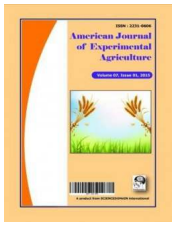

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Degradation of Crude Oil by Indigenous Edible Mushrooms

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Authors' contributions

This work was carried out in collaboration between both authors. Author OMA designed the study, wrote the protocol, managed the analyses of the study, performed the statistical analysis and wrote the first draft of the manuscript. Author AEA reviewed all drafts of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The ability of three indigenous fungi, *Pleurotus pulmonarius* (Fries) Quelet, *Pleurotus tuber-regium* (Fries) Singers and *Lentinus squarrosulus* (Mont.) Singer 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.
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

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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.

Keywords: Myco-remediation; oil spillage; aliphatic hydrocarbon; polycyclic- aromatic hydrocarbon; native fungi.

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. Organic compounds such as crude oil and by-products have been reported as hazardous to plants [2,3]. They also deter emergence of seed, plant development and 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 damages the soil in the 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 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 edible fungi, *Pleurotus pulmonarius*(PP), *Pleurotus tuber-regium*(PT) and *Lentinus s quarrosulus* (LS) used in the present study, 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 mycoremediation of oil polluted environment.

2. MATERIALS AND METHODS

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 PP, PT and LS were obtained from a research Institute. Modified method of Adedokun et al. [12] was used for assessing crude oil degradation. Briefly in 100 mL distilled water containing 5% w/w of calcium carbonate ($CaCO_3$), 50 g cotton waste was soaked for 30 minutes, drained and squeezed to about 60% wet weight. The cotton, shreds was treated with 20% v/w crude oil and placed in transparent bottles (7.5 × 17 × 7.5 cm) and sterilized in an autoclave. Spawn of each fungus, 10%, was used to inoculate samples in triplicate. Experimental design was completely randomized design. Control samples with pollutant but not inoculated with any of the fungi were set up. Treated samples were incubated at room temperature (28±2℃) for 15 weeks. Remnant hydrocarbons -aliphatic (AH) and poly- cyclic-aromatic (PAH) hydrocarbon were determined at interval of 5 weeks for each of the treatments.

2.2 Sample Preparation and Extraction (Sonication Water Bath Method)

Polluted 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); $Pyrened_{10}$ (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) [13].

2.4 Statistical Analysis

Data was analyzed using two way Analysis of Variance (ANOVA)

3. RESULTS AND DISCUSSION

Tables 1, 2 and Fig. 1 show crude oil degradation by the three fungal species for 15 weeks. Crude oil was degraded at different rates by the three fungi. At 5 weeks, for the AH profile, there was significant differences (P≤0.05) in the degradation of crude oil between treatments with and without the test fungi as well as among the different 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 Figs. 1a-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≤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 10weeks however, at 15 weeks; there was significant difference between the control and LS as well as between LS and PT, PP.

There was significant difference P≤0.05 in oil degradation among the weeks. Oil degradation increased from 5 weeks and reached the peak at 15 weeks (Tables 1, 2). 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.

Table 1. Aliphatic hydrocarbon contents after degradation by fungi at 5, 10 and 15 weeks

Treatment	5 weeks	10 weeks	15 weeks
Control	5114.0^{a*}	5111.8^{a}	5110.0^{a}
Lentinus squarrosulus	3889.5^{b}	3491.71^{b}	2027.4^{b}
Pleurotus tuber-regium	1298.84^{c}	894.8^{c}	301.01^{c}
Pleurotus pulmonarius	2388.4^{d}	382.96^{cd}	16.18^{cd}

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

Table 2. Polycyclic aromatic hydrocarbon contents after degradation by fungi at 5, 10 and 15 weeks

Treatment	5 weeks	10 weeks	15 weeks
Control	328.3^{a*}	329.9^{a}	329.9^{a}
Lentinus squarrosulus	325.0^{a}	304.45^{a}	146.9^{b}
Pleurotus tuber-regium	95.5^{bc}	80.2^{bc}	45.96^{c}
Pleurotus pulmonarius	55.5^{c}	20.73^{c}	16.18^{cd}

