

Original Research Article

Mycoremediation: Monitoring Crude Oil Degradation by indigenous mushrooms over a period of 15 weeks

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Abstract

Aim: The ability of three indigenous fungi, *Pleurotus pulmonarius* (Fries) Quelet (PP), *Pleurotus tuber-regium* (Fries) Singers (PT) and *Lentinus squarrosulus* (Mont.) Singer (LS) to degrade Crude oil polluted substrate over a period of 15 weeks was investigated. The aim was to assess the extent to which each of the fungi could degrade the hydrocarbon profiles of the crude oil for further use in mycoremediation.

Study design: The study design was completely randomized design (CRD)

Place and Duration: The research was carried out at the Department of Plant Science and Biotechnology, University of Port-Harcourt between March 2005 and September 2005.

Methodology: The spawns of the three mushrooms were used to inoculate polluted substrate. At 5 weeks intervals for a period of 15 weeks, samples were removed and analyzed for remnant hydrocarbon.

Results: Oil degradation by the three fungi was observed at different rates. *P. pulmonarius* degraded crude oil (Aliphatic Hydrocarbon profile (AH)) by 51.8% after 10 weeks; 87.4% after 15 weeks and 62.7% after 10 weeks; 71.2% after 15 weeks (Polycyclic aromatic Hydrocarbon profile (PAH)). *P. tuber-regium* degraded crude oil by 31.1% after 10 weeks; 73.7% after 15 weeks (AH) and 16.1% after 10 weeks; 51.9% at the end of 15 weeks (PAH). Also *L. squarrosulus* degraded crude oil by 11.0% after 10 weeks; 50% after 15 weeks (AH) and (PAH) by 5.7% and 57.0% after 10 and 15 weeks respectively.

There was significant difference ($p \leq 0.05$) in substrates inoculated with fungi, but no significant difference in the control which was not inoculated with fungi.

Conclusion: The tests mushrooms are potential mycoremediation agents.

Introduction

The soil is a key component of natural ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem [1]. When soil is polluted, the ecosystem is altered and agricultural activities are affected. Crude oil and petroleum products such as gasoline, fuel oils and diesel fuels are complex mixtures of organic compounds and have been shown to be toxic to plants [2], [3]. It also inhibits seed germination and plant growth [4]. In Nigeria, most of the terrestrial ecosystem and shorelines in oil-producing communities are important agricultural land under continuous cultivation. Any contact with crude oil results in damage to soil conditions in these agricultural lands, including adverse effects on micro-organisms and plants [5], [6]. It was

also reported that the exploration of oil contributed to Nigeria becoming one of the five most petroleum damaged ecosystems of the world [6]. As a means of remediation of soil polluted with these substances, various technologies have been employed some of which constitute further damage to the environment. An environment friendly approach which cleans up oil pollution and does not jeopardize environmental health is preferred. Such approach uses microorganisms as bioremediation agents. From the report of [7], fungi are able to degrade many hazardous substances which may be toxic to plants and animals. He reasoned that naturally produced agricultural and industrial wastes are best degraded by natural resources. The test fungi, PP, PT and LS are described as white rot, [8] [9], [10], [11] and are able to produce different enzymes. This study investigated the degradation of crude oil by three indigenous fungi in order to assess their potentials for myco remediation of oil polluted environment.

Materials and methods

The crude oil used in this study was a Nigerian Bonny light blend obtained from Shell Petroleum Development Company Limited Port-Harcourt, Nigeria. Waste cotton (secondary wastes from the mechanical processing of raw cotton prior to spinning) was obtained from Atlantic Textile Mills, Lagos, Nigeria. Cultures of *Pleurotus pulmonarius* (PP), *P. tuber-regium* (PT) and *Lentinus squarrosulus* (LS) were obtained from the Federal Institute of Industrial Research Oshodi, Nigeria. Fifty grams of waste cotton was weighed and soaked in 100 mL distilled water containing 5% w/w of calcium carbonate for 30 minutes. The water was drained and the cotton squeezed to about 60% wet weight. The cotton was shredded and treated with 20% v/w crude oil. Samples in transparent bottles of dimensions 7.5 × 17 × 7.5 cm were autoclaved at 121°C for 15 minutes. After cooling, 10% spawn of PP, PT and LS was used to inoculate the samples. Negative Control samples with pollutant but not inoculated with any of the fungi were set up as well as positive control samples, inoculated with the mushroom but with no pollutant added. Samples were incubated for 15 weeks at room temperature (28±2°C). At five-week intervals (5, 10, 15 weeks), samples were removed and remnant hydrocarbons (aliphatic (AH) and polycyclic-aromatic hydrocarbon fractions) determined.

Determination of remnant hydrocarbons fractions

Determination of AH and PAH

Mushroom samples harvested from the polluted soil were analyzed for aliphatic and aromatic hydrocarbons according to [12] using Agilent 6890 GC (Flame Ionization Detector). Hydrocarbon profile was tested for by the following steps:

Sample Preparation and extraction (sonication water bath method)

Polluted mushroom sample (2.0 ± 0.1g) was weighed into a clean extraction bottle and dried with anhydrous sodium sulphate after thorough mixture of sample. One mL of 60 µg/mL 1 – Chlorooctadecane (AH); Pyrened₁₀ (PAH) surrogate standard was added and 40 mL extraction

solvent. The mixture was placed in a shaking water bath for 5 hours. The extract was allowed to settle for one hour, filtered through funnel fitted with cotton wool, and sodium sulphate was added into an amber coloured extraction bottles washed with methylene chloride. The residue was washed with 20 mL of extracting solvent and filtered through the funnel. Sample extract was concentrated to 1 mL using a rotary evaporator, cleaned up with cyclohexane and fractionated for AH and PAH.

