FUPRE Journal of Scientific and Industrial Research
Vol.3, (1), 2019
ISSN: 2579-1184 (Print) ISSN: 2578-1129 (Online)

Degradation Potential of some Hydrocarbon Compounds of Petroleum and Plant Origin by *Aspergillus Oryzae* (JQ675305.1)

[1]*Sanyaolu, Adeniyi A.A., [2]Adekunle, Adedotun A. And [3]Osuntoki, Akinniyi A.
[1]Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Nigeria
[2]Department of Botany, Faculty of Science, University of Lagos, Nigeria
[3]Department of Biochemistry, College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria
Corresponding author: (adeniyisanyaolu@uniuyo.edu.ng ; +234805-309-1616)

Abstract
A good way of restoring an environment polluted with hydrocarbon is the application of methods that exploit the metabolic activity of microorganisms. Twenty five isolates of known fungi from three species of the genus *Aspergillus* isolated from five different soil environments and necrotic seeds of *Irvingia gabonensis* from four open markets in Lagos, Nigeria were studied for their ability to degrade hydrocarbons of petroleum and plant origin. These fungi were screened (in a preliminary test) for ability to grow under crude oil fume. Thereafter, using the Gas Chromatography technique, the fungal isolate adjudged to have performed best in the preliminary screening was evaluated for its ability to degrade 5 different petroleum hydrocarbon compounds and one vegetable hydrocarbon compound by measuring Total Petroleum Hydrocarbon (TPH) and Free Fatty Acid (FFA) profile respectively. Results from the preliminary screening showed that *Aspergillus oryzae* (from necrotic seeds of *I. gabonensis*) thrived best under crude oil fume. Also, results from the degradation studies showed a reduction in the Total Petroleum Hydrocarbon (TPH) in each of the petroleum based oil and a change in the fatty acid levels in the oil from *I.gabonensis* seed. This confirms the ability of this fungus to degrade petroleum and vegetable hydrocarbon compounds. This work has thus contributed to literature on the identity and sources of some fungi that are capable of causing disease on *I. gabonensis* seed and the attendant deterioration in its FFA as well as having the potential for remediating a petroleum hydrocarbon contamination. This work is probably a first report at comparing the efficiency of *A. oryzae* from an oilseed at utilizing petroleum and vegetable hydrocarbon compounds.

Keywords: **Biodegradation, Petroleum hydrocarbon, Plant pathogenic fungi and *Irvingia gabonensis* seed.**

1. Introduction

Man and the environment relate to each other dialectically because of the duo of population explosion and environmental pollution. One veritable connection between these twin maladies is the quest for

development. Developmental strides as encapsulated in industrialization bring with it a growing pressure on the exploitation of natural resources. Generally, industrialization (if not properly managed) is known to have an inverse relationship with the overall quality of the environment. Today, an offshoot of the industrialization process is the consumption of petroleum, its refined constituents and by-products as fuel. Microorganisms with the ability to degrade xenobiotics have been severally reported in literatures as been ubiquitously distributed (Yassin and Almouqatea, 2010). Microorganisms because of close contact with the soil environment are in many ways ideal as test organisms to monitor soil health (Bento *et al*., 2005).

Fungi are nature's original recyclers. Their capabilities to utilize natural and synthetic chemicals for their metabolism and growth suggests that biological processes, which are less expensive and more environment friendly (Sasikumar and Papinazath, 2003) can potentially replace the other methods of environmental cleanup. Biodegradation has been intensively studied in controlled conditions (Sugiura *et al*., 1997; Chaillana *et al.,* 2004) and in open field experiments (Chaıneau *et al.,* 2003; Gogoi *et al.,* 2003; Sanyaolu *et al*., 2018), and has acquired a new significance as an increasingly effective and potentially inexpensive cleanup technology.

This work is aimed at contributing to information on how man can potentially turn a problematic fungus associated with the loss and deterioration in the quality of his valued food item into an ally that can potentially help him maintain a sane environment. The specific objectives however are to verify whether or not a phytopathogenic fungal species is capable of degrading hydrocarbons of petroleum and plant origin and also to present a comparative picture of the efficiency of this plant pathogenic fungus at degrading both types of hydrocarbon.

