IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 11, Issue 10 Ver. I (October. 2017), PP 49-57
www.iosrjournals.org

Comparative bioremediation potential of *Mucor racemosus* and *Paecilomyces variotii* on crude oil spill site in Gio Tai, Ogoni land.

Nrior, R. R. and Jirigwa, C. C.
Department of Microbiology, Rivers State University of Science and Technology, Port Harcourt.
**Corresponding author: Nrior, Renner Renner (Nrior, R. R.),*

Abstract: *Evaluation of bioremediation potential of two fungi species: Paecilomyces variotii and Mucor racemosus were carried out on soil sample collected from crude oil spill site in Gio Tai Ogoni land. Soil samples were inoculated and pure culture of Mucor racemosus and Paccilomyces variotii were obtained from the soil, the soil samples were also analyzed for Total fungi count, hydrocarbon-degrading bacteria count and total heterotrophic bacteria count. Residual oil was measured in terms of Total Hydrocarbon Content (THC).The result for Total Hydrocarbon Content (THC) for surface soil control was 202792.9mg/kg and subsurface soil was 18690.71mg/kg in day 1.The residual crude oil content of soil with Paecilomyces variotii was less (136364.29mg/kg) than Mucor racemosus (176292.83mg/kg). Also for the subsurface soil the residual crude oil content at the end of 28 days was less with Paecilomyces variotii (10707.86mg/kg) than Mucor racemosus (14577.86mg/kg). The bioremediation rates were as follows: surface soil: crude oil polluted soil with Paecilomyces variotii (32.56%) > crude oil polluted soil with Mucor racemosus (13.07%) > control (8.10%). Sub-surface soil: crude oil polluted soil with Paecilomyces variotii (42.71%) > crude oil polluted soil with Mucor racemosus (22:01%) > control (0.79%). In this study, it was also observed that fungi count decreased in both surface soil and subsurface soil from $1.0x10^8$ to $5x10^7$cfu/g for the control (polluted soil without added microorganism), polluted soil with Mucor racemosus decreased from $8x10^7$ to $4x10^7$ and polluted soil with Paecilomyces variotii from $7x10^7$ to $3x10^7$cfu/g for the surface soil. In subsurface soil, total fungi count decreased from $1.6x10^8$ to $2x10^7$cfu/g for the control, $1.1x10^8$ to $4x10^7$ for polluted soil with Mucor racemosus and $8x10^7$ to $3x10^7$ for polluted soil with Paecilomyces variotii. Conclusively crude oil polluted soil incubated with Paecilomyces variotii has the highest bioremediation potential.*

Keywords*: Bioremediation, Hydrocarbon utilizing fungi, Paecilomyces variotii, Mucor racemosus*

Date of Submission: 12-09-2017 Date of acceptance: 06-10-2017

I. Introduction

Contamination of soil by oil spills is an environmental problem in Nigeria that often requires cleaning up of the contaminated sites (Bundy *et al.,* 2002). Oil spillage is the release of petroleum substances or product into the streams, lakes, rivers, beaches, seas, oceans and lands, which becomes poisonous and thus make the water and land fouled and threatened the rich coastal habitat.

Oil spillage is a major environmental concern in Nigeria. It is common in oil producing areas. During a spill, oil floats on land and water surface and forms an oil slick that is 0.1mm thick continuing to spread; the stick becomes 0.01mm thick. This environmental concern is because petroleum hydrocarbons in soil adversely affect the germination and growth of plant (Samina *et al.,* 2001) by creating conditions which makes essential nutrients like nitrogen and oxygen unavailable. This problem after oil spill also affect lives (microorganisms and higher organisms) in the river. Bioremediation of areas degraded by pollutants or otherwise damaged through mismanagement of ecosystem is a form of biotechnological approach aimed at rehabilitating the area for positive uses. In their studies on the environmental implications of oil exploitation and production activities Adejoke and Ojesina (2000), observed that oil spillage constitute by far the most significant source of pollution. These spill often occur in pipelines, flow line delivery line failure (Udo and Fayemi, 2005). It has been commercially explored since the middle of the 19th century; petroleum has been used for many decades for Illumination and on a smaller scale, as lubricant. The invention of the internal combustion engine is its best adaptation in all transport forms with enlarged resources, thus increasing its demand production, transports stockpiling, and distribution, as well as the raw oil and its by-products. All these activities involve pollution risks that can be minimized but not totally eliminated, causing several problems for the environment (Pala *et al.,* 2006). The application of biotechnological processes involving microorganisms, with the objective of solving environmental pollution problem, is rapidly growing in recent decides where petroleum and its by-product are concerned. Bioremediation process, which takes advantage of microbial degradation of organic and inorganic substances, can be defined as the use of microorganism to remove environmental pollutants of soils, water and

sediments (Pala *et al.,* 2006; Nrior and Echezolom, 2016). The bioremediation process presents countless advantages in relation to other processes employed to remove pollution such as extraction with solvent addition chemical oxidizers (Van Gestel *et al.* 2007; Gogoi *et al.,* 2003; Nano *et al.*,2003; Morelli *et al*., 2005; Demnerova *et al.,* 2005; Nrior and Echezolom, 2016). The use of microorganisms that are able to degrade toxic chemicals in bioremediation process is one of the major approaches used to restore contaminated soil. Therefore, hydrocarbon biodegradation in soil can be influenced by many factors, such as type of microorganism, temperature, oxygen, pH, moisture, nutrient soil properties, and contaminant concentration (Bradi *et al.,* 2000; Semple *et al.,* 2001; Sabele *et al.,* 2004; Ghazali *et al.,* 2004; Walter *et al.,* 2005; Atlas and Bartha, 2006). These researchers have concluded that the disappearance of crude oil from seawater could be accelerated by the addition of nutrients: such as nitrogen or phosphorus or both.

