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Biodegradation of Crude-oil by Fungi Isolated from Cow Dung-contaminated soils

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Abstract

This study assayed oil-degrading potential of fungi isolated from cow dung-contaminated soils. The samples were collected aseptically from four different cow dung dumping sites with no history of crude-oil pollution in Akure metropolis. These samples were analyzed for fungal loads and oil-degrading fungi using potato dextrose agar and mineral salt medium respectively. The biodegradation of crude oil was observed spectrophotometrically using the broth culture of the fungal isolates for a period of 15 days on mineral salt medium. 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. The sixteen (16) fungi identified from the contaminated soils include; Bdellospora helicoides, Aspergillus fumigatus, Gonadobotricum apiculata, Aspergillus niger, Trichoderma viridae, Pleurothecium recurvatum, Streptothrix atra, Thysarophora longispora, Candida albicans, Aspergillus flavus, Helminthosporium velutinum, Botrytis cinerea, Zoophage nitospora, Varicosporium elodeae, Articulospora inflata and Neurospora crassa. All fungi showed degradation of the crude oil, with Trichoderma viridae, Aspergillus flavus and Varicosporium elodeae demonstrating best degradation ability. Trichoderma viridae exhibited highest degradation (66.2%) while Varicosporium elodeae exhibited least degradation (40%). The measurement of the rate of biodegradation of crude oil by the three fungi was further confirmed using Gas Chromatography and Mass Spectrophotometer (GC-MS). The GC-MS analysis showed that the fungi degraded the hydrocarbon compounds when compared to that of the control. The result obtained revealed that oil-degrading fungi can be isolated from cow dung dumping sites and they are competent mycoflora for the biodegradation of crude oil polluted soils. They can be used as a better approach to restoring oil contaminated environments through bioremediation process.

Keywords: Biodegradation, Mycoflora, Crude-oil, Gas chromatography and Mass spectrophotometer (GCMS).

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Introduction

The common problems associated with petroleum industry are accidental and deliberate discharge of oil spills. Oil spillage is known to be a major environmental problem in Nigeria, most especially in the Niger-Delta region, attracting considerable attention in the recent years. Many approaches (physical, chemical and biological methods) have been employed in order to reduce or eliminate the effect of oil spillage on the environment and living organisms. However, most of these efforts have limitations in their applications, either as being too expensive or posing threats to the ecosystem. The most promising of many researches carried out to deal with large-scale oil spills is the use of microorganisms to provide an effective alternative (Singh et al., 2001). This approach is referred to as 'bioremediation', and it is one of the most rapidly growing areas of environmental microbiology, which has been used for cleaning up pollutants. This is because of its low cost, safety and its public acceptability (Grazyna et al., 2001). Microbial degradation represents the major route responsible for the ecological recovery of oil spills (Johnsen, et al., 2005).

Many species of bacteria and fungi isolated from oil spilled sites have been shown in recent years to have the abilities to use petroleum hydrocarbon as sole source of carbon and energy (Olukunle, et al., 2012; Boboye et al., 2010 and Ojo, 2006). Fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria (Batelle, 2000). Although, hydrocarbon degraders may be expected to be readily isolated from a petroleum oil-associated environment, the same degree of expectation may be anticipated for microorganisms isolated from a totally unrelated environment (Ojo, 2006).

The source of fungi for the biodegradation of petroleum oil becomes the issue. In most previous researches, the source of fungi is either selected from crude oil contaminated soils or water (their natural environments), rhizoplanes, phyloplanes, food or crops and root tubers. Some other fungal source for biodegradation of petroleum oil, are the experimental use of mycorrhizal fungi and wood rotting fungi in the biodegradation of petroleum. In this study, attention is paid to source for fungi from cow dung-contaminated soils. Bioremediation techniques involves biostimulation or bioaugmentation i.e. addition of nutrients or addition of oil-degrading microorganisms respectively (Samantha, 2002). The objective of this study therefore was to isolate fungi from cow dung-contaminated soils and assay for their degrading activities on crude oil using turbidity growth and GC-MS with the view to providing them for bioaugumentation so that soil could be enhanced for productivity.

Materials and Methods

The soil samples used were collected at cow dung-contaminated sites at a depth of about 10-15cm in various locations in Akure, Ondo State for the cultivation of fungal isolates while the petroleum sample was a Bonnylight crude oil collected from the Shell Petroleum Development Corporation refinery in Port Harcourt, Rivers State.

Pour plate technique was used for the microbiological analysis of samples collected from cow dung-contaminated sites (Song and Bartha, 1990). One gram of the soil and 1 ml of the water sample was mixed with 9ml of normal saline. One millilitre of the soil and water each was diluted from 10^{-1} to 10^{-10}. An aliquot of (1ml) dilution 10^{-5} was seeded into potato dextrose agar (PDA). The inoculated plates were incubated at 28^{o}C for 72 hr. The fungal spore forming units (Sfu/g) were then counted. Repeated subculturing was done for each isolate to get pure culture of the fungi for easy identification.

Pure Fungal cultures were observed while still on plates and the isolates were characterized using cultural characteristics such as the colour of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spore, presence of foot cell, sporangiophore or conidiophores and the characteristics of spore head using microscopic and macroscopic methods. The identities of the organisms were determined by comparing their characteristics with those of known taxa as described by Domsch and Gams (1970).

