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Research Article

Keywords: Biodegradation, Petroleum, indigenous microorganism, Bacteria, Fungi

Posted Date: January 27th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1267852/v1>

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BIODEGRADATION OF SELECTED PETROLEUM HYDROCARBONS USING INDIGENOUS MICROORGANISMS

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ABSTRACT

Bioremediation make use of microorganisms to remove contaminants from the environment, the use of indigenous microorganisms in the bioremediation of hydrocarbon pollutant to clean up environment has become a valuable technique. This study was aimed to biodegrade some petroleum hydrocarbon using indigenous microorganism from the petroleum contaminated soil. Top soil sample was collected from petroleum contaminated site in Minna, Nigeria. The petroleum was obtained from a local petrol bunk. The indigenous microorganism with biodegrading potential was obtained by plating the aliquot of the soil sample on minimal salt medium containing petroleum as the only source of carbon. The bacterium was identified by cultural and biochemical test and the fungi were identified by both macroscopic and microscopic examinations. The isolates with optimal potential were then subjected to biodegradation for 25 days. A control was setup without the isolate. Three bacterial isolate and four fungi isolate were isolated from the petroleum contaminated site, the bacterial isolates were *Corynebacterium xerosis* with maximal rate of degradation on day 20 (86%), the control had 12%, *Proteus vulgaris* maximal rate of biodegradation was on day 35 (80%) while that of control is 40%, and *Bacillus subtilis* maximal rate of biodegradation was on day 10 (49.73%) while that of control is 2.2 %. The fungi isolates were identified as *Aspergillus niger*, *A. versicolor*, *Trichophyton verrucosum* Bodin and *Saccharomyces cerivisiae*. *Trichophyton verrucosum* Bodin on day 25 had the highest percentage (88%) of biodegradation in comparison with the control 41%. While *A. niger* highest biodegradation was observed on day 15 (24%). There was a gradual increase in the count of all the isolate during the course of biodegradation. The result of this study showed that these isolates were able to degrade petroleum hydrocarbon and can be useful for large scale bioremediation of petroleum contaminated soils.

Keywords: Biodegradation, Petroleum, indigenous microorganism, Bacteria, Fungi

INTRODUCTION

Crude oil and it derivatives is one of the most common contaminants in the environment and due to their wide spread, they pose severe risks to human health and water bodies, hence, intense remediation practices are required at the contaminated sites. Environmental contamination problems from petroleum extraction, transportation and storage are becoming more widely

36 recognized around the world. Nigeria's crude oil production has increase to approximately 2.8
37 million barrel per day. Nigeria is presently Africa tops oil producer and the world sixth largest
38 (Oyewole et al. 2021). The continued usage of petroleum products around the world has resulted
39 in a massive increase in pollution from diesel, spent engine oil, and jet fuel in the environment. As
40 a result, petroleum pollution is one of the most common environmental problems, as it disrupts
41 human metabolism and produces fatal mutations in genetic materials even at low levels of
42 pollution due to their persistence and biological toxicity When crude oil is separated via fractional
43 distillation, diesel fuel is produced; other distillates include aviation fuel, kerosene, and petrol. The
44 amount of carbon in diesel fuel varies between 11 and 25, and the distillation temperature is
45 between 180 and 380 °C. Diesel contains hydrocarbons in the range of 2000 to 4000, which cannot
46 be separated by gas chromatography. It is made up of 64 percent aliphatic hydrocarbons, 2%
47 olefinic hydrocarbons, and 35% aromatic hydrocarbons. It's made up of a variety of hydrocarbons,
48 including n-alkanes, isoalkanes, cycloalkanes, and aromatic hydrocarbons (Ahmed and
49 Fakhruddin 2018). Kerosene is a component of crude oil that is utilized as an energy source.
50 Kerosene is used as a fuel in some jets, as well as an illuminating oil for wicks and lamps.
51 Researchers discovered that kerosene-polluted soil has fewer microorganisms than non-polluted
52 soil. Kerosene toxicity to biota ranges from mild to severe, with product toxicity dependent on the
53 kind and concentration of aromatic compounds. Spills of kerosene have the potential to cause acute
54 poisoning in aquatic life (Hasan 2014).