Bioremediation was defined by Allen, (1998) as the return of soil to a condition of ecological stability together with establishment of plant communities it supports or supported prior to disturbance. Among hydrocarbon pollutants, diesel oil is a complex mixture of alkanes and aromatic compounds that are frequently reported as soil contaminants leaking from storage tanks released in accidental spills (Gallego *et al.,* 2001). Soil contamination results in damage of crop growth; depending on the degree of contamination, the soil may remain unsuitable for plant growth for months or several years. The effect of contamination of soil with petroleum product on plants ranges from chlorosis, bleaching, sporting of leaves, necrosis, malformations to epidermal cells and mesophyl layers, yield reduction and impaired fecundity (Quinone-Aquilar *et al.,* 2003). Achuba (2006) reported that toxic hydrocarbon molecules could inhibit the activities of amylase and starch phosphorylase and thereby effect the assimilation of starch. Henner *et al.,* (1999) reported that petroleum hydrocarbons consisting of small molecules and those that are water soluble are more phytotoxic to seed germination.

Heavier oil can wash to the shore causing serious short-term harm to shell fish and also make the soil unsatisfactory for the plant growth. This is due to insufficient aeration of the soil due to blockage of air from the spaces between the soil particles by oil spillage. Tarry lumps, float or sink covers habitats of shallow water organism. They may also foul beaches. Weather is key factor for oil spills; wind can cause an emulsion which can last for weeks and be difficult to pump. During the early stages, coming in contact with the soil, may kill sea birds and mammals, because of the toxic chemicals. Oil on feathers hinders water repellency and poisons the birds from the oral consumption of the toxic materials. Bearing animals, seabirds and seals lose their buoyancy and insulation when oil fouls their fur; they either drown or suffer hypothermia and die. More so, fishes may simply swim away but floating eggs can be destroyed.

The Gio Tai and environmental costs of oil production have been extensive. They include destruction of wildlife and biodiversity, loss of fertile soil, pollution of air and drinking water, degradation of farmland and damage to aquatic ecosystem all of which caused serious health problems for the inhabitants of areas surrounding oil producing zones. In fact, the occurrence of oil spillage in Ogoni is frequent in February 2001, and still on till date.

II. Materials And Methods

Soil Collection

The materials selected for this study were in the same environment but in different location from Ogoni land. Contaminated soils were collected directly from the crude oil polluted site in Gio Tai community in Ogoni Land. The soil samples were collected from the surface and sub-surface at the depth 30cm from different locations and kept in perforated sterilized black polythene bags. The surface soil was highly polluted than the underground and it was transported to the laboratory. Processing of soil sample began two (2) days upon the arrival at the laboratory. They were sieved through a (<2mm) filter sieve and air dried, the moisture content was analyzed after sieving.

***Source of organisms* (*Mucor racemosus* and *Paecilonyces variotii*)**

Pure cultures of these organisms were obtained from inoculation and incubation of the soil sample using SDA (Sabouroud Dextrose Agar). After the pure cultures of the organisms were obtained, a broth culture was also prepared of the organism.

Scientific classification:

For ***Mucor racemosus*** Kingdom: Fungi, Division/Phylum: Zygomycota, Class: Zygomycetes, Order: Mucorales, Family: Mucoraceae, Genus: *Mucor* Specie: *racemosus*

For ***Paecilimyces variotii*** Kingdom: Fungi, Phylum: Ascomycota, Class: Eurotiomycetes, Order: Eurotiales, Family: Trichocomaceae, Genus: *Paecilomyces* Specie: *variotii*

Preparation of soil and application of the organism (contamination of organisms)

Soil (crude oil spill polluted soil from site) were collected in two locations, one batch is surface soil while the other is sub-surface soil about 3 feet deep. About 1000 grams of contaminated surface soil were weighed into 3 batches while 1000g of the contaminated sub-surface soil were also weighed into another 3 batches. However, different treatments were considered for each soil batch.

Illustration for easy interpretation

(A) Crude Oil Polluted Soil (Surface)
- (i) Control; nothing was added.
- (ii) Polluted soil + *Mucor racemosus*
- (iii) Polluted soil + *Paccilomyces variotii*

(B) Crude Oil Polluted Soil (Sub-surface)
- (i) Control; nothing was added
- (ii) Polluted soil + *Mucor racemosus*
- (iii) Polluted soil + *Paccilomyces variotii*

Media Preparation

Nutrient Agar

Nutrient Agar of L:S – Brotech company was used for the isolation and enumeration of total Heterotrophic bacteria. The media was suspended by weighing 28gm into 1000ml of distilled water according to the manufacturer's specification. It was mixed thoroughly and autoclaved at 15 psi, 121^0C for 15min and was aseptically poured into Petri-dishes.

Sabouroud Dextrose Agar

Sabouroud dextrose agar of Titan Biotech limited was used for the isolation of total fungal. 65 grams of the medium was suspended in 1000ml of distilled water according to the manufacturer's specification it was mixed thoroughly and autoclaved at 15psi, 121^0C for 15min and was aseptically poured into petri dishes.