The screening of fungal isolates for the ability to utilize petroleum was done according to the method of Ijah and Antai (2005) by growing the isolates in potato dextrose broth for 3 days before they were transferred to oil broth (1.2g K_2HPO_4, 1.8g K_2PO_4, 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, 0.01g $FeSO_4.7H_2O$, in 1000ml distilled water, 1percent crude oil) and incubated at room temperature. After every 3 days, 1ml of the oil broth was extracted to know the extent of oil degradation by measuring the turbidity on a spectrophotometer at wavelength of 540nm and the best and most efficient oil degraders were selected for further studies using Gas Chromatography and Mass spectrophotometry (GC-MS). The best and most efficient oil degraders were later grown in 5mls of Potato dextrose broth in test tubes for three days. At the end of 3 days of incubation, the fungi were introduced into 7mls of sterile oil broth in McCartney bottles and incubated at room temperature. This was done in duplicates for each of the fungal isolates. Negative control was also prepared without adding any fungal isolates. After every 4 days, residual crude oil was extracted using diethyl ether. The diethyl ether was then allowed to evaporate overnight after which the remnant crude oil was taken for Gas Chromatography and Mass Spectrophotometry analysis. The analysis was conducted at the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria. Four microlitres (4µl) of the extractable crude oil

was diluted with 4µl of n-hexane and analyzed on a 19091J-413HP-5 capillary column installed in a Gas Chromatograph (Agilent Technologies, 7890 GC system) equipped with a Mass spectrophotometer (5975 series MSD). Split injection was used with helium as carrier gas. The oven temperature was initially set at 35°c for 2 minutes and increased at a rate of 5°c/min to 280°c and ran for 60 minutes.

Results

Microscopic and macroscopic features of the hyphal cells and morphology of cells and spores revealed diverse microbial community, such as Bdellospora helicoides, Aspergillus fumigatus, Aspergillus niger, Trichoderma viridae, Pleurothecium recurvatum, Streptothrix atra, Gonadobotricum apiculata, Candida albicans, Aspergillus flavus, Helminthosporium velutinum, Botrytis cinerea, Zoophage nitospora, Varicosporium elodeae, Articulospora inflata, Neurospora crassa and Thysarophora longispora, as shown in Table 1.

The turbidity growth of the fungi species are shown in Fig.1. Trichoderma viridae, Aspergillus flavus and Varicosporium elodeae had the best growth in the mineral salt broth after 15 days of incubation. From the Gas Chromatography and Mass Spectrophotometric analysis on the three isolates, Fig.2 shows the gas chromatogram of undegraded (incubated control) crude oil. Fig.3 shows gas chromatographic patterns of crude oil degraded by Aspergillus flavus after 4, 8, 12 and 16 days of incubation, while Fig.4 and Fig.5 show the gas chromatographic patterns of crude oil degraded by Varicosporium eloedea and Trichoderma viridae respectively after 4, 8, 12 and 16 days of incubation.

Light chain alkanes were probably lost by volatilization during the 16 days of incubation. Comparing the chromatograms in Fig.3, Fig.4 and Fig.5 with those of the control (Fig.2), it was observed that the fungi were slow in attacking or degrading the crude oil on the 4^{th}, 8^{th} and 12^{th} day of incubation but were later able to degrade the oil effectively. This is due to the fact that the fungi are slow growers, so it took them longer time to start attacking the crude oil. It was also observed that after 4, 8 and 12 days of incubation the organisms did not show any significant degradation but after the 16^{th} day of incubation, it was observed that Trichoderma viridae degraded the crude oil best while Varicosporium eloedea was the least in degrading it. This may be due to the differences in competence of degradative enzymes and pathways of degradation used by the organisms. It was also observed that there were reductions in the quality of some compounds in the crude oil. Therefore, according to the results obtained, Trichoderma viridae and Aspergillus flavus should be preferred to Varicosporium eloedea when microbial seeding of oil spills in tropical soils is intended.

The percentage depletion of Decahydro-4,4,8,9,10-pentamethylnaphthalene, Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene, ,6-Dimethyl-Decane, 1-Methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl] Cyclohexene and 16.beta,18.alpha,19.alpha-Urs-20-en-16-ol by V. elodea, T. viride and A. flavus are presented in Table 2. There was complete degradation of 1-Methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl] Cyclohexene, with retention time of 49.857 by Trichoderma viride.

Table 1: Suspected fungi from cow dung contaminated soils

Isolates	Cultural appearance	Microscopic features	Name of Probable organism
1.	White mycelium growth	The hyphae is aerial, slender and non-septate; a short haustoria, branched, conidia hyaline and elongate as well as catenulate	Bdellospora helicoides
2.	Brown mycelia growth	An upright conidiophores that terminates in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose	Aspergillus fumigatus
3.	Black mycelia growth and fully extended in the growth medium	An upright conidiophores that terminates in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose	Aspergillus niger
4.	Green mycelia growth	A branched conidiophore, hyaline, not verticillate; phialides single, conidia hyaline, 1-celled, ovoid, borne in small terminal clusters.	Trichoderma viridae
5.	Dark mycelia growth	A single, simple, dark and narrow conidiophores	Pleurothecium recurvatum
6.	Dark mycelia growth	A branched conidiophore, conidia branches spirally coiled (appearing wavy), and the conidia is single.	Streptothrix atra
7.	Cotton-like mycelia at 24 hours turning dirty with development of black spores on mycelium	Non-septate hyphae, thin sporagiophore with a sporangium in club-like form	Thysarophora longispora
8.	Greenish mould	Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid, forming short chains by budding which are produced on mycelium epically or laterally.	Candida albicans
9.	Yellow mycelia growth	An upright conidiophores that terminates in a davate swelling bearing phialides at the apex or radiating from the entire surface; conidia are 1-celled and globose	Aspergillus flavus
10.	Dark mycelium	A single conidiophore, tall erect brown and simple conidia	Helminthosporium velutinum
11.	Grey/Green mould	Long conidiophores which are septate, conidia are borne in clusters on branched sterigmata.	Botrytis cinerea
12.	Light grey mycelia	Sparse mycelia, non-septate hyphae, conidia hyaline single	Zoophage nitospora
13.	White mycelium	Conidiophore sparingly branched near apex, conidium elongated axis with 2 laterals on one side, septate lateral, hyaline conidia.	Varicosporium elodeae
14.	White mycelium	Conidiophore hyaline,slender with sparing upper part, branched conidia and septate hyphae	Articulospora inflacta
15.	White mycelium when young but turn yellow at maturity	Branched septate conidiophore, produced golden yellow conidia in masses	Neurospora crassa
16.	Cotton-like mycelia at 24 hours turning dirty with development of black spores on mycelium	Non-septate hyphae, thin sporagiophore with a sporangium in club-like form	Gonadobotricum apiculata