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

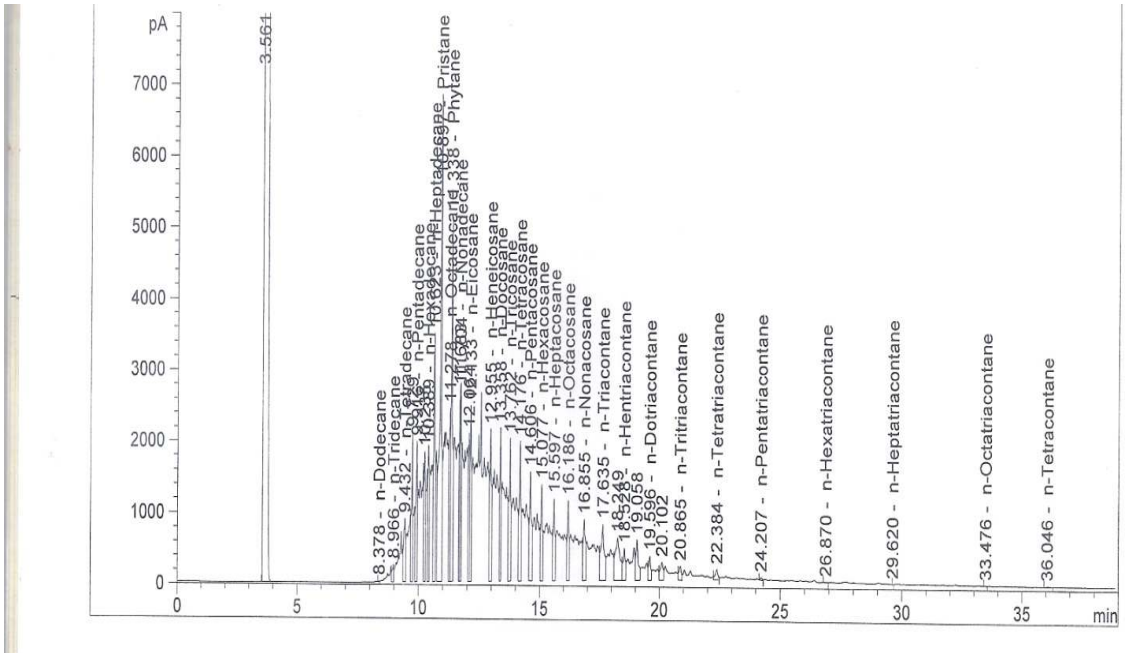

Fig. 1a. Chromatogram of control- Aliphatic hydrocarbon profile of crude oil polluted cotton in the absence of fungal inoculation

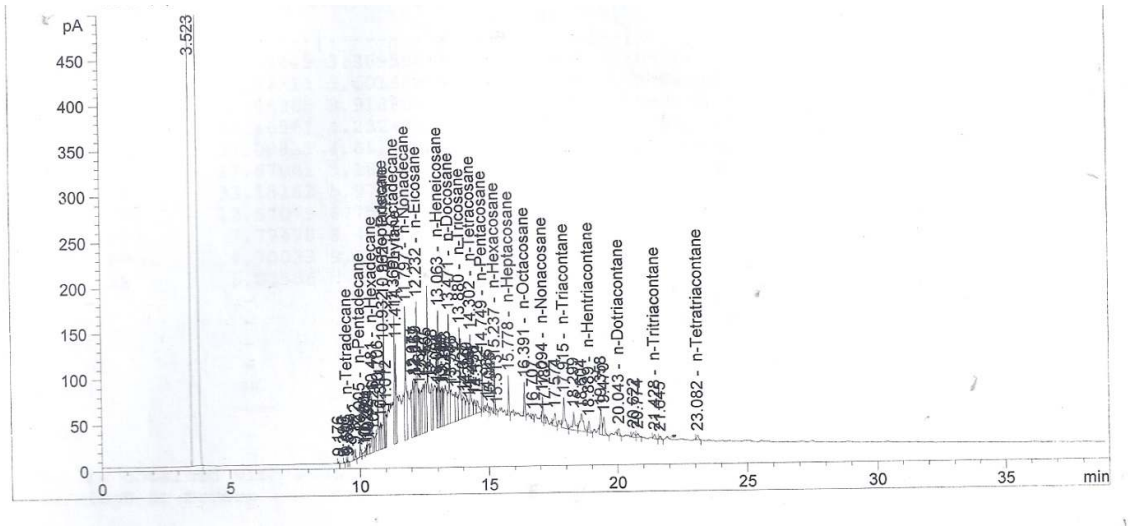

Fig. 1b. Chromatogram of aliphatic hydrocarbon profile of crude oil polluted cotton inoculated with *Pleurotus pulmonarius*