Results and discussion

The results of the degradation of crude oil by the three fungi species for a period of 15 weeks are shown in Tables 1&2 and figure 1. The three fungi degraded crude oil but at different rates. At 5 weeks, for the AH profile, there was significant difference $P \leq 0.05$ in the degradation of crude oil between the control and the test fungi as well as between the test fungi. At 10 weeks however, the trend was the same between the control and test fungi but no significant difference in oil degradation between PT and PP although oil degradation was more pronounced by PP (Table 1 and figure 1(a-h)). The trend at 15 weeks was same as 10 weeks with further oil degradation. Oil degradation for PAH at 5 weeks was not significant $P \leq 0.05$ between the control and LS. There was significant difference however, in oil degradation between the control, LS and PT, PP but no significant difference between PP and PT. The trend was the same at 10 weeks however, at 15 weeks; there was significant difference between the control and LS as well as between LS and PT, PP. The chromatograms also corroborate the results.

P. pulmonarius degraded crude oil (AH) by 51.8% after 10 weeks; 87.4% after 15 weeks and 62.7% after 10 weeks; 71.2% after 15 weeks (PAH). *P. tuber-regium* degraded crude oil by 31.1% after 10 weeks; 73.7% after 15 weeks (AH) and 16.1% after 10 weeks; 51.9% at the end of 15 weeks (PAH). Also *L. squarrosulus* degraded crude oil by 11.0% after 10 weeks; 50% after 15 weeks (AH) and PAH by 5.7% and 57.0% after 10 and 15 weeks respectively.

The results indicated the AH contents are quantitatively higher than the PAH contents (Tables 1 and 2). There was no significant degradation of crude oil observed in the control set-up to which no fungi was added.

The different ways in which PP, PT and LS degraded crude oil for the 15-week period may be attributed mainly to enzyme production since other parameters such as substrate and pollutant were uniform. Although the three test fungi are recognized as white-rot fungi as observed from previous research reports: [8], [9], [10], [11], their responses to crude oil degradation differed. Degradation of crude oil at 5, 10 and 15 weeks of analysis was highest by PP, followed by degradation by PT and lastly degradation by LS for both aliphatic and poly cyclic aromatic crude oil fractions. [14] observed that white-rot fungi can be divided into groups depending on the type of lignolytic enzymes they produced while [10] reported that PP produced low manganese peroxidase in both low- and high-nitrogen conditions, together with laccase and cellulase. It was also reported that the fungus secreted aryl-alcohol oxidase (AAO), cytochrome P-450 mono-oxygenase and epoxide hydrolase [15] which were found to initially oxidise and further hydrate PAH compounds.

A preliminary work on enzymes produced by PT by [8] stated that the fungus has a higher manganese peroxidase activity in low nitrogen nutrient than in higher nitrogen nutrient. It also

produced laccase, but no cellulase was recorded. On the other hand, the production of ligninase by the LS was stated by [16]. The enzyme combinations of PP might have given it an edge over the two other fungi in degradation of crude oil in this work. It is generally recognized that fungal degradation of crystalline cellulose to glucose requires a number of enzymes with different activity profiles, often acting in synergy. The different activities of the various enzymes produced by these fungi must have been responsible for the difference in crude-oil degradation. The low concentrations of PAH as indicated in the result agrees with the reports of [13] that most petroleum hydrocarbon mixtures contain very low concentrations of PAHs. However, [17] observed that the major concern regarding PAHs is the potential carcinogenicity of some

Table 1: Aliphatic Hydrocarbon Contents after Degradation by Fungi at 5, 10 and 15weeks

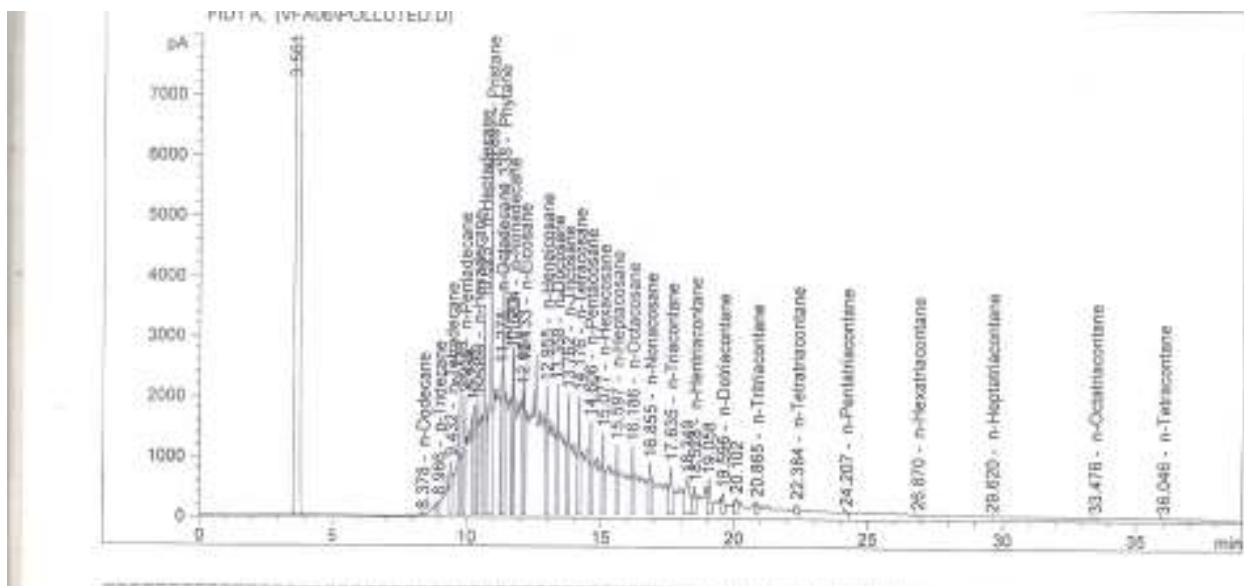
Treatment	5weeks	10weeks	15weeks
control	5114.0 ^{a*}	5111.8 ^a	5110.0 ^a
<i>L.squarrosulus</i>	3889.5 ^b	3491.71 ^b	2027.4 ^b
<i>P.tuber-regium</i>	1298.84 ^c	894.8 ^c	301.01 ^c
<i>P.pulmonarius</i>	2388.4 ^d	382.96 ^{cd}	16.18 ^{cd}

