*Journal of Advances in Microbiology*

***21(9): 68-78, 2021; Article no.JAMB.73969***
***ISSN: 2456-7116***

# Bioremediation of Soil Contaminated with Spent Engine Oil using Pig Dung

**A. A. Ibiene[1], O. Aleruchi[2*], L.C. Nnodim[2] and R. U. Ihunwo[1]**

*[1]Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Nigeria.*
*[2]Department of Microbiology, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JAMB/2021/v21i930385
Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Vijayadeep C, Bangalore City University, India.
(2) S. Muthukrishnan, University Of Kerala, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/73969

***Original Research Article***

***Received 05 July 2021***
***Accepted 15 September 2021***
***Published 21 September 2021***

## ABSTRACT

Studies were carried out to investigate the bioremediation potential of pig dung in a soil contaminated with spent engine oil. Soil samples were obtained from the Ofrima complex, University of Port Harcourt. The soil samples were contaminated with various concentrations (50 ml and 100 ml) of spent engine oil and allowed for 21 days for proper exposure, mimicking natural spill. This was followed by the addition of the pig dung. The experimental setup was labeled sample A (1 kg soil + 100 g pig dung + 50 ml spent engine oil) and sample B (1 kg soil + 100 g pig dung + 100 ml spent engine oil). The physicochemical parameters and the microbiological analysis were done using standard methods. The total petroleum hydrocarbon was analyzed using gas chromatographic methods. Analyses were carried out at 14 days intervals for 28 days. The physicochemical parameter results showed a reduction in pH values in the contaminated soil samples, ranging from 6.21 - 6.65 in sample A and 6.57 - 6.87 in sample B. Temperature values were constant at $23^0$C from day 1 to day 14 in sample A and increased at day 28 to 24 $^0$C, also for sample B, the temperature was constant at $23^0$C from day 1 to day14 and increased at day 28 to 26 $^0$C. The amount of heavy metal (Lead) content decreased from 4.3645 - 1.93676 (mg/kg) and 6.18361 - 3.89654 (mg/kg) for samples A and B, respectively. There was also a significant reduction in the amount of Total Petroleum Hydrocarbon, from 16631.86 - 3280.83 mg/kg for sample A and 18464.73 - 6784.60 mg/kg for sample B. The THB counts for samples A and B ranged from 7.73 - 7.91 and 7.05-8.20 (Log cfu/g), respectively. The fungal counts ranged from 3.99–4.58 and 5.12 -

**Corresponding author: E-mail: owhonka.aleruchi@gmail.com;*

7.93 (Log cfu/g) for samples A and B respectively. HUB counts ranged from 4.52–5.09 and 4.93-5.55 (Log cfu/g) for samples A and B, respectively. The HUF counts ranged from 4.12 - 5.49 and 4.13 - 4.70 (Log cfu/g) for samples A and B, respectively. The results clearly showed that microorganisms capable of utilizing total petroleum hydrocarbon were present, also the pig dung showed both bio-stimulation and bio-augmentation tendency to attract high microbial load which supported the bioremediation of the spent engine oil contaminated soil.

*Keywords: Bioremediation; pig dung; spent engine oil; contaminated soil; total petroleum hydrocarbon.*

## 1. INTRODUCTION

Mechanic workshops within Nigeria are poorly managed and are the source of constant release of spent engine oil discharged from the engine of cars and motorcycles which causes serious environmental pollution [1]. Cleanup of mechanic sites is still elusive as operators of such sites are usually ignorant of the deleterious effects on the environment [1]. The disposal of spent engine oil into gutters, water drains, open vacant plots, and farms is a common practice in Nigeria, especially by motor mechanics. This oil, also called spent lubricant or waste engine oil, is usually obtained after servicing and subsequently draining from automobile and generator engines [2], and much of this oil is poured into the soil. There are relatively large amounts of hydrocarbons in the used oil, including the highly toxic polycyclic aromatic hydrocarbons [3]. Also, most heavy metals such as Pb, Al, Ni, and Fe, which were below detection in unused lubricating oil, have been reported by Uhor, [4] to give high value (ppm) in used oil. These heavy metals may be retained in soils in the form of oxides, hydroxides, carbonates, exchangeable cations, and/or bound to the organic matter in the soil [5]. Nevertheless, this is dependent on the local environmental conditions and the kind of soil constituents present in the soil-water system.

Oil pollution of soil leads to the build-up of essential (organic C, P, Ca, Mg) and non-essential (Mg, Pb, Zn, Fe, Co, Cu) elements in soil and the eventual translocation in plant tissues [6]. Although some heavy metals at low concentrations are essential micronutrients for plants, at high concentrations they may cause metabolic disorders and growth inhibition for most of the plant species [7]. Used engine oil from the motor engine is released indiscriminately into free land space, drainage systems which carry them to inland and coastal waters. An engine oil-polluted land is unhealthy for plant proliferation and microbial survival. When it seeps into underground water, it renders it unfit for human consumption. When in coastal waters, it affects fish and other aquatic animal growth and health meaning a significant drop in fish population, bioaccumulation, and biomagnifications as well.