The pattern of crude oil degradation for the 15-week period differed for the fungi studied. A plausible explanation for this may be production of combination of enzymes by the fungi other factors- substrates and pollutants being uniform. Reports from previous research [8-11] indicate that the fungi degraded crude oil in various manners despite the fact that they are all white-rot fungi. Consequently, LS least degraded aliphatic and poly cyclic aromatic crude oil fractions at 5, 10 and 15 weeks of analyses, degradation by PT was next and maximum for PP. Generally, white-rot fungi can be divided into groups depending on the type of lignolytic enzymes they produce under different conditions [14]. For example, PP produces low manganese peroxidase at 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 under low nitrogen condition than in higher nitrogen condition. Further, this fungus produced laccase, but not cellulase. The presence of aryl-alcohol oxidase (AAO), cytochrome P-450 mono-oxygenase and epoxide hydrolase secreted by PP furthermore enabled it initially oxidise and hydrate PAH compounds [15]. The production of ligninase by the LS has already been reported [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

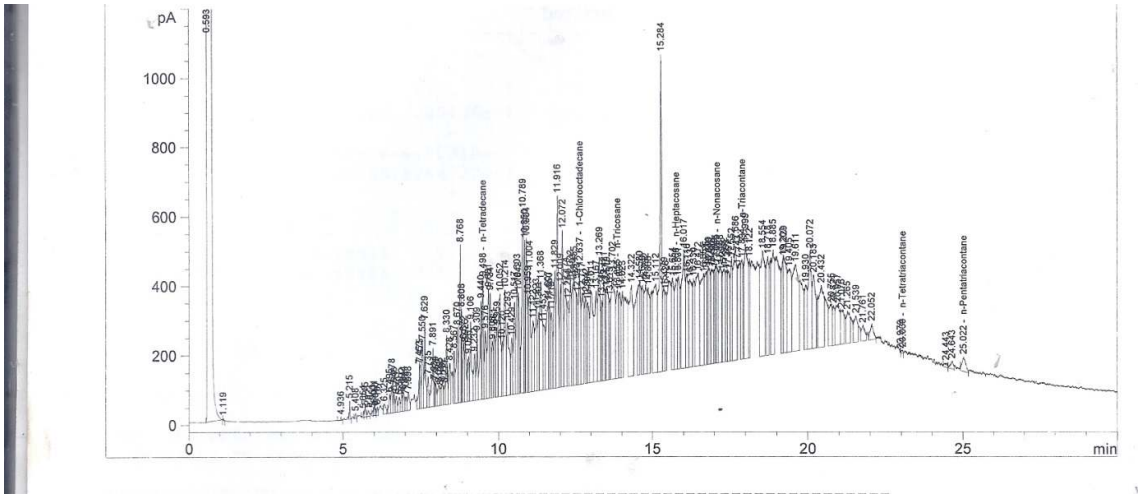

Fig. 1c. Chromatogram of aliphatic hydrocarbon profile of crude oil polluted cotton with *Pleurotus tuber-regium*