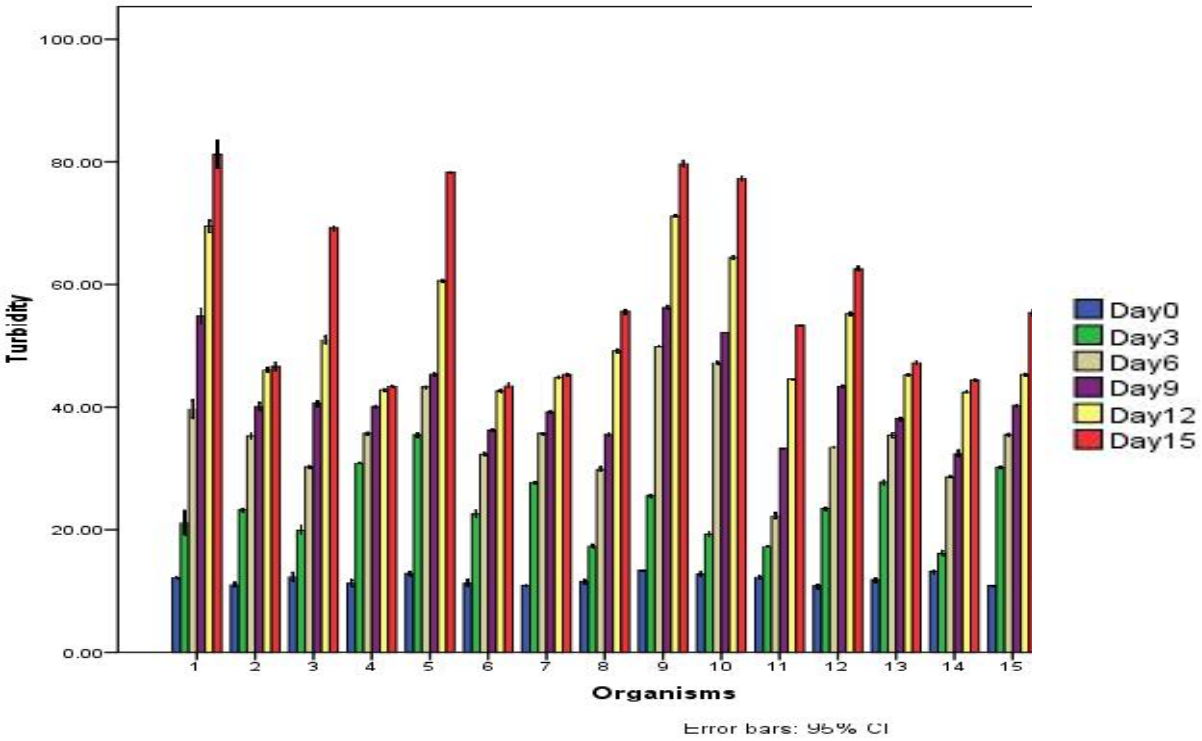
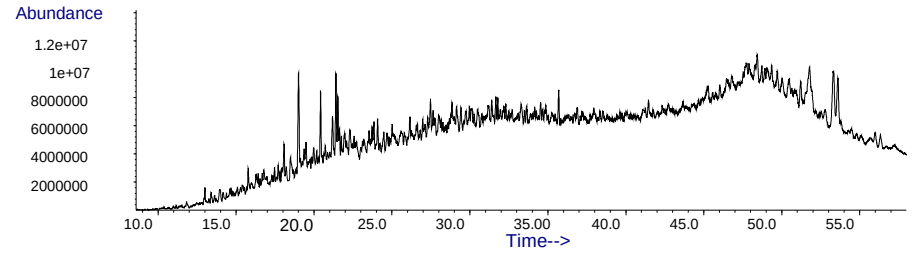
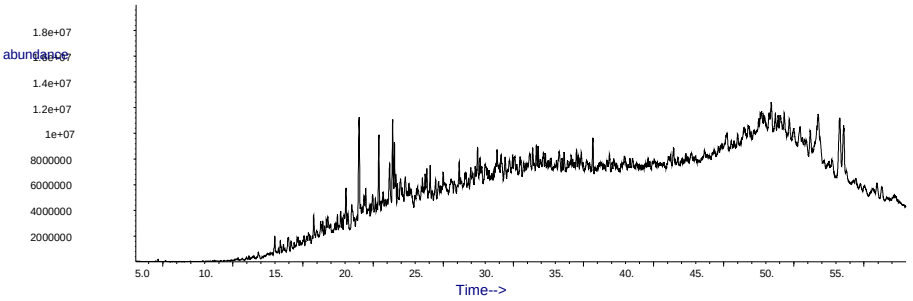


Figure 1: Turbidity of each organism after 15 days of incubation.