Oil Agar Medium

Oil agar medium was prepared according to the modified mineral salts medium (MSM) composition of Mild *et al.*, (1978). The composition of this media is as follows:

$K_2HPO_4.7H_2O$ (0.5g), $MgSO_4$ (0.3g), $NaCl_2$ (0.3g), $MnSO_4 H_2O$ (0.2g), $FeSO_4.H_2O$ (0.02g), $NaNO_3$ (0.03g), $ZnCl_2$ (0.03g), Agar Agar (16g), Distilled water (200ml).

The media was prepared by adding 1% of crude oil to this mineral salt medium. The medium was used for the isolation of total petroleum degrading bacteria. The medium was mixed thoroughly and autoclaved at 15psi at 121^0C for 15 minutes and it was allowed to cool to 45^0C and was aseptically poured into Petri dishes.

Culturing, isolation and enumeration of total fungi

Isolation and enumeration of total fungi was done by serial dilution, sterile normal saline i.e. 0.85% of sodium chloride was used as diluents for inoculum preparation. 1.0g soil sample was aseptically transferred into a sterile test tube containing 9.0ml of the diluents. This gave 10^{-1} dilution subsequently 10^{-6} serial solution were prepared from the 10^{-1} dilution. Then 0.1ml aliquot of 10^{-6} dilution of each soil sample was aseptically removed with a sterile pipette and separately spread plated with flame sterilized glass spreader on Sabouroud dextrose agar plates. The cultured plates were incubated at 35^0C for 5 days. After incubation, the colonies that appeared on the Sabouroud dextrose agar plates were recorded as counts of total fungi count for all the six soil samples respectively.

Isolation and enumeration of Total Heterotrophic Bacteria (THB)

For isolation of total heterotrophic bacteria, nutrient agar medium was used. Nutrient agar plates were inoculated in quintuple with 0.1ml a liquids of 10^{-6} dilutions of each soil sample and incubated at 35^0C for 24 hours. Colonies that appeared on the nutrient agar plates were counted and recorded as the count of total heterotrophic bacteria for all seven soil samples, applying the appropriate correction factors. The colonial morphology of bacteria isolate was carried out on the basis of their shape, edge, color, elevation, surface, opacity and their consistency while biochemical assay were based on Gram reaction, motility, catalase, oxidase, coagulase, indole, methyl red, Voges Proskauer, citrate, sugar fermentation tests.

Isolation and enumeration of Total Hydrocarbon Utilizing Bacteria

For isolation of total hydrocarbon utilizing bacteria, oil agar medium was used. The oil agar plates were inoculated in quintuple with 0.1ml aliquots of 10^{-6} dilution if each soil sample and incubated at 35^0C for 7 days. The colonies that appeared on the agar plate were counted after a week and result were recorded as total heterotrophic degrading bacteria. The colonies counted were expressed as the colony forming unit (CFU) per gram soil after applying the appropriate correction factor.

Characterization of fungi

Identification of fungi isolates was based on the type of mycelium, pigmentation, type of sporulating structures and sexual reproduction (if present). They are examined using hand lens to determine those morphological characteristics.

Microscopy: Several coloured colonies were selected from the incubated plate for identification using the following procedure. A wet mount slide was prepared by transferring a small amount of the culture with a dissecting needle or inoculating loop to make a slide. This was covered with a cover slip and examined under low power (x10) or high power (x100) objective.

Stock solution: Ten percent glycerol solution was prepared dispensed in McCartney bottles and autoclaved at 121^0C for 15 minutes, allowed to cool, then the pure cultures were inoculated into each McCartney bottle, until the clear colourless solutions turns turbid and were stored in the refrigerator. This served as pure cultures for subsequent characterization.

Petroleum Hydrocarbon Analysis

The soil samples were analyzed for total hydrocarbon content (THC). The procedure was undertaken 5 times (interval of 5 days) to form five replicates.

About 10g of soil from each soil sample was weighed into 250ml Erlenmeyer flask containing 20ml of Xylene. It was covered with aluminium foil and was thoroughly shaked and extracted with cotton plug/filter paper in glass funnel inserted into a sterile conical flask. The extract was immediately analyzed using spectrophotometer at 620nm wavelength to determine the level of degradation by the organism (*Mucor racemosus* and *Paccilomyces variotii*).

III. Result And Discussion

Total heterotrophic bacteria, fungi and total petroleum utilizing bacteria analysis using spread plate method were shown in Table 1-2, 4-7. Microbial counts were taken (Total heterotrophic bacteria counts, fungi counts and petroleum utilizing bacteria counts) with a view to evaluate the difference in the microbial numbers between the composite soil samples in their different locations.