2. Materials and Methods

2.1 Collection of Samples

Diseased seeds of *I. gabonensis* (from 4 open markets) and sandy soil samples (from 5 sources) were cultured for fungal species. All the sampling sites were in Lagos metropolis, Lagos State, Nigeria.

The following hydrocarbons were used in this work: Spent engine oil (SEO), Crude Oil (CO), Kero (Dual Purpose Kerosine - DPK), Diesel (Automotive Gas Oil- AGO), Fresh Engine Oil (unused engine oil - FEO) and the Oil extracted from both healthy and diseased *I. gabonensis* seeds (I.O). The diseased seeds were those inoculated with *A.oryzae* as described by Sanyaolu *et al*. (2014) for less than 24 hours (Day 0) and for 40 days duration (Day 40) after which their oil was extracted. One thousand millilitre each of all the petroleum hydrocarbon compounds (with the exception of crude oil) were collected from Forte Oil Service Station, Moshalashi Bus Stop, Mushin, Lagos, while the same quantity of crude oil was collected from one of Chevron Nigeria Limited's Platform in Warri, Delta State, Nigeria.

Oil extracted from healthy *I. gabonensis* seeds (I.O) but thereafter inoculated at with *A.oryzae* for less than 24 hours (day 0), for

Isolation of Fungi from *I. Gabonensis* Seed Samples

The method of Adekunle and Oluyode (2005) was employed in isolating fungi from the diseased seed.

Identification of fungi

Morphological and photomicograph presentation as described by Samson *et al.* (2011) and molecular identification as reported by Sanyaolu *et al*. (2018) were used in identifying the isolated fungal species.

2.2 Hydrocarbon Utilization Studies

This was done in 2 stages namely:

A. **Preliminary experiment for petroleum hydrocarbon utilizing ability of the isolated fungal samples:** Here, the method of Adekunle and Oluyode (2005) was adopted, where the petroleum hydrocarbon utilizing ability of the fungal isolates was evaluated by culturing them under crude oil fume

B. Confirmatory (final) experiment of the petroleum hydrocarbon utilizing ability of the phytopathogenic fungal sample

Here, a modified method of AOAC (2000) by means of a GC was used. The GC test served as a confirmatory and a comparative measurement (at days 0 and 40 for each of the oils) of the ability of the fungal isolate to degrade the different hydrocarbons - petroleum hydrocarbon and plant hydrocarbon.

In confirming the extent to which this fungal isolate degraded each of the hydrocarbons, a comparison of the initial Total Petroleum Hydrocarbon (TPH) at day 0 and the final TPH at day 40 and the free fatty acid profile (at days 0 and 40) was conducted for the media of petroleum origin and plant origin respectively.

Three levels of GC profiling were carried out on each of the oil. The 3 levels were: Media + fungus at day 0, media + fungus at day 40 and media only (with no fungus added) - Control. The values of the TPH and fatty acid were obtained after triplicate analysis from electronic integration measurements using flame ionization detector.

The GC model and its condition for the Fatty Acid Methyl Esters (FAMEs) analysis are as detailed below:

GC: HP 6890 powered with HP Chemstation Rev. A 09.01{1206} software.

Injection temperature : Split Injection; Split Ratio: 20:1; Carrier Gas: Nitrogen; Inlet temperature: 250^{o}C; Column type: HP INNOW ax; Column dimensions: 30m X 0.25m X 0.25µm; Oven programme: Initial temperature @ 60^{o}C; First Ramping @ 12^{o}C/min for 20min, maintained for 2 min; Second Ramping @ 15^{o}C/min for 3 min, maintained for 8min;

Detector: Flame Ionization Detector- FID; Detector temperature: 320^{o} C; Hydrogen pressure: 22 psi and Compressed air: 35 psi.

