

Isolation and Characterization of Hydrocarbon-Degrading Fungi from Ruminant Dung and Poultry Dropping-Impacted Soils

Elewu, H.A¹., Omowaye, O.S¹., Orji, M.U^{2*}., Agu, K.C².,

¹Department of Biosciences, College of Natural and Applied Sciences, Salem University, Lokoja, Kogi State, Nigeria

²Department of Applied Microbiology, Nnamdi Azikiwe University, PMB 5025, Awka, Nigeria.

*Corresponding Author

Orji, M.U.

Department of Applied
Microbiology, Nnamdi
Azikiwe University,
PMB 5025, Awka,
Nigeria.

Article History

Received: 05.05.2025

Accepted: 09.06.2025

Published: 28.06.2025

Abstract: Hydrocarbon pollution of the environment due to oil spilled has been a major environmental problem in Nigeria in the recent past. The need to continuously seek to find consortium of organisms that will be used to remediate the oil contaminated environment is on the increase. The study assayed oil-degrading potential of fungi isolated from cow dung, goat dung and poultry dung contaminated soils. The samples were collected aseptically from nine different locations with no history of crude oil pollution in Lokoja metropolis. These samples were analyzed for fungal loads and oil-degrading fungi using potato dextrose agar (PDA) and mineral salt agar (MSA) respectively. The biodegradation of engine oil was observed for a period of fourteen days on PDA and MSA respectively. The fungi were identified based on the microscopic and macroscopic features of the hyphal mass, nature of the fruiting bodies and the morphology of cells and spores using standard charts. The fungi identified from the contaminated soil include *Aspergillus fumigatus*, *Rhizopus stolonifer*, and *Mucor ramosissimus*. All fungi showed degradation of the crude oil, with *Aspergillus fumigates* demonstrating the best degradation ability. The result obtained revealed that oil-degrading fungi can be isolated from cow, goat and poultry dung soils and they are competent mycoflora for the biodegradation of crude oil polluted soils. They can thus be used as a better approach to restoring oil contaminated environments through bioremediation process.

Keywords: Hydrocarbon, Fungi, Soils, Animal Dung, Deposits.

Cite this article:

Elewu, H.A., Omowaye, O.S., Orji, M.U., Agu, K.C., (2025). Isolation and Characterization of Hydrocarbon-Degrading Fungi from Ruminant Dung and Poultry Dropping-Impacted Soils. *ISAR Journal of Science and Technology*, 3(6), 62-70.

Introduction

Recently, microbial ecologists have identified numerous microbial strains capable of effectively breaking down hydrocarbons across diverse ecosystems. Many of these successful microbial communities originate from heavily contaminated coastal areas, primarily due to their metabolic versatility in utilizing various carbon sources, including aliphatic, aromatic, and chlorinated hydrocarbons. These effective organisms are typically enriched using selective culture methods, with growth rate and cell density serving as key selection criteria (Agu & Odibo, 2021; Anaukwu et al., 2016).

Microorganisms, encompassing bacteria, fungi, yeast, and microalgae, possess the metabolic capacity to degrade fossil hydrocarbons. Their inherent ability to metabolize hydrocarbons for both growth and energy powers the process of petroleum biodegradation. Generally, low molecular weight alkanes degrade more rapidly, and mixed microbial cultures often demonstrate

broader degradation capabilities compared to pure cultures (Mbachu et al., 2014; Agu et al., 2022).

In most natural settings, indigenous microbial populations already possess hydrocarbon-degrading capabilities. When environmental conditions, particularly the availability of essential nutrients like nitrogen and phosphorus, are optimized, these populations can efficiently remediate oil contamination (Chaineau et al., 2005; Okafor et al., 2016). Supplying these nutrients, often through fertilizers at optimal Carbon:Nitrogen:Phosphorus (C:N:P) ratios, can significantly boost microbial activity (Ferguson et al., 2003). Aerobic degradation, which uses oxygen as the final electron acceptor, typically proceeds faster and more efficiently than anaerobic processes due to its higher energy yield (Agu et al., 2014). The widespread release of petroleum and its derivatives, particularly prevalent in Nigeria's Niger Delta region, has driven interest in bioremediation as an eco-friendly, cost-effective, and sustainable solution to oil pollution (Okeke et al., 2023; Orji et al., 2022).

While bacteria have historically been the primary focus of bioremediation research, fungi are gaining increasing recognition for their robust hydrocarbon-degrading potential, frequently outperforming bacterial counterparts (Batelle, 2000; Agudosi et al., 2023). Several fungal species, including *Aspergillus flavus*, *Penicillium chrysogenum*, and *Aspergillus niger*, have exhibited strong performance in breaking down hydrocarbons (Ifediegwu et al., 2015; Ojiagu et al., 2018).

Although studies have evaluated organic amendments like cow and poultry manure to enhance bioremediation, few have specifically investigated the fungal populations naturally present in such dung-contaminated soils (Obire & Akinde, 2006; Okafor et al., 2016). These organic wastes not only supply vital nutrients but also serve as reservoirs for potentially effective hydrocarbon-degrading fungi, positioning them as promising candidates for microbial bioremediation (Uwanta et al., 2023).

Research Aim

This research therefore aims to isolate, identify, and assess the hydrocarbon utilization potential of fungi from soils collected at cow, poultry, and goat dung deposit sites within Lokoja Metropolis.

