

Original Research Article

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

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 a Crude oil polluted substrate over a period of 15 weeks was investigated.

Method: 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. After a period of 15 weeks, degradation of crude oil in substrates inoculated with PP was 87.4% for aliphatic hydrocarbon (AH) and 71.2% for polycyclic aromatic hydrocarbon (PAH). Crude oil degradation in substrate inoculated with PT was 73.7% for AH and 51.9% for PAH. However, degradation of crude oil in substrate inoculated by LS was 50.0% for AH and 57.0% for PAH. There was significant difference ($p < 0.05$) in substrates inoculated with fungi, but no significant difference in the control which was not inoculated with fungi.

Conclusion: The tests