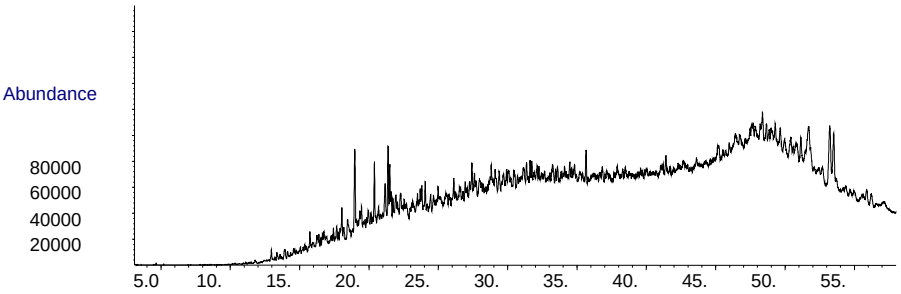
1 – Trichoderma viridae 2 – Bdelospora helicoide 3 – Aspergillus fumigates
4 – Streptothrix atra 5 – Varicosporium elode 6 – Botrytis cinerea
7 – Articulosporium inflate 8 – Gonadobotricum apiculata 9 – Aspergillus flavus
10 – Aspergillus niger 11 – Neurospora crassa 12 – Candida albicans
13 – Zoophage nitospora 14 – Thysarophora longispora 15 – Aspergillus rupens
16 – Helminthosporium velutrium 17 – Control