Table 1: Total Heterotrophic Bacteria Count (cfu/g) – Surface soil

Days	Control	Polluted soil + *Mucor racemosus*	Polluted soil + *Paecilomyces variotii*
1	1.3×10^8	$1.2\ 10^8$	1.3×10^8
7	1.3×10^8	1.0×10^8	9.0×10^8
14	7×10^7	5×10^7	6×10^7
21	5×10^7	3×10^7	4×10^7
28	4×10^7	2×10^7	3×10^7

Table 2: Total Heterotrophic Bacteria Count (cfu/g) - Sub-Surface Soil

Days	Control	Polluted soil + *Mucor racemosus*	Polluted soil + *Paecilomyces variotii*
1	2.3×10^8	1.8×10^8	1.4×10^8
7	2.0×10^8	9.0×10^7	1.0×10^8
14	$1.2\ 10^8$	5×10^7	6×10^7
21	9×10^7	4×10^7	6×10^7
28	7×10^7	2×10^7	4×10^7

Fungi have been used effectively in bioremediation of crude oil contamination. Different species of fungi has played a significant role in the study of bioremediation, *Pleurotus pulmonarius* has been used to remediate contaminated soil by spent diesel oil (Adenipekun *et al.,* 2013.).

Table 3: Microscopic morphologies of the fungal isolate and their identities

Species	Best growth temp.	Growth rate	Colour on SDA	Colour on reverse side	Texture	Special feature
Rhyzopus arrhizus	40^0C	Very rapid	Initially white, quickly becoming pale gray and then developing small black dots in the mycelium	White	Cottony candy	Develops small black dots in mycelium which are mature sporangia
Penicillium marneffei	35^0C	Slow	Creamy to slightly pink	Red	Glabrous to convoluted	Filamentous, flat, radially sulcate colonies
Mucor racemosus	25^0C	Rapid	White	White	Cottonly candy	Fluty appearance.
Aspergellus niger	25^0C	Rapid	Deep brown to black	Light gray or buff colon	Densely stippled surface	Conidia on surface
Penicillium chrysogenum	25^0C	Rapid	White to blue-green	Pale to yellow	Velocity wooly	The colonies initially white and become blue-green, pinkish
Paccilonyces variotii	25^0C	Rapid	Yellow brown	Green yellow	Granular	Unusually granular

Adenipekan and Kessim, (2006) opted that the reduction in stem girth with increase in concentration of spent oil in soil could be due to the reduction of nutrient uptake by the plants. The plant in the remediated soil performed better than the control plants because fungus was able to bioaccumulate heavy metals. Malcova *et al.,* (2003) confirmed the role of mycorrhiza fungi in alleviating nutritional stress and that this may have helped to protect hydrides against harmful effect of crude oil pollution. It was also observed that the plants cultivated on crude oil-polluted soil treated with *P. Pulmonarius* mycelia grew well at the different crude oil concentration, this was reported by Isikhuemhen *et al.,* (2003).

Table 4: Total Fungal count (cfu/g) – surface soil

Days	Control	Polluted soil + *Mucor racemosus*	Polluted soil + *paecilomyces variotii*
1	1.0×10^8	6×10^7	7×10^7
7	8×10^7	8×10^7	4×10^7
14	7×10^7	4×10^7	7×10^7
21	6×10^7	4×10^7	3×10^7
28	5×10^7	4×10^7	3 x 10

Table 5: Total Fungal count (cfu/g) – sub-surface soil

Days	Control	Polluted soil + *Mucor racemosus*	Polluted soil + *Paccilomyces variotii*
1	1.6×10^8	1.1×10^8	8×10^7
7	9×10^7	3×10^7	4×10^7
14	1.0×10^7	7×10^7	3×10^7
21	2×10^7	6×10^7	2×10^7
28	2×10^7	4×10^7	3×10^7

In this study petroleum utilizing count in the sub-surface soil and surface were higher in the day I than in day 2. In the surface soil the average count for of petroleum utilizing fungi in the control were higher than crude oil polluted soil + *Mucor racemosus* and crude oil polluted soil + *Paccilomyces variotii*, still in the surface soil the Total fungi count in polluted soil + *Mucor racemosus* were also higher than polluted soil + *Paccilomyces variotii* as well as in the sub-surface soils (that is) sub-surface soil – fungi count in control > polluted soil +*Mucor racemosus* > polluted soil + *Paccilomyces variotii*.

Table 6: Petroleum utilizing bacteria (PUB 10^{-6}) – surface soil

Days	Control	Polluted soil + *Mucor racemosus*	Polluted soil + *Paccilomyces variotii*
1	1.9×10^8	1.7×10^8	1.0×10^8
7	1.7×10^8	1.2×10^8	8×10^7
14	7×10^7	3×10^7	6×10^7
21	5×10^7	3×10^7	5×10^7
28	4×10^7	2×10^7	3×10^7

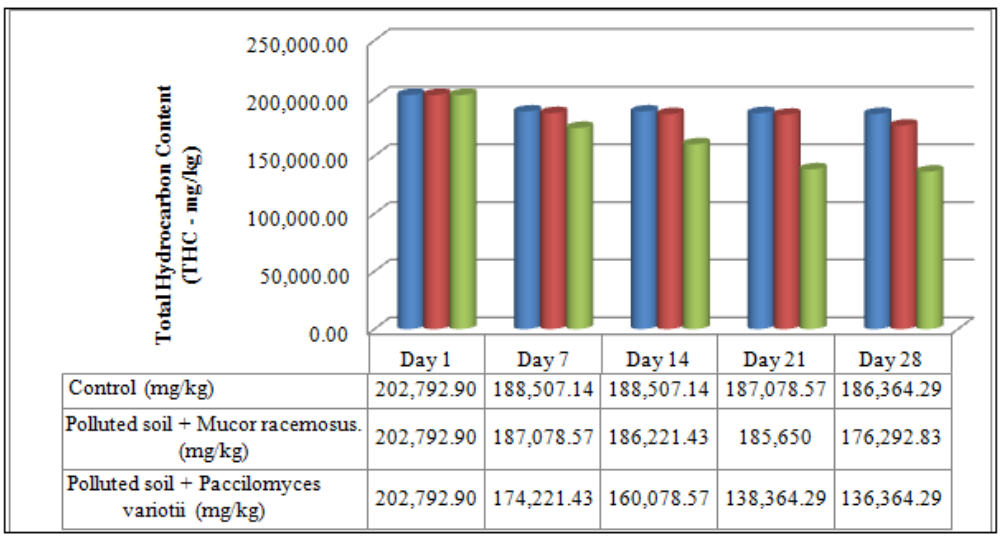
Table 7: Petroleum utilizing bacteria (PUB cfu/g) – sub-surface soil