Materials and Methods

Study Area

Soil samples (approximately 5-10cm deep) were collected from various dung accumulation sites where animals (cows, goats, and poultry) are reared. The specific dung sites chosen for this study are located in Adankolo, Kpata, Ganaja Mount Patti road, and Felele, all situated within the Lokoja Metropolis.

Sample Collection

A total of nine samples were collected, with three samples taken from each selected dung site. The samples were placed into sterile polythene bags, appropriately labeled, and transported to the laboratory for analysis within 24 hours.

Sterilization

All glassware designated for laboratory analysis, including disposable petri dishes, pipettes, measuring cylinders, McCartney bottles, and conical flasks, underwent thorough washing and rinsing with distilled water before air-drying. These items were then wrapped in aluminum foil or canisters and sterilized in a hot air oven at 160°C for one hour.

Media Preparation

Potato Dextrose Agar (PDA) and Mineral Salt Agar (MSA) were utilized for this laboratory work. Both media were prepared strictly following the manufacturer's instructions.

Preparation of PDA:

Nineteen point five grams (19.5g) of Potato Dextrose Agar powder was dissolved in 500 ml of distilled water as per the manufacturer's guidelines. The mixture was gently heated until the medium completely dissolved. It was then sterilized in a conical flask by autoclaving at 121°C for 15 minutes. After cooling, the medium was aseptically poured into sterile petri dishes to solidify.

Preparation of Mineral Salt Agar:

Mineral Salt Agar was prepared by dissolving 1.8g of K_2HPO_4 , 4g of NH_4Cl , 0.2g of $MgSO_4$, 0.1g of $NaCl$, and 0.01g of $FeSO_4$ in 1 liter (1000ml) of distilled water. For solid basal medium, 20g of bacteriological agar (agar-agar) was added to the solution.

Procedure

Soil samples collected from cow, goat, and poultry dung sites underwent preliminary processing. One gram of each sample was introduced into separate sterile test tubes containing 9ml of sterile distilled water and thoroughly mixed. Subsequently, 1ml of the soil solution was added to a petri dish, followed by the addition of 20ml of Potato Dextrose Agar. The plates were then incubated at 27°C for seven days.

Upon obtaining initial isolates, they were subcultured onto freshly prepared PDA medium and re-incubated for another seven days at 27°C to ensure pure cultures. Each pure culture was then transferred to a freshly prepared PDA slant in a bijou bottle for stock storage. These pure isolates were subsequently inoculated onto freshly prepared Mineral Salt Agar. Engine oil, infused onto a Whatman filter paper, was placed on the inner surface of the petri dish lid, which then covered the inoculated Mineral Salt Agar. These setups were incubated for seven days at 27°C and observed daily. Fungal growth on the plates indicated that the organism was a potential hydrocarbon-utilizing fungus, as it successfully utilized the engine oil as its sole carbon source.

Putative petroleum-utilizing fungal isolates were streaked onto plates of Agar medium (one isolate per plate). A sterile Whatman No. 1 filter paper saturated with filtered, sterilized engine oil was placed on the inside of each petri dish cover. This setup was designed to provide hydrocarbons as the sole carbon and energy source for fungal growth on the PDA medium. All plates were inverted and incubated at room temperature for seven days (Okpokwasili and Amanchukwu, 1988). Uninoculated plates served as controls. Fungi exhibiting growth on the MSA plates were isolated and further purified on PDA plates. They were then transferred to PDA slants, at which point they were confirmed as petroleum-utilizing fungi.

Isolation and Characterization of the Fungi

Fungal isolation and characterization were performed based on the visual assessment of fungal colony morphology on SDA culture medium. Microscopic evaluation was conducted using the modified slide culture technique with lactophenol cotton blue stain, viewed under X10 and X40 magnification (Agu and Chidozie, 2021). Identification was further guided by reference to various Manuals of Fungal Atlases (Barnett and Hunter, 2000; Ellis et al., 2007).

Results and Discussion

Microscopic and macroscopic features of hyphal and morphology of cells and spores of fungi isolated are shown in table 4.1

The fungi isolated includes: *Aspergillus fungatus*, *mucor ramosissimus*, *Rhizopus stolonifer*. were isolated from a soil of poultry dung sites, cow dung sites and goat dung sites.

Mucor was isolated from cow dung sites.

Table 4.1: Cultural and Microscopic Properties of Fungi Isolated from Cow Dung Sites, Poultry Dung Sites and Goat Dung Sites

