

Original Research Article

Mycoremediation: Monitoring Degradation of Crude Oil Degradation by indigenous edible 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 myco-remediation.

Study design: The study design was completely randomized design (CRD)_[R1]

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 myco-remediation agents.

Key words_[R2]: Myco-remediation, Crude oil, Degradation, Aliphatic hydrocarbon, Polycyclic-aromatic hydrocarbon

1. 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], [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], [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 Moore et al. [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 *white rot* fungi, *Pleurotus pulmonarius* (PP), *P. tuber-regium* (PT) and *Lentinus squarrosulus* (LS) [PP,PT]_[R3] and LS- used in the present study are described as white rot, [8][9], [10], [11], and are capable to producing different enzymes [8-11]. This study investigated the degradation of crude oil by three indigenous fungi (PP, PT, LS) in order to assess their potentials for myco remediation of oil polluted environment.

2. MATERIALS AND METHODS_[R4]

2. 1. Experimental design

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 _[R5] of dimensions 7.5 × 17 × 7.5 cm were autoclaved at 121°C for 15 minutes. After cooling, 10% spawn _[R6] 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_[R7]. 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 Oil Degradation

Oil degradation was monitored by evaluating the remnant aliphatic and polycyclic-aromatic hydrocarbon fractions according to [12] using Agilent 6890 GC (Flame Ionization Detector). Hydrocarbon profile was determined by the following steps:

2.2 Sample Preparation and extraction (sonication water bath method)

Polluted sample ($2.0 \pm 0.1\text{g}$) was weighed into a clean extraction bottle and dried with anhydrous sodium sulphate after thorough mixture of sample. One mL of $60\text{ }\mu\text{g/mL}$ 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.

2.3 Determination of Oil Degradation

Oil degradation was monitored by evaluating the remnant aliphatic and polycyclic-aromatic hydrocarbon fractions using Agilent 6890 GC (Flame Ionization Detector) [12].

2.4 Statistical analysis

3. 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 differences ($P \leq 0.05$) in the degradation of crude oil between ~~treatments with and without the control~~ and the test fungi as well as ~~between~~ among the ~~different test~~ fungi ~~tested~~. 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 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: [8-11], [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 Generally, white-rot fungi can be divided into groups depending on the type of lignolytic enzymes they produced under different conditions [14]. For example, while [10] reported that PP produced low manganese peroxidase in both low- and high-nitrogen conditions, together with laccase and cellulase [10]. In contrast, a preliminary work on enzymes produced by PT [8] suggests that the fungus has a higher manganese peroxidase activity in low nitrogen condition than in higher nitrogen condition. Further, this fungus produced laccase, but not cellulase. It was also reported that PP 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 [R8] 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 has already been reported 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 study reports of [13] that where most petroleum hydrocarbon mixtures contained very low concentrations of PAHs. However, [17] observed that the major concern regarding PAHs is the potential carcinogenicity of some

Table 1: [R9] Aliphatic Hydrocarbon Contents after Degradation by Fungi at 5, 10 and 15 weeks

Treatment	5weeks	10weeks	15weeks
control	5114.0 ^{a*}	5111.8 ^a	5110.0 ^a
<i>L.squarrosulus</i> [R10]	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}