55 The term Engine oil can be defined as a thick mineral liquid applied to a machine or engine so as
56 to reduce friction between the moving parts of the machine (Shahida et al. 2015). Mostly in the
57 World today especially in developing countries, lands contaminated with spent engine oil are
58 found due to the ineffective environmental laws which directly affects the rate at which the spent
59 engine oil enters and pollutes the environment. Engine oil was defined as a pollutant or
60 contaminant in the environment that causes damage to the ecosystem as well as health hazard to
61 human beings (Ajiboye et al. 2020). The chemical and physical approach of remediation can create
62 another kind of pollution and are very expensive to implement, while bioremediation make use of
63 microorganism that are environmentally friendly and cost effective compare to physical and
64 chemical treatments. This technology makes use of the capacity of microorganisms to metabolize
65 toxic compounds as source of carbon and energy, it also uses enzymatic and metabolic activities
66 to convert hydrocarbons into less harmful molecules. Bacteria, yeast, and fungi are mostly

responsible for the cleanup (Farber et al. 2019, Oyewole et al. 2021). Bachmann et al. (2014) refers to biodegradation to be a process that has the ability to change the chemical structure and nature of a pollutant, which is enforced by a biological activity that produces naturally metabolites.

Microorganism are used in bioremediation to remove hydrocarbon pollutant from the environment, the indigenous microbial population play significant role in biodegradation, but their performance decline when the pollution concentrations are high, to effectively remove the pollutant, engineered approach such as bio-stimulation and bio-augmentation are used. For the breakdown of aliphatic hydrocarbons, *Acinetobacter*, *Pseudomonas*, *Rhodococcus*, *Stenotrophomonas*, *Alcanivorax*, *Bacillus* and *Arthrobacter* species have been widely used (Chaudhary et al. 2020), *Amorphoteca*, *Talaromyces*, *Neosartorya*, and *Graphium*, as well as *Candida*, were also isolated from petroleum-contaminated soil and found to be potential degraders of hydrocarbons. It was also reported that *Aspergillus*, *Penicillium* and *Cephalosporium* has the ability to degrade hydrocarbon. After being isolated from contaminated water, *Candida lipolytica*, *Rhodotorula mucilaginosa*, *Geotrichum* sp, and *Trichosporon mucoides* were discovered to degrade petroleum compounds (Das and Chandran 2010). Growth enhancement of the indigenous microorganism, inoculation of foreign oil-degrading bacteria and bio-stimulation is one of the approach of accelerating the detoxifying and degrading activities at the oil contaminant site with a very minimal impact on the ecological system (Mukred et al. 2008).

Most organic pollutants are broken down by microorganism under aerobic condition, oxygen is required for the initial intercellular attack of the pollutant and also required for the activation of enzymatic process carried out by oxygenases and peroxidases. Peripheral degradation pathways convert the organic contaminant step by step into intermediates of the central intermediary metabolism, such intermediate include tricarboxylic acid cycle. Cell biomass synthesis occurs from the central precursor metabolites, examples are succinate, pyruvate and acetyl-CoA. Gluconeogenesis produced the sugar necessary for growth and biosynthesis. Enzymatic process by microorganism can be used for the breakdown of petroleum hydrocarbons (Das and Chandran 2011). The amount of oil spilled, the chemical composition of the crude oil or petroleum product, and the biodegrading potential of the microorganism present in the affected area all play a role in the recovery of water and soil after an oil spill. Many environmental elements have an impact on the bioremediation process and should be kept in mind. On the afflicted site, these include pH,

temperature, pollutant kind and concentration, oxygen, nutrient availability, and microbe concentration (Laurelta et al. 2017).

The aim of this study was to isolate, identify and screened microorganism with degrading potential from petroleum contaminated soil.

MATERIALS AND METHODS

Collection of soil sample and petroleum

Top soil samples was collected from petroleum contaminated site in Minna, Nigeria, in a sterilized plastic container and taken to the microbiology laboratory of Federal University of Technology Minna, Nigeria. Petroleum fuel (kerosene, diesel and spent engine oil) used was obtain from local petrol bunk, Minna, Nigeria.