Isolates	Dung Source	Cultural Appearance	Microscopic features	Name of probable organism
1	PDS	Initially white yellowish colour that become deep black on sporulation.	Hyphae are septate and hyaline. The conidiophore originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex	<i>Aspergillus fumigatus</i>
2	CDS	Woolly white centre with brown edge	Has a broad hyphae which are scarcely or non septate, sporangiophores are long, may be branched and terminates in a round spore-filled sporangia.	<i>Mucor ramosissimus</i>
3	PDS	Woolly white centre with brown edge	Has a broad hyphae which are scarcely or non septate, sporangiophores are long, may be branched and terminates in a round spore-filled sporangia.	<i>Mucor ramosissimus</i>
4	GDS	Woolly white centre with brown edge	Has a broad hyphae which are scarcely or non septate, sporangiophores are long, may be branched and terminates in a round spore-filled sporangia.	<i>Mucor ramosissimus</i>
5	CDS	Initially white yellowish colour that become deep black on sporulation.	Hyphae are septate and hyaline. The conidiophore originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex	<i>Aspergillus fumigatus</i>
6	CDS	Initially white yellowish colour that become deep black on sporulation	Hyphae are septate and hyaline. The conidiophore originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex	<i>Aspergillus fumigatus</i>
7	CDS	Woolly white centre with brown edge	Has a broad hyphae which are scarcely or non septate, sporangiophores are long, may be branched and terminates in a round spore-filled sporangia.	<i>Mucor ramosissimus</i>
8	PDS	Initially white yellowish colour that become deep black on sporulation	Hyphae are septate and hyaline. The conidiophore originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex	<i>Aspergillus fumigatus</i>
9	PDS	black powdery inside with whitish edge	Characterized by a body of branched mycelia composed of three types of hyphae stolon, rhizoids and usually unbranching sporangiophores. The black sporangia at the tip of sporangiophores are rounded and produces numerous non motile multinucleate spores for asexual reproduction.	<i>Rhizopus stolonifer</i>

Key: PDS - Poultry Dung Soil (Adankolo, Lokoja)
CDS - Cow Dung Soil (Mount Patti Road, Fefe and Kpata)
GDS - Goat Dung Soil (Ganaja village, Lokoja)