*Means followed by the same letter in the same column are not significantly different at P=0.05.

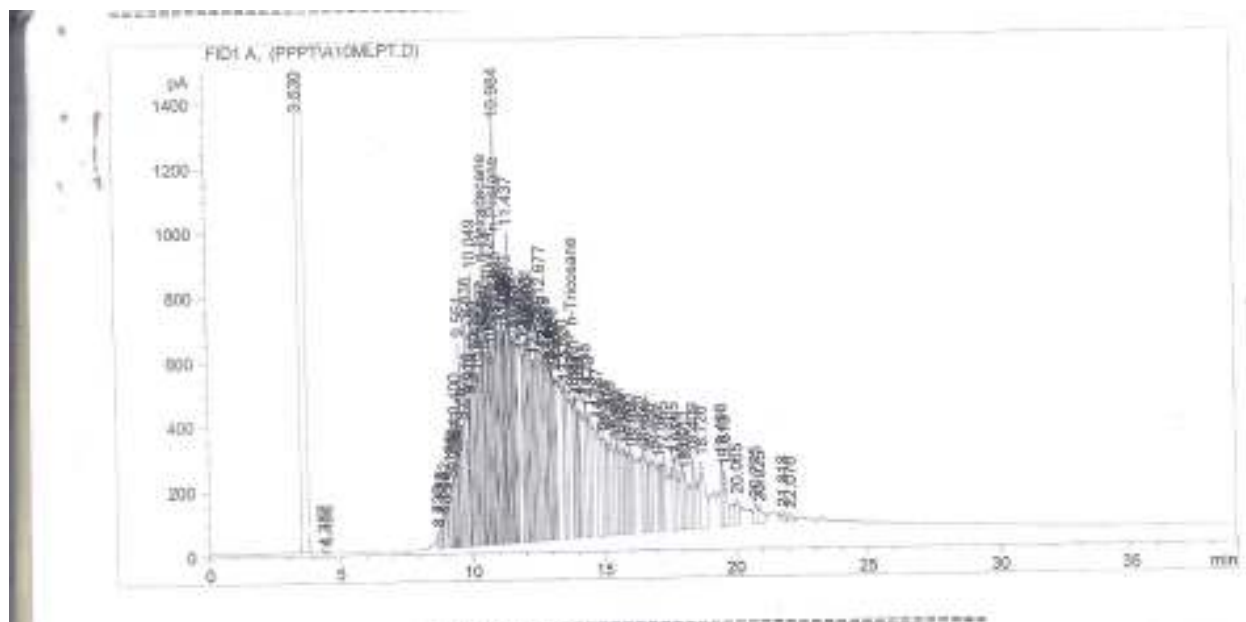
Table 2: PAH Contents after Degradation by Fungi at 5, 10 and 15weeks

Treatment	5weeks	10weeks	15weeks
control	328.3 ^{a*}	329.9 ^a	329.9 ^a
<i>L.squarrosulus</i>	325.0 ^a	304.45 ^a	146.9 ^b
<i>P.tuber-regium</i>	95.5 ^{bc}	80.2 ^{bc}	45.96 ^c
<i>P.pulmonarius</i>	55.5 ^c	20.73 ^c	16.18 ^{cd}

*Means followed by the same letter in the same column are not significantly different at P=0.05.