Isolation and Screening of Microorganism

Contaminated soil sample (1g) was collected, serial dilution was performed, and 0.1 ml aliquot of the dilution 10^{-2} was dispense into petri-dish and Mineral salt medium + agar agar was poured on it and incubated for 6 days at 30 ± 2 °C, for bacterial isolation the mineral salt medium consist of 1.8 g K HPO₄, 1.2g K₂HPO₄ ,4.0g NH₄Cl, 0.2g MgSO₄.7H₂O, 0.1g NaCl, 0.01g FeSO₄. 7H₂O in 1L of distilled water, while for fungi, it consists of 1.8g K₂HPO₄, 1.2g KH₂PO₄, 4.0g NH₄Cl, 0.2g MgSO₄.7H₂O, 0.01g FeSO₄.7H₂O in 1Liter of distilled water, petroleum was added as the only energy and carbon source (1.0 ml per L).

Identification of the Bacterial Isolates

Isolated and screened bacteria were identified by cultural, morphological and biochemical characteristics, following the methods of Cheesbrough (2010).

Identification of the Fungi Isolates

The observed characteristics of the isolates were recorded. Morphological identification was performed for the selected fungal strains. Both macroscopic (cultural characteristics) and microscopic (lactophenol cotton blue staining) examinations were performed on the isolates. The

findings were verified by comparing their morphological and cultural traits to descriptions provided by Tsuneo (2007).

Biodegradation studies with the Bacteria and Fungi isolates

It was done by inoculating 2 ml of 24hours culture into 18 ml of sterilized minimal salt medium that also contained 2000 mg of petroleum and another control flask was prepared not containing the organism. The conical flasks were incubated at 30°C for 25days, and shaken regularly. At 5 day interval, two conical flasks were removed (organism and control).

Determination of Biodegradation activities

The extent of petroleum fuel biodegradation in the mixture was determined by emptying the mixture with diethylether in a separating funnel, after shaking vigorously, the separating funnel was put on the retort stand and allow to separate the organic phase from the liquid phase, the tap was open to remove the MSM while the solvent oil-mixture was collected into a beaker of a known weight. The solvent was evaporated using a water bath. The new weight of the beaker consisting of residual oil was recorded (Dadrasnia and Agamuthu 2013). The rate of degradation of the hydrocarbons were calculated using the formula of Abioye et al. (2013).

$$\% \text{ Biodegradation} = \frac{\text{weight of initial oil} - \text{weight of residual oil}}{\text{weight of initial oil}} \times 100$$

RESULTS

Microorganisms isolated from the petroleum hydrocarbon contaminated soil

The cultural, morphological and biochemical characteristics of the bacterial isolates indicated that the species were: *Corynebacterium xerosis* was isolated from diesel, *Proteus vulgaris*, *Trichophyton verrucosum* Bodin and *Saccharomyces cerevisiae* were isolated from kerosene while *Bacillus subtilis*, *Aspergillus niger* and *A. versicolor* were isolated from the spent engine oil (Table 1).

Table 1. Bacterial and fungi isolates obtained from the petroleum hydrocarbon contaminated soil

Petroleum hydrocarbon	Bacteria	Fungi
Diesel	<i>Corynebacterium xerosis</i>	-
Kerosene	<i>Proteus vulgaris</i>	<i>Trichophyton verrucosum Bodin</i> <i>Saccharomyces cerevisiae</i>
Spent Engine oil	<i>Bacillus subtilis</i>	<i>Aspergillus niger</i> <i>A. versicolor</i>

Rate of biodegradation

The diesel biodegradation by *Corynebacterium xerosis* for 25 days is shown in Table 2. the percentage of diesel degradation by the bacterial on day 20 had the highest percentage (86%) of biodegradation in comparison with the un-inoculated control 12%, while the total kerosene of kerosene contaminated soil treated by the isolate for 25 days is shown in Table 4. That of kerosene maximal biodegradation percentage was on day 25 with 80% while that of control is 40% by *Proteus vulgaris* while percentage of kerosene degradation by the *Trichophyton verrucosum Bodin* on day 25 had the highest percentage (88%) of biodegradation in comparison with the control 41%. The total spent engine oil degradation on day 10 had the highest rate of degradation with a value of 49.73% and control 2.2 by *Bacillus subtilis* and The biodegradation rate of spent engine oil by *A. niger* had the highest biodegradation on day 15 having percentage degradation of 24% shown in Table 4

166 **Table 2 Diesel biodegradation by *Corynebacterium xerosis***

Isolate	Days	0	5	10	15	20	25
% degradation							
% degradation of isolate		26± 0.003	67± 0.003	67± 0.006	80± 0.003	86± 0.003	34± 0.003
% degradation of control (Uninoculated)		10± 0.003	24± 0.003	12± 0.003	13± 0.003	12± 0.003	14± 0.003