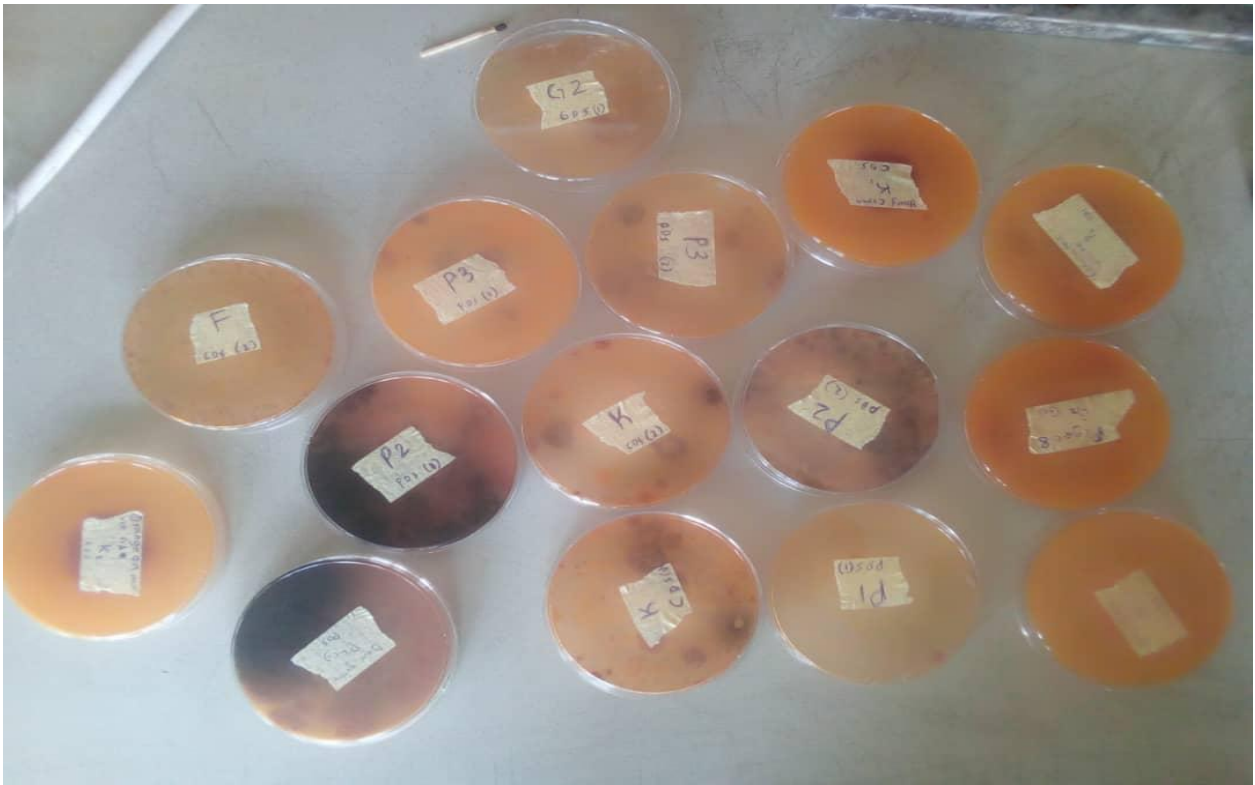


Plate 1: PDA Culture Plates of the Dung (Cow, Goat and Poultry) Soil Samples

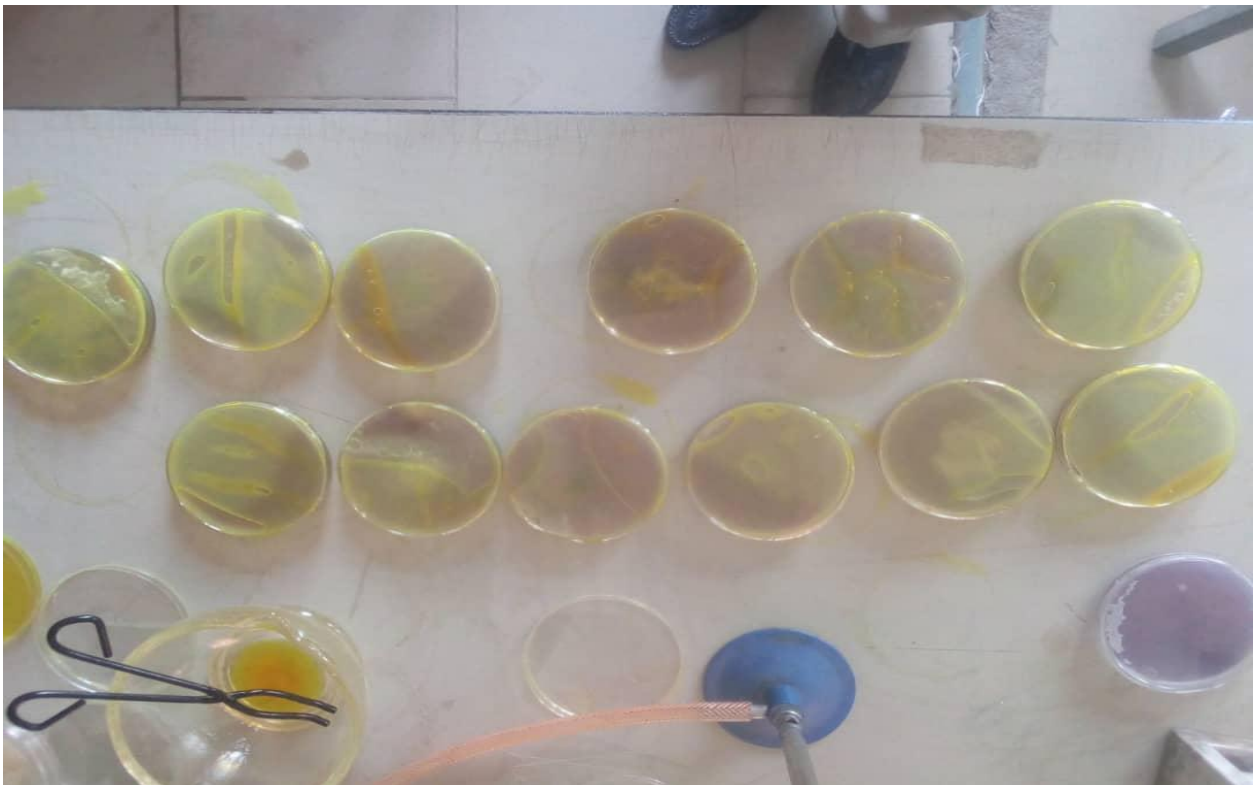


Plate 2: Front View of Mineral Salt Agar Cultured Plates with Infused Engine oil with Whatman Filter Paper



Plate 3: Pure Culture of the Fungi Isolated from the Dung Soil Samples



Plate 4: Back View of Mineral Salt Agar Cultured Plates with Infused Engine oil with Whatman Filter Paper

Discussion

This study explored the capacity of fungi, isolated from animal dung-affected soils, to metabolize engine oil as their sole carbon source in a time-course experiment. Microorganisms thriving in such contaminated environments offer potential as tools for environmental cleanup, enhancing safety at these sites. Substances that can be biodegraded by microbes almost universally serve as their carbon sources. Once assimilated, these microorganisms progressively break down the original complex molecules. Various studies have documented microbially enhanced degradation of persistent environmental compounds (Akinnibosun and Ashegbare, 2018).

Microorganisms, including bacteria, fungi, yeast, and microalgae, possess the metabolic flexibility necessary to break down fossil hydrocarbons. Their ability to use hydrocarbons for growth and energy production is central to petroleum biodegradation. Lighter alkanes are typically degraded more quickly, and diverse microbial consortia frequently demonstrate broader degradation capabilities than isolated pure cultures (Mbachu et al., 2014; Agu et al., 2022).

In most natural settings, indigenous microbial populations capable of hydrocarbon degradation are already present. When environmental conditions, particularly nutrient availability like nitrogen and phosphorus, are favorable, these microbial communities can effectively remediate oil pollution (Chaineau et al., 2005; Okafor et al., 2016). Supplying these nutrients, often through fertilizers at optimal Carbon:Nitrogen:Phosphorus (C:N:P) ratios, can significantly boost microbial activity (Ferguson et al., 2003). Aerobic degradation, which relies on oxygen as the terminal electron acceptor, is generally more rapid and efficient than anaerobic processes due to its higher energy yield (Agu et al., 2014). The widespread release of petroleum and its byproducts, especially prominent in Nigeria's Niger Delta, has spurred interest in bioremediation as an eco-friendly, cost-effective, and sustainable approach to managing oil contamination (Okeke et al., 2023; Orji et al., 2022).

While bacteria have traditionally dominated bioremediation research, fungi are increasingly recognized for their robust hydrocarbon-degrading potential, frequently outperforming bacterial counterparts (Batelle, 2000; Agudosi et al., 2023). Several fungal species, including *Aspergillus flavus*, *Penicillium chrysogenum*, and *Aspergillus niger*, have demonstrated strong capabilities in hydrocarbon degradation (Ifediegwu et al., 2015; Ojiagu et al., 2018).

Although research has assessed the use of organic amendments like cow and poultry manure to enhance bioremediation, fewer studies have specifically examined the fungal populations inherently present in such dung-contaminated soils (Obire & Akinde, 2006; Okafor et al., 2016). These organic wastes not only provide crucial nutrients but also harbor potentially effective hydrocarbon-degrading fungi, making them promising candidates for microbial bioremediation strategies (Uwanta et al., 2023).