3. Results and Discussion

3.1 Results

3.1.1 Preliminary Screening of Fungal Isolates for Petroleum Hydrocarbon Utilizing Ability

The results for the fungal isolates that 'passed' the preliminary hydrocarbon utilizing test (i.e. were able to grow within 5 days of inoculation under crude oil fume) their location and origin are summarized in

Table 1. This Table showed the identities as well as the method deployed in confirming of the identities of the 25 fungal isolates encountered from the different (nine) sources

Table 1: Fungal isolates (location and origin) suspected to be capable of utilizing petroleum Hydrocarbon.

S/n	Location	Origin	Identity
1	Olushosun dump site, Ojota	Soil	*A. niger**
2	"	"	*A. oryzae****
3	"	"	*A. oryzae.****
4	"	"	*A. oryzae****
5	"	"	*A. niger*.*
6	"	"	*A. niger**
7	Mechanic village, Onitiri- Akoka	"	*A. niger****
8	"	"	*A. oryzae****
9	Mechanic village, Sabo-Yaba.	"	*A. tubingensis****
10	"	"	*A. niger**
11	"	"	*A. tubingensis****
12	"	"	*A. niger**
13	"	"	*A. oryzae****
14	"	"	*A. niger****
15	"	"	*A. niger*
16	"	"	*A. niger****
17	Biological Gardens, Unilag.	Soil	*A. niger****
18	"	"	*A. niger**
19	Shodex Gardens, Anthony- village.	"	*A. oryzae****
20	Bariga market	*I. gabonensis* seed	*A. niger**
21	Oyingbo market.	"	*A. niger.**
22	"	"	*A. oryzae****

23	Alayabiagba market, Ajegunle.	"	*A. oryzae****
24	"	"	*A. niger**
25	Agege market	"	*A. niger**

*** Identity determined using Morphological, photomicrographic and molecular (DNA sequence) techniques

* Identity determined using morphological characteristics and photomicrograph

3.1.2 Confirmation Studies of Fungal Isolates for Petroleum Hydrocarbon Utilizing Ability

On a general note, there was a reduction in the TPH levels of the petroleum hydrocarbon compounds at day 40 when compared to day 0. However, there was no significant difference ($P< 0.05$) in the TPH levels between the two Control samples i.e. day 0 with *A. oryzae* and the day 0 without *A. oryzae* for each of the oils containing Petroleum hydrocarbon (Figures 1 - 5).

With respect to the GC confirmatory studies of the oil extracted from *I gabonensis* seed, in all cases, there was an increase in the value of the saturated free fatty acid components and a corresponding decrease in the value of the unsaturated free fatty acid components of the oil at day 40, when compared to the Control (two day zero) samples (Table 2).