Days	Control	Polluted soil + *Mucor* sp.	Polluted soil + *Paccilomyce* spp
1	1.7×10^8	8×10^7	1.2×10^8
7	1.5×10^8	7×10^7	1.0×10^8
14	1.2×10^8	5×10^7	4×10^7
21	1.0×10^8	2×7^8	4×10^7
28	9×10^7	4×10^7	2×10^7

Table 7 showed that the sub-surface soil has more number of hydrocarbon utilizing bacteria than in the surface soil this may be due to high concentration of the crude oil spilled on the surface soil and also more growth were observed on the day I than in day 28, this is because, on the first day, there was suitable feeding materials or nutrient for this microorganisms to feed on, but with increasing time, the lack of organic matter appeared little by little limiting the growth of this microorganism in the soil Sang-Haw *et al.*, (2007) made a similar observation and concluded that hydrocarbon microbial population increased rapidly during the first day.

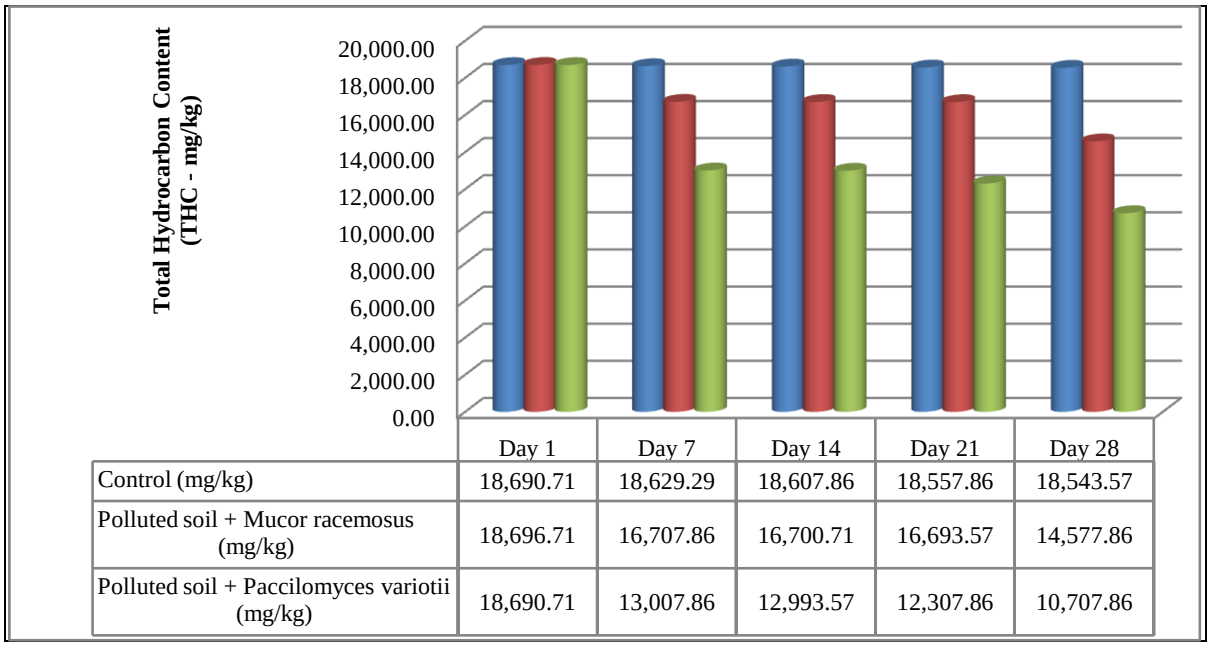
Bioremediation of Petroleum Hydrocarbon using spectrophometric assay for Total Hydrocarbon Content (THC – mg/kg) carried out on soil samples (crude oil contaminated soil) collected in two location surface and subsurface were shown in fig. 1-2.

It is observed in this study that the total degradation rate of crude oil contaminated soil using *Mucor racemosus* and *Paccilomyces variotii* was successful. In the surface soil which is highly polluted with crude oil, the degradation potential of *Mucor recemosus* was higher than the control mean while degradation potential of crude oil polluted soil using *Paccilomyces variotii* was higher than using *Mucor recemosus*.