172

173 **Table 3 Mean of kerosene biodegradation by the isolate**

Isolate	Days	0	5	10	15	20	25
% degradation							
<i>Proteus vulgaris</i>		18± 0.001 ^d	29.0± 0.009 ^b	36± 0.001 ^d	37± 0.000 ^a	56± 0.006 ^b	80± 0.001 ^c
Control (Uninoculated)		10± 0.000 ^a	25± 0.003 ^a	23± 0.003 ^b	28± 0.000 ^b	32± 0.006 ^a	40± 0.003 ^a
<i>Trichophyton verrucosum</i>		12± 0.001 ^c	49± 0.003 ^c	12± 0.003 ^a	80± 0.000 ^b	81± 0.001 ^c	88± 0.003 ^d
Control (Uninoculated)		0.05± 0.00 ^b	23± 0.003 ^a	27± 0.005 ^c	27± 0.000 ^b	31± 0.000 ^a	41± 0.003 ^b

182

183 **Table 4 Mean of spent engine oil biodegradation by the isolate**

Microbial Isolate(Days)	0	5	10	15	20	25	184
							185
<i>Bacillus subtilis</i>	0.00± 0.000 ^a	0.00± 0.000 ^a	49± 0.001 ^c	45± 0.001 ^b	41± 0.000 ^c	46± 0.000 ^c	186
Control (Uninoculated)	7.46± 0.000 ^c	18.41± 0.000 ^c	2.2± 0.006 ^b	3.86± 0.007 ^c	3.03± 0.006 ^b	3.23± 0.003 ^b	187
<i>Aspergillus niger</i>	4± 0.000 ^b	7± 0.000 ^b	21± 0.003 ^a	27± 0.006 ^a	24± 0.003 ^a	18± 0.006 ^a	188
Control (Uninoculated)	0.00± 0.000 ^a	2± 0.000 ^b	4± 0.006 ^b	5.5± 0.007 ^b	6.5± 0.006 ^b	7.5± 0.003 ^b	189 190

191

192 **Growth of the isolates during the course of biodegradation**

193 The growth of the isolate during biodegradation is shown in Table 6, 7, 8. Figure 1 shows that

194 *Corynebacterium xerosis* on day 0 had a count of 4×10^6 and the highest on day 25 with a count

195 of 270×10^6 CfU g⁻¹, Figure 2 shows that *Bacillus subtilis* had the minimum count of 1.6×10^4

196 on day 5 and maximal of 3.2×10^4 on day 25 and *Aspergillus niger* had 1.5×10^2 on day 0 and 6.6

197 $\times 10^2$ g⁻¹ on day 15. Figure 3 shows *Proteus vulgaris* having the highest on day 25 with a viable

198 count of 56×10^5 CfU g⁻¹, with the minimum on day 5 of 1×10^5 CfU g⁻¹ and *Trichophyton*

199 *verrucosum* having the highest on day 25 with a viable count of 5×10^6 CfU g⁻¹ with the minimum

200 on day 5 of 1×10^6 cfu g⁻¹

Figure 3. Growth profile of the organism at 30 °C for 25 days during diesel biodegradation

Figure 4. Growth profile of the organism at 30 °C for 25 days during spent engine oil biodegradation

Figure 5. Growth profile of the organism at 30°C for 25 days during kerosene biodegradation

226 DISCUSSION

227 After the screening, a total of 3 bacterial genera and 4 fungi species were isolated from the various
228 petroleum contaminated soil. Petroleum hydrocarbon biodegradation by natural population of
229 microorganisms represents one of the primary mechanisms through which these pollutant can be
230 eliminated from the environment, hydrocarbon-degrading microorganism are many in the
231 environment, even in pristine ones, however, prior exposure to hydrocarbons largely influence
232 their abundance in various environments and communities exposed to hydrocarbon become
233 adapted, the phenomenon of adaption is a consequence of three interrelated mechanism such as
234 induction or depression of specific enzymes that degrade hydrocarbon, selective enrichment of
235 microorganisms able to transform the hydrocarbons and genetic change which result in new
236 metabolic capabilities (Lima et al. 2020).

