Sambo, S., & Salau, I. A. (2015). Biodegradation of used engine oil by fungi isolated from mechanic workshop soils in Sokoto metropolis, Nigeria. *Sky Journal of Soil Science and Environmental Management, 4*(6), 54–69.

Smith, P., Adams, J., Beerling, D.J, Beringer, T., Calvin, K.V, Fuss, S,. Griscom, B., Hagemann, N., Kammann, C., Kraxner, F., Minx, J.C, Popp, A., Renforth, P., Vicente, J.L.V,, Keesstra, S (2019) Impacts of land-based greenhouse gas removal options on ecosystem services and the United Nations Sustainable Development Goals. Annu Rev Environ Resour 44:255-286. [Crossref]

Sun, K., Song, Y., He, F., Jing, M., Tang, J., & Liu, R. (2021). A review of human and animals exposure to polycyclic aromatic hydrocarbons: Health risk and adverse effects, photo-induced toxicity and regulating effect of microplastics. *Science of the Total Environment, 773*, 145403. [Crossref]

Tirmizhi, M., Faggo, A. A., & Gulumbe, B. H. (2022). Species of *Pseudomonas* and *Bacillus* isolated from refined oil-contaminated soil showed the potential to efficiently degrade diesel. *Journal of Biochemistry, Microbiology and Biotechnology, 10*(1), 72–75. [Crossref]

Ugoh, S. C., & Moneke, L. U. (2011). Isolation of bacteria from engine oil contaminated soils in auto-mechanic workshops in Gwagwalada, Abuja, FCT-Nigeria. *Academia Arena, 3*(5), 28–33.

Umar, Z. D., Nor Azwady, A. A., Zulkifli, S. Z., & Muskhazli, M. (2018). Effective phenanthrene and pyrene biodegradation using *Enterobacter* sp. MM087 (KT933254) isolated from used engine oil contaminated soil. *Egyptian Journal of Petroleum, 27*(3), 349–359. [Crossref]

Uriyo, A. P. and Singh, B. R. (2001). Practical Soil Chemistry Manual. Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture and Forestry University of Dar Es Salam - Morogoro.

Uriyo, A. P. and Singh, B. R. (2001). Practical Soil Chemistry Manual. Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture and Forestry University of Dar Es Salam – Morogoro.

Vidalii, M. (2001). Bioremediation. An overview. Pure Appl Chem. 2001,73,11-1172. [Crossref]

Ziag, A. G., Saadoun and Shakah, A.A. (2005): Selection of bacteria and plant seeds for potential use in the remediation of diesel contaminated soils. J. Basic. Micribiol., 45: 251-256 (2005). [Crossref]