IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 12 Ver. I (December. 2018), PP 49-57
www.iosrjournals.org

Biodegradation potential of Aspergillus Niger and Rhizopus arrhizus isolated from crude oil spilled site in Rivers State.

Samuel A. Wemedo, Renner R. Nrior, Anita A. Ike
Department of Microbiology, Rivers State University, Port Harcourt.
Corresponding Author: Samuel A. Wemedo

Abstract : *Evaluation of biodegradation potentials of two fungal species (Aspergillus niger and Rhizopus arrhizus) isolated from a crude oil spilled site in Rivers State. The experimental set-up was done to determine degradation rates of fungal species during an incubation period of 28days. Total Petroleum Hydrocarbon content was taken on a bi-weekly basis while the Total Viable Count and pH readings were taken on a weekly basis respectively. Four experimental set-ups were used: mineral salt broth with crude oil (Control), Aspergillus species in mineral salt broth with crude oil (Set-up 1), Rhizopus species in mineral salt broth with crude oil (Set-up 2) and Aspergillus species +Rhizopus species in mineral salt broth with crude oil (Set-up 3) respectively. After the experiment there was an evident change in percentage degradation: Control 4.80%, Set-up1 29.10%, Set-up2 26.32% and Set-up3 48%. The pH reading was observed to reduce from almost neutral to almost acidic: Control 6.4 to 4.8, Set-up1 6.3 to 4.2, Set-up2 6.4 to 4.0 and Set-up3 6.0 to 4.1. Total Viable Counts (Log10sfu/ml) increased progressively across each week: Control 0 to 4.11±0.09, Set-up1 2.11±0.07 to 4.18±0.08, Set-up2 1.85±0.00 to 4.05±0.05 and Set-up3 2.0±0.04 to 4.05±0.02. Total Petroleum Hydrocarbon reduced from an initial of 74.80mg/l to 71.21mg/l Control, 53.03mg/l Set-up 1, 55.11mg/l Set-up 2 and 38.89mg/l Set-up 3. This study concludes that crude oil contaminants can indeed be remediated using microbiological life forms, in this case, the fungal consortium (Aspergillus +Rhizopus) which showed the highest hydrocarbon degradation percentage.*

Keywords–*Biodegradation potential, Aspergillus, fungal consortium, Rhizopus, mineral salt broth, pH, Total Petroleum Hydrocarbon.*

Date of Submission: 10-12-2018 *Date of acceptance: 25-12-2018*

I. Introduction

The huge presence of petroleum products in the world today, either used for power generation (motor spirits, engine oils, kerosene, diesel and aviation fuel) or used as raw materials in the manufacturing industries (plastics, pharmaceuticals or cosmetics); pose a great threat in the amount of hydrocarbons and oil sludge distributed across countries and continents coupled with its inherent health risks [1]. Crude oil is a naturally occurring compound with a complex mixture of hydrocarbons and non-hydrocarbon components; which at varying concentrations possess a degree of toxicity to the living organisms in the spilled environment. The toxicity of spilled crude oil or petroleum products greatly depends on their composition, concentration, environmental factors and on the biological state of the organisms at the time of contamination [2]. There is a continuous link of thousands of various hydrocarbon molecules some of which include paraffinic (straight and branched-chain alkanes), naphthenic (cycloalkanes or cycloparaffins), alkenes (olefins) and aromatic hydrocarbons [3]. Oil spillage is the unintentional discharge of liquid hydrocarbons into the soil or aquatic environment, which ultimately leads to disruption of life forms, damage to human health since these compounds are mutagenic and highly potent immunotoxicants, including all other public health hazards and economic losses [4].

Several works have been done on the biodegradation potential of fungi on crude oil polluted sites and petroleum products, with several fungal genera being isolated from such sites. Examples include: *Alternaria*, *Aspergillus*, *Candida*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pleurotus*, *Polyporus*, *Rhizopus*, *Rhodotolura*, *Saccharomyces*, *Talaromyces* and *Torulopsis* [5][6][7][8][9][10][11][12][13]. Fungi play a crucial role in aquatic ecosystems, with their ability to remove hazardous compounds. The readiness of most fungi to produce extracellular enzymes for the assimilation of complex carbohydrates makes possible the breakdown of a wide range of pollutants [14]. Due to the irregular structure of lignin, lignolytic fungi produce extracellular enzymes with low substrate specificity making them suitable for degradation of different compounds. The lignolytic system comprise three main enzyme groups with lignin peroxidase, manganese dependent peroxidase, phenoloxidases (lacases, tyrosinases) and H_2O_2-producing enzymes [5].

The Niger Delta region of Nigeria is known for its fertile soil, dense tropical forest, abundant resources and rich cultural heritage; however, it has been plagued with the reoccurrence of pipeline vandalization, wellhead explosion, gas flaring and oily sludge discharge, on almost monthly basis for about 50years [15]. Oil exploration, having started in Oloibiri (1956) in present day Bayelsa State, began the slow but eventual decay of the soil, air and water environments of this region with its all-important resource: Crude Oil [16]. There has been government's effort to stem the public health effects of this pollutant but to little avail [17]. Physiochemical and mechanical methods of pollution remediation exist, especially in soil, but tends to worsen the mutagenic effect in the already contaminated environment, thus the need to explore the advantageous biological method of remediation [18]. It has become imperative that this method, which has fast gained relevance in the sphere of microbiology and biotechnology, be harnessed to help rid the environment of such toxic compounds; to which this research seeks to find.