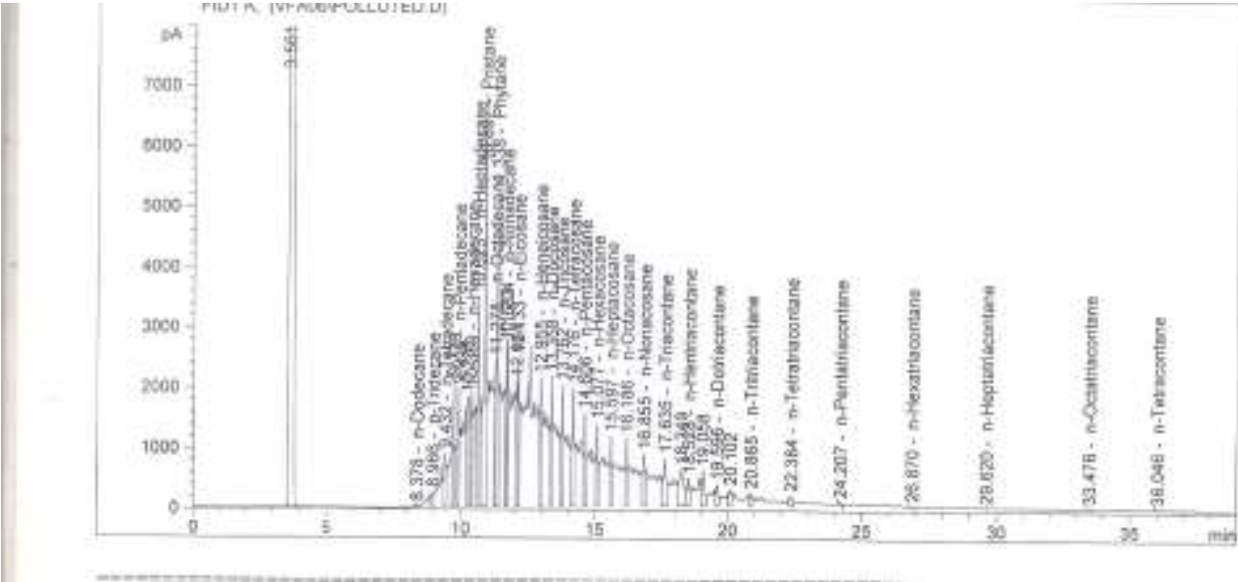
*Means in a column followed by the same letter in the same column are not significantly different at P=0.05.

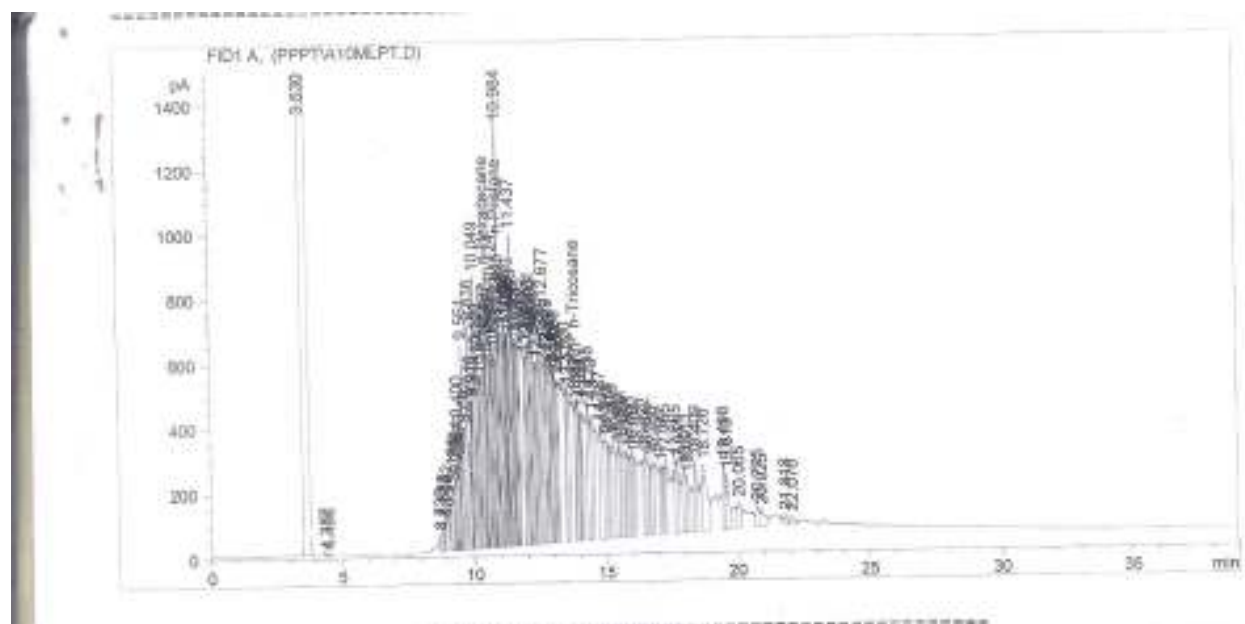
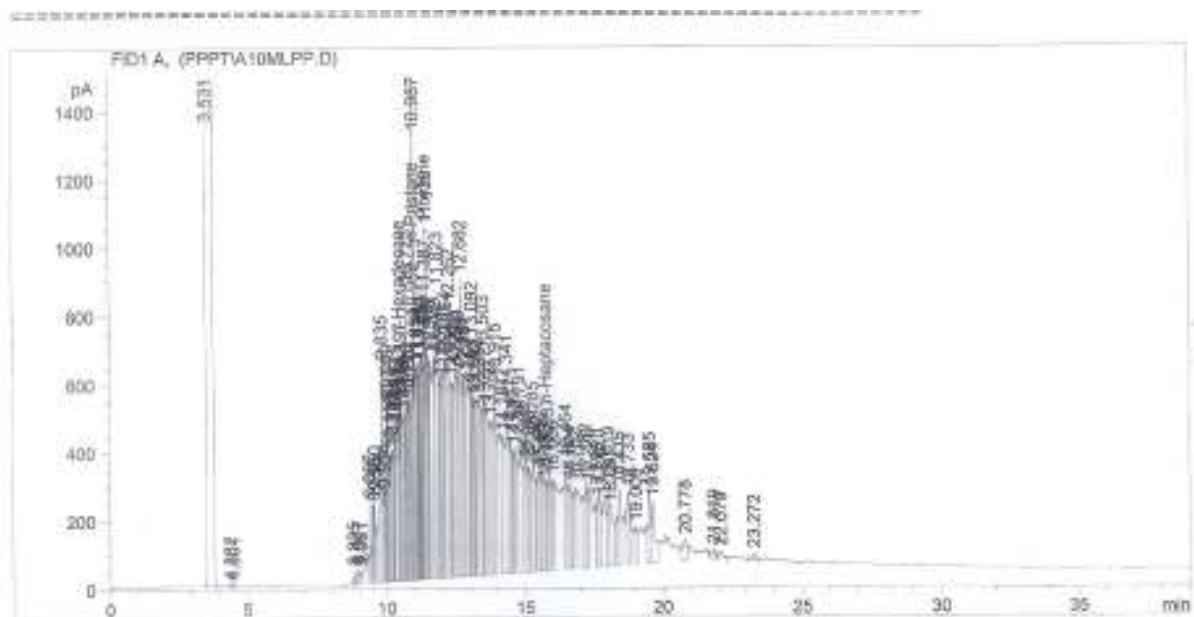
Table 2: PAH [R11] Contents after Degradation by Fungi at 5, 10 and 15 weeks