DAY 4

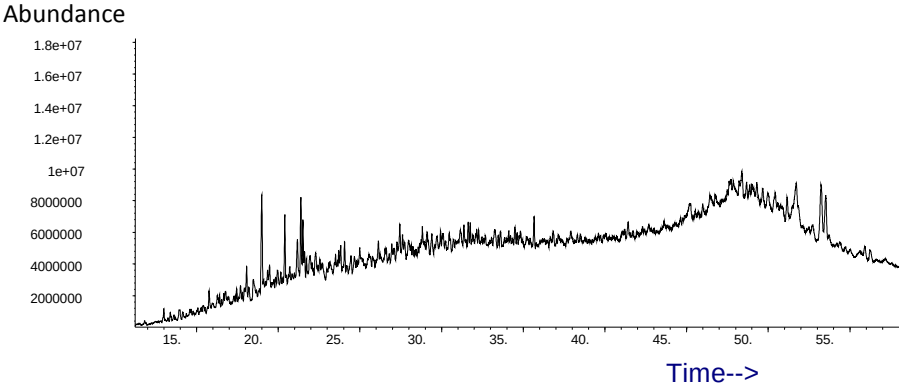




DAY 8



DAY 12



DAY 16

Fig. 2: Chromatograph of undegraded (incubated control) crude oil

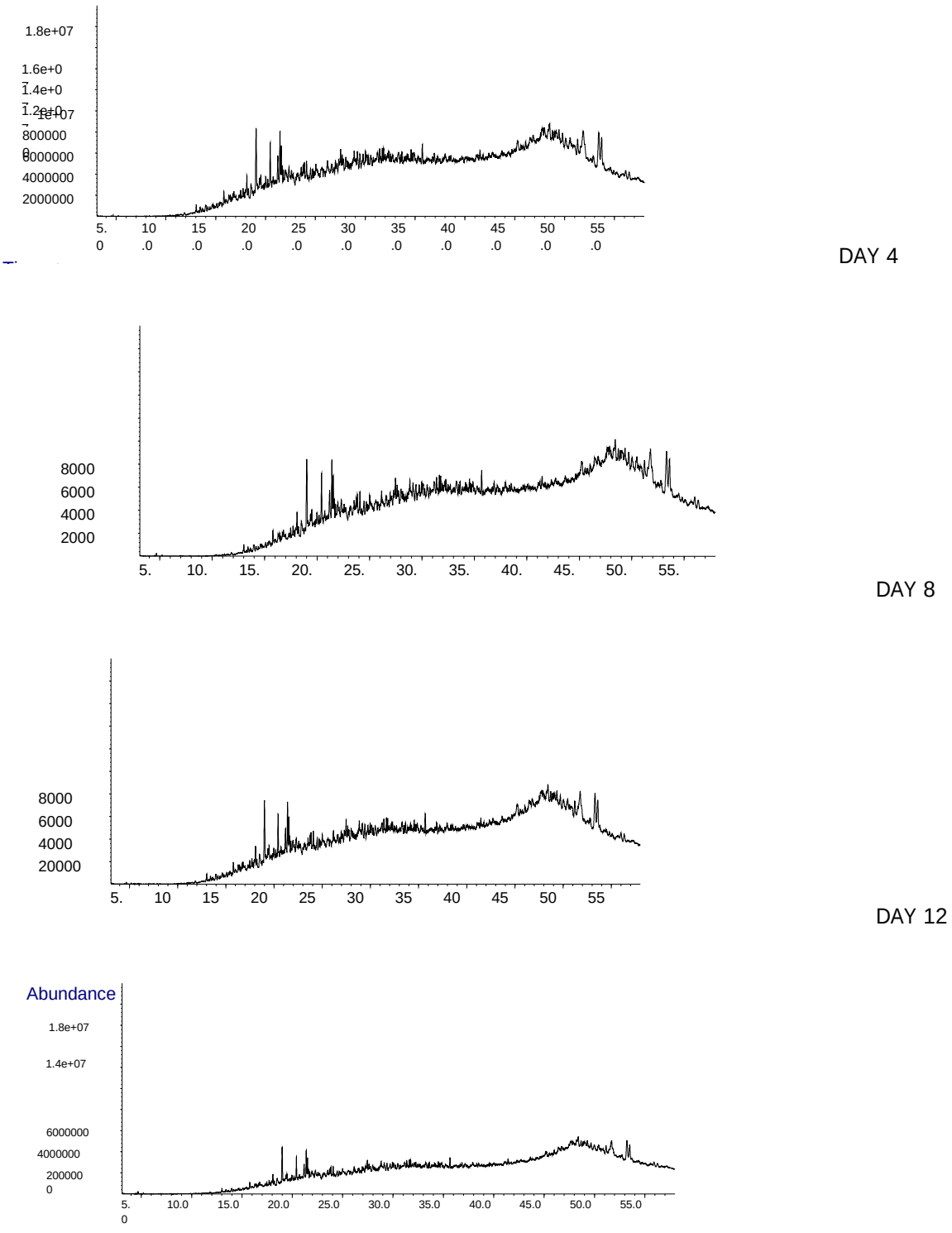


Fig. 3: Chromatograph of degraded crude oil by Aspergillus flavus

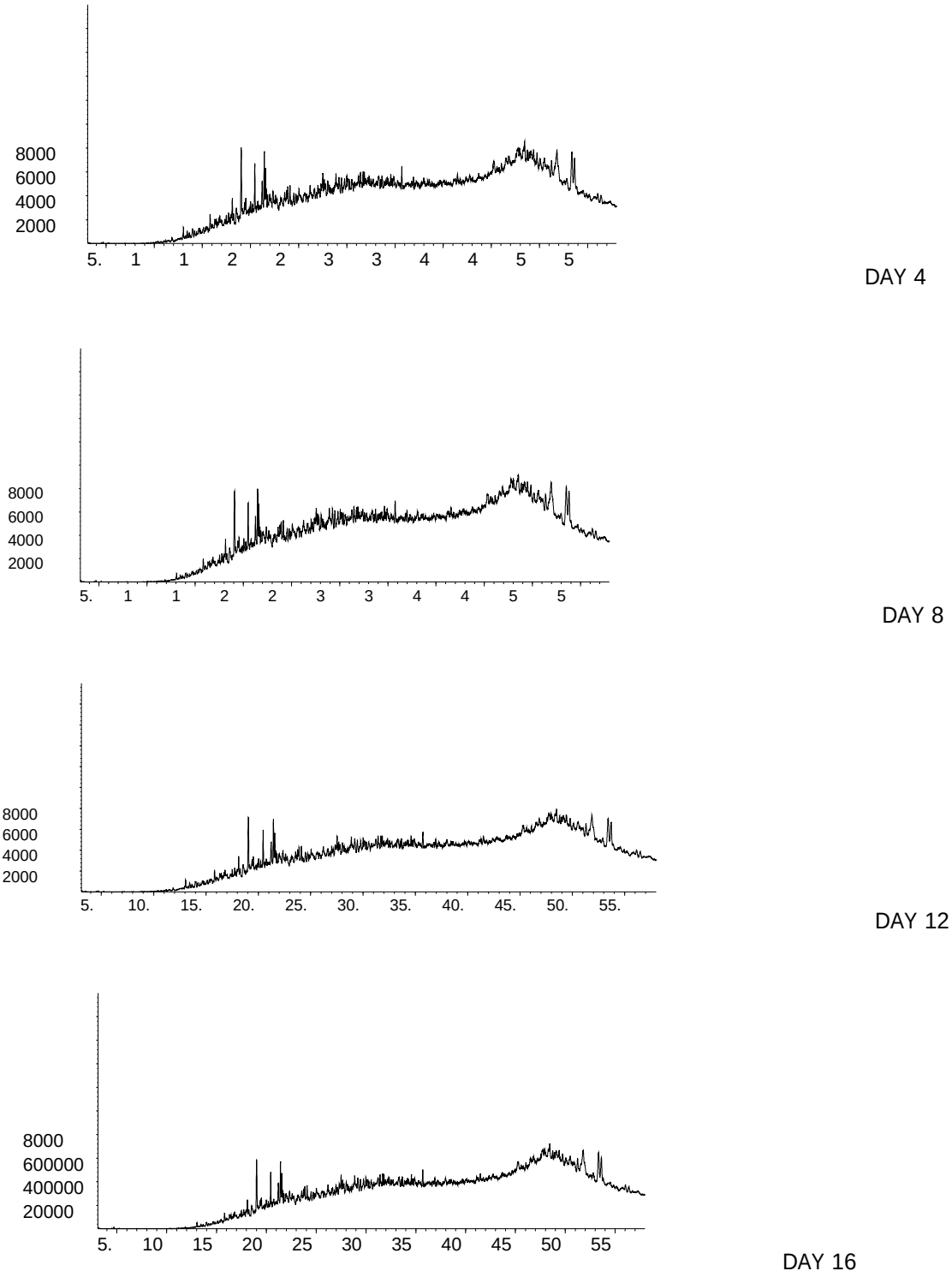


Fig. 4: Chromatograph of degraded crude oil by Varicosporium eloedea

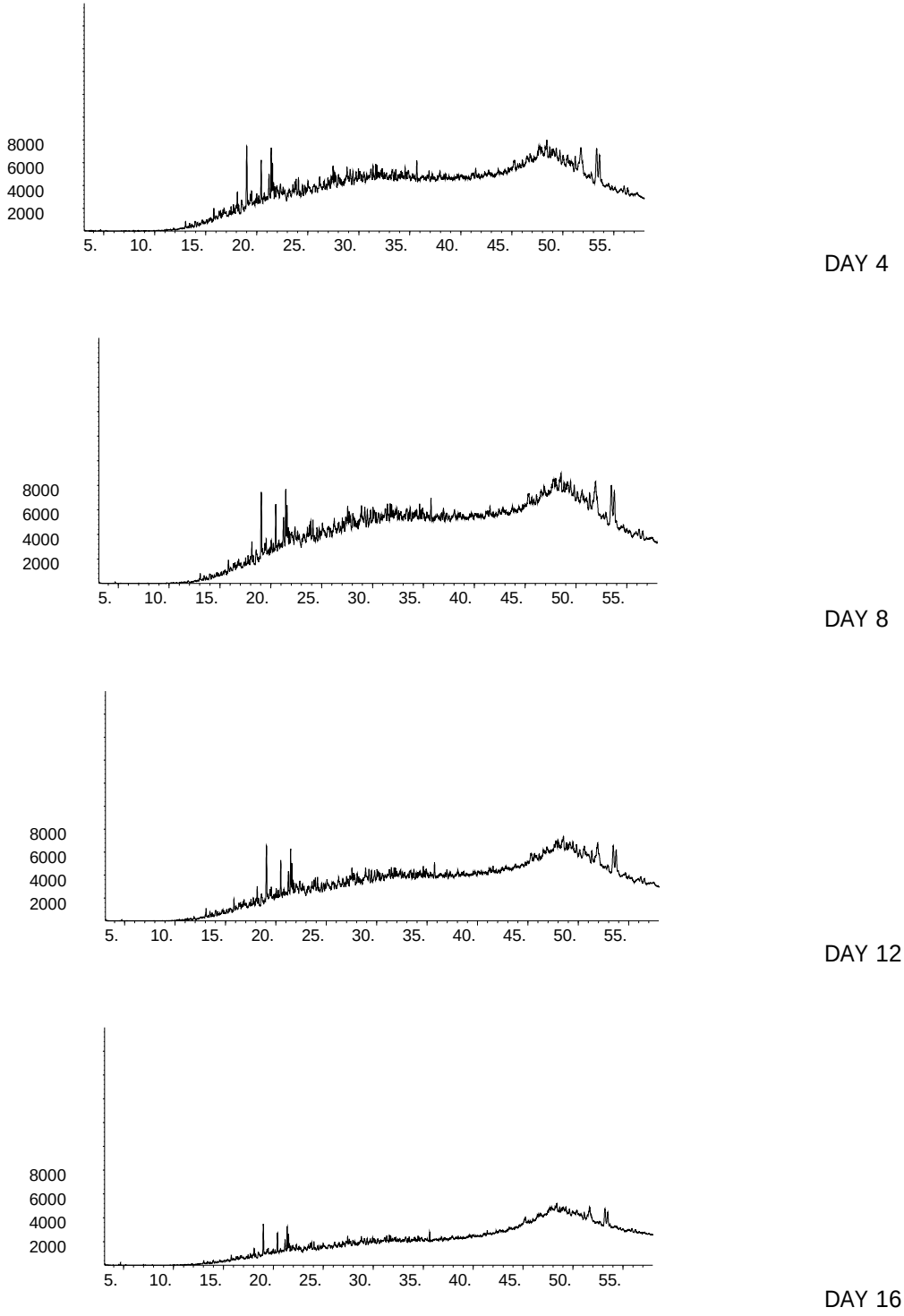


Fig. 5: Chromatograph of degraded crude oil by Trichoderma viride

Table 2: The percentage depletion of some selected compounds by the fungal isolates

	Retention Time/Amounts in Compounds			
Ungraded percent depletion (%)	Control	V. elodea	T. viride	A. flavus
19.053 Decahydro-4,4,8,9,10-pentamethylnaphthalene	11.25	39	51	42
21.575 Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene	4.6	39	48	47
35.731 3,6-Dimethyl-Decane	3.12	34	67	46
49.857 1-Methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl] Cyclohexene	3.4	39	100	56
53.709 16.beta,18.alpha,19.alpha-Urs-20-en-16-ol	9.1	49	65	61

Discussions

Various fungal genera have been isolated from non-hydrocarbon contaminated soils, playing important ecological role in removal of hydrocarbons (Van Beilen et al., 2006; Challan et al., 2004). The high microbial count from the various cow dung polluted sites indicated that cow dung polluted soil is a very rich source of mycoflora, (coprophilous fungi) as shown in Fig.1. This supports the work of Richardson (2001) that the animal faeces provide an environment rich in nitrogenous material suitable for the proliferation of microorganisms. It is also in line with the work of Obire, et al (2008) that the presence of cow dung in the soil resulted in an increase in the population of total saprophytic fungi and an increase in the population of petroleum-utilizing fungi in soil.