II. Materials And Methods

2.1 Study Area

Soil samples were collected from spilled site in Kegbara dere Creek in Gokana Local Government Area within coordinates 4.30.03N & 7.13.46E 19km and 4.28.45N & 7.14.42E 19km of Rivers State.

2.2 Sample Collection

Soil samples were collected by adopting the Food and Agriculture Organisation [19] guideline, using a sterile soil auger to make a depth of 0-15cm of topsoil. The soil samples were collected into fresh unused black polythene bags. The samples were transported within 2 hours of collection to the Microbiology Laboratory of the Rivers State University for analysis.

2.3 Isolation and Identification of fungal species

Two fungal species used for this study were isolated from the top 5cm of homogenously mixed and sieved crude oil spilled soil from Kegbara dere Creek in Gokana Local Government Area of Rivers State. One gram (1.0g) of soil sample was weighed and introduced into sterile normal saline (8.5g of NaCl in 1000ml of distilled water) under aseptic conditions. Ten-fold serial dilution was carried out up to 10^{-3} and 10^{-4} dilutions. Afterwards, 0.1ml aliquot of each dilution was spread plated onto sterile solidified Sabouraud Dextrose Agar (SDA), and Mineral Salt Agar (MSA) using the vapour transfer method in duplicates. The plates were incubated at 28^0C for 2-7days. Fungal colonies on SDA were counted to obtain the colony forming unit per gram (cfu g^{-1}) of the soil sample. Thereafter, colonies suspected to be *Aspergillus species* and *Rhizopus species*, were picked and sub-cultured from MSA onto freshly prepared SDA plates which were incubated at 28^0C for 5-7days.

Macroscopic and microscopic identification of fungal species were carried out. Wet Preparation and Slide Culture Technique were used to microscopically identify the fungal species [20].

2.4 Biodegradation test of the fungal species

The two species of fungi (*Aspergillus niger* and *Rhizopus arrhizus*) isolated from the soil samples were used for the biodegradation set up. Each set up had Mineral Salt Broth containing 1000ml distilled water, 0.5g K_2HPO_4, 0.3g $MgSO_4.7H_2O$, 0.3g NaCl, 0.2g $MnSO_4.H_2O$, 0.02g $FeSO_4$. $6H_2O$, 0.03g $NaNO_3$, 0.3g ZnCl; all measured into four 1000ml conical flasks and autoclaved; with 5ml of sterilized crude oil aseptically added into each flask and plugged with cotton wool [10]. For the single fungi set up, serial dilutions from nutrient broth cultures were made and aliquots plated on SDA using the spread plate method to determine the number of fungal cells in each aliquot. It was within the range of 30-300 cells. Consequently, 1ml from 10^{-3} dilution was aseptically added into each set up; while 0.5ml of each organism was added in the mixed culture set up. Each flask was agitated using of a rotatory shaker for 30minutes daily to allow for aeration and even distribution of nutrients and the pollutant. During the incubation period, pH readings were taken with a pH meter in duplicates; while total viable counts were monitored in triplicates using the spread plate method on SDA. Total Petroleum Hydrocarbon readings were taken using Gas Chromatography method with Flame Ionisation Detector (Agilent HP 5890 series II). Total Viable Counts and pH readings were monitored on a 7-day basis (day 1, 7, 14, 21, 28) while the Total Petroleum Hydrocarbon readings were taken on a 14-day basis (day 1, 14, 28).

2.5 Percentage Biodegradation

Percentage biodegradation was calculated using:

Step 1: Amount of pollutant remediated equals to Initial concentration of pollutant (Day 0 or 1) minus Final concentration of pollutant at end of experiment (Last day). ***Step2***: Percentage (%) Bioremediation equals to Amount of pollutant remediated divided by Initial concentration of pollutant (Day 0 or 1) multiplied by 100.

BC =IC – FC

Where;

BC = Amount of pollutant remediated
IC = Initial concentration of pollutant (Day 0 or 1)
FC = Final concentration of pollutant at end of experiment (Last day)
% Bioremediation = BC x100 [6]

III. Results And Discussion

Two fungal species, *Aspergillus niger* and *Rhizopus arrhizus*, were used for the biodegradation study. Fig. 1 shows the gradual and steady decrease of the hydrogen ion concentration (pH) of all experimental set-ups: Control 6.4 to 4.8, Set-up1 6.3 to 4.2, Set-up2 6.4 to 4.0 and Set-up3 6.0 to 4.1; with Set-up2 showing the lowest value. There was a significant increase in the Total Viable Count (TVC), from the beginning to the end of the experiment: Control 0 to 4.11±0.09, Set-up1 2.11±0.07 to 4.18±0.08, Set-up2 1.85±0.00 to 4.05±0.05 and Set-up3 2.0±0.04 to 4.05±0.02; as seen in Fig. 2. This decline in pH readings with corresponding increase in fungal growth can be attributed to the high concentration of metabolites and increased metabolism by fungal species using the hydrocarbons present as sole carbon and energy source [21]. However, there was decrease in fungal growth between day 21 and day 28, probably due to the decrease in pollutants concentration.