Results and Discussion

The high microbial counts observed in the various cow dung-polluted sites suggest that these environments are rich sources of diverse microflora, particularly coprophilous fungi. This aligns with Richardson's (2001) findings that animal faeces offer a

nitrogen-rich environment conducive to microbial proliferation. Furthermore, it corroborates the work of Obire et al. (2008), which indicated that the presence of cow dung in soil leads to increased populations of both total saprophytic fungi and petroleum-utilizing fungi.

The fungal species isolated from the animal dung soil samples were identified as *Aspergillus fumigatus*, *Rhizopus stolonifer*, and *Mucor ramosissimus*. The overall findings demonstrate that the microflora from cow, goat, and poultry dung sites possess the capacity to utilize engine oil as a carbon source. All isolated fungal strains exhibited the ability to degrade or utilize engine oil as a carbon source. This observation is consistent with studies by Wemedo et al. (2002), who reported genera like *Penicillium*, *Aspergillus*, and *Rhizopus* in petroleum-polluted soils. Similarly, Oboh et al. (2006) found that all their isolates could grow on crude petroleum as a sole carbon and energy source during hydrocarbon utilization screening.

An intriguing aspect revealed by this research is the increased rate of fungal growth observed in media containing engine oil. This heightened growth likely occurs because the fungi use engine oil as a primary substrate, employing extracellular enzymes to break down recalcitrant hydrocarbon molecules. By dismantling the long chains of hydrogen and carbon, they convert the engine oil into simpler, absorbable products that support their growth and nutrition, as demonstrated by Adekunle and Adebambo (2007). This finding also supports Akinde and Obire's (2008) work, which identified potential hydrocarbon degraders within cow dung.

This study's results reinforce the reports by Odu (1978) and Ijah (1998) that crude oil-degrading microorganisms are widely distributed throughout the Nigerian environment. The organisms' efficient crude oil degradation capabilities can likely be attributed to their robust degradative enzyme systems.

The dominance of filamentous fungi, such as *Aspergillus* species, in crude oil-polluted environments has been widely documented by several researchers (April et al., 2000; D'Annibale et al., 2006; George-Okafor et al., 2009; Das and Chandran, 2011). Ihsan (2014) investigated the ability of four fungal species, indigenous to polluted soil, to utilize petroleum hydrocarbons. His results showed that *A. niger*, *A. fumigatus*, *F. solani*, and *P. funiculosum* were commonly present, with *A. fumigatus* reaching a frequency of up to 83%. It appears that petroleum contamination did not inhibit the growth or diversity of fungal strains in the polluted soil; rather, these fungal species seemed to utilize oil compounds as nutrients, suggesting that crude oil pollution could even stimulate fungal growth. Concurrently, organic compounds in the soil appeared to activate and increase fungal growth and extracellular enzyme excretion, potentially leading to a decrease in soil pH compared to liquid media, ultimately enhancing crude oil biodegradation.

Ihsan (2014) also assessed the efficiency of kerosene utilization by *Rhizopus stolonifer* in a mineral salts medium for biodegradation purposes. This study found that *R. stolonifer* was capable of consuming kerosene as a sole carbon source, with substantial reductions in kerosene amounts observed in its presence. Kerosene comprises paraffins, cycloparaffins, aromatic, and olefinic hydrocarbons, predominantly in the C9 to C16 range, along with asphaltic compounds of varying molecular weight, complexity, and susceptibility to microbial oxidation.

Szewczyk (2003) isolated *Mucor ramosissimus* IM 6203, a fungal strain from spent cutting fluid, which demonstrated the ability to degrade spent engine oil. Deeper insights into the capabilities of this tested strain, as presented in their work, could serve as a foundation for its future application in removing engine oil from contaminated sites.

Conclusion and Recommendation

This study concludes that the fungal isolates from soils at cow dung, goat dung, and poultry dung sites—specifically *Aspergillus fumigatus*, *Rhizopus stolonifer*, and *Mucor ramosissimus*—possess the ability to utilize engine oil as a carbon source for growth. Given their isolation from hydrocarbon-impacted environments, and under optimal environmental and nutritional conditions, these fungi hold significant potential for the bioremediation of hydrocarbon-polluted sites.

Recommendation:

Based on these findings, the use of engine oil as a carbon source for fungal growth is recommended due to its demonstrated effectiveness, lower cost, and ability to promote faster and reliable fungal proliferation.