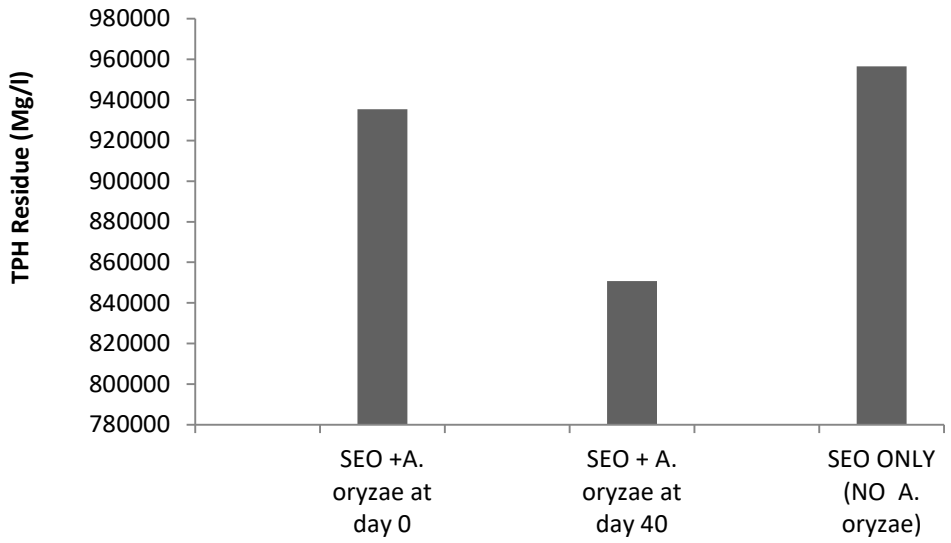

Figure 1: Comparative level of TPH Residue in SEO Samples

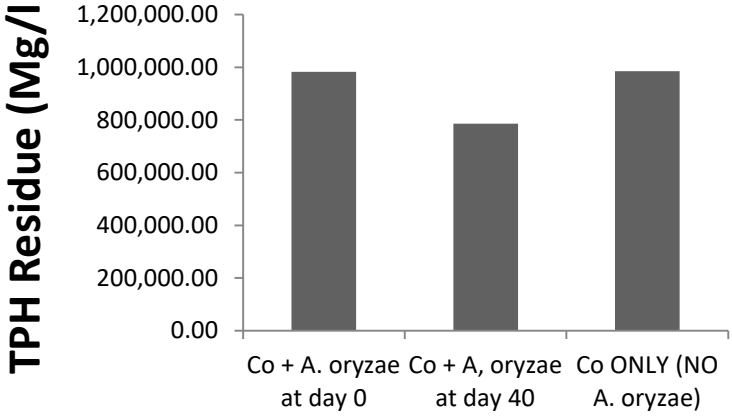

Figure 2: Comparative level of TPH Residue in Co Samples

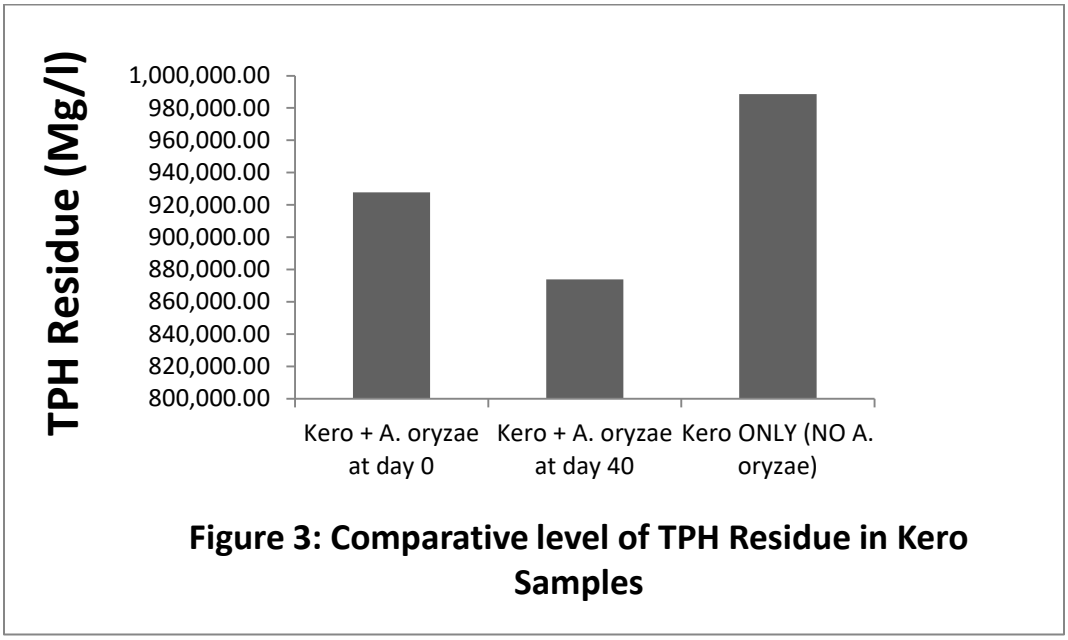

Figure 3: Comparative level of TPH Residue in Kero Samples