Treatment	5weeks	10weeks	15weeks
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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 in a column followed by the same letter in the same column are not significantly different at P=0.05.





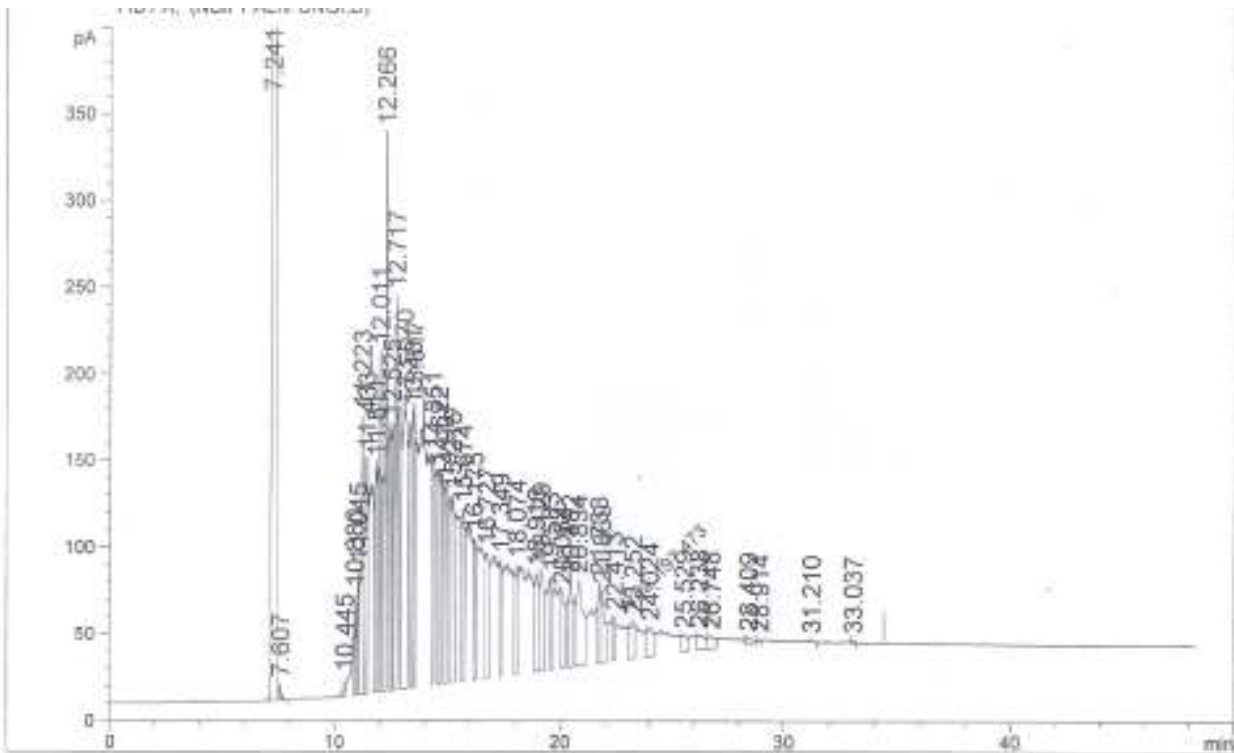
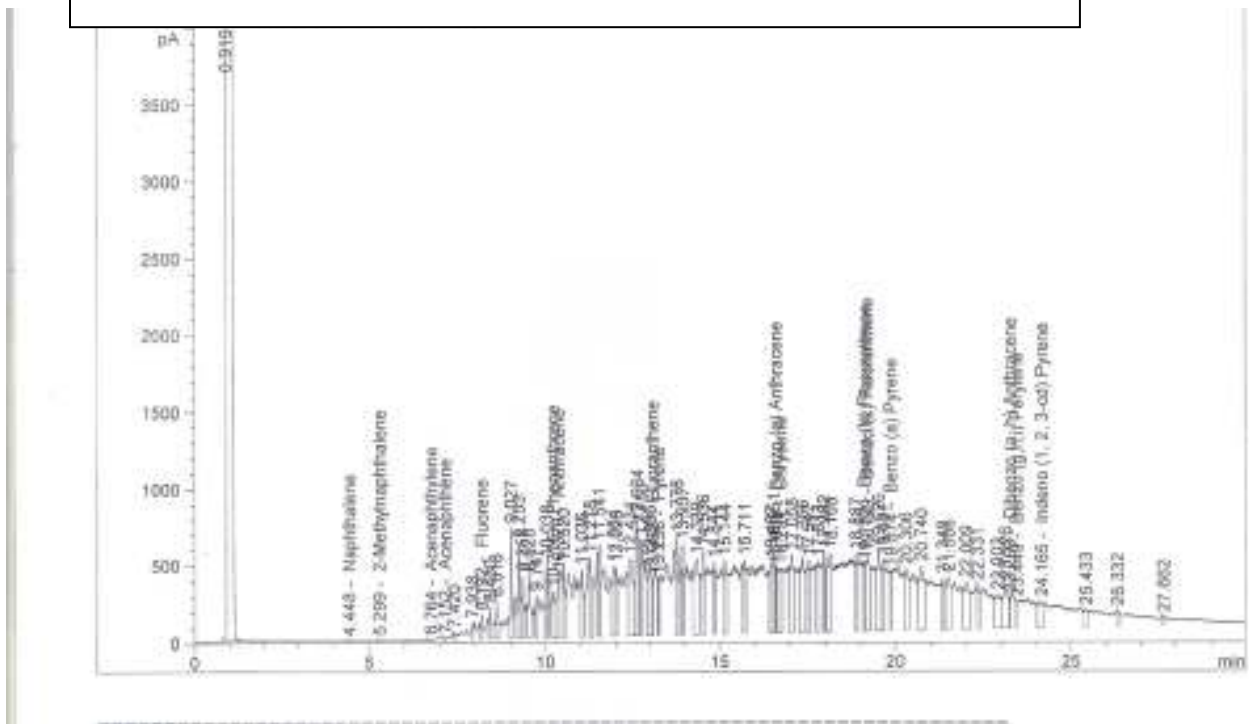
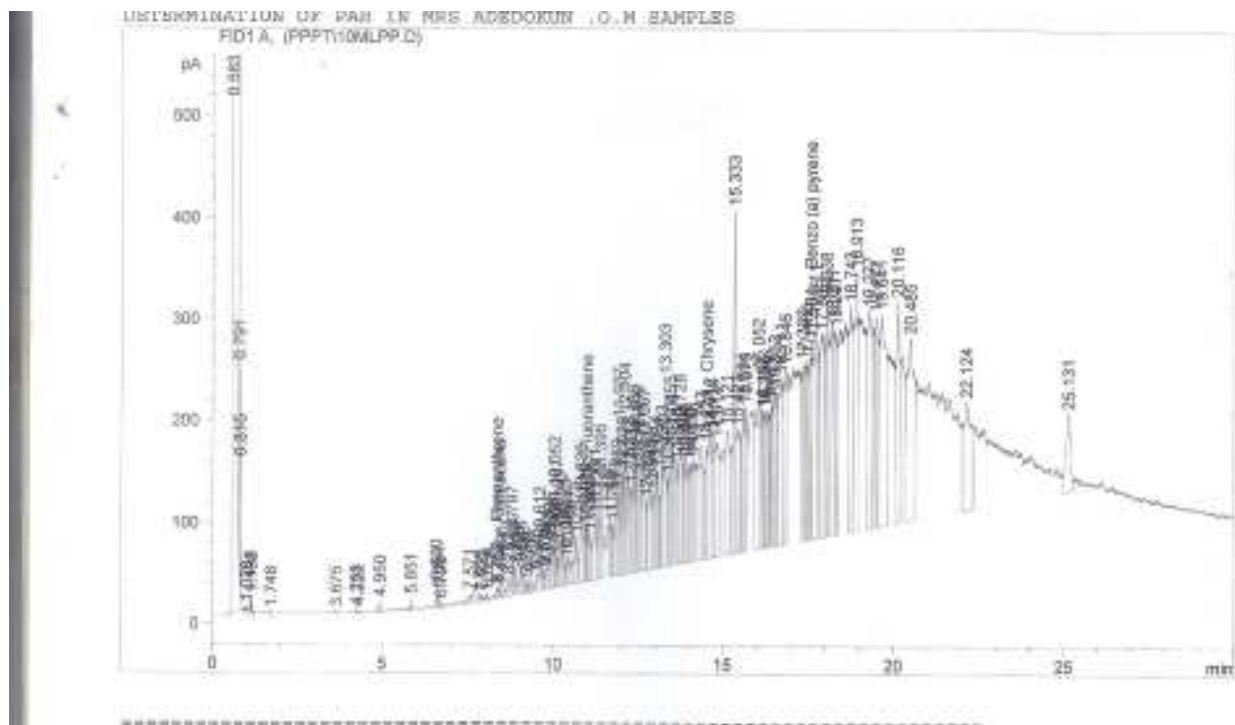