The fungi isolated from the soil samples are Bdellospora helicoides, Aspergillus fumigatus, Aspergillus niger, Trichoderma viridae, Pleurothecium recurvatum, Streptothrix atra, Gonadobotricum apiculata, Candida albicans, Aspergillus flavus, Helminthosporium velutinum, Botrytis cinerea, Zoophage nitospora, Varicosporium elodeae, Articulospora inflata, Neurospora crassa and Thysarophora longispora as shown in Table 1. Arotupin and Akinyosoye (2001), Ekundayo (2004) and Obire (1988) separately reported some of these microbial isolates to be an autochthonous inhabitant of soil while Orji, et al., (2012) mentioned some of the fungi isolates from cow dung contaminated soils.

Results (Fig. 2 - 5) showed that the mycoflora of cow dung possess the ability to utilize crude oil. All the fungal isolates showed varying degree of biodegradation of crude oil in Mineral salt medium indicating that they all utilize crude oil as their main source of carbon. An interesting demonstration generated in this work shows an increase in rates of fungal growth in the media containing crude oil which might be due to the fact that the fungi used crude oil as a substrate for their growth using extra cellular enzymes to break down the recalcitrant hydrocarbon molecules, by dismantling the long chains of hydrogen and carbon, thereby, converting crude oil into simpler forms or products that can be absorbed for the growth and nutrition of the fungi as shown by Adekunle and Adebambo, (2007). It also agreed with the work of Akinde and Obire (2008), which revealed the presence of potential hydrocarbon degraders in cow dung.