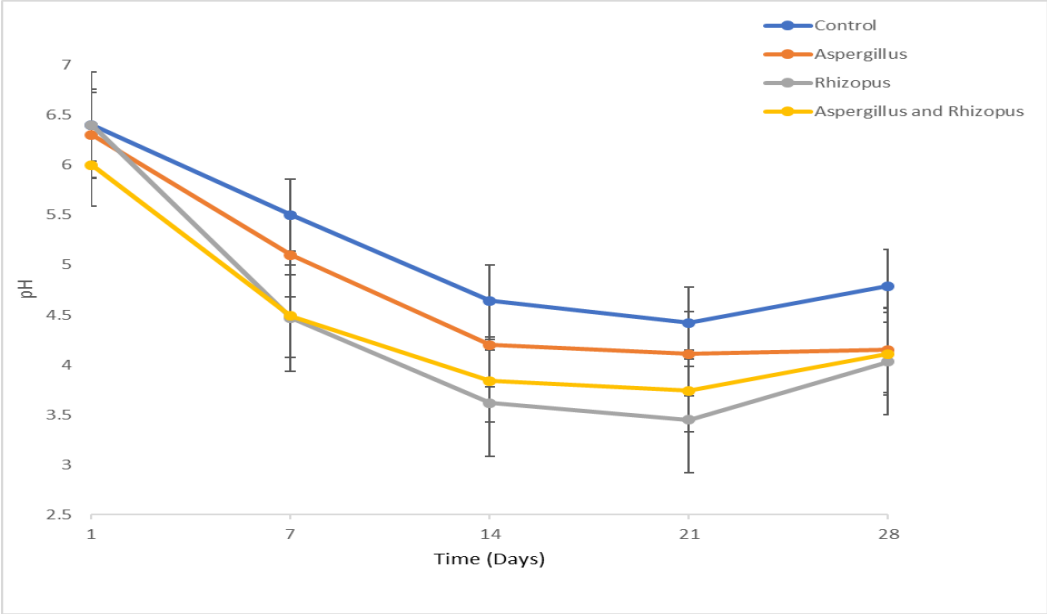


Fig 1: Hydrogen ion concentration (pH) by fungal species during biodegradation of crude oil polluted mineral salt broth.

Furthermore, the fungal species due to their lignolytic features are able to produce extracellular enzymes that breakdown the pollutants; releasing CO_2 and reducing the pH of the mineral salt broth. Fig. 3, shows the Total Petroleum Hydrocarbon (TPH) which decrease over the 28days incubation from an initial of 74.80mg/l to 71.21mg/l Control, 53.03mg/l Set-up1, 55.11mg/l Set-up2 and 38.89mg/l Set-up3. Although *Aspergillus niger* degraded the hydrocarbon significantly for single strains, the mixed culture (*Aspergillus niger+Rhizopus arrhizus*) was a better degrader than the individual strains as corroborated by [22]. This showed that a synergy of mixed culture worked better than individual strains because each organism has varying degradative capabilities on the various hydrocarbon chain lengths. Using ANOVA from SPSS 22, shown significant difference that there was a correlation between the various treatment with the increase in fungal growth and the decrease in Total Petroleum Hydrocarbon and pH readings. At the end of experiment the percentage degradation were: Control 4.80%, Set-up1 29.10%, Set-up2 26.32% and Set-up3 48%.

Fig. 4-12 shows the chromatograph peaks and values for each set-up on days 1, 14 and 28.