Soil is a key component of natural ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem [8]. When soil is polluted, the ecosystem is altered, and agricultural activities are affected. Contamination of the soil by spent engine oil creates an unsatisfactory condition for life in the soil, which is due to the poor aeration it causes in the soil, immobilization of soil nutrients, loss of water-holding capacity, lowering of soil pH, and reduction in soil catalase enzyme activity [9].In recent times, a lot of effort has been made toward reducing environmental pollution, by using natural processes such as bio-augmentation, bio-stimulation, mycoremediation, phyto-remediation, bio-sparging, bio-venting and composting. Among the natural processes, bio-remediation is the most common method used for hydrocarbon removal for 30 years [10]. Bioremediation is defined as a biological response to environmental abuse [11], using living microorganisms or microbial processes to detoxify and degrade environmental pollution. In other words, it is a technology for removing pollutants from the environment, thus restoring the original natural environment [12]. Pig dung serves as manure in soil and they stimulate the growth of soil microbes, which can be effective in crude oil polluted soil.

This study is therefore aimed at evaluating the effectiveness of pig dung in the bioremediation of spent engine oil-polluted soil.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The spent engine oil was collected from a mechanic village located at Ikoku, Port Harcourt,

Rivers State. The portion of soil used for this experiment was obtained from the Ofrima complex, University of Port Harcourt, Choba, Rivers State. The soil was not cultivated at the time of the investigation. The soil was collected from the surface horizon (0-20cm depth) using a clean trowel to obtain the sample, and put in a plastic bag which was taken to the laboratory for soil analysis, and pig dung was collected from the Faculty of Agriculture, Rivers State University.

## 2.2 Preparation and Treatment of Soil Samples

The soil samples were obtained and sieved to remove debris. Then 2 kg of sieved soil obtained were distributed into 2 plastic bowls (1 kg each). The 2 bowls containing the soil sample were labeled A and B and were polluted with 50 ml and 100 ml of spent engine oil, respectively, and allowed to fallow for 21 days to mimic the natural oil spill environment. The experimental setup labeled sample A contained 100 g of pig dung, 1 kg of soil, and 50 ml of spent engine oil. Sample B contained 100 g of pig dung, 1 kg of soil, and 100 ml of spent engine oil. Samples were mixed wearing gloves. The soil samples A and B were watered with sterile water every week after treatment application to keep the soil moist.

## 2.3 Physicochemical Analysis of Soil Samples

Various analyses such as pH, temperature, heavy metals (Lead), total petroleum hydrocarbon were carried out on the soil samples. The lead concentration was then analyzed using a solar thermal elemental Atomic Absorption Spectrophotometer (Flame AAS) mode: S4 = 71096.

The total petroleum hydrocarbon (TPH) concentrations were ascertained using the EPA 8015 method with modification as reported by Stewart *et al.* [13] and Barathi and Vasudevan [14]. The soil was air-dried and ground separately. 10 g of the soil samples were transferred into a small amber bottle and mixed with 50 g of sodium sulphate to remove its water content. The mixture was then stirred thoroughly until it was friable, dry, and powder-like. 50 ml of dichloromethane was added to extract the hydrocarbon present. The mixture was allowed to stand for 1 hour after stirring, then it was filtered. The filtrate was passed through a column packed with sodium sulphate and silica gel separated with cotton wool, evaporated with nitrogen, and diluted with 1 ml of the dichloromethane solvent. 1 µl aliquot of the mixture was syringed into Agilent 6890 Nitrogen gas chromatography (HP 5890, Hewlett Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID) (Agilent, 7890A).

## 2.4 Microbiological Analysis of Samples

Microbiological analysis was carried out. These include: isolation and enumeration of total heterotrophic bacteria, total fungi, hydrocarbon-utilizing bacteria, and hydrocarbon-utilizing fungi.

## 2.5 Isolation of Total Heterotrophic Bacteria

Exactly 1 g of the sample was serially diluted using normal saline and 0.1 ml of the serially-diluted sample was plated out on a sterile nutrient agar plate containing 0.05 mg/ml of Ketoconazole to inhibit fungal growth and accurately spread on the agar using a glass spreader. Duplicate plates were prepared and incubation was carried out in an inverted position at $30^0$C for 24-48 hours. Colonies that developed after incubation were counted and the total heterotrophic bacteria expressed as a log of colony-forming units per gram of sample.

## 2.6 Isolation of Total Fungi

Exactly 1g of the sample was diluted serially using distilled water and 0.1 ml aliquot of the serially-diluted sample was spread-plated on potato dextrose agar containing 0.05 mg/ml of chloramphenicol to inhibit bacterial growth and accurately spread on the agar using a hockey stick. Duplicate plates were prepared which were incubated at 30 $^0$C for 3-4 days in an inverted position. The colonies that grew after incubation were counted and the total fungi expressed as a log of colony-forming units per gram of the sample.

## 2.7 Isolation of Hydrocarbon-Utilizing Bacteria

Exactly 1 g of the sample was serially diluted with distilled water and 0.1 ml aliquot of the serially-diluted sample was plated out on nutrient agar containing nystatin at a concentration of 0.05 mg/ml to inhibit fungal growth and accurately spread on the agar using a glass spreader. Sterile filter paper impregnated with crude oil was placed on the cover of the petri

dish to supply hydrocarbons to the organisms through the vapor transfer phase. Duplicate plates were prepared and incubated at $30^0$C for 24-48 hours after which the colonies that developed were counted and the hydrocarbon utilizing bacteria expressed as a log of colony-forming units per gram of the sample.