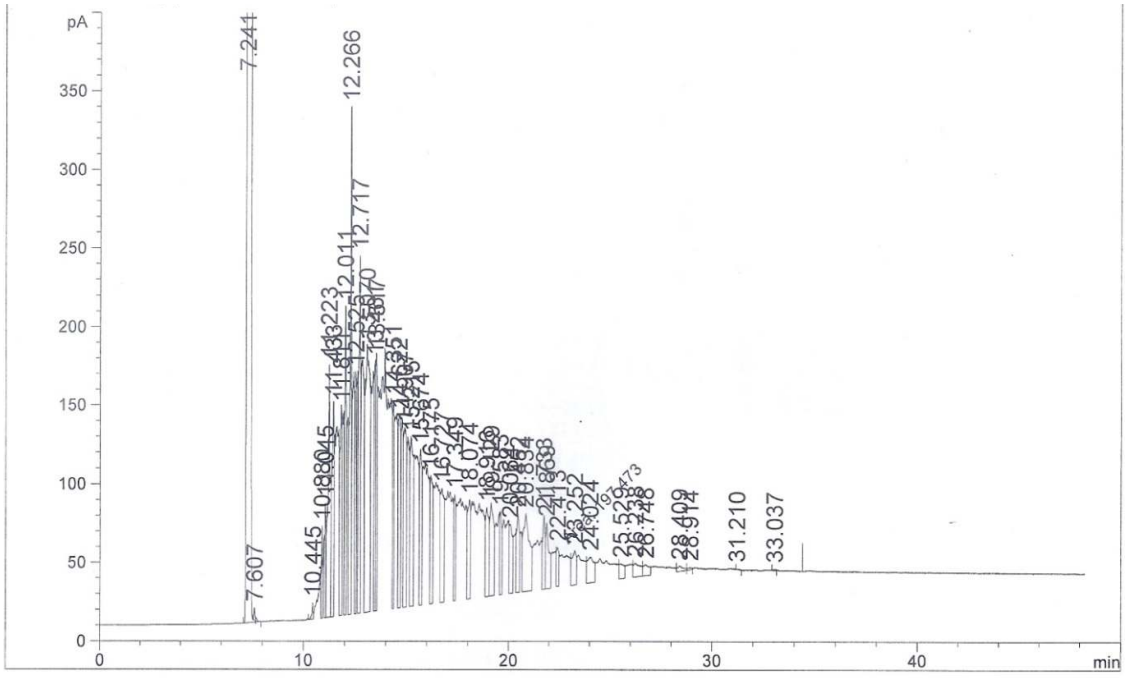

Fig. 1d. Chromatogram of aliphatic hydrocarbon profile of crude oil polluted cotton with *Lentinus squarrosulus*

crude-oil degradation. The low concentrations of PAH as indicated in the result agrees with the study [14] where most petroleum hydrocarbon mixtures contained very low concentrations of PAHs. However, the major concern regarding PAHs is the potential carcinogenicity of some molecules [17]. It is however interesting to note that some of these toxic fractions were broken down while concentrations of others were reduced as a result of the activities of the fungi by the end of the 15 weeks.