	Day 1	Day 7	Day 14	Day 21	Day 28
Control (mg/kg)	202,792.90	188,507.14	188,507.14	187,078.57	186,364.29
Polluted soil + Mucor racemosus. (mg/kg)	202,792.90	187,078.57	186,221.43	185,650	176,292.83
Polluted soil + Paccilomyces variotii (mg/kg)	202,792.90	174,221.43	160,078.57	138,364.29	136,364.29

Fig. 1: Total Hydrocarbon Content (THC – mg/kg) during bioremediation of crude oil spill polluted soil (surface soil)



	Day 1	Day 7	Day 14	Day 21	Day 28
Control (mg/kg)	18,690.71	18,629.29	18,607.86	18,557.86	18,543.57
Polluted soil + Mucor racemosus (mg/kg)	18,696.71	16,707.86	16,700.71	16,693.57	14,577.86
Polluted soil + Paccilomyces variotii (mg/kg)	18,690.71	13,007.86	12,993.57	12,307.86	10,707.86

Fig. 2: Total Hydrocarbon Content (THC – mg/kg) during bioremediation of crude oil spill polluted soil (sub-surface soil)

The total petroleum hydrocarbon concentration of crude oil contaminated soil incubated with *Mucor recemosus* and *Paecilomyces variotii* for 5 weeks. The rate of biodegradation increases effectively while values decreased after 1 month of incubation. That is the effect of time on petroleum degradation was significant. The petroleum degradation was significant. The petroleum degradation rate decreased with an increasing time.

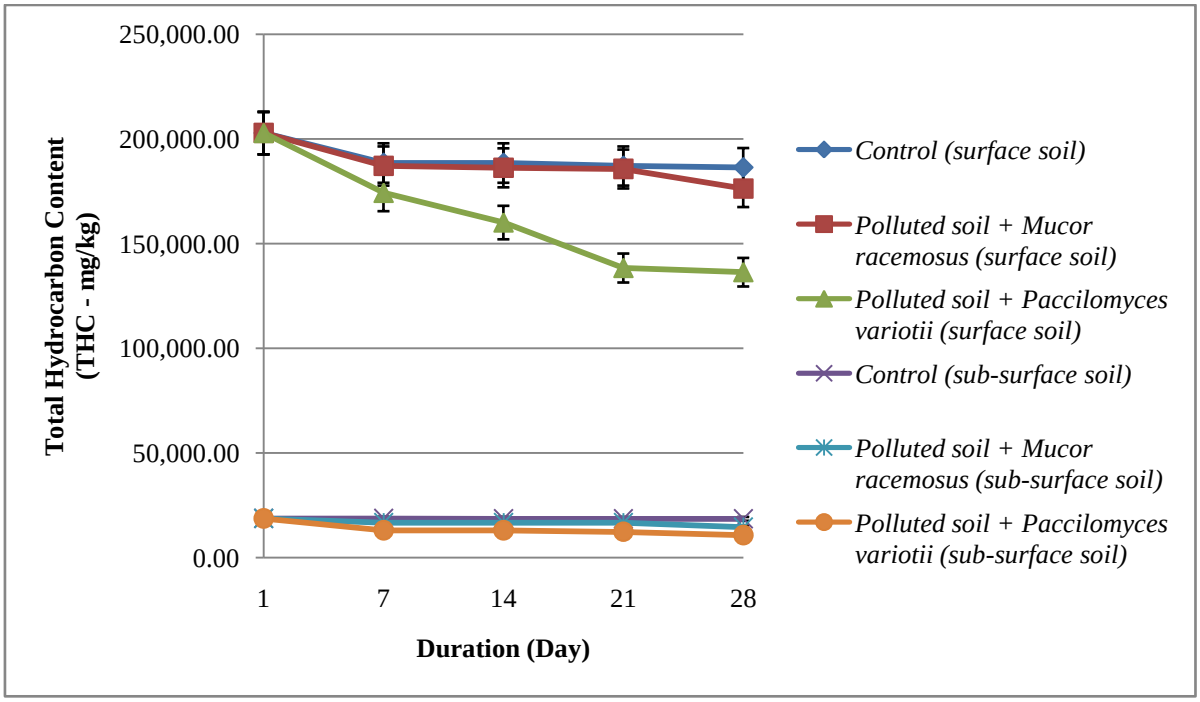


Fig. 3 Comparative Total Hydrocarbon Content (THC – mg/kg) during bioremediation of crude oil spill polluted soil (surface and sub-surface soil)

Moreover, in the underground soil, the degradation rate of polluted soil with crude oil using *Mucor racemosus* was higher than the control while degradation rate of *Paccilomyces variotii* were higher than the *Mucor racemosus* and the control.

IV. Conclusion And Recommendation

Petroleum hydrocarbon pollution is a worldwide threat to the environment and the remediation of oil contaminated soil is a major challenge for environmental research. The use of microorganism is a useful method of soil remediation, and less expensive. The bioremediation consist bioaugumentation strategy of *Mucor racemosus* and *Paccilomyces variotii.* The study shows that the soil was highly contaminated by crude oil spill.

However, the addition of organism *Mucor racemosus* and *Paccilomyces variotii* showed the ability to enhance petroleum hydrocarbon of microbial degradation, with *Paccilomyces variotii* having greater petroleum hydrocarbon reduction than *Mucor racemosus*. The soil samples analyze for microbial count for total heterotrophic bacteria count, total fungi count and total heterotrophic degrading bacteria count showed that the rate of microbial population in all the treatment samples decreased with an increasing time.

However, the bacteria and fungi that were identified in the soil samples are of the following genera: pseudomonas species, *Arthrobacter* species, *Penicillium* species, Bacillus species, *Mucor Racemosus*, *Paccilomyces* species, *Rhyzopus arrhizus, penicillium marneffei Aspogellus Niger, Penicillium chrysogenum, Phialophora verrocusum*.