From the biodegradation study, it was observed that Aspergillus flavus, Varicosporium elodeae and Trichoderma viridae had the best growth in the oil broth. This is in line with the work of other researchers such as Vidali, (2001) and Bhattacharya et al., (2003), who reported similar findings which made their application for bioremediation efforts suitable. This study also revealed that crude oil-degrading fungi are not restricted to oil contaminated soil as Aspergillus

flavus, Varicosporium elodeae and Trichoderma viridae were isolated from cow dung-contaminated soil with no history of oil pollution. This finding supports the report of Odu (1978) that crude oil degrading microorganisms are widely distributed in the Nigerian environment (Ijah, 1998). The efficient crude oil degrading ability of the organisms may be due to the competent degradative enzyme system of the organisms. The high degradation ability of these organisms especially Aspergillus flavus and Trichoderma viridae may be due to the higher production of extracellular enzymes and organic acids that enable the organisms to utilize the hydrocarbon faster, (Damisa et al., 2013). This agrees with the findings of Stamets (1999), that mycelial mats are used for bioremediation because they produce extracellular enzymes and acids that break and dismantle the long chains of hydrocarbon, the base structure common to oils, petroleum products and many other pollutants. The fact that the species of Aspergillus, Trichoderma and Varicosporium could degrade crude oil efficiently point to their potential usefulness in cleaning oil spills in tropical soils.

This study showed that there were reductions in the quality of some compounds in the crude oil according to the GMSC analysis. By comparing the GMSC profiles for the different days, it was observed that there was a significant decrease in the peaks corresponding to the hydrocarbon fractions of the oil. According to April et al., (2000), the reduction in peak heights of the different hydrocarbon fractions of the crude oil indicates the degradation of the components. This is well observed in all of the profiles which showed that the fungal isolates are capable of degrading the crude oil. This study supports the findings by April et al., (2000) that reported 22 species of Penicillium and 5 species of Aspergillus isolated from the flare pit soils in Northern and Southern Canada. These isolates showed the ability to degrade hydrocarbons on solid medium amended with crude oil. It was also observed that after 4, 8 and 12 days of incubation the organisms did not show any significant degradation but after the 16th day of incubation it was observed that Trichoderma viridae degraded the crude oil best while Varicosporium eloedea was the least in degrading it. This may be due to the differences in competence of degradative enzymes and pathways of degradation used by the organisms. This could also be as a result of the fact that hydrocarbons differ in their susceptibility to microbial attack, (Ulrici et al., 2000).

It was observed from Table 2 that there was a significant degradation of some of the selected compounds from the GCMS result. There was complete degradation of 1-Methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl] Cyclohexene, with retention time of 49.857 by Trichoderma viride. There is scarce information about the genus Trichoderma under petroleum hydrocarbon-contaminated systems in order to make comparisons about the tolerance of Trichoderma to either crude oil or Polycyclic Aromatic Hydrocarbon. Although there has been some research about the utilization of PAHs by this fungus (Li, et al., 2008; Atagana, 2006). Elad et al., (1981) also referred to the utilization of a PAH as a selective medium for the growth of Trichoderma viride. Nevertheless, the present study demonstrates that Trichoderma viride is capable of degrading PAHs and can be seeded for the bioremediation of some recalcitrant hydrocarbons.

It is widely believed that microorganisms isolated from crude oil contaminated environment degrade crude oil more efficiently than their counterparts from uncontaminated environments. It is also generally upheld that bacteria are better crude oil degraders than fungi, (Ijah, 1998). These should not be held as absolute rules since the present study has revealed that Aspergillus flavus and Trichoderma viridae isolated from crude oil free soil caused a high extensive degradation of crude oil. The information provided by this study in regards to the rate at which oil is metabolized by fungi from crude oil unpolluted tropical soil is crucial especially now that the emphasis is on finding efficient hydrocarbon degrading microorganisms for seeding oil contaminated environments. It also contributes to the limited information in literature on the potentials of fungi in degrading oil pollutant in soil when used in microbial seeding process, (Ijah and George, 2003). This study also showed that the cow dung contaminated soil has relatively high populations of crude oil-degrading fungi. This however means that enhanced biodegradation of crude oil in soil through microbial seeding should not be ignored. It is essential for speedy recovery of oil spills in the environment.

The results obtained in this study indicate that Trichoderma viride was the best candidate in degrading the crude oil which means that it should be preferred to either Aspergillus flavus or Varicosporium eloedea when microbial seeding of oil spills in tropical soils is intended. Moreover, the mixed culture of petroleum degrading fungi present in cow dung can be harnessed by researchers in the search for mixed culture of microorganisms with naturally enhanced oil degrading capabilities or which could be genetically engineered for enhanced bioremediation of contaminated sites, (Obire et al., 2008).

Conclusion

It can be concluded from this study that the rate of biodegradation of petroleum hydrocarbon mixtures in soil could be enhanced by the addition of cow dung in soil media. The fungi isolated and tested for biodegradation were very effective and since they were isolated from cow dung-contaminated soils, the use of cow dung to stimulate petroleum hydrocarbon mixtures degradation in the soil could be one of the severally sought environmentally friendly ways of remediating natural ecosystem contaminated with crude oil and the organisms Aspergillus spp, Trichoderma spp and Varicosporium spp may be pointed out as potential fungi to degrade petroleum hydrocarbons, especially those spilled out over tropical soil.