References

1. Agu, K.C. and Chidozie, C.P. (2021). An Improved Slide Culture Technique for the Microscopic Identification of Fungal Species. *International Journal of Trend in Scientific Research and Development*, 6 (1): 243-254, URL: www.ijtsrd.com/papers/ijtsrd45058.pdf
2. Agu, K.C. and Frederick J. C. Odibo (2021). Biodegradation Potentials of *Aspergillus Flavipes* Isolated from Uburu and Okposi Salt Lakes. *International Journal of Trend in Scientific Research and Development*, 5 (5): 1160-1170. URL: www.ijtsrd.com/papers/ijtsrd44949.pdf
3. Agu, K.C., Orji, M.U., Ikele, M.O., Uwanta, L.I. and Onyeneho, V.I. (2022). Hydrocarbon Biodegradation Potential of Cyanobacteria in Oil Polluted Soil. *International Journal of Trend in Scientific Research and Development*, 6 (7): 733-737. URL: www.ijtsrd.com/papers/ijtsrd52397.pdf
4. Agu, K.C., Orji, M.U., Onuorah, S.C., Egrefa, S.O., Anaukwu, C.G., Okafor, U.C., Awah, N.S., Okafor, O.I., Mbachu, A.E. and Anyaegbunam, B.C. (2014). Influence of Solid Waste Dumps Leachate on Bacteriological and Heavy Metals Contamination of Ground Water in Awka. *American Journal of Life Science Researches*, 2 (4): 450-457.
5. Agudosi S.O, Chukwura E. I. and Uwanta L.I. (2023). Monoculture Degradation of *Aspergillus flavus* and *Penicillium Chrysogenum* of Varying Concentration of Spent Engine Oil (Spent Motor Oil) in Oba, Anambra State-Nigeria. *International Journal of Research Publication and Reviews*. 4 (8): 747-754
6. Akinnibosun, F. I. and Ashegbare, A. (2018). Isolation and Characterization of Hydrocarbon-degrading Fungi from Ogbe-Ijoh Greek, Warri, Delta State, Nigeria. *Ovidius University Annals of Chemistry*, Vol. 29 (2), pp. 72-76.
7. Anaukwu, C.G., Ezemba, C.C., Anakwenze, V.N., Agu, K.C., Okeke, B.C., Awah N.S., Ekwealor, I.A. (2016). Effect of biosurfactant produced by *Citrobacter murlinae* AF025369 and a synthetic surfactant on degradation of crude oil. *Edorium Journal of Microbiology*, 2: 1–6
8. April, T. M., Foght, J. M. and Currah, R. S. (2000). Hydrocarbon-degrading Filamentous Fungi Isolated from Flare Pit Soil in Northern and Western Canada. *Canadian Journal of Microbiology*. 46(1), 38-49.
9. Atlas, R. M. and Bartha R. (1998). *Fundamentals and Applications*. 4th edition, Benjamin/Cummings Publishing Co. Inc., California, USA., 523-530
10. Barnett, H.L. and Hunter, B.B. (2016). C.R.C. Press, West Palm Beach, Florida. *Illustrated Genera of Imperfect Fungi*, pp, 1- 197.
11. Batelle, C.D. (2000). *Mushrooms: Higher Macrofungi to Clean Up the Environment*. Batelle Environmental Issues, Fall 2000.
12. Bento, F. M., Camarago, F. A. O., Okeke, B. C. and Frankenberger, W. T. (2005). Comparative Bioremediation of Soil Contaminated with Diesel Oil by Natural Attenuation, Biostimulation and Bioaugmentation. *Bioresour. Technol.* 96, 1049-1055
13. Bidwell, J.R., Donald, S.C. and Merski, T. (2002). Toxicity Evaluation of a Commercial Bioremediation Agent Mixed with Crude Oil. *Environ. Toxicol. and Chemistry* 22(1):84–91.
14. Boboye, B., Olukunle, O. F. and Adetuyi, F. C. (2010). Degradative Activity of Bacteria Isolated from Hydrocarbon-Polluted Site in Ilaje, Ondo State, Nigeria. *African Journal of Microbiology Research* Vol. 4(23), pp. 2484-2491.
15. Bragg, J.R., Prince, R.C., Harner, E.J. and Atlas, R.M. (1994). Effectiveness of Bioremediation for the Exxon Valdez Oil Spill. *Nature*, 368:413–418.
16. Bundy, J.G., Paton, G.I. and Campbell, C.D. (2004) Combined Microbial Community Level and Single Species Biosensor Responses to Monitor Recovery of Oil Polluted Soil. *Soil Biology and Biochemistry* 36 1149 - 1159.
17. Chaineau, C.H., Rougeux, G., Yepremian, C. and Oudot, J. (2005). Effect of Nutrient Concentration on the Biodegradation of Crude Oil and Associated Microbial Populations in the Soil. *Soil Biology and Biochemistry* :37 1490-1497.
18. Chhatre, S., Purohit, H., Shankar, R. and Khanna, P. (1996). Bacteria Consortia for Crude Oil Spill Remediation. *Water Sci. Technol.* 34:187–193.
19. Cohen, A.M., Nugeguda, D. and Gagnon, M.M. (2001). The Effect of Different Oil Spill Remediation Techniques on Petroleum Hydrocarbon Elimination in Australian Bass (*Macquaria novemaculeata*). *Arch. Environ. Contam. Toxicol.* 40:264–270.
20. D'Annibale, A., Rosetto, F., Leonardi, V., Federici, F. and Petruccioli, M. (2006). Role of Autochthonous Filamentous Fungi in Bioremediation of a Soil Historically Contaminated with Aromatic Hydrocarbons. *Appl. Environ. Microbiol.* 72(1):28-36.