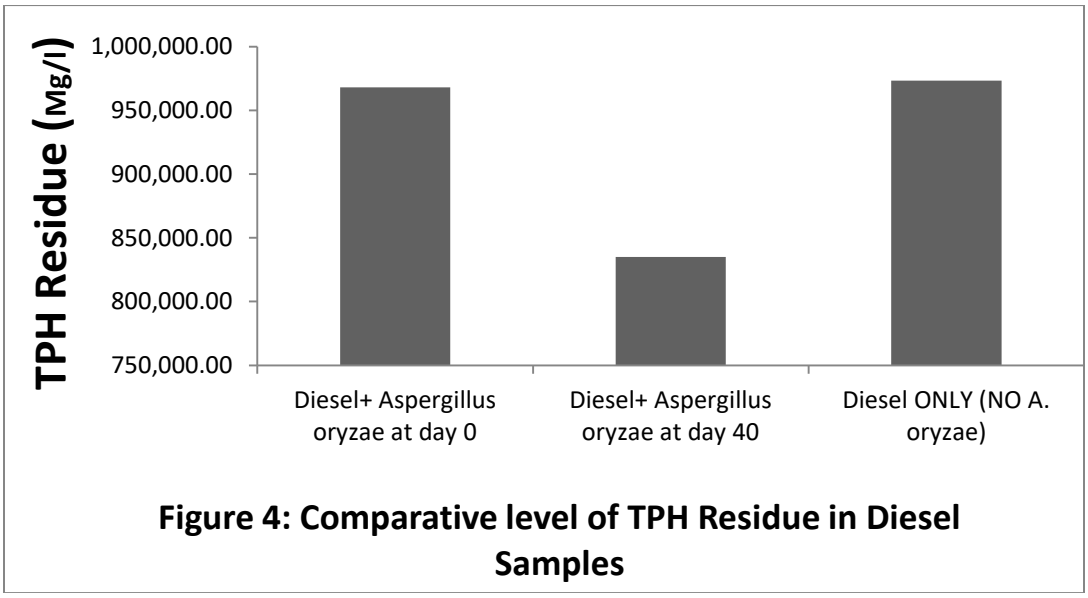

Figure 4: Comparative level of TPH Residue in Diesel Samples

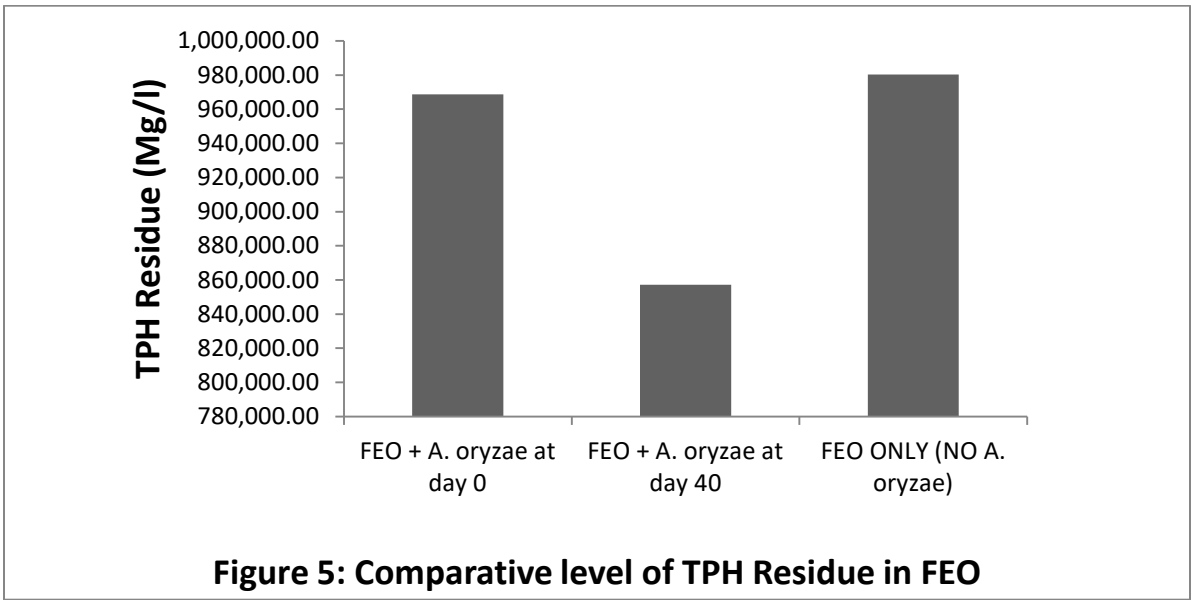

Figure 5: Comparative level of TPH Residue in FEO

Table 2: Comparative rate of degradation of extracted *Irvingia* Oil (I.O.) by *A. oryzae*