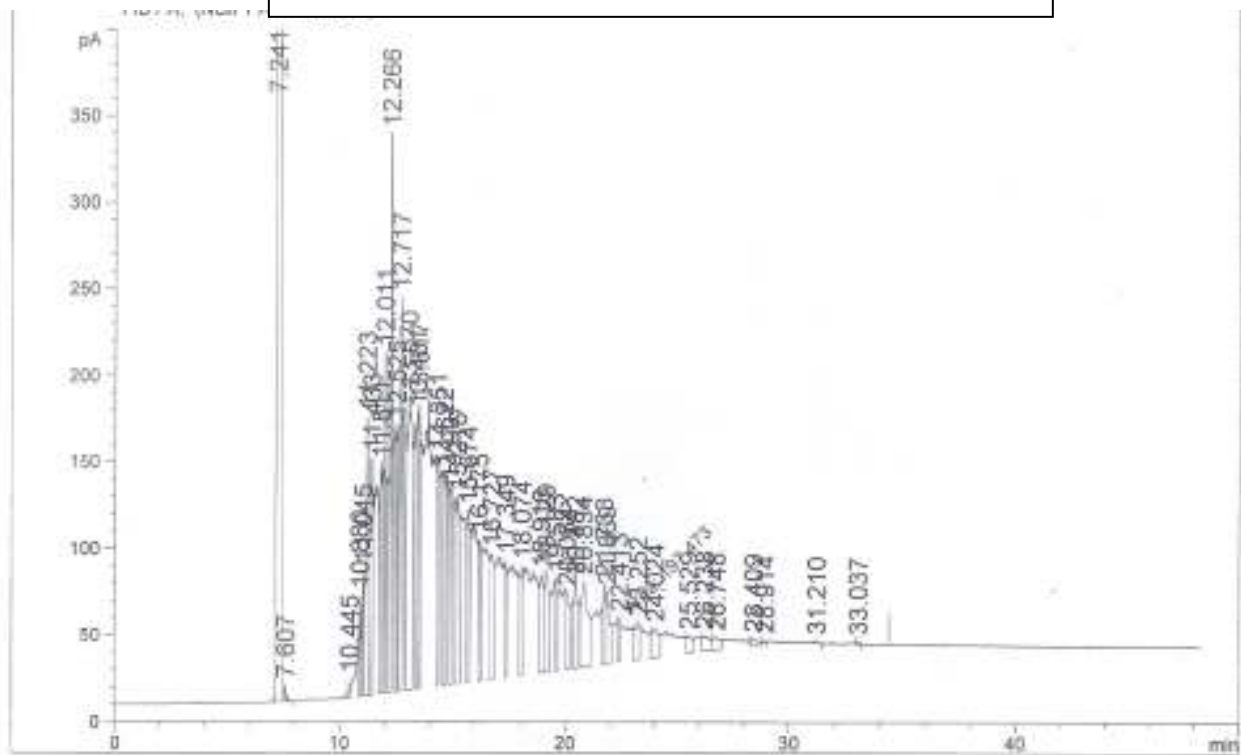
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Figure 1c: Chromatogram of Aliphatic Hydrocarbon Profile of Crude oil polluted cotton with *P. tuber-regium*



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Figure 1d: Chromatogram of Aliphatic Hydrocarbon Profile of Crude oil polluted cotton with *L.squarrosulus*