## 2.8 Isolation of Hydrocarbon-Utilizing Fungi

Exactly 1g of each sample was serially diluted using distilled water and 0.1 ml aliquot of it was spread-plated on potato dextrose agar which contained 0.05 mg/ml of chloramphenicol to inhibit bacterial growth and accurately spread on the agar using a glass spreader. Sterile filter paper saturated with crude oil was placed on the cover of the petri dish to supply hydrocarbons to the organisms in the sample through the vapour transfer phase. Duplicate plates were prepared per sample and incubated at $30^0$C for 3-4 days. Colonies that developed after incubation were counted and the hydrocarbon-utilizing fungi were expressed as a log of colony-forming units per gram of the sample.

## 2.9 Identification of Bacterial Isolate

The isolates were obtained and subjected to various characterization procedures. Pure isolates of bacteria were identified based on their cultural, morphological, and physiological characteristics [15, 16]. The following standard characterization tests were performed, Gram staining reaction, motility test, oxidase test, catalase test, coagulase test, starch hydrolysis, methyl red, and voges proskauer test, urease test, including carbohydrate fermentation test (glucose, lactose, maltose, mannitol) and indole test.

## 3. RESULTS

The pH values of the soil sample contaminated with 50 ml and 100 ml of spent engine oil, on day 1 recorded 6.65 and 6.87, respectively. The pH value was reduced to 6.46 on day 14 and 6.21 on day 28 from the sample contaminated with 50ml. The 100 ml sample recorded 6.73 and 6.57 pH values on days 14 and 28, respectively. The temperature recorded $23^0$C on both samples on days 1 and 14. On day 28, the temperature increased to 24 $^0$C and 26 $^0$C in samples A and B, respectively. The lead (P) values recorded 4.36452 mg/kg and 6.18361 mg/kg from the sample with 50ml and 100 ml, respectively on day 1 and reduced on day 28 to 3.58365 mg/kg and 3.8954mg/kg, respectively.

The carbon groups shown in the samples were from C8 to C23. The Pr and Ph were also identified. C30 was not recorded in sample A (50ml). On day 28, sample A (50ml) showed clearance of C8, C9, C10, C11, C15, C17, Pr, Ph, C21, C22, C26, C28, C29, C32, and C33. Sample B (100ml) on day 28 had a clearance of C9, C10, C29, and C30. The total petroleum hydrocarbon obtained from sample A (50ml) was 16631.85 mg/kg on day 1, 5921.15 mg/kg on day 14, and 3280.83 mg/kg on day 28. Sample A (50ml) also recorded 80.3% removal of the total petroleum hydrocarbon. Sample B (100ml) had a total petroleum hydrocarbon of 18464.73 mg/kg on day 1, 11034.56 on day 14, and 6784.60 mg/kg on day 28. Sample B (100ml) recorded a percentage removal of 63.3% at the end of the experiment.

Gas chromatograms were obtained on days 1, 14, and 28 accordingly for samples A and B respectively. The peaks represent residual carbon fractions in the samples during the study.
The microbial counts from the experimental samples (A-50ml and B-100ml) are shown in Table 3. The total heterotrophic bacterial count in sample A (50 ml) recorded 7.73 $Log_{10}$ cfu/g on day 1, and increased progressively on days14 and 28 to 7.91 and 7.81 $Log_{10}$ cfu/g, respectively. Sample B (100 ml) obtained a total heterotrophic bacteria count of 7.93 $Log_{10}$ cfu/g on day 1, on day 14 recorded 8.20 $Log_{10}$ cfu/g, the counts decreased to 7.05 $Log_{10}$ cfu/g on day 28. The total fungal counts from samples A and B recorded 4.58 and 7.93 $Log_{10}$ cfu/g, respectively on day 1. The total fungal counts decreased to 3.99 and 4.58 $Log_{10}$ cfu/g, in samples A and B, respectively on day 14, which later increased to 4.15 and 5.12 $Log_{10}$ cfu/g, respectively. Hydrocarbon utilizing bacteria count in sample A recorded 4.52, 5.09, and 4.77 $Log_{10}$ cfu/g on days 1, 14, and 28, respectively. Sample B recorded 5.55, 5.35, and 4.93 $Log_{10}$Cfu/g on days 1, 14, and 28, respectively. On day 1 the hydrocarbon utilizing fungi from sample A was 4.39 $Log_{10}$ cfu/g, the counts increased to 5.49 $Log_{10}$ cfu/g on day 14 and later decreased to 4.12 $Log_{10}$ cfu/g on day 28. Sample B recorded high counts of 4.70 $Log_{10}$ cfu/g on day 1 which decreased to 4.13$Log_{10}$ cfu/g on day 14 and later increased to 4.96 $Log_{10}$ cfu/g on day 28.