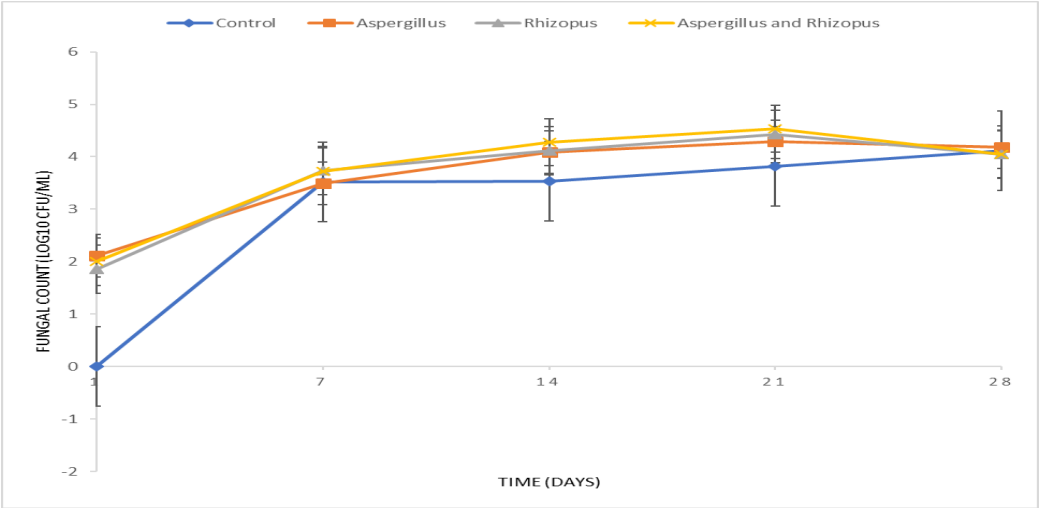
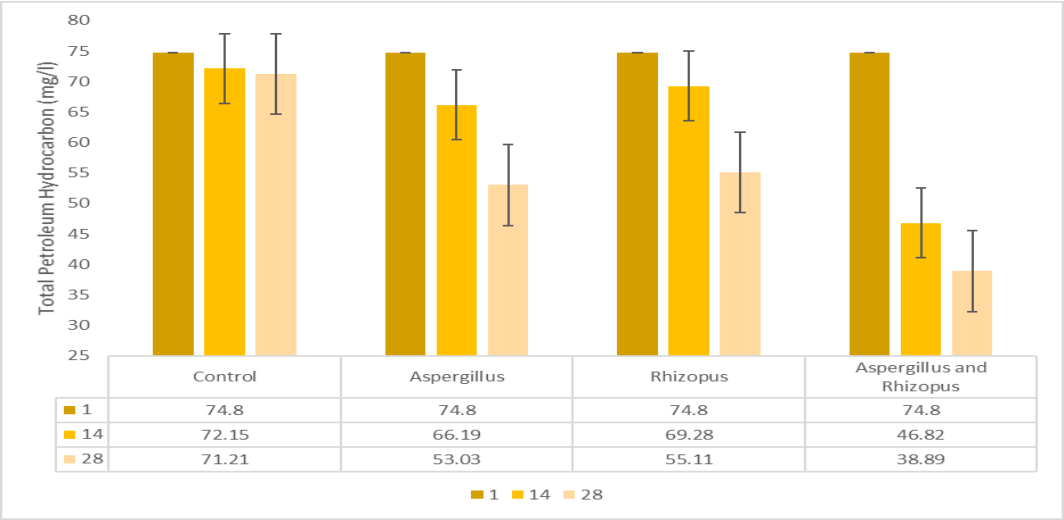
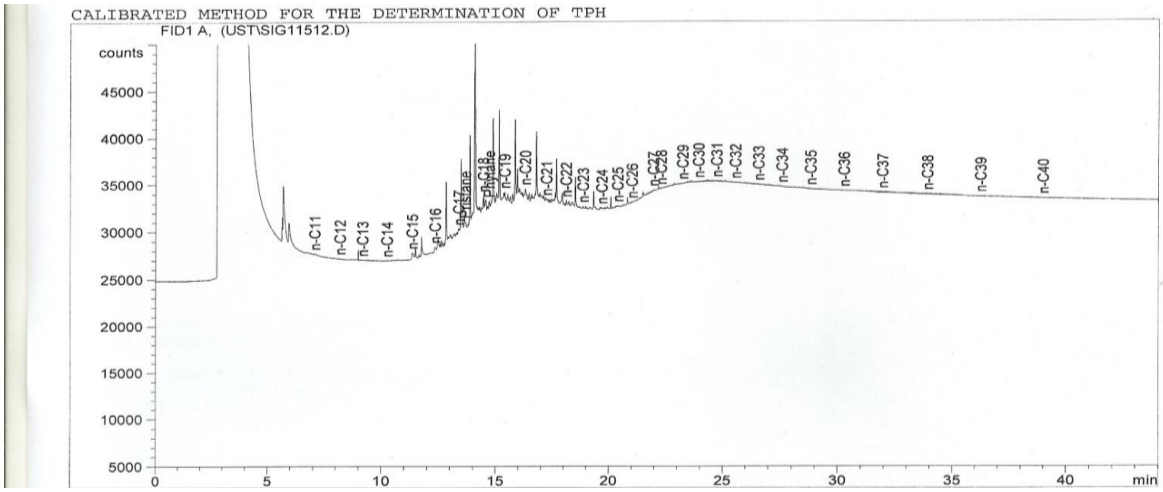


Fig 2: Petroleum Utilizing Fungi (log10 cfu/ml) during biodegradation of crude oil polluted mineral salt broth.



	Control	Aspergillus	Rhizopus	Aspergillus and Rhizopus
1	74.8	74.8	74.8	74.8
14	72.15	66.19	69.28	46.82
28	71.21	53.03	55.11	38.89

Fig 3: Total Petroleum Hydrocarbon readings of fungal species during biodegradation of crude oil polluted mineral salt broth.