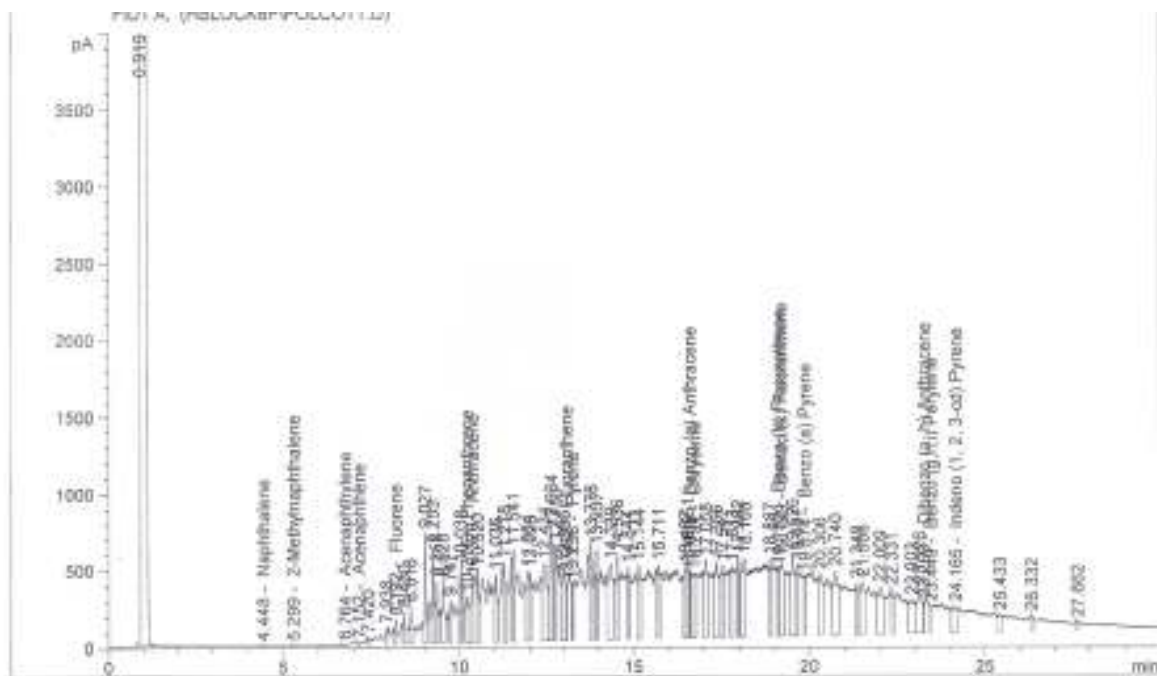
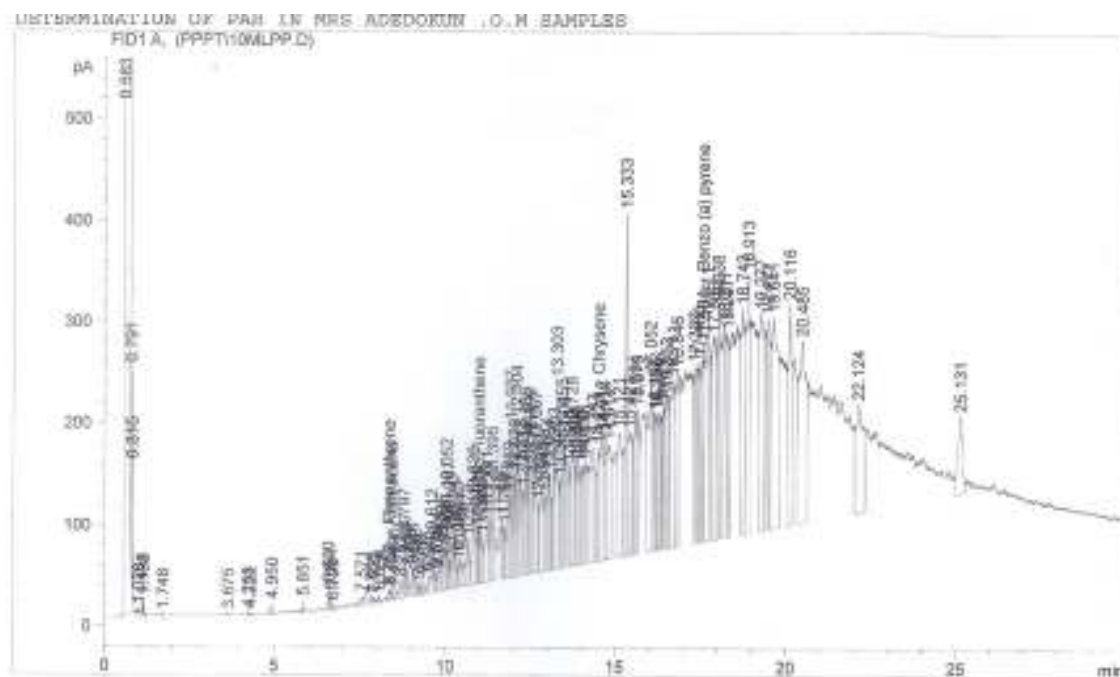
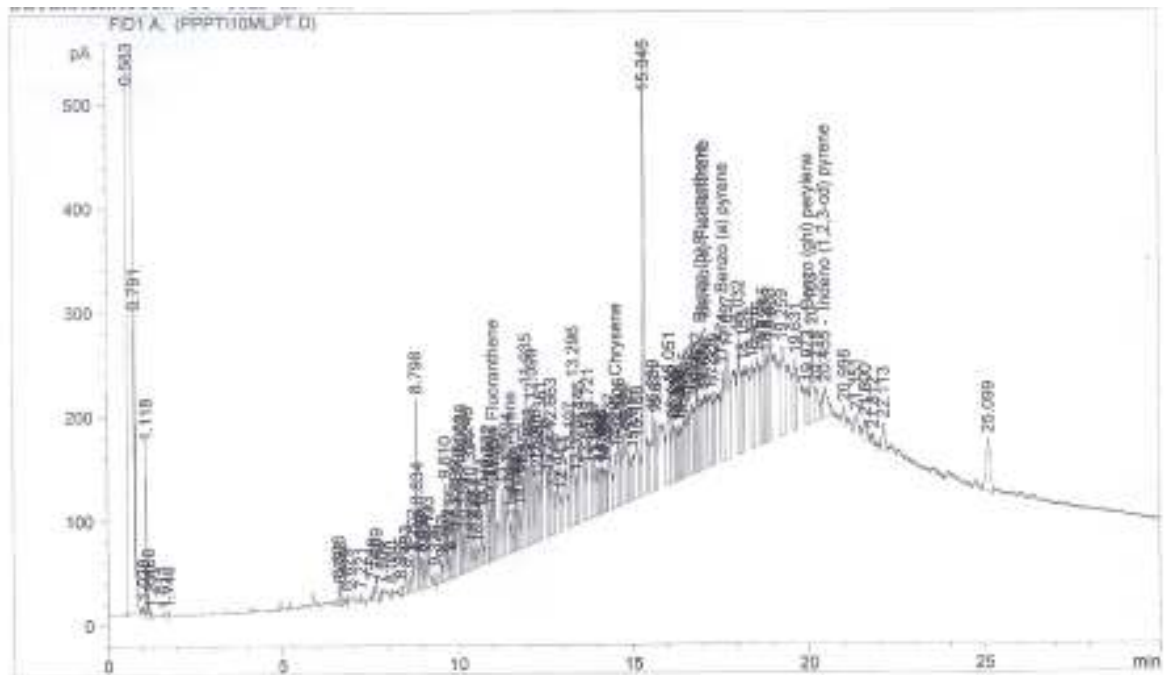
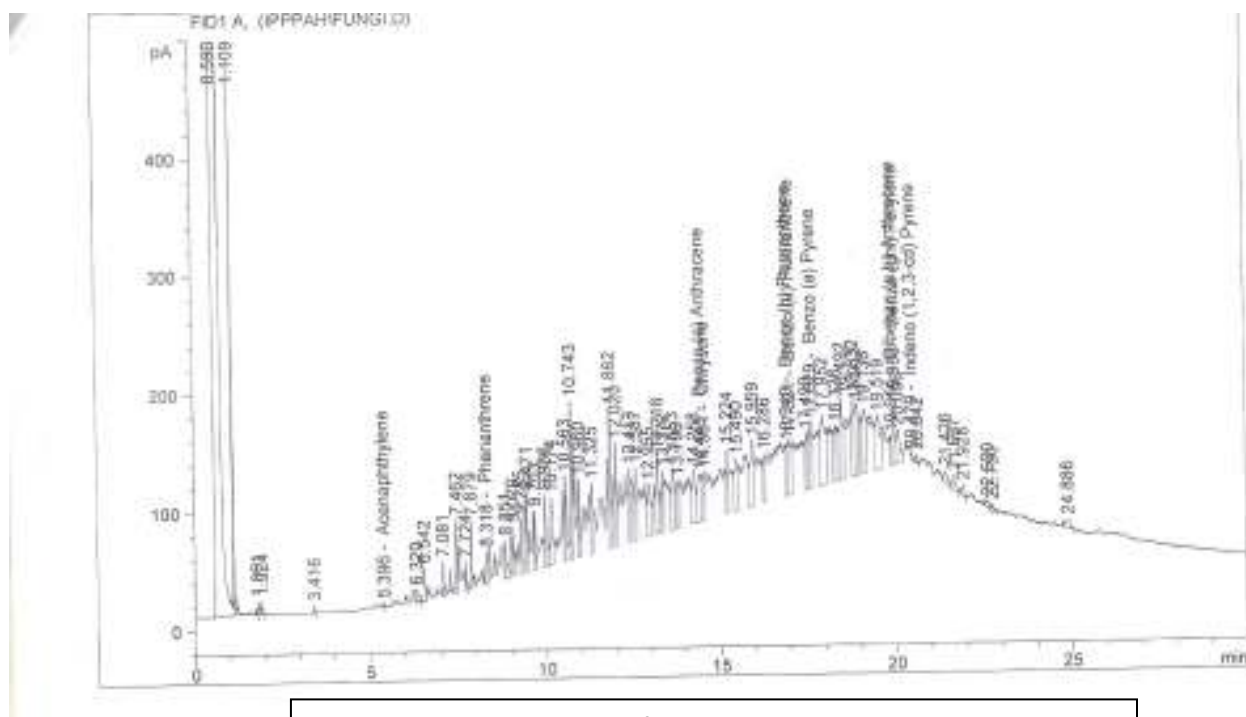