**Table 1. Some physicochemical parameters of samples**

| Samples | pH | Temp. | Lead |
|---|---|---|---|
| 50ml day 1 | 6.65 | $23^0$C | 4.36452 mg/kg |
| 50ml day 14 | 6.46 | $23^0$C | 3.58365 mg/kg |
| 50ml day 28 | 6.21 | $24^0$C | 1.93676 mg/kg |
| 100ml day 1 | 6.87 | $23^0$C | 6.18361 mg/kg |
| 100ml day 14 | 6.73 | $23^0$C | 5.04629 mg/kg |
| 100ml day 28 | 6.57 | $26^0$C | 3.89654 mg/kg |

**Table 2. Total petroleum hydrocarbon concentration of experimental samples**

| Carbon groups | TPH concentrations of Sample A (50ml) at Day 1 | Day 14 | Day 28 | TPH concentration of Sample B (100 ml) at Day 1 | Day 14 | Day 28 |
|---|---|---|---|---|---|---|
| C8 | 874.35 | 283.81 | | 948.18 | 625.94 | 485.29 |
| C9 | 513.94 | | | 541.42 | 276.01 | |
| C10 | 169.14 | | | 218.30 | | |
| C11 | 726.80 | 210.55 | | 764.50 | 392.98 | 128.95 |
| C12 | 1211.54 | 728.73 | 538.27 | 1310.26 | 984.64 | 740.57 |
| C13 | 935.63 | 392.86 | 210.55 | 979.74 | 702.28 | 510.94 |
| C14 | 1101.94 | 643.27 | 437.20 | 1151.51 | 876.82 | 676.01 |
| C15 | 579.71 | | | 635.20 | 313.37 | 192.98 |
| C16 | 1172.95 | 705.05 | 541.42 | 1218.05 | 905.79 | 702.28 |
| Pr | 1154.12 | 816.32 | 643.27 | 1194.40 | 862.31 | 624.08 |
| C17 | 727.02 | 243.27 | | 785.91 | 410.17 | 240.14 |
| C18 | 961.34 | 437.20 | 131.64 | 993.35 | 499.72 | 213.31 |
| Ph | 483.31 | | | 551.63 | 371.84 | 162.72 |
| C20 | 1074.71 | 631.64 | 501.21 | 1103.82 | 893.14 | 647.08 |
| C21 | 553.34 | | | 618.76 | 424.08 | 105.82 |
| C22 | 659.71 | 128.53 | | 731.49 | 408.85 | 212.42 |
| C23 | 997.14 | 443.72 | 185.22 | 1052.14 | 612.42 | 481.41 |
| C25 | 679.87 | 256.22 | 92.06 | 730.93 | 456.27 | 209.85 |
| C26 | 581.24 | | | 621.48 | 293.67 | 96.40 |
| C28 | 376.84 | | | 423.79 | 98.42 | 47.83 |
| C29 | 238.27 | | | 308.65 | | |
| C30 | | | | 217.02 | | |
| C32 | 345.28 | | | 594.88 | 228.40 | 112.05 |
| C33 | 513.63 | | | 769.31 | 397.42 | 194.47 |
| Total | 16631.85 | 5921.15 | 3280.83 | 18464.73 | 11034.56 | 6784.60 |

**Table 3. Microbial counts during the experimental period**

| Experimental Sample/Day | Total Heterotrophic Bacteria Count (Log cfu/g) | Total Fungal Count (Log cfu/g) | Hydrocarbon Utilizing Bacteria Count (Log cfu/g) | Hydrocarbon Utilizing Fungal Count (Log cfu/g) |
|---|---|---|---|---|
| 50ml day 1 | 7.73 | 4.58 | 4.52 | 4.39 |
| 50ml day 14 | 7.91 | 3.99 | 5.09 | 5.49 |
| 50ml day 28 | 7.81 | 4.15 | 4.77 | 4.12 |
| 100ml day 1 | 7.93 | 7.93 | 5.55 | 4.70 |
| 100ml day 14 | 8.20 | 4.58 | 5.35 | 4.13 |
| 100ml day 28 | 7.05 | 5.12 | 4.93 | 4.96 |