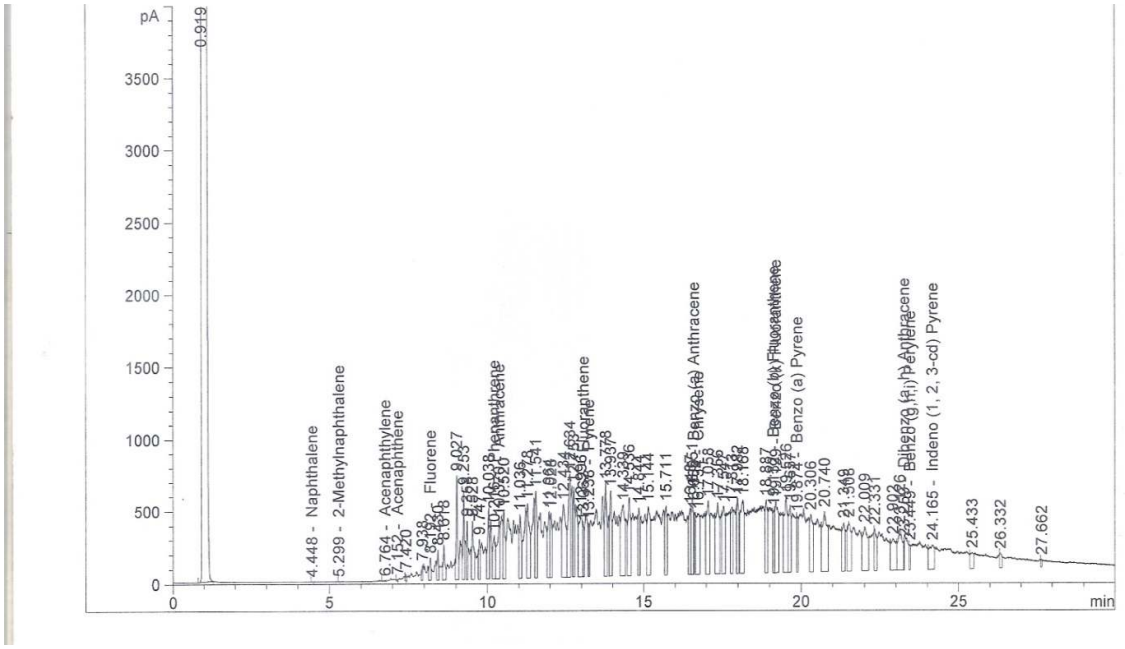

Fig. 1e. Chromatogram of poly cyclic aromatic hydrocarbon profile of crude oil polluted cotton with no fungus

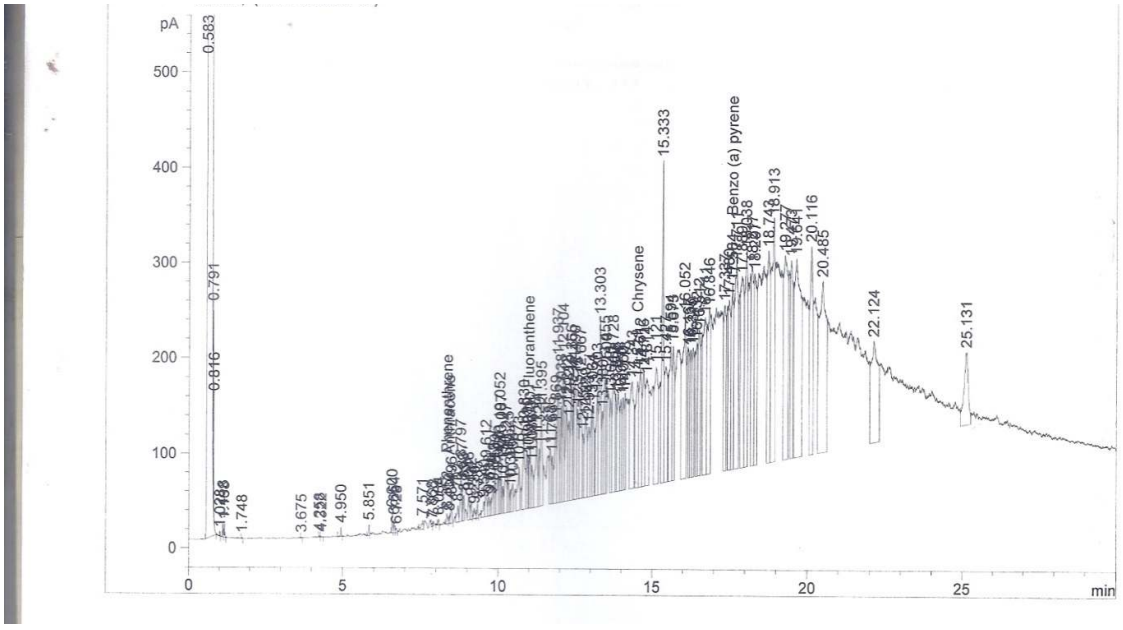

Fig. 1f. Chromatogram of poly cyclic aromatic hydrocarbon profile of crude oil polluted cotton with *Pleurotus pulmonarius*