Figure 1e: Chromatogram of Poly cyclic aromatic Hydrocarbon Profile of Crude oil polluted cotton with no fungus



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molecules. For instance, benzo[a]pyrene, benzo[a]anthracene and fluoranthene are classified as probable human carcinogens, and a small number have been shown to induce skin tumours in skin painting studies in laboratory mice. Interestingly, some of these toxic fractions were degraded by the fungi at the end of the 15 weeks and concentrations of others were reduced. A longer duration of experimental period might have resulted in total degradation of the hydrocarbon fraction.

Conclusion: Indigenous mushrooms, *Pleurotus pulmonarius*, *Pleurotus tuber-regium* (PT) and *Lentinus squarrosulus* (LS) were able to degrade crude oil over a period of 15 weeks although at various degrees. These fungi are potential mycoremediation agents and could be useful in environmental related studies and for industrial purposes.

PDegradation of crude oil fractions was described in this

Disclaimer: - This manuscript was presented in the conference.

Conference name: "International Society for Mushroom Science"

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["http://www.researchgate.net/profile/Olutayo_Adedokun/publication/269334762_Degradation_of_crude_oil_by_Pleurotus_pulmonarius_Pleurotus_tuber-](http://www.researchgate.net/profile/Olutayo_Adedokun/publication/269334762_Degradation_of_crude_oil_by_Pleurotus_pulmonarius_Pleurotus_tuber-)