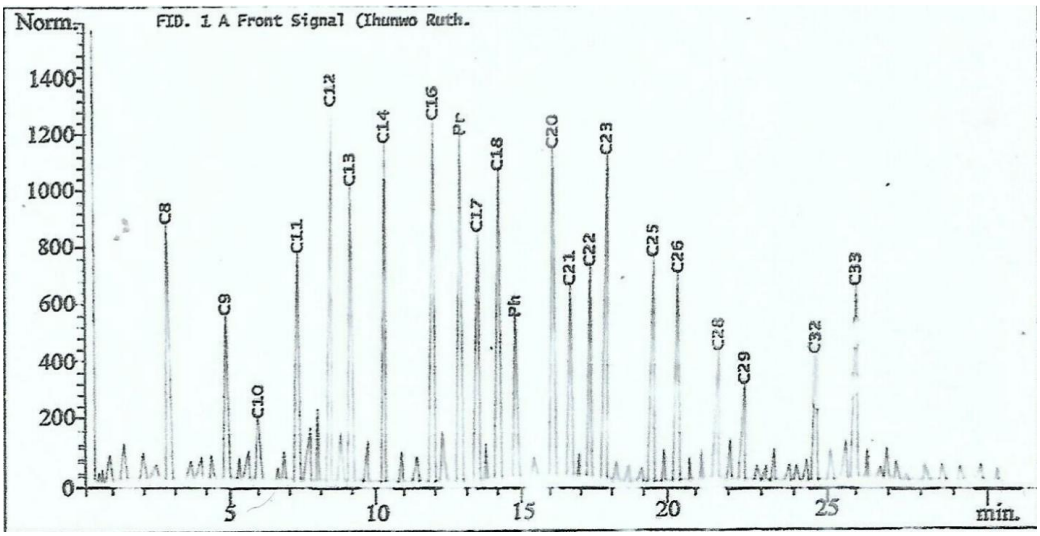


**Fig. 1. Gas chromatography obtained at day 1 from sample A**

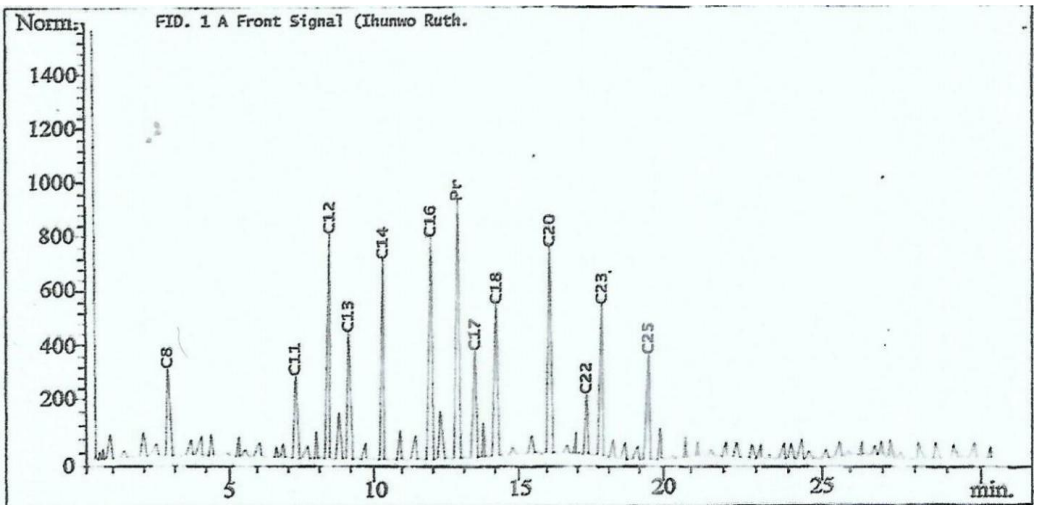


**Fig. 2. Gas chromatography obtained at day 14 from sample A**

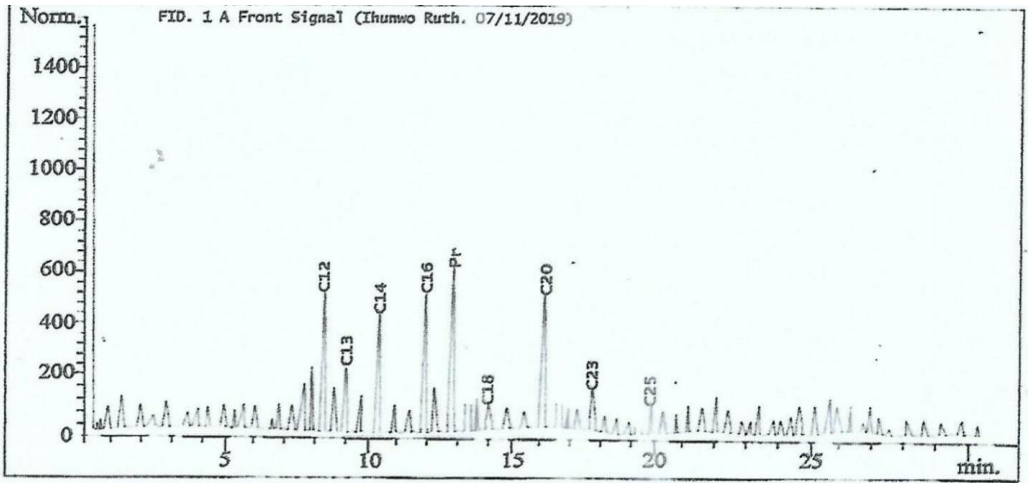


**Fig. 3. Gas chromatography obtained at day 28 from sample A**

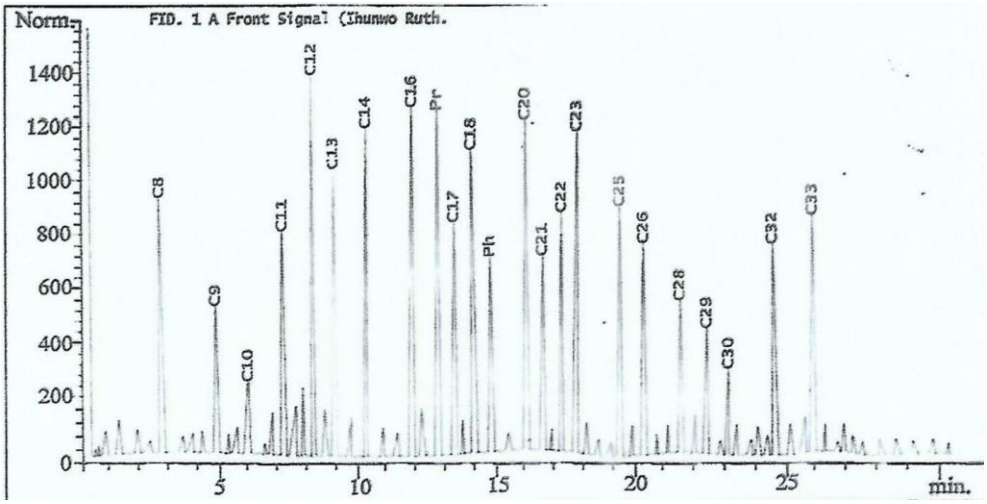


**Fig. 4. Gas chromatography obtained at day 1 from sample B**

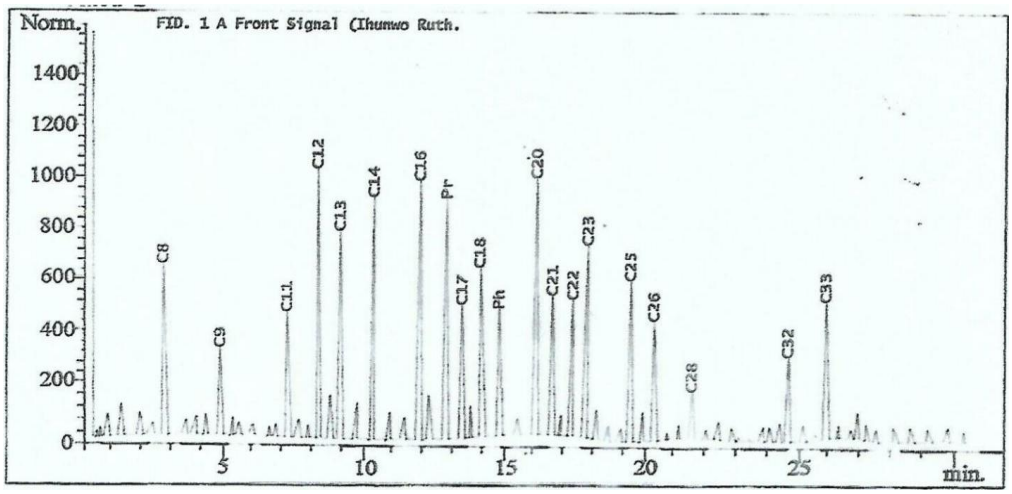


**Fig. 5. Gas chromatography obtained at day 14 from sample B**

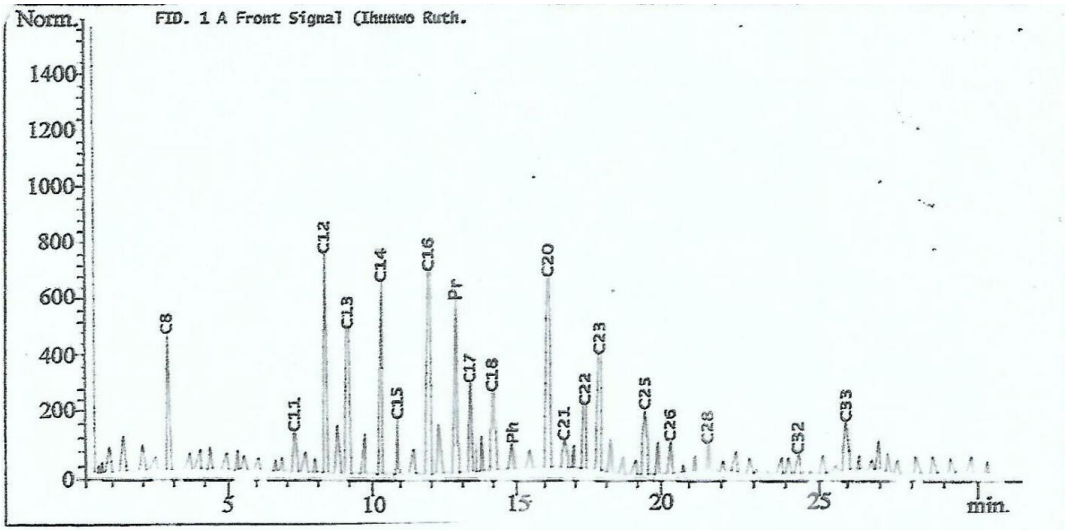


**Fig. 6. Gas chromatography obtained at day 28 from sample B**

**Table 4. Heterotrophic bacteria and fungi Isolated from Pig dung**

| Heterotrophic Bacteria | Fungi |
|---|---|
| *Escherichia* sp. | *Aspergillus niger* |
| *Enterococcus* sp. | *Penicillium* sp. |
| *Micrococcus* sp. | *Candida* sp. |
| *Staphylococcus* sp. | *Trichoderma* sp. |
| *Bacillus* sp. | *Cladosporium* sp. |
| *Aeromonas* sp. | *Mucor* sp. |
| *Arthrobacter* sp. | *Rhizopus* sp. |
| *Sarcina* sp. | |
| *Pseudomonas* sp. | |

A total of twenty-eight (28) bacterial isolates were obtained from this research. The bacterial genera obtained from the samples include; *Escherichia* sp., *Enterococcus* sp., *Micrococcus* sp., *Staphyloccocus* sp., *Bacillus* sp., *Aeromonas* sp., *Arthrobacter* sp., *Sarcina,* and *Pseudomonas* sp. The fungi isolated include; *Aspergillus niger, Penicillium sp., Candida sp., Trichoderma sp., Cladosporium sp., Mucor* sp., and *Rhizopus* sp.