Figure 1d: Chromatogram of Aliphatic Hydrocarbon Profile of Crude oil polluted cotton with *L.squarrosulus*



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163 Figure 1f: Chromatogram of Poly cyclic aromatic Hydrocarbon Profile of
164 Crude oil polluted cotton with *P. pulmonarius*

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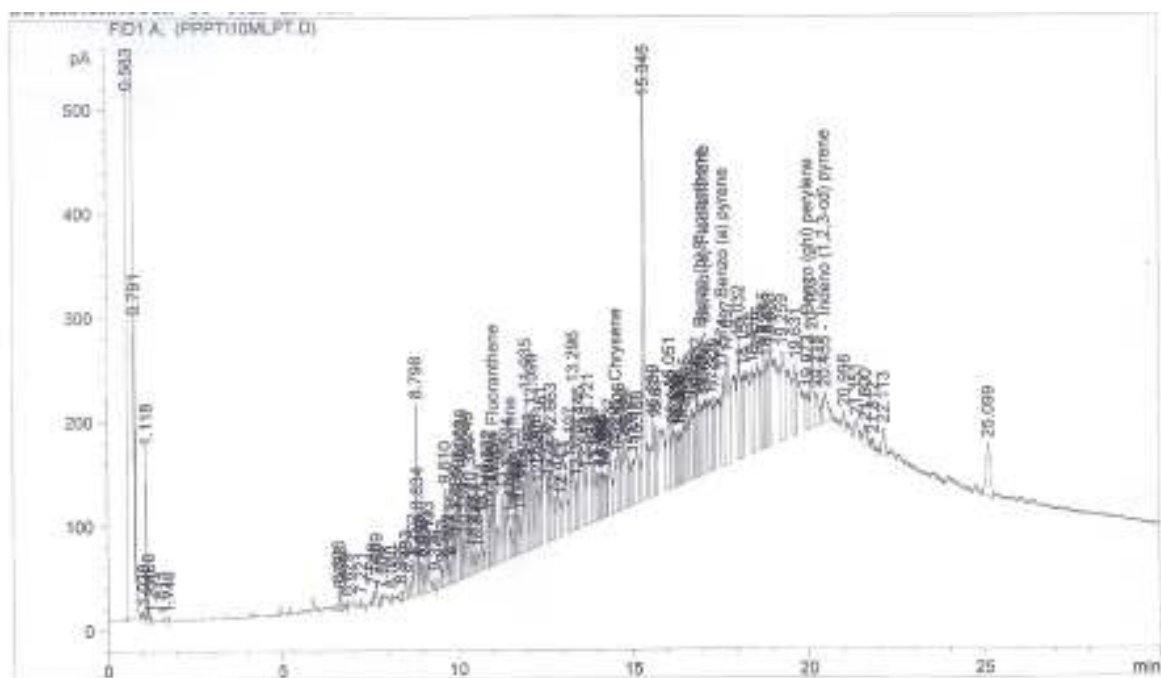