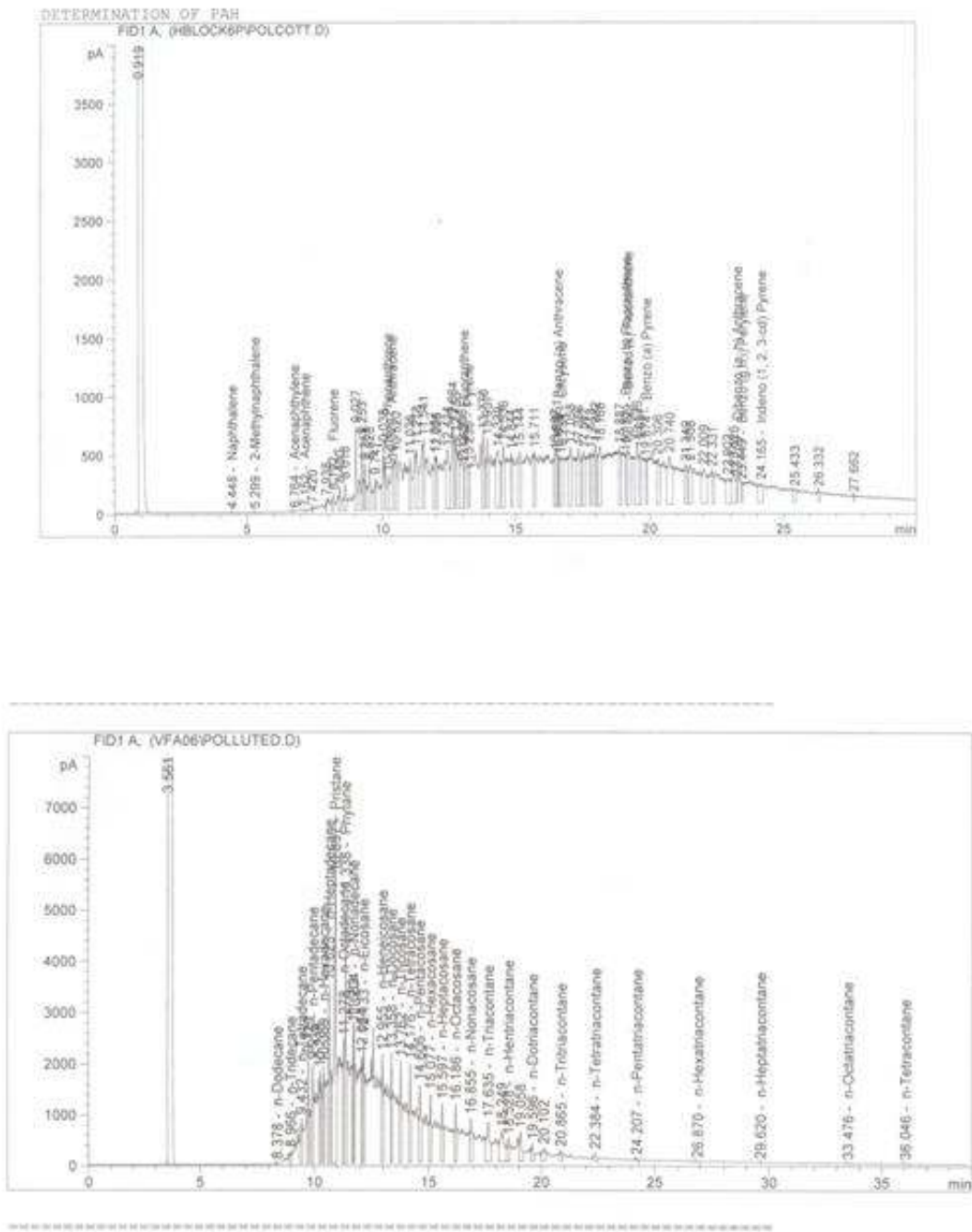
[regium and Lentinus squarrosulus/links/548718eb0cf268d28f070bc7.pdf?inViewer=true&&origin=publication_detail&inViewer=true](http://regium_and_Lentinus_squarrosulus/links/548718eb0cf268d28f070bc7.pdf?inViewer=true&&origin=publication_detail&inViewer=true) ”

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References

1. Adriano DC, Chlopecka A, Kaplan KI. Role of soil chemistry in soil remediation and ecosystem conservation. Soil Science Society of America. 1998; Madison, WI, Special Publication.
2. Anonymous. Bioremediation: an alternative tool for remediation of petroleum-contaminated media [online]. 2003; At: <<http://www.xlenvironmental.com/library/winter.htm>>. Accessed 15 June 2006.
3. Achuba FI, Okoh PN. Effects of Petroleum Products in Soil on α -Amylase, Starch Phosphorylase and Peroxidase Activities in Cowpea and Maize Seedlings. *American Journal of Experimental Agriculture*. 2015; 6(2): 112-120.
4. Odjegba VJ, Sadiq AO. Effect of spent Engine Oil on the growth parameters, chlorophyll and protein levels of *Amarathus hybridus* L. *The Environmentalist*. 2002, 22:23 – 28.
5. Onuoha CI, Arinze AE, Ataga AE. Evaluation of growth of some fungi in crude oil polluted environment. *Global Journal of Agricultural Sciences*. 2003, 2(2): 80-81
6. Kadafa AA. Oil Exploration and Spillage in the Niger Delta of Nigeria. *Civil and Environmental Research*. 2012; 2(3): 38-51.
7. Moore JN, Castro JM. Pit lakes: their characteristics and the potential for their remediation. *Environmental Geology*. 2000; 39:11.
8. Isikhuemhen OS, Nerud F. Preliminary studies on the ligninolytic enzymes produced by the tropical fungus *Pleurotus tuber-regium* (Fr.) Sing. *Antonie van Leeuwenhoek*. 1999; 75, 257–260.
9. Shide EG, Wuyep PA, Nok AJ. Studies on the degradation of wood sawdust by *Lentinus squarrosulus* (Mont.) Singer. *African Journal of Biotechnology*. 2004; 3(8), 395–398.
10. Buswell JA, Cai YJ, Chang ST, Peberdy JF, Fu SY, Yu HS. Ligno-cellulolytic enzyme profiles of edible mushroom fungi. *World Journal of Microbiology & Biotechnology*. 1996; 12, 537–542.
11. Ghosh M, Mukherjee R, Nandi B. Production of extracellular enzymes by two *Pleurotus* species using banana pseudostem biomass. *Acta Biotechnologica*. 1998; 18(3), 243–254.
12. United States Environmental Protection Agency (USEPA). Sonication Extraction Procedure—Method 3550. 1998; 3rd Edition, US EPA, Washington DC.
13. Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG). Composition of petroleum mixtures. TPHCWG Series. 1998; Volume 2. Amherst Scientific Publishers, Amherst, MA, USA.