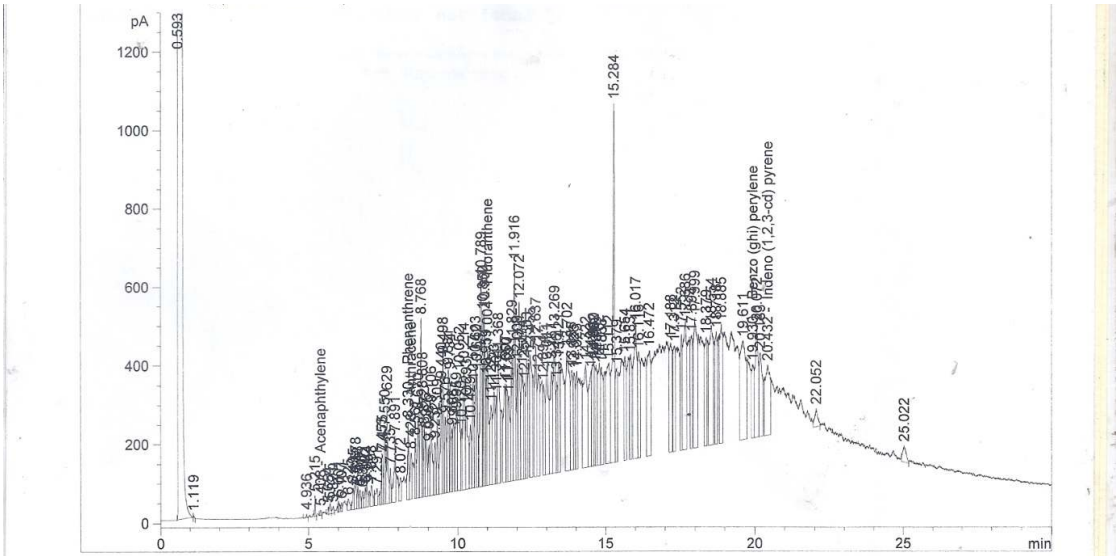

Fig. 1g. Chromatogram of Poly cyclic aromatic Hydrocarbon Profile of Crude oil polluted cotton with *Pleurotus tuber-regium*

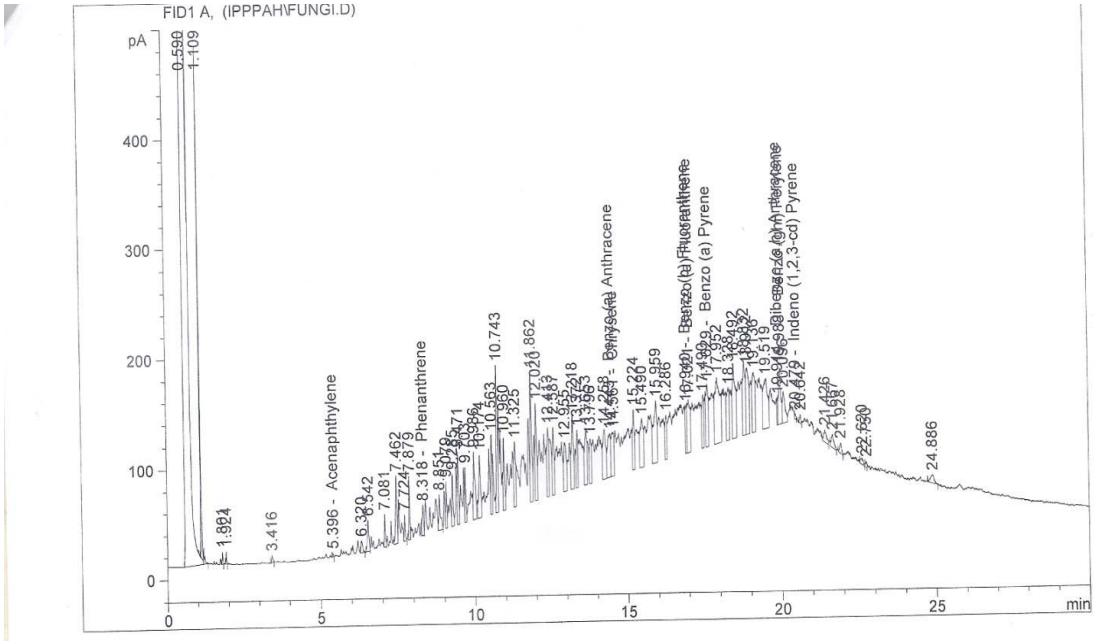

Fig. 1h. Chromatogram of Poly cyclic aromatic Hydrocarbon Profile of Crude oil polluted cotton with *Lentinus squarrosulus*

4. CONCLUSION

Indigenous mushrooms, *P. pulmonarius*, *P. tuber-regium* and *L. squarrosulus* 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 research was presented in the International Society for Mushroom Science in 2008. It has been updated significantly for the purpose of this publication and the Society has granted permission for peer review journal publication.

http://www.researchgate.net/profile/Olutayo_Adedokun/publication/269334762_Degradation_of_c

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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