S/N	Fatty acid parameter	Level of Saturation/Unsaturation of fatty acid in i.o.	% conc. of FFA. in oil containing *A.oryzae* at day 0*	% conc. of FFA. in oil containing *A.oryzae* at day 40*	%conc. of FFA. in oil without *A.oryzae**
1	Myristic acid	C14:0	1.29	1.67	0.98
2	Palmitic acid	C16:0	14.69	16.06	12.47
3	Palmitoleic acid	C16:1	5.71	5.11	6.58
4	Stearic acid	C18:0	10. 00	14.10	9.44
5	Oleic acid	C18:1	19.85	17.37	19.94
6	Linoleic acid	C18:2	20.94	20.08	21.18
7	Linolenic				

	acid	C18:3	24.23	21.82	24.78
8	Arachidic acid	C20:0	0.41	0.44	0.38
9	Arachidonic acid	C20:4	1.42	1.27	1.81
10	Behenic acid	C22:0	0.44	0.64	0.29
11	Erucic acid	C22:1	0.88	0.69	1.62
12	Lignoceric acid	C24:0	0.48	0.54	0.36

***The percentages of oil components were obtained as the mean value of triplicate analysis from electronic integration measurements using flame ionization detector**

3.2 Discussion

With respect to the hydrocarbon utilizing ability of fungi species, the results from this work show that the *A. oryzae* associated with pathogenic conditions in the seed of *I.gabonensis*, is capable of degrading petroleum hydrocarbon and vegetable hydrocarbon. This finding is in agreement with many previous reports, where filamentous fungi in were reported to have degraded a whole array of hydrocarbon containing compounds (Adekunle and Oluyode, 2005; Saratale *et al.,* 2007, George-Okafor *et al*., 2009, Reuben *et al*., 2011) by producing capable enzymes. On account of their aggressive growth, greater biomass production and extensive hyphal growth in soil, fungi offer potential for mycoremediation technology (Kenneth, 1995; Saadoun 2002, Obire and Anyanwu, 2009). It is of interest that. *A.oryzae* is being shown, probably for the first time in any report to have the ability to utilize different types of petroleum and vegetable hydrocarbon compounds.

It is known that communities of microorganisms have been shown as petroleum hydrocarbon utilizers. George-Okafor *et al*. (2009) and Reuben *et al*. (2011) commented that soil pollution either due to oil spillage or indiscriminate dumping of refuse as a common phenomenon in many places in Nigeria, has resulted into the wide spread presence in our environment, of many species of bacteria, fungi and algae that have the enzymatic capability to utilize petroleum hydrocarbons as food. A report by Sanyaolu *et. al.* (2018) showed a marked

change in properties of soils polluted with petroleum hydrocarbon, thus affecting the physical, chemical, biological and microbiological properties of the soil.

Conclusion

The fungi isolated from diseased *I.gabonensis* seeds and the different soils were all found to be capable of growing under crude oil fume, thereby suggesting their ability to utilize hydrocarbons of petroleum origin. To the best of our knowledge this is probably a first report at comparing the efficiency of pathogenic fungal species from an oilseed at degrading/utilizing petroleum and vegetable hydrocarbon compounds.

Acknowledgement: The assistance received from the following is thankfully acknowledged: Chevron Nigeria Limited (CNL) and Nigeria Conservation Foundation (NCF) for their part sponsorship of this work through the provision of the Chief S.L. Edu Memorial Research grant; Dr. Richardson Okechukwu of the International Institute of Tropical Agriculture (IITA) Ibadan for some aspects of the statistical analysis of the data .