- 214 14. Nerud F, Misurcova Z. Distribution of ligninolytic enzymes in selected white rot fungi.
215 Folia Microbiologica. 1996; 41(3), 264–266.
- 216 15. Cohen R, Persky L, Hadar Y. Biotechnological applications and potential of wood-
217 degrading mushrooms of the genus *Pleurotus*. Applied Microbiology and Biotechnology.
218 2002; 58, 582–594.
- 219 16. Wuyep PA, Khan AU, Nok AJ. Production and regulation of lignin degrading enzymes
220 from *Lentinus squarrosulus* (Mont.) Singer and *Psathyrella atroumbonata* Pegler.
221 African Journal of Biotechnology. 2003; 2(11), 444–447.
- 222 17. International Programme on Chemical Safety (IPCS). Selected non-heterocyclic
223 polycyclic aromatic hydrocarbons. Environmental Health Criteria 202. 1998; World
224 Health Organization, Geneva.

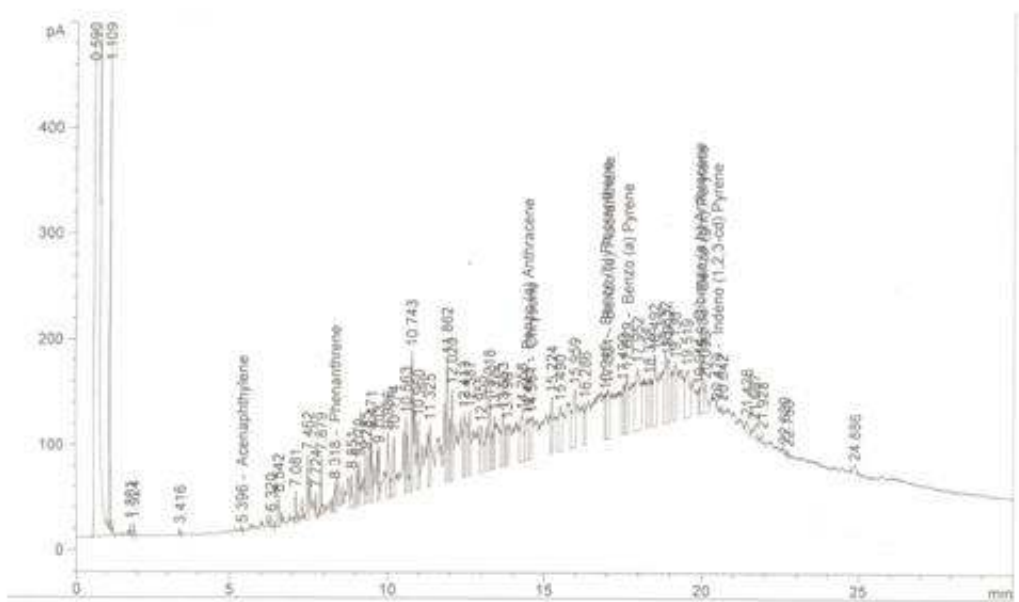
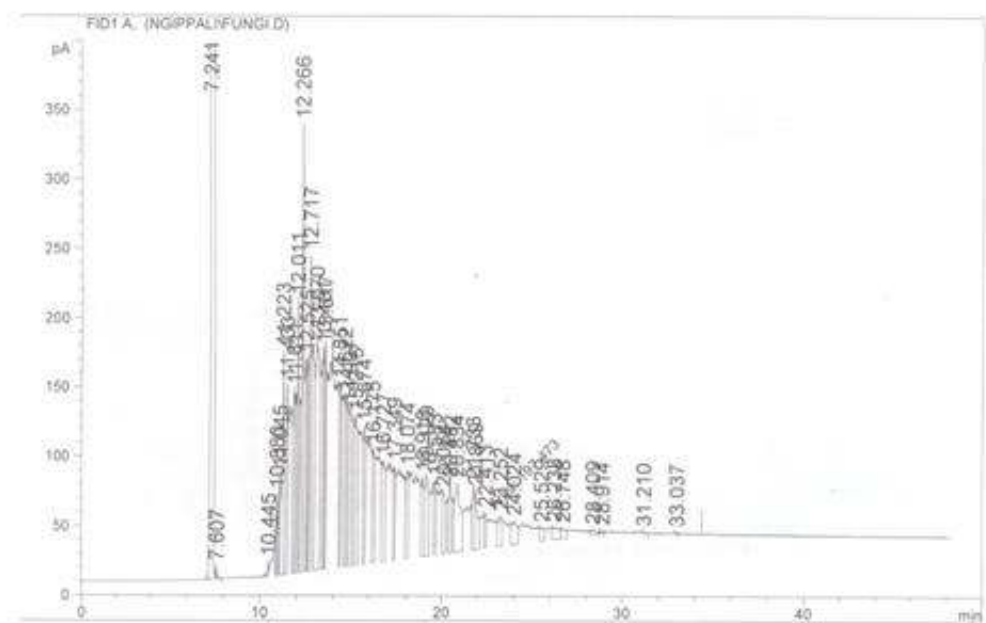


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Figure 2a. Chromatogram of control at 15 weeks: polyaromatic hydrocarbon (PAH), upper plot;
aliphatic hydrocarbon (AH), lower plot



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Figure 2c. Chromatogram of *Lentinus squarrosulus* at 15 weeks: aliphatic hydrocarbon, upper plot; polyaromatic hydrocarbon, lower plot