21. Das, N. and Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotech. Res. Int.*2001: 1-13.
22. Ellis, D., Davis, S., Alexiou, H., Handke, R., and Bartley, R. (2007). Descriptions of Medical Fungi. www.mycology.adelaide.edu.au, accessed on September 13, 2013.
23. Ewulo, B.S. (2005). Effect of poultry dung and cattle manure on chemical properties of clay and sandy clay loam soil. *J. Anim. Vet. Adv.* 4(10):839–841.
24. Fayad, N.M., Edora, R.I., EL– Mubarak, A.H. and Polankos, A.B. (1992). Effectiveness of a Bioremediation Product in Degrading the Oil Spill in the 1991 Arabian Gulf war. *Bull. Environ. Contam. Toxicol.* 49:787–796.
25. Ferguson, H.S. , Franzmann, D.P., Reville, S.I. and Rayner, L.J. (2003). The Effects of Nitrogen and Water on Mineralisation of Hydrocarbons in Diesel-Contaminated Terrestrial Antarctic Soils. *Cold Regions Science and Technology*: 37 197 - 212.
26. George-Okafor, U., Tasié, F. and Muotoe-Okafor, F. (2009). Hydrocarbon Degradation Potentials of Indigenous Fungal Isolates from Petroleum Contaminated Soils. *J. Phy. & Nat. Sci.* 3(1): 1-6.
27. Ghazali, M.F., Zaliha, N.R., Abdul, R.N. Salleh, A.B. and Basri, M. (2004). Biodegradation of Hydrocarbons in Soil by Microbial Consortium. *International Biodeterioration and Biodegradation* 54 :61 - 67.
28. Hoff, M. (1991). Types of Bioremediation and Case Histories In: Background Information, Chevron EFT response plan. *Biorem.* 8:23–32.
29. Ibekwe, V.I., Ubochi, K.C. and Ezeji, E.U. (2006). Effect of organic nutrient on microbial utilization of hydrocarbons on crude oil contamination soil. *Afr. J. of Biotech.* 5(10):983-986.
30. Ifediegwu, M.C., Agu, K.C., Awah, N.S., Mbachu, A.E., Okeke, C.B., Anaukwu, C.G., Uba, P.O., Ngenegbo, U.C., Nwankwo, C.M. (2015). Isolation, Growth and Identification of Chlorpyrifos Degrading Bacteria from Agricultural Soil in Anambra State, Nigeria. *Universal Journal of Microbiology Research* 3 (4): 46-52
31. Ihsan Flayyih Hasan (2014). Biodegradation of Kerosene by *Aspergillus niger* and *Rhizopus stolonifer*. *Journal of Applied & Environmental Microbiology*, vol. 2, no. 1 (2014): 31-36.
32. Ijah, U. J. J. and Antai, S. P. (2005). Changes in Physicochemical Properties and Fungal Population after Application of Crude Oil to Soil. *Journal of Environmental Sciences* 9:64-72.
33. Ijah, U. J. J. and George, B. G. (2003). Potentials of Yeast Isolates in Decontamination of Crude Oil Spilled Soil. *Nigerian Journal of Science and Technology* 1:189-195.
34. Ikuesan F. A., Boboye B. E. and Adetuyi F. C. (2016). The Microbiological and Physicochemical Properties of Some Crude Oil Contaminated and Uncontaminated Agricultural Soils in Ondo State, Nigeria. *Pyrex J. Microbiol Biotechnol.* 2(1), 1-8.
35. Jain P. K., Gupta V. K., Gaur R. K., Lowry M., Jaroli D. P. and Chauhan U. K. (2011) Bioremediation of Petroleum Oil Contaminated Soil and Water. *Res. J. Environ.Toxicol.*, 5(1), 1- 26
36. Johnsen, A. R., Wick, L. Y. and Harms, H. (2005). Principles of microbial PAH-degradation in soil. *Environmental. Pollution.* 133:71-84.
37. Lee, K. and Demora, S. (1999). In-situ Bioremediation Strategies for Oiled Shoreline Environments. *Environ. Technol.* 20:783–794.
38. Lee, K. and Levy, E.M. (1991). Bioremediation Waxy Crude oil Stranded on Low-Energy Shoreline. *Proc. 1991 Oil Spill Conf. American Petroleum Institute, Washington DC.*
39. Mbachu, A.E., Onochie, C.C., Agu, K.C., Okafor, O.I. and Awah, N.S. (2014). Hydrocarbon Degrading Potentials of Indigenous Bacteria Isolated from Auto-Mechanic Workshops at Mgbuka-Nkpor, Nigeria, *Journal of Global Biosciences*, 3 (1): 321-326
40. Obire, O. and Akinde, S. B (2006). Comparative Study of the Efficiency of Cow Dung and Poultry Manure as Alternative Nutrient Sources in Bioremediation of Oil – Polluted Soil. *Niger Delta Biologia*, Vol. 5(2) 82-91.
41. Oboh B.O, M.O Ilori, Akinyemi J.O and Adebusoye S.A, (2006). Hydrocarbon Degrading Potentials of Bacteria Isolated from a Nigerian Bitumen (Tarsand) Deposit. *Nature and Science*, 4(3): 51-57.
42. Odeyemi J. O. (2014) Two Centuries of Oil and Gas (1860 - 2060). www.universalacademicservices.org.
43. Ogboghodo, I.A., Erebor, E.B., Osemwota, J.O. and Isitekhole, H.H. (2004). The Effects of Application of Poultry Manure to Crude Oil Polluted Soils on Maize (*Zea mays*) Growth and Soil Properties. *Environ. Monitor and Assess.* 96(3):153-161.
44. Ojiagu, K.D., Odibo, F.J.C, Ojiagu, N.C., Agu, K.C., Okafor, A.C. (2018). Biosorption of Hexavalent Chromium by *Pseudomonas aeruginosa* Strain ANSC: Equilibria Isothermic, Kinetic and Thermodynamic Studies. *Bioengineering and Bioscience*,6(1): 1-10. DOI: 10.13189/bb.2018.060101
45. Okafor, U.C., Orji, M.U., Agu, K.C., Awah, N.S., Okeke, B.C., Okafor, O.I. and Okoro N.C.N. (2016). Bioremediation of Crude Oil-polluted Soil Using Broiler-Chicken Droppings. *Journal of Applied and Environmental Microbiology*, 4, (4): 75-84, DOI:10.12691/jaem-4-4-2
46. Okafor, U.C., Orji, M.U., Nwankwegu, A.S. Anaukwu, C.G., Onuorah, S.C., Archibong, E.J., Obika, I.E. and Agu, K.C. (2016). Effect of Chicken droppings amendment on bioremediation of crude oil polluted soil. *European Journal of Experimental Biology*, 6 (4): 62-68.
47. Okeke, B. C., Uwanta L. I., Odibo, F. J. C., Agu, K. C. and Victor-Aduloju A. T. (2023). Monoculture Solid Phase Degradative Potential of Congo red by *Aspergillus Niger* and