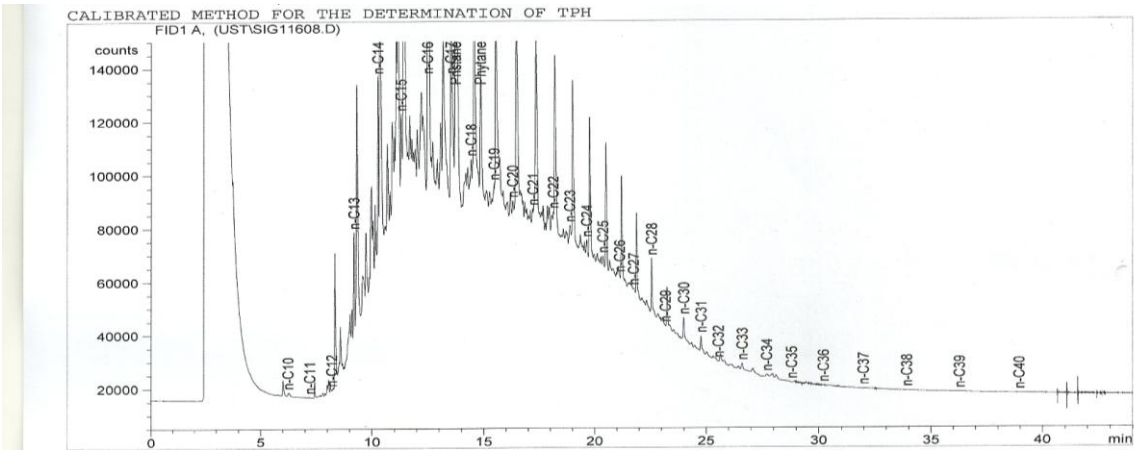


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		2.48199e4	74.80115	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig 4: Total Petroleum Hydrocarbon value and peaks on day 1 of crude oil polluted aquatic ecosystem using mineral salt broth by bacterial isolates.

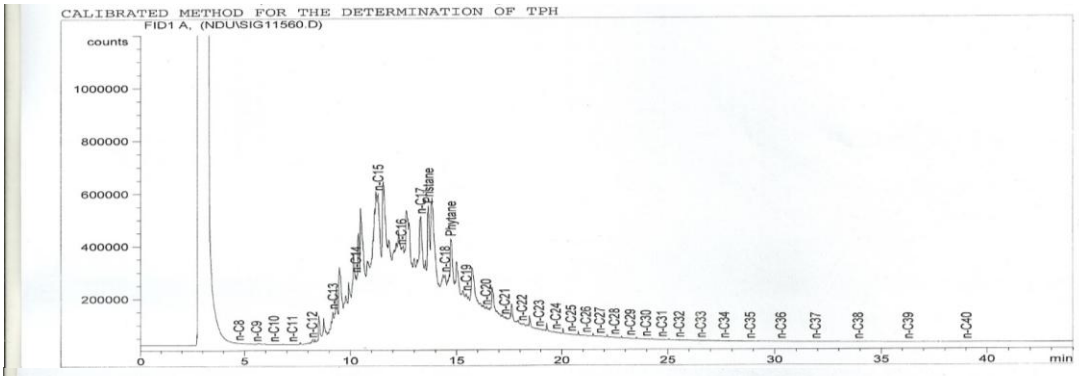


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		2.37930e6	72.15486	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig 5: Control on day 14 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.

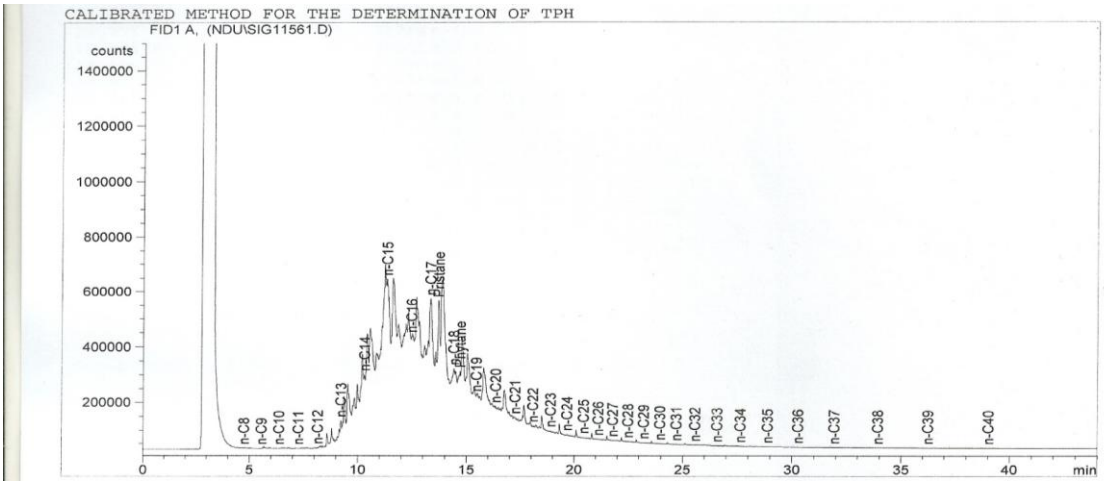


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		6.08455e6	66.18721	TPH

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Fig 6: *Aspergillus* species on day 14 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.