## 4. DISCUSSION

The pH of the samples was alkaline for sample A (50 ml) and sample B (100 ml) respectively on the first day of bioremediation, the pH finally decreased in both sample A (50 ml) and B (100 ml), and the least pH value was recorded on day 28 in both samples. However, sample A had the least value. This corresponds with Dalyan*et al.,* [17] study, where they concluded that the decrease may have been as a result of $CO_2$ evolution, a decrease in pH of the treated soil must have been due to the pH content of the pig dung coupled with the actual soil pH. The observed reduction in pH was also similar to the findings of Osuji and Nwoye [18].

In this research, the temperature was constant at $23^0$C for day 1 and day 14, with the highest recorded for day 28 in both samples (A and B). The activities of microbes increased with the increase of temperature in the appropriate range because it enhances the microbial metabolism as well as the enzyme activities.

Sample B (100 ml) recorded the highest value of lead on day 1 than sample A. The values of lead in both samples decreased during the experimental period and the least value was recorded on day 28. The result showed that the amount of lead decreased with an increase in time and also decreased with an increase in concentration. This agrees with the findings of Stephen *et al. (*2016) where he stated that the reduction in the amount of lead could be due to the sorption of heavy metals by soil microorganisms bacteria and fungi in the formation of oxides, hydroxides, carbonates, and exchangeable cations in the soil.

There was a marked decrease in the amount of hydrocarbon for total petroleum hydrocarbon results per retention time, the amount of hydrocarbon drastically reduced from day 1 to day 14, some groups of carbon such $C_9$, $C_{10}$, $C_{15}$, Ph, $C_{21}$, $C_{26}$, $C_{28}$, $C_{29}$, $C_{30}$, $C_{32}$ and $C_{33}$ had disappeared at day 14 for sample A (50 ml), $C_{10}$, $C_{29}$ and $C_{30}$ on day 14 disappeared completely in sample B (100ml). On day 28, the additional group of carbon that cleared in sample A was $C_8$ and $C_{17}$, while $C_9$ was cleared in sample B. This investigation clearly showed that spent engine oil contaminated soil with the addition of pig dung as an organic nutrient supplies microorganisms that speed up the rate at which spent engine oil is broken down and increases the rate at which hydrocarbon pollution is cleaned off the environment [19].

The total heterotrophic bacteria count in sample A increased progressively from day 1 to 28. In sample B, the total heterotrophic bacteria count increased on days 1 and 14 but later decreased on day 28. Total fungal counts in both samples (A and B) decrease on day 14 but slightly increased on day 28. Hydrocarbon utilizing bacteria count in sample A increased on day 14 and later increased on day 28. In sample B the hydrocarbon utilizing bacteria decreased progressively during the experimental period. Hydrocarbon utilizing fungi count in sample A increased on day 14 but later decreased on day 28, reverse was the case in sample B. The increase in counts of the heterotrophic population is in agreement with results obtained by other researchers that hydrocarbon pollution does not

enrich only hydrocarbon utilizers but also other populations that utilize breakdown products of hydrocarbons [20]. There was a decrease in the counts of fungal populations in the varying samples of spent engine oil contaminated soil at day 1 to day 14 and a slight increase at day 28. This observation agrees with that of Atlas, who reported that the drop in the fungal counts in the contaminated soil in the first week can be attributed to selective inhibition of members of the microbial community as a result of the toxic components of spent engine oil and also as a result of reduced aeration and upset of carbon/inorganic nutrient balance for the indigenous population caused by the presence of petroleum. The counts of the HUFs increased at day 28 after a slight reduction at day 14. This finding is consistent with the work done by William *et a*l. who used poultry litter to enhance the degradation of petroleum hydrocarbons in the soil. The counts for THB and HUB increased slightly at day 14 and then reduced at day 28.

Margesin and Schinner [21], reported that fluctuations in counts of microorganisms might be caused by specific mutual interactions of microorganisms in soil contaminated with spent motor engine oil.

This study showed that pig dung contains a teeming population of heterotrophic bacteria, bacteria isolated were *Pseudomonas* sp*., Escherichia* sp*., Enterococcus* sp*., Micrococcus* sp., *Staphylococcus* sp., and *Bacillus* sp. Udebuani *et al*. [22] report is in agreement with present findings of this study that animal dung inhabits *Micrococcus* sp*., Pseudomonas* sp*.,*and *Proteus* sp. Yakubu, [23] studied the biodegradation of Lagoma crude oil using pig dung and reported the presence of the species of *Pseudomonas, Proteus,*and *Micrococcus* in the dung. The fungi isolated from the pig manure were *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp., *Trichoderma* sp., and *Candida* sp. Benal *et al.*2014 in their study of the prevailing fungi on hydrocarbon polluted soil also isolated *Aspergillus, Penicillium, and Cladosporium* and reported that they had hydrocarbon-degrading capabilities. Chikere and Azubike, [24] in their work on the characterization of hydrocarbon-utilizing fungi from hydrocarbon polluted sediments and water isolated *Aspergillus, Penicillium, Cladosporium, and Candida.* Omotayo *et al.* [25] also isolated Candida tropicalis from tropical polluted soils and reported that it degraded aviation fuel while *Candida* sp. has been reported by Amund and Nwokoye, [26] to utilize oil.