References

Adekunle, A.A. and T.F. Oluyode (2005). Biodegradation of Crude Petroleum and Petroleum Products by fungi isoloated from two oil seeds (Melon and soybean seeds). *Journal of Environmental Biology* 26(1): 37-42.

AOAC (2000). *Official Methods of Analysis of AOAC International*. William Horwitz (Ed.), volume 1. AOAC International, Suite 500, 481 North Frederick Avenue, Maryland – USA. 658pp.

Bento, F.M., Camargo, F.A.O., Okeke, B.C. and W.T. Frankenberger (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology* 96(9): 1049 – 1055.

Chaillana, F., Flechebe, A., Burya, E., Phantavoga, Y-hui, Saliot, A. and J. Oudot (2004). Identification and biodegradation potentials of tropical aerobic hydrocarbon degrading microorganisms. *Resource Microbiology* 155(7): 587-595.

Chaineau, C.H., Yepremian, C., Vidali, J.F., Ducreux, J. and D. Ballerini (2003). Bioremediation of a crude oil polluted soil: biodegradation, leaching and Toxicity assessments. *Water, Air, and Soil Pollution* 144: 419-440.

George-Okafor, U., Tasie, F. and F. Muotoe-Okafor (2009). Hydrocarbon degradation potentials of indigenous fungi isolated from petroleum contaminated soils. *Journal of Physical and Natural Sciences* 3(1): 1-6.

Gogoi, B.K., Dutta, N.N., Goswami, P. and T.R. Mohan (2003). A case study of bioremediation of Petroleum hydrocarbon contaminated soil at a crude oil spill site. *Advances in Environmental Research* **7**: 767-782. doi: 10.1016/S1093-0191(02)00029-1

Kenneth, E.H. (1995). Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi. *Environmental Health Perspective* 103: 41–43.

Obire, O. and E.C. Anyanwu (2009). Impact of various concentrations of crude oil on fungal populations of soil. *International Journal of Environmental Science & Technology* 6(2): 211-218.

Reuben, N. O., Josiah, M. A., Davidson, D. D. and M. Allagoa (2011). Mycoremediation of polycyclic aromatic hydrocarbons (PAH) contaminated oil-based drill-cuttings. *African Journal of Biotechnology* 10(26): 5149-5156.

Saadoun, I. (2002). Isolation and characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel fuel. *Journal of Basic Microbiology* 42: 422– 430.

Sanyaolu, A.A.A., Adekunle, A.A. and A.A. Osuntoki (2014). The Effects of Post Harvest Mycodeterioration on the Proximate Composition of *Irvingia gabonensis* seeds. *International Journal of Phytopathology* 03(01): 41 – 48.

. Sanyaolu, A.A.A., Adekunle, A.A. and A.A. Osuntoki (2018). Bioremediation Potentials of a Petroleum Hydrocarbon Polluted Soil by *Aspergillus oryzae* (JQ675305.1) Isolated from Diseased *Irvingia gabonensis* Seeds. *The Pacific Journal of Science and Technology* 19(1): 321-333

Saratale, G., Kalme, S., Bhosale, S. and S. Govindwar (2007). Biodegradation of kerosene by *Aspergillus ochraceus* NCIM – 1146. *Journal of Basic Microbiology* 47: 400 – 406.

Sasikumar, C.S. and T. Papinazath (2003). Environmental Management and Bioremediation of Polluted Environment. In: Burch, M.J., Suresh, V.M. and Kumaran, T.V. (Eds). *Proceedings of the Third International Conference on Environment and Health*, Chennai-India. 469pp.

Sugiura, K., Ishihara, M., Shimauchi, T. and S. Harayama (1997). Physicochemical properties and biodegradability of crude oil. *Environmental Science & Technology* 3**:** 45-51.

Yassin, M.F. and S. Almouqatea (2010). Assessment of airborne bacteria and fungi in an indoor and outdoor environment. *International Journal of Environmental Science and Technology* 7(3): 535-544.