237 The use of indigenous microorganism facilitate the identification of microorganism with optimal
238 biodegradation potential for petroleum hydrocarbon, the added Petroleum provide nutrient for the
239 species that have the inherent petroleum hydrocarbon utilizing potential, while the less adapted
240 species among the heterotrophic population are gradually eliminated, hence resulting to only one
241 isolate that was chosen based on its ability to grow on Mineral salt agar with 1% (v/v) of diesel
242 oil at 37 °C after 5 day, Mukred et al. (2008) also used a similar approach for their isolation and
243 came up with 3 bacterial isolates, Most biodegradation studies have shown that the potential for
244 microbial degradation isolated from petroleum hydrocarbon polluted soil was higher than those
245 isolated from non-polluted sites (Ali 2018). Various petroleum utilizing bacteria and fungi were
246 isolated from the oil contaminated soil, of which most of these as been genera had already been
247 found in oil contaminated soil and identify as petroleum oil degrader. The bacterial isolate used
248 in this study was identified has *Corynebacterium xerosis*, *Proteus vulgaris* and *Bacillus subtilis*,
249 in similar to Ekanem and Ogunjobi (2017) who also isolate *Corynebacterium* sp, *Proteus* sp,
250 *Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp from oil contaminated soil. *Corynebacterium*
251 genera was the only microorganism from diesel contaminated soil suggesting that it has the ability
252 to resist the toxic effect of diesel oil in similar to Rahman et al. (2002) who also reported
253 *Corynebacterium* sp has the dominance bacteria in diesel station soil, the dominance of this genus
254 revealed that it has developed the ability to utilize the oil as a sole carbon and energy sources.

The genus *Bacillus* sp is another outstanding bacterial genus reported as petroleum degrader, their ability to form spores when nutrients are limiting makes species of *Bacillus* self-sustainable bioremediation tools, the strains, *Bacillus thurengiensis* and *Bacillus pumilus* were able to on solid media containing naphthalene, phenanthrene, anthracene and pyrene as the sole source carbon (Lima et al. 2020). The relatively low number of bacterial count observed in oil contaminated soil can be attributed to the toxic effect of petroleum contamination, and their ability to survive can be due to the presence of hydrocarbon degrading enzyme system and the presence of catabolic genes involved in biodegradation (Ekanem and Ogunjobi 2017).

The fungi isolated from the petroleum contaminated soil are *A. niger*, *A. versicolor*, *Trichophyton verrucosum* Bodin and *Saccharomyces cerevisiae*, the ability of the four fungi isolate to be able to grow on the mineral salt medium with 1% of some petroleum compound showed they are all capable of using the petroleum fuel has their sole source of carbon and energy, Oyewole et al. (2021) also use similar approach to screen fungi isolate for diesel oil degrading potential. It was also reported that *Aspergillus*, *Pencillium* and *Cephalosporium* has the ability to degrade petroleum hydrocarbon. After being isolated from contaminated water, *Candida lipolytica*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae*, *Geotrichum* sp., and *Trichosporon mucoides* were discovered to degrade petroleum compounds (Das and Chandran 2010). This could be due to the presence of hydrocarbon degrading enzymes such as laccases, manganese peroxidases lignin peroxidases and lipase. Yusoff et al. (2020) reported that lipase is involved in inducing the lipolytic reactions of emulsified hydrocarbonat the lipid–water interface, thus assisting the hydrocarbon uptake and showed showed the presence of lipase intracellularly or extracellularly produced by hydrocarbon degraders Joutey et al. (2014) reported that fungi are effective, especially when it comes to breaking down natural polymeric materials, because they have extracellular multi-enzyme complexes. They can also colonize and penetrate substrates quickly, because of their hyphal networks, they can transfer and redistribute nutrients throughout their mycelium. Alkane-using yeasts include *Candida lipolytica*, *Candida tropicalis*, *Rhodoturularubra*, *Aureobasidion*, and *Trichosporon pullulan*. Diesel oil degradation was discovered in *Rhodotorula aurantiaca* and *C. ernobii*.