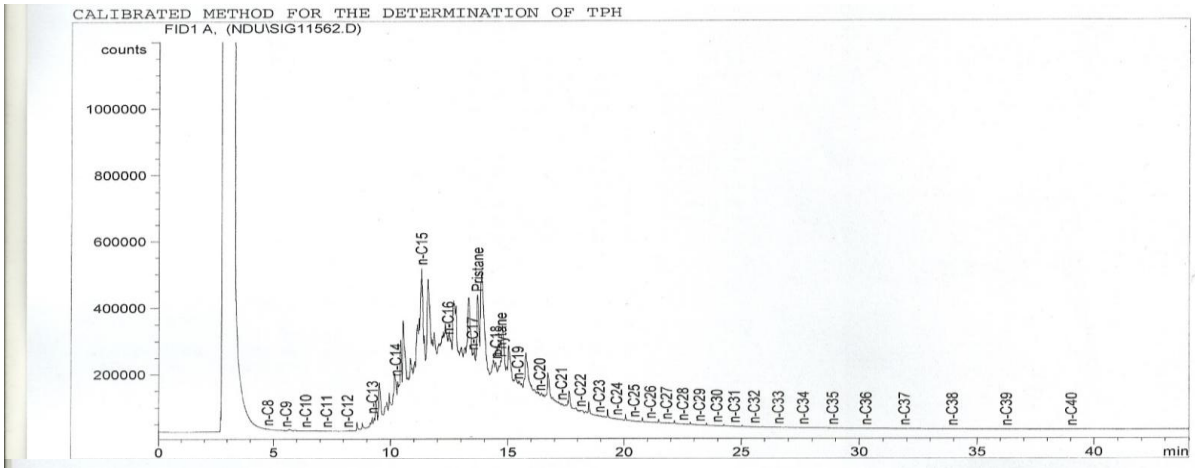


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		4.40177e6	69.27949	TPH

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Fig 7: *Rhizopus* species on day 14 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.

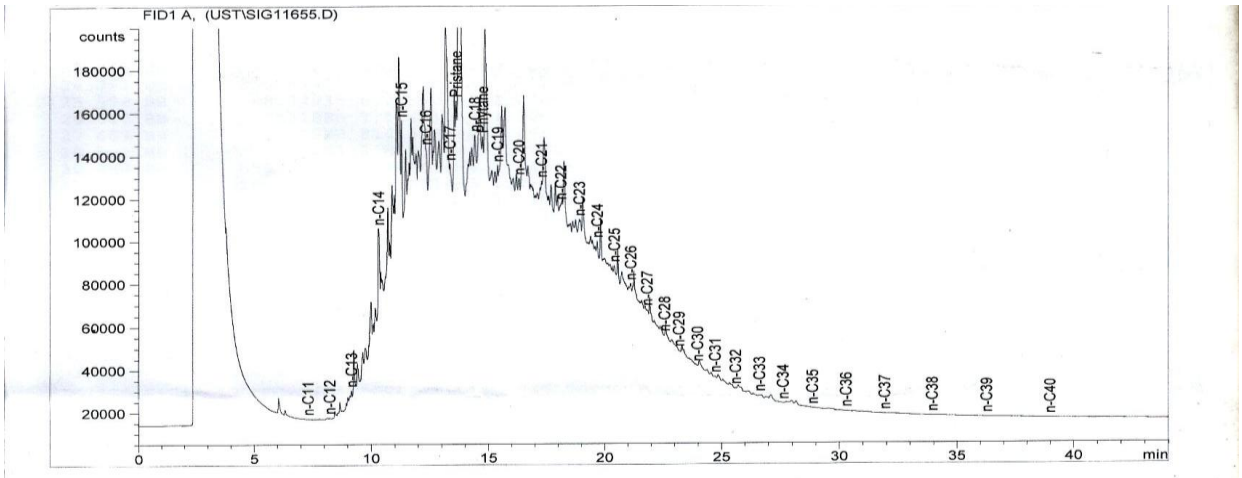


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		2.94820e6	46.82207	TPH

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

Fig 8: *Aspergillus and Rhizopus* species on day 14 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.

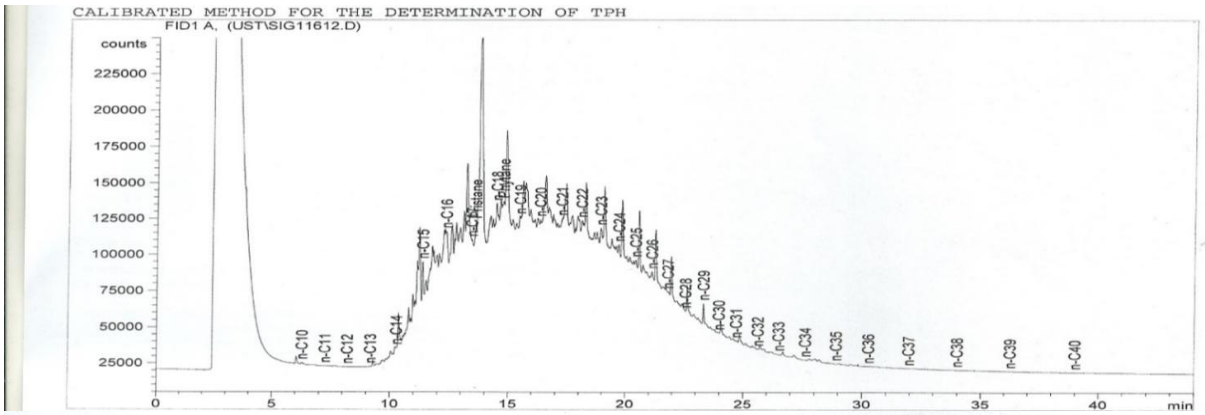



```
Totals :                                71.21043

 Results obtained with enhanced integrator!
Group summary :

 Group  Use    Area       Amount       Group Name
  ID          counts*s     [PPM]
|------|---|----------|----------|--------------------------|
  1          8.28641e5    71.21043   TPH
```

Fig 9: Control on day 28 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.