Figure 1g: Chromatogram of Poly cyclic aromatic Hydrocarbon Profile of Crude oil polluted cotton with *P. tuber-regium*

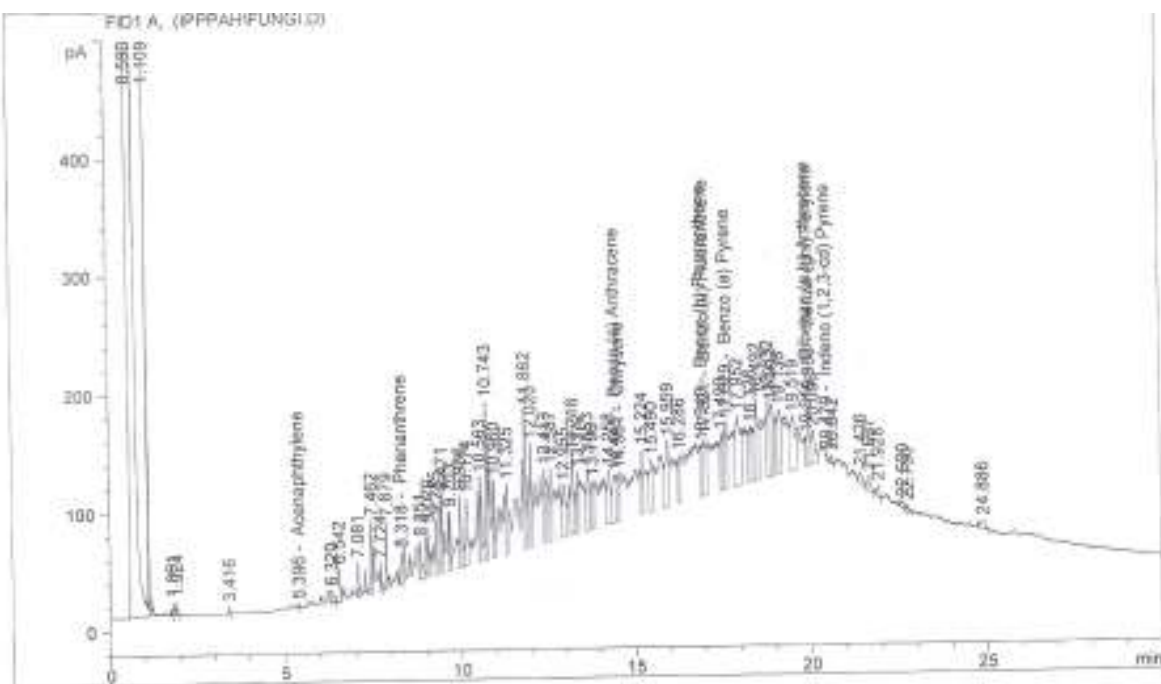


Figure 1h: Chromatogram of Poly cyclic aromatic Hydrocarbon Profile of Crude oil polluted cotton with *L. squarrosulus*

Molecules [17]. For instance, benzo[a]pyrene, benzo[a]anthracene and fluoranthene are classified as probable human carcinogens, and a little quantity has been shown to induce skin tumors in skin painting studies in laboratory mice [R12]. 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 fractions.

4. 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.