The result of the percentage of biodegradation of petroleum by bacterial isolate and colony forming unit increase with increase in incubation period. *Corynebacterium xerosis* had the maximal percentage of degradation on day 20 (86%), *Proteus vulgaris* on day 25 with 80%. and *Bacillus*

subtilis maximal biodegradation percentage was on day 10 with 49.73%. *Trichophyton verrucosum* Bodin on day 25 had the highest percentage (88%) of biodegradation in comparison with the control 41% suggesting that other factor was responsible for the decrease in kerosene. While *Aspergillus niger* highest biodegradation was observed at day 15 having percentage degradation of 24%. *Corynebacterium xerosis* increase from 4×10^6 cfu g⁻¹ (day 0) to 270×10^6 cfu g⁻¹ (day 25), *Bacillus subtilis* had the minimum count of 1.6×10^4 on day 5 and maximal of 3.2×10^4 on day 25, *Proteus vulgaris* increase from 1×10^5 cfu g⁻¹ (day 5) to 56×10^5 cfu g⁻¹ (day 25), *Aspergillus niger* had 1.5×10^2 on day 0 and 6.6×10^2 on day 15 and *Trichophyton verrucosum* had 1×10^6 cfu g⁻¹ on day 5 and 5×10^6 cfu g⁻¹ on day 25. This revealed the potential of *Corynebacterium* species to use diesel oil as a sole carbon source, *Bacillus subtilis* to utilize spent engine oil as carbon source and *Proteus vulgaris* to use kerosene has carbon source. This might be due to the ability to produce biosurfactant, efficient hydrocarbon assimilation by receptor site for binding hydrocarbon and might have feature that enhance the emulsification and transport of hydrocarbon into the cell and the presence of enzymes such as oxygenase and peroxidase that introduce molecular oxygen into the hydrocarbon and produce intermediate that will later enter common energy yielding catabolic pathway (Abioye et al. 2013).

Aspergillus fungi utilize spent engine oil better than other fungi. This is in agreement with George-Okafor et al. (2014) who reported that *A. niger* and *A. versicolor* degrade 98 percent of selected polycyclic aromatic hydrocarbons in liquid culture medium.

The percentage of degradation of the hydrocarbon increase with increase in incubation period with the highest on day 25 (88%), the highest rate of biodegradation was seen by bacteria suggesting that bacteria has higher potential for biodegradation than fungi which might be due to the rapid growth of bacterial or short generation time compared to fungi in similar to Ali (2018) who reported from his study that bacteria were more active than fungi in the hydrocarbon biodegradation. The count monitored during the experiment is indicative of a steady increase in the number of fungal spores and a subsequent drop in the treatments, by the end of the incubation period. The eventual drop may be due to the number of spores which could be as a result of depletion of the only source of carbon in the medium, which is the Spent Engine Oil (Odili et al. 2020).

The study shows that the *Trichophyton verrucosum* Bodin has the ability unlike other fungus to degrade kerosene fuel, however this species has not been cited as oil degrader by other authors. Aboiye et al. (2013) however reported that the rate of the biodegradation of oil by *Saccharomyces cerevisiae* also increase with increase in incubation period from 5 days to 25 days, this shows that the isolate can utilize the hydrocarbon as their source of carbon.

CONCLUSION

This study was able to isolate bacteria and fungi with biodegrading potential from petroleum products' contaminated soils. The isolates were identified as *Corynebacterium* sp, *Proteus vulgaris*, *Bacillus subtilis*, *Aspergillus niger*, *A. versicolor*, *Trichophyton verrucosum* Bodin and *Saccharomyces cerevisiae*. This study shows that it is possible to isolate bacteria and fungi capable of degrading complex hydrocarbon compound from contaminated soil and can be exploited for large scale clean-up of petroleum contaminated soil.

Ethical Approval: Not applicable

Consent to Participate: All the experiments were carried out *in vitro* by the researchers as undergraduate project work, under my supervision.

Consent to Publish: All the authors approved of the publication of this manuscript in ESPR

Authors Contributions: Oluwafemi A Oyewole: Supervised the research. Clement S Olusanya: Wrote the manuscript. Olusegun I Akinlade: carried out the research on biodegradation of spent engine oil using bacteria isolated from soil contaminated with spent engine oil. Yusuf O Abdulwasiu: carried out the research on the biodegradation of spent engine oil using fungi isolated from contaminated spent engine oil contaminated site. Anthony O Kelechi: carried out the research on the biodegradation of kerosene using fungi isolated from kerosene contaminated soil and Michael E Okoro carried out the research on the biodegradation of kerosene using indigenous bacterial isolates

Funding: No funding was received for this research

Competing Interests: No competing interest in the course of writing up this manuscript

Availability of data and materials: Data and Materials are available for this research

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