The fungus *Paccilomyces variotii* has been found from this study to be a potential bioremediation agent in sites contaminated with crude oil. It is recommended that companies and refineries that contaminate soil with petroleum products should be encouraged to simply employ the strategies of *Paccilomyces variotii* on sites contaminated with crude oil.

References

[1]. Adejoke, O. S. and Ojesina, A. O. (2000). Environmental Implications of oil exploration and production activities. Proceedings of capacity building workshop for environmental impact Assessment in the Benue Trough, Hill Station Hotel, Jos 22nd – 25th June 2000. Pp 162 – 174.

[2]. Adenipekun, C. O. and Kassim, L. (2006). Effects of spent engine oil on the growth parameters and moisture content of *Celosia argentca. Nigerian Journal of Botany.* 19(2):318-324.

[3]. Adenipekun, C.O., Ayanleye, O. O. and Oyefunji, O. J. (2013).Bioremediation of Soil Contaminated by spent diesel oil using *Pleurotus pulmonarius* Fries (Quelet) and its effect on the growth of *Corchorus olitorius* (L) *Journal of Applied Bioscience (2013).* 68:5366-5373.

[4]. Achuba, F. I. (2006). The effect of Sublethal Concentrations of Crude Oil on the Growth and Metabolism of Cowpea (*Vigna unguiculata*) Seedlings. *The Environment* 26:17-20.

[5]. Alexander M. (1995) How Toxic are toxic Chemicals in Soil? *Environmental Science and Technology*, Pp 2936 – 3936x.

[6]. Allen, M. F. (1988). Below ground structure: a key to reconstructing a productive ecosystem, p. 133-135. In: E. B. Allen (Eds), The reconstruction of disturbed arid lands. Westview Press, Boulder, Colorado, Arizona.

[7]. Atlas, R. M. and Bartha, R. (2006). "Fate and effect of polluting petroleum in the marine environment" *Residue Review*. 49 (1): 49 – 83.

[8]. Atlas, R. M., and Bertha, R., (2006). "Fate and effects of Polluting Petroleum in the Marine Environment". *Residue Rev.*, 49(1):49 – 83.

[9]. Bradi, L., Mottei, A. Steffan, S. and Marzona M. (2000),. "Hydrocarbon degradation by a soil microbial population with B – cyclodeztrin as surfactant to enhance bioavailability" *Enzyme and microbial technology*: 27(3); 709 – 713.

[10]. Bundy, J. G., Paton G. I., and Campbell C. D., (2002). Microbial Communities in different soil types do not coverage after diesel contamination" *J. Appl. Microbial*, 92: 276 – 288.

[11]. Coulon, F., Pelletier, E., Gourhant, L., and Delille, D., (2005). Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil. *Chemosphere*: 58: 1439 – 14448.

[12]. Das, N., Chandran, P., (2011). Biodegradation of Petroleum Slude and Microbial Degradation of Petroleum Hydrocarbon contaminants; An overview, Review Article, Sage-Hindawi Access to Research Biotechnology Research International, Article ID 941810, Pp13.

[13]. Debey R. C. (2004). A text book of biotechnology 3rd edition, S. Chand and company Ltd, New Delhi, India. Pp 365 – 375.

[14]. Demnerova, K., Mackova, M., Spevakova, V., Beranova, K., Kochankova, L., Lovecka, P., Ryslava, E., Macek, T., Alexander M. (1995). How Toxic are toxic Chemicals in Soil? *Environmental Science and Technology* 1:2936 – 3936x.

[15]. Henner, P., Schiavon, M., Druelle, V. and Lichtfouse, E. (1999). Phytotoxicity of ancient gaswork soils. Effect of a polycyclic aromatic hydrocarbons (PAHs) on plant germination. *Organic geochemistry* 30:963-969.

[16]. Gallego, J. R., Loredo, J., Llamas, J. F., Vazquez, F. and Sanchez, J. (2001). Bioremediation of diesel-contaminated soils: evaluation of potential in-situ techniques by study of bacterial degradation. *Biodegradation* 12:325-335.

[17]. Ghazali, F. M., Rahman, R. N. Z. A., Salleh, A. B., Basri, M., (2004). "Biodegradation of hydrocarbons in hydrocarbons in soil by microbial consortium" *J. Int. Biodeter and Biodegradation*; 54(5): 61 – 67.

[18]. Gogoi, B. K, Dutta, N. N., Krishnamohn, T. R. (2003). A case study of bioremediation of petroleum hydrocarbon contaminated soil at a crude oil spill site. *Advances in Environmental Research, 7*(4), 767-782.

[19]. Isikhuemhen, O. S., Anoliefo, G. & Oghale, O. 2003. Bioremediation of Crude Oil polluted soil by the white rot fungus, P. tuberregium (Fr) sing – *Environmental Science and pollution Research* 10: 108 – 112.

[20]. Mann, D. K., T. M. Hurt. E. Malkos, J. Swis, S. Twait and G. Wachter (2000). Onsite treatment of petroleum oil and lubricant (pol) contaminated soils at Illinois corps of engineers lake sites.

[21]. Molcova, R., Vosatka, M. & Grynder, M. 2003. Effects of inoculation with Glomus intraradices on lead uptake by zea mays L. and Agrostis capillaries L. *Applied soil Ecology* 239, 55 – 67.