- Aspergillus Flavus. International Journal of Trend in Scientific Research and Development, 7 (3): 684-688
48. Okolo, J.C., Amadi, E.N. and Odu, C.T.I. (2005). Effects of Soil Treatment Containing Poultry Manure on Crude Oil Degradation in Sandy Loam Soil. Appl. Ecol Environ. Res. 3(1): 47-53.
 49. Okpokwasili, G.C. and Amanchukwu, S.C. (1988). Petroleum hydrocarbon degradation by Candida species. Environment International 14: 243 - 247.
 50. Olukunle, O. F., Boboye, B. and Ikuomola, O. T. (2012). Indigenous Bacteria and Fungi Responsible for Bioremediation of Oil-polluted Soils in Ondo Soils in Ondo State, Nigeria. Environtropica, 8: 138-148.
 51. Orji, M.U., Agu, K.C., Ikele, M.O., Uwanta, L.I. and Ugwuoke, G. (2022). Bioremediation of Glyphosate Polluted Soil using Fungal Species" Published in International Journal of Trend in Scientific Research and Development, 6 (7): 726-732, URL: www.ijtsrd.com/papers/ijtsrd52396.pdf
 52. Oteyza, T.G., Lopez, J.F., Teixidor, P. and Grimalt, J.O. (2005) . Long Chains Alkenones in Hypersaline and Marine Coastal Microbial Mats. Organic Geochemistry, 36:861 - 872.
 53. Rainbow, P.S. (2002). Trace Metal Concentration in Aquatic invertebrates; Why and so what? Environ Pollut. 120:497-507.
 54. Samantha, S. K., Singh, O. V. and Jain, R. K. (2002). Polycyclic Aromatic Hydrocarbons: Environmental Pollution and Bioremediation. Trends in Biotechnology. 20:243-248.
 55. Shukry W. M., Al-Hawas G. H. S., Al-Moaikal R. M. S. and El-Bendary M. A., (2013). Effect of Petroleum Crude Oil on Mineral Nutrient Elements, Soil Properties and Bacterial Biomass of the Rhizosphere of Jojoba. British J. Environ. and Climate Change., 3(1), 103- 118.
 56. Singh, A., Mullin, B. and Ward, O. P. (2001). Reactor-based Process for the Biological Treatment of Petroleum Wastes. In: Petrotech (ed) Proceedings of the Middle East Petrotech 2001 Conference. Petrotech, Bahrain, pp 1-13.
 57. Singh, H. (2006). Mycoremediation: Fungal Bioremediation, p. 592, Wiley - Interscience, New York.
 58. Szewczyk R., Bernat P., Milczarek K., Dlugonski J., 2003. Application of microscopic fungi isolated from polluted industrial areas for polycyclic aromatic hydrocarbons and pentachlorophenol reduction. Biodegradation 14, 1-8.
 59. Timmis, K.N. and Peper, D.H. (1999). Bacteria Designed for Bioremediation. Trends Biotechnol. 17:201- 204.
 60. Udebuani, A.C. and Ozoh, P.T. (2007). Aspects of the Chemistry of Soils and Elicine indica Growing on Seven Years Old Spill Site. Inter. J. of Trop. Agric and Food Syst. 1(2): 187-192.
 61. Uwanta L.I., Orji. M.U., Agu K.C., Udenweze E.C., Umeoduagu N. D., Egurefa, S. O. and Awari, V. G. (2023). Pseudomonas saeruginosa Isolated from Vermicompost in the Degradation of Varying Concentration of Polyvinyl Chloride (PVC) and Polyvinyl Alcohol (PVA). International Journal of Research Publication and Reviews, 4 (8): 547-553
 62. Venosa, A.D., Haines, J.R., Nisamanepong, W., Govind, R., Pradlhan, S. and Siddique, B. (1992). Efficacy of Commercial Products in Enhancing Oil Degradation in Close Laboratory Reactors. J. Ind. Microbiol. 10:13-23.
 63. Vidali, M. (2001). Bioremediation: An overview. Journal of Applied Chemistry, 73 (7): 1163-1172.
 64. Wemedo SA, Obire O, Dogubo DA, 2002. Myco-flora of a kerosene-polluted soil in Nigeria. J Appl Sci Environ Manag, 6:14-77.