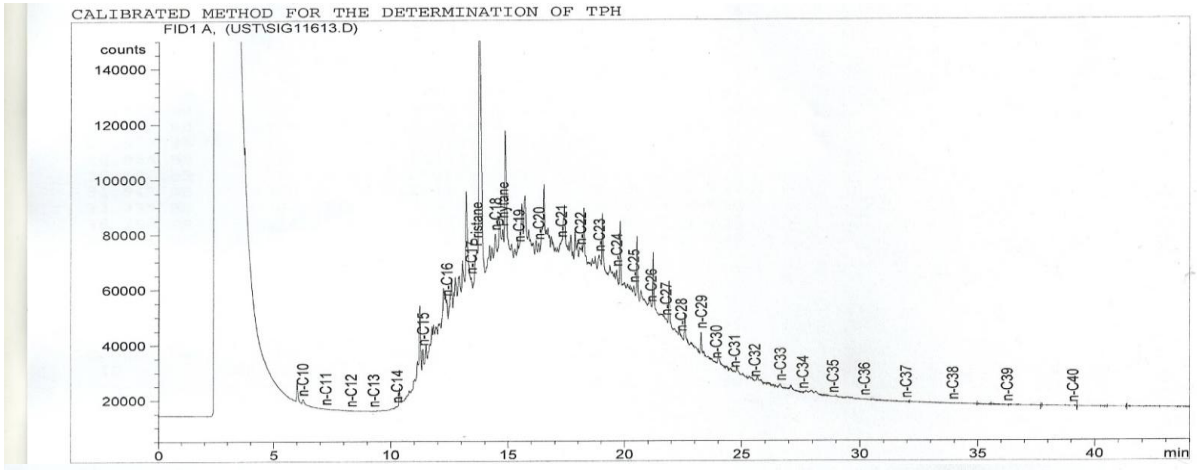

```
 Group  Use    Area       Amount       Group Name
  ID          counts*s     [PPM]
|------|---|----------|----------|--------------------------|
  1          6.29922e5    53.03359   TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

_______________________________________________________________________
```

Fig 10: *Aspergillus* species on day 28 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.

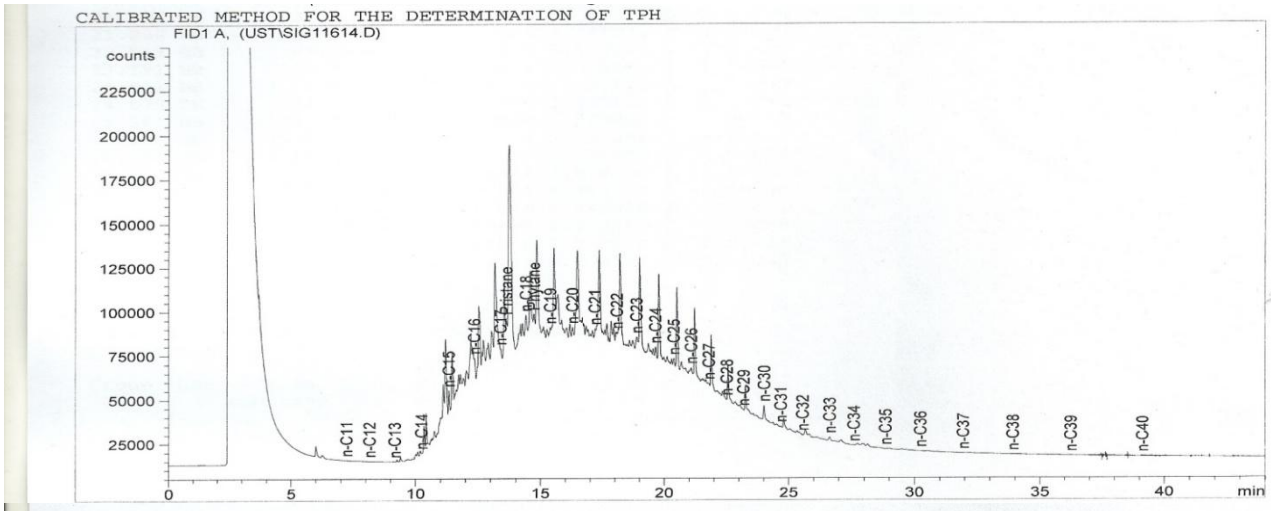


Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		3.22777e5	55.10642	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig 11: *Rhizopus* species on day 28 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.



Group ID	Use	Area counts*s	Amount [PPM]	Group Name
1		4.41166e5	38.89043	TPH

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

Fig 12: *Aspergillus and Rhizopus* species on day 28 of Total Petroleum Hydrocarbon value and peaks of crude oil polluted mineral salt broth.

IV. Conclusion

It is worthy to note that the somewhat decaying environment of the Niger Delta region in Nigeria is a constant reminder of the effects of an ill-managed natural resource; as such as crude oil which is free-flowing, toxic and mutagenic. Results from this study showed that fungal species are relevant in the scheme of bioremediation and depolluting the environment of toxic and carcinogenic substances; in this case petroleum hydrocarbon. The study showed that degradation was best achieved by the mixed culture of the fungal species which can be harnessed and used in the field. However, more research needs to be done on improving the

degradative potential of these organisms and how they can be seeded into a field environment while delivering effective results.

References

[1]. Ojumu, T., Bello, O., Sonibare, J., & Solomon, B. (2004). Evaluation of microbial systems for ioremediation of petroleum refinery effulents in Nigeria. *African Journal of Biotechnology, 4*, 31-35.