[22]. Morelli, A., Fillipi, S. and Zhang, X. H. (2005). Peripherial regulatory mechanism in erection. *Int. J. Androl.* 28(2):23-27.

[23]. Nano, G., Borroni, A., Rota, R., (2003). Combined Slurry and Solid-phase bioremediation of diesel contained soils. *Hazardous Materials* 100(4): 91 – 94.

[24]. Nrior, R. R. and Echezolom, C. (2016) Assessment of percentage bioremediation of Petrole- um Hydrocarbon contaminated soil with biostimulating agents. *Journal of International Society of Comparative Education, Science and Technology (ICEST)* 3(1):203-215.
[25]. Oh, Y. S., Sim, D. S. Kim, S. J., (2001). Effects of nutrients on crude oil biodegradation. In the upper intertiadal zone. *Marine Pollution Bulletin*, 12: 1367 – 1372.
[26]. Pala, M. D., Decarvalho, D. Pinto, J. C. Sant Anna Jr, G. L., (2006). "A suitable model to describe bioremediation of a petroleum contaminated soil". J. Int. Biodet and Biodegr., 58 (6): 254 – 260
[27]. Prescott, L. M., Harley, J. P. and Klein, D. A. (2002). Microbiology, 5th Edition, McGraw-Hill, New York, pp. 1014.
[28]. Quinones-Aquillar, E. E., Ferra-Cerrato, R., Gavi, R. F., Fernandez, L. and Rodriguez, V. R. (2003). Emmergence and growth of maize in a crude oil polluted soil. *Agrociencia* 37:585-594.
[29]. Rahman, H. Y., Husain, F. I. Khan (2003). Land Farming Operatoin in aird region-human health risk assessment. *Journal of Hazardous Materials*, 43: 1124 – 3894.
[30]. Sabele, J., Vinas, M., Solanas, A. M., (2004). "Laboratory Scale bioremediation expereiments on hydrocarbon contaminated soil" *J. Int. Biodet*., 54(3): 19 – 25.
[31]. Salmina B. E., Glaser J. A., Baveye P. V., (2001). The utilization of bioremediation to reduce soil contamination: problems and solutions. Dordrecht: Wuwere Academic Publishers.
[32]. Semple, K. T., Reid, B. J., Fermor, T. R., (2001). "Impact of compositing strategies on the treatment of soils contaminated with organic pollutants". *Environmental Pollution* 112(1): 269 – 283.
[33]. Sanchez, M. A., Campbell, L. M., Brinker, F. A., Owens, D., (2002) Attenuation the natural way. A former wood-Preserving site offers a case study for evaluating the potential of monitored natural attenuation, *industrial Waste Water*, 5:37 – 42.
[34]. Sang-Haw, I., Seokho, I., Dae Yaeon, K., Jeong-gyu, K., (2007). Degradation Characteristics of waste lubricants under different nutrient condition *Journal of hazard materials* 243: 65 – 72.
[35]. Sarkar, D., Ferguson, M., Datta, R., Birnbaum, S., (2005). Bioremediation of Petroleum Hydrocarbons in Contaminated soils: Comparison of Biosolids addition, carbon supplementation, and monitored natural attenuation, *Environmental Pollution*. 136:187 – 195.
[36]. Sims, G. K. (2006). Nitrogen starvation promotes biodegradation of N-Heterocyclic compounds in soil" soil biology and Biochemistry 38: 2478 – 2480. doil: 10. 1016/j. soilbio. 2006. 01.006.
[37]. Udo, E. J. and Fayemi, A. A. A. (2005). The effect of oil pollution on soil germination, growth and nutrient uptake of corn. *Journal of environmental quality* 4,537 – 540.
[38]. Van Gestel, K., Margret, J., Swings, J., Coosemans, J., Ryckebore, J., (2001). "Bioremediation of diesel oil contaminated soil by comporting with biowaste". *Environmental pollution*, 125(5): 361 – 368.
[39]. Vasudevan, N., and Rajaram, P., (2001). Bioremediation of oil sludge-contaminated soil. *Environment International*: 26: 409 – 411.
[40]. Venosa, A. D., Zhu, X., (2003). Biodegradation of Crude oil contaminating marine shorelines and Freshwater Westlands. *Spill Science and Technology Bulletin*, 8(2): 163 – 178
[41]. Vidali, M., (2001) Bioremediation An overview, pure applied Chemistry, Vol. 73, No.7, pp 1163 – 1172.
[42]. Walter, M., Boyd-Wilson, K.S.H., McNaughton, D., Northcott, G., (2005). "Laboratory Frals on the bioremediation of aged pentachlorophenol residue". *Journal Inter. Biodeter & Biodegradation.*, 55(3): 121 – 130.
[43]. Yerushalmi, L., Rocheleau, R. Cimpola, M. Sanazin. G. Sunahara, A. Peisajovich, G. Leclair, S. R. Guiot, (2003). Enhanced Biodegradation of Petroleum Hydrocarbons Hydrocarbons in Contaminated Soil. *Bioremediation Journal*, 29:7138 – 9868.

Nrior, R. R. "Comparative bioremediation potential of Mucor racemosus and Paecilomyces variotii on crude oil spill site in Gio Tai, Ogoni land." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), vol. 11, no. 10, 2017, pp. 49–57.