Disclaimer: - This manuscript was presented in the International Society for Mushroom Science conference at 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

Conference name: "International Society for Mushroom Science"

Conference link is

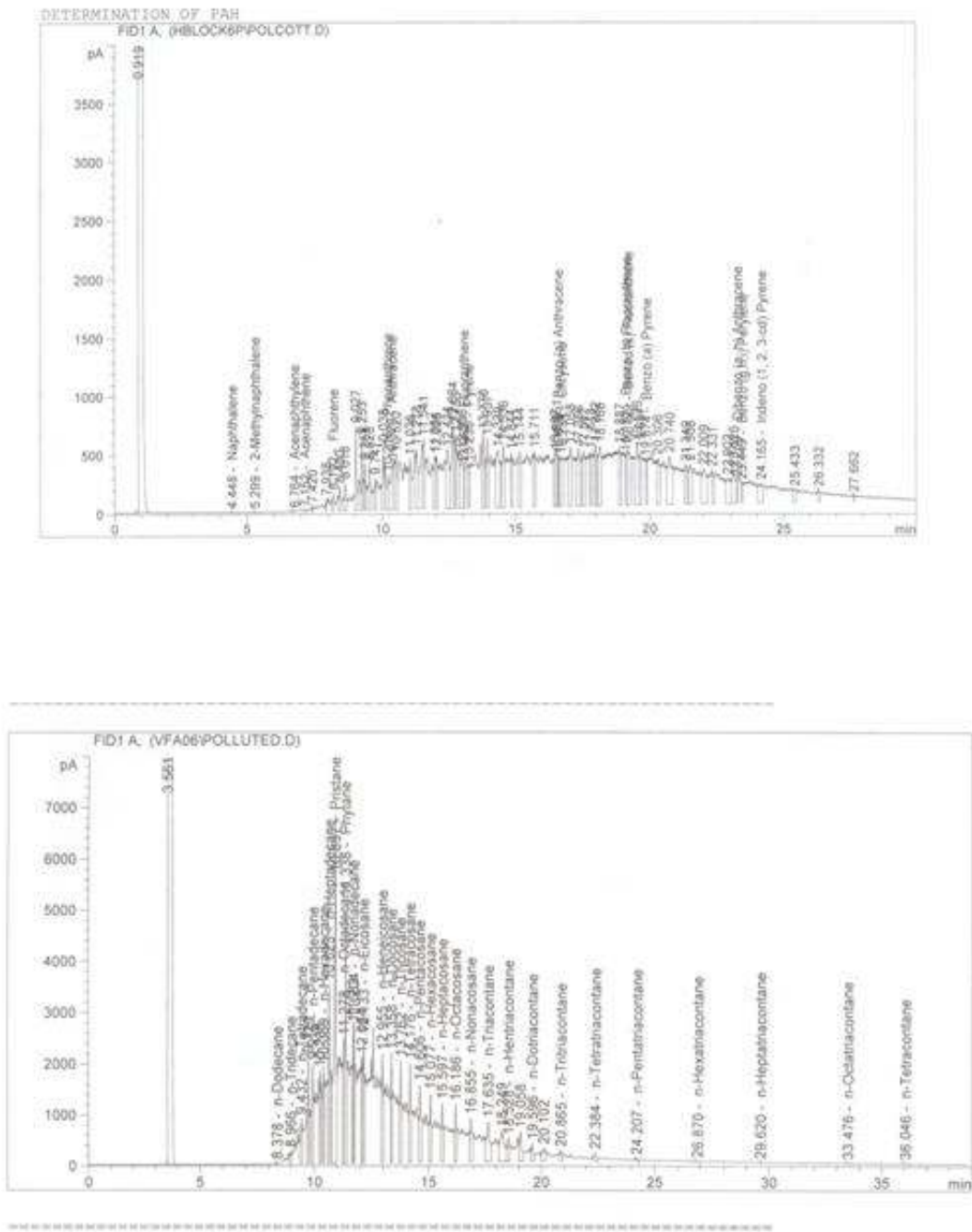
"http://www.researchgate.net/profile/Olutayo_Adedokun/publication/269334762_Degradation_of_crude_oil_by_Pleurotus_pulmonarius_Pleurotus_tuber-regium_and_Lentinus_squarrosulus/links/548718eb0cf268d28f070bc7.pdf?inViewer=true&&origin=publication_detail&inViewer=true"

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