## 5. CONCLUSION AND RECOMMENDATION

The results obtained in this study also showed that bioremediation using pig dung can increase the rate at which the microorganisms utilized spent oil. The study revealed that oil pollution stimulated the growth of certain organisms and inhibited the growth of others. The study also revealed that many bacterial genera were present in spent motor engine oil amended soil, which suggests that bacteria can grow and utilize engine oil as a source of carbon. Environmental pollution in Nigeria has been a major threat. This study has revealed that bioremediation using pig dung can effectively clean up spent engine oil-contaminated sites. Consideration can be given to cheap organic fertilizers like the use of organic nutrients because it is less expensive and has a higher growth rate of microorganism. Organic fertilizers like pig dung are good, since they do not have any side effects and are environmentally friendly.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Adams GO, Tawari-Fufeyin P, Igelenyah E. Bioremediation of spent oil-contaminated soil using poultry litter. Research Journal in Engineering and Applied Sciences. 2014;2:124-130.
2. Anoliefo GO, Vwioko DE. “Tolerance of *Chromolaena odoranta* (L) K and R. grown in soil contamination with spent lubrication

oil". Journal of Tropical Biosciences. 2001;1:20-224.

3. Wang J, Jia CR, Wong CK. Characteristics of polycyclic aromatic hydrocarbon created in lubricating oils, Water, Air, Soil Pollution. 2006;120:381-396.
4. Uhor AS. Nigeria delays search for super oil spill agent. Daily sunray.1993;4.
5. Okpokwasili GC, Amanchukwu SC. Petroleum hydrocarbon degradation by *Candida* species. Environment International. 1988;14:243-247.
6. Vwioko DE, Anoliefo GO, Fashemi SD. Metals concentration in plant tissues of *Ricinus communis L.* (Castor oil) grown in soil contaminated with spent lubricating oil. Journal of Applied Science and Environmental Management. 2006;10:127-134.
7. Fernandes JC, Henriques FS. Biochemical, physiological, and structural effects of excess copper in plants. The Botanical Review. 1991;57:246-273.
8. Van Hamme JD, Singh A, Ward OI. Recent advances in petroleum microbiology. Microbial Biology Revision. 2003;67:503-549.
9. Dollah SA. Bioremediation of Petroleum hydrocarbon polluted soil: potentials, principles, and applications. Journal of Environmental Management and Education. 2004;1(2):171-180.
10. Lee K, Tremblay GH, Levy EM. Bioremediation application of slow-release fertilizers on low-energy shorelines. Proceedings of the 1993 oil spill conference. American petroleum institute, Washington D. C. 1993;449-454.
11. Hammer G. Bioremediation: a response to gross environmental abuse. Trends Biotechnology. 1993;11:317-318.
12. Sasikumar CS, Papinazeth T. Environmental Management. Bioremediation of polluted Environmental. Proceedings of the Third International Conference on Environment and health, Chennai, India, 2003;456-469.
13. Stewart DJ, Manley TR, White DA, Harrisson DL. Fluorine residues on pasture, in soil, and sheep urine, resulting from topdressing with superphosphate. New Zealand Journal of Experimental Agriculture. 1974;2:129-133.
14. Barathi S, Vasudevan N. Utilization of petroleum hydrocarbons by Pseudomonas fluorescens isolated from petroleum-contaminated soil. Environmental inte, 2001;26:413-416.
15. Buchanan RE, Gibbons NE. Bergey's Manual of Determinative Bacteriology 8th Edition. Williams and Wilkins Co. Baltimore, USA;1974.
16. Cowan ST. Manual for the identification of medical bacteria. Cambridge University Press, Cambridge. 1974;1-82.
17. Daylan U, Harder H, Hoepner T. Hydrocarbon biodegradation in sediments and soils: A systematic examination of physical and chemical conditions – part II pH values. Wissenschaft and Technical Science and Technology. 1990;43:337-342.
18. Osuji L, Nwoye I. An appraisal of the impact of petroleum hydrocarbons on soil fertility: the Owaza experience. African Journal of Agricultural Research. 2007;2(7):318-324.
19. Odokuma LO, Okpokwasill GC. Role of Composition in the degradation of dispersant waste management. Global Journal on Environmental Science. 1992;12:39-43.
20. Aleruchi O, Abu GO. Aerobic biodegradation of petroleum hydrocarbons in laboratory contaminated groundwater. British Microbiology Research Journal. 2015;7(6):313-321.
21. Margesin R, Schinner F. Bioremediation of diesel oil-contaminated alpine soils at low temperatures. Applied Microbiology and Biotechnology. 1997;47:462-468.
22. Udebuani AC, Okoli CI, Nwaigwe H, Ozoh P. Effect of spent engine oil pollution on arable soil of Nekede mechanic village Owerri, Imo State, Nigeria. International Journal Natural Applied Science. 2011;7:257-260.
23. Yakubu MJ. Biodegradation of lagoma crude oil using pig dung. African Journal of Biotechnology. 2007;6:2821-2825.
24. Chikere CB, Azubike CC. Characterization of hydrocarbon-utilizing fungi from hydrocarbon-polluted sediments and water. Nigerian Journal of Biotechnology. 2014;27:49-54.
25. Omotayo AE, Efetie AO, Oyetibo GO, Ilori MO, Amund. Degradation of aviation fuel by microorganisms isolated from tropical polluted soils. International Journal of Biological and Chemical Sciences. 2011;5:698-708.

26. Amund OO, Nwokoye N. Hydrocarbon degrading potentials of a yeast isolate from a polluted lagoon. Journal of Scientific Research and Development. 1993;1:65-69.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*https://www.sdiarticle4.com/review-history/73969*