References

Adekunle, A. A. and Adebambo, O. A. (2007). Petroleum Hydrocarbon Utilization by Fungi Isolated From Detarium Senegalense (J. F Gmelin) Seeds. Journal of American Science 3: 69-76.

Akinde, S. B. and Obire, O. (2008). Aerobic heterotrophic bacteria and petroleum utilizing-bacteria from cow dung and poultry manure. World Journal of Microbiology and Biotechnology. 24, 1999-2002.

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.

Arotupin, D. J. and Akinyosoye, F. A. (2001). Evaluation of microbial isolated from sawdust for cellulose hydrolysis. Nigerian Journal of. Microbiology, 15: 97-105.

Atagana, H. I. (2006). Fungal bioremediation of PAHs in the presence of heavy metals in soil. Proceedings of the International Conference on Remediation of Chlorinated and Recalcitrant Compounds, United States.

Batelle, C. D. (2000). Mushrooms: Higher Macrofungi to clean up the environment. Batelle Environmental Issues, Fall.

Bhattacharya, D., Sarma, P. M., Krishnan, S., Mishra, S. and Lal, B. (2003). Evaluation of genetic diversity among Pseudomonas citronellolis strains isolated from oily sludge-contaminated sites. Applied Environmental Microbiology. 69 (3): 1435-1441.

Boboye, B., Olukunle, O. F. and Adetuyi, F. C. (2010). Degradative activity of bacteria isolated from hydrocarbon-polluted sites in Ilaje, Ondo State, Nigeria. African Journal of Microbiology Research, 4(23): 2484 – 2491.

Chaillan, F., Flèche, A. L., Bury, E., Phantavong, Y., Grimont, P., Saliot, A. and Oudot, J. (2004) Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Research Microbiology 155: 587-595.

Damisa, D., Oyegoke, T. S., Ijah, U. J. J., Adabara, N. U., Bala, J. D. and Abdulsalam, R. (2013). Biodegradation of petroleum by fungi isolated from unpolluted Tropical soil. International journal of applied biology and pharmaceutical technology 4(2): 136-140.

Domsch, K. H. and Gams, W. (1970). Fungi in Agricultural Soil. Longman Group Limited, London, pp.120-152.

Ekundayo, E. O. (2004). Fungi in the rhizosphere and non-rhizosphere soil of Okra cultivated in Southern Nigeria. Nigerian Journal of Soil Science, 14: 48-55.

Elad, L., Chet, I. and Henis, Y. (1981). A Selective Medium for Improving Quantitative

Isolation of Trichoderma Sp. from Soil. Phytoparasitica 9(1): 59-67.

Grazyna, P., Krzysztof, U., Terry, C. H. and Robin, L. B. (2001). Use of Molecular Technique in habitats. Applied Microbiology and Biotechnology, 76, 287–308.

Ijah, U. J. J. (1998). Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. Waste Management 18:293-299.

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.

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.

Johnsen, A. R., Wick, L. Y. and Harms, H. (2005). Principles of microbial PAH-degradation in soil. Environmental. Pollution. 133:71-84.

Li, S., Li, F., Zhang, Z., Luo, Y., Yang, S., and Wei, B. (2008). In-situ remediation of microorganisms in frozen and thawed petroleum contaminated soil from Liaohe Oil Field, Liaoning Province. Liaoning Gongcheng Jishu Daxue Xuebao, 27(4), 599-601.

Obire, O. (1988). Studies on the biodegradation potentials of some microorganisms isolated from water systems of two petroleum producing areas in Nigeria. Nigerian Journal of Botany 1: 81- 90.

Obire, O., Anyanwu, E. C. and Okigbo, R. N. (2008). Saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bioremediating agents. Journal of Agricultural Technology 4(2): 81-89.

Odu, T. I. (1978). The effect of nutrient application and aeration on oil degradation in soil. Environmental Pollution 15:235-239.

Ojo, O. A. (2006). Petroleum-hydrocarbon utilization by native bacterial population from a wastewater carnal Southwest Nigeria. African Journal of Biotechnology 5 (4) 333-337.

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.

Orji, F. A., Ibiene, A. A., Uzomba, P. C., Itoandon, E. E. and Nwachukwu, N. C. (2012). Cow Dung and Water Hyacinth Nutrient Powder: Good sources of Limiting Nutrients for Bioremediation of Hydrocarbon Polluted Mangrove Swamps in the Niger Delta, Nigeria. Nigerian Journal of Agriculture, Food and Environment. 8(2):52-58.

Samantha, S. K., Singh, O. V. and Jain, R. K. (2002). Polycyclic aromatic hydrocarbons: Environmental pollution and bioremediation. Trends in Biotechnology. 20:243-248.

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.

Song, H. G. and Bartha, R. (1990). Effects of jet fuel spills on the microbial comm-unity of soil. Applied Environmental Microbiology, 56 (3), 646-651.

Stamets, P. (1999). Helping the ecosystem through mushroom cultivation In: Growing gourmet and medical mushroom. Batellet, A. (edition). Ten speed press, Berkeley, California. p. 452.

Ulrici, W., Rehm, H. J. and Reed, G. (2000). Contaminant soil areas, different countries and contaminant monitoring of contaminants. Environmental Process II. Soil Decontamination Biotechnology. 11:5–42.

Van-Beilen, J. B., Funhoff, E. G., and Van-Loon, A. (2006). Cytochrome P450 alkane hydroxylases of the CYP153 family are common in alkane-degrading eubacteria lacking integral membrane alkane hydroxylases. Applied and Environmental Microbiology; 72 (1):59–65.

Vidali, M. (2001). Bioremediation: An overview. Journal of Applied Chemistry, 73 (7): 1163-1172.