[2]. Obire, O., & Anyanwu, E. (2009). Impacts of various concentrations of crude oil on fungal populations of soil. *Int. J. Environ. Sci. Tech, 6*(2), 211-218.

[3]. Ogbolosingha, A., Essien, E., & Ohiri, R. (2015). Variation of Lipase, Catalase and Dehydrogenase Activities during Bioremediation of Crude Oil Polluted Soil. *Journal of Environmental and Health Science*, Vol.5, No.14, 128-141.

[4]. Gesinde, A., Agbo, E., Agho, M., & Dike, E. (2008). Bioremediation of some Nigerian and Arabian crude oils by fungal isolates. *International Journal of Pure and Applied Science, 2*, 37-44.

[5]. Haritash, A., & Kaushik, C. (2009). Biodegradatiion aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. *Journal of Hazardous Materials, 169*, 1-15. doi:10.1016/j.jhazmat.2009.03.137

[6]. Nrior, R. R., & Mene, G. B. (2017, August). Assessment of bioaugmentation efficiency of Penicillium chrysogenum and Aspergillus nudilans in bioremediation of crude oil spill soil. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*.

[7]. Nrior, R. R., Odokuma, L., & Tete., E. (2017). Ultimate Biodegradation of Industrial Detergent Used in the Upstream Sector of the Nigeria Petroleum Industry in Fresh water, Brackish water and Marine water. *International Journal of Ecotoxicology and Ecology.*, Vol. 2, No. 4, 134-144. doi:10.11648/j.ijee.20170204.11

[8]. Nrior, R. R., Ogbonna, D. N., & Alabo, A. E. (2017). Biodegradation of Drilling Fluid Used in Upstream Sector of the Nigeria Petroleum Industry in Marine Water Environment. *International Journal of Waste Resources, 7*(4), 1-5. doi:10.4172/2252-5211.1000302

[9]. Nrior, R., & Jirigwa, C. (2017). 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), 11*(10), 49-57. doi:10.9790/2402-1110014957

[10]. Mohsenzadeh, F., Rad, A., & Akbari, M. (2012). Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum polluted soils. *Iranian Journal of Environmental Health Science and Engineering.*, 9(26), 222-230.

[11]. Allinnor, I., & Nwachukwu, M. (2013). Determination of total petroleum hydrocarbon in soil and groundwater samples in some communities in Rivers State, Nigeria. *Journal of Environmental Chemistry and Ecotoxicology, 5*(11), 292-297.

[12]. Nrior, R., & Onwuka, N. (2017). Bioremediation of Crude oil Contaminated Marshland muddy soil By Augumentation Approach Using Candida Tropicalis and Penicillium Chrysogenum. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, Vol. 11, No. 10, 57-64.

[13]. Burghal, A. A., Abu-Mejdad, N. M., & Al-Tamimi, W. H. (2016, February). Mycodegradation of Crude Oil by Fungal Species Isolated from Petroleum Contaminated Soil. *International Journal of Innovative Research in Science, Engineering and Technology, 5*(2), 1517-1524. doi:10.15680/IJIREST.2016.0502068

[14]. Vanishree, M., Thatheyus, A., & Ramya, D. (2014). Biodegradation of Petrol Using the Fungus Penicillium sp. *Science International, 2*(1), 26-31. doi:10.17311/sciintl.2014.26.31

[15]. Ordinioha, B., & Brisibe, S. (2013). The human health implications of crude oil spills in the Niger Delta, Nigeria: An interpretation of published studies. *Nigerian Medical Journal, 54*(1), 10-16. doi: 10.4103/0300-1652.108887

[16]. Usang, E., & Ikpeme, N. (2015). The Niger Delta Oil Rich Region: The Paradox of Fascination and Horror. *Academic Journal of Interdisciplinary Studies, 4*(1), 117-123.

[17]. Vaughan, A. (2011, August Thursday). Oil in Nigeria: a history of spills, fines and fights for fights. *The Guardian*, p. 1.

[18]. [18] Das, N., & Chandran, P. (2010). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International, 2011*, 1-13. doi:10.4061/2011/941810

[19]. Food, & Organisation, A. (2006). *Guidelines for Soil Description.* Rome: F.A.O. 4th Edition, 97.

[20]. Kidd, S., Halliday, C., Alexiou, H., & Ellis, D. (2016). *Descriptions of Medical Fungi* (3rd ed.). Adelaide, Australia: Authors.

[21]. Spellman, F. R. (2008). *Ecology for Nonecologists.* Rockville: Government Institutes Inc.

[22]. Fritsche, W., & Hofrichter, M. (2005). Aerobic degradation of recalcitrant organic compounds by microorganisms, in environmental technology: Concepts and applications. In D. H.- J. Jördening, P. D. Winter, D. H.- J. Jördening, & P. D. Winter (Eds.), *Environmental Biotechnology: Concepts and Applications* (pp. 203-227). Wiley- VCH Verlag GmbH & Co. KGaA. doi:10.1002/3527604286.ch7

Samuel A. Wemedo. " Biodegradation potential of Aspergillus Niger and Rhizopus arrhizus isolated from crude oil spilled site in Rivers State. " IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 12.12 (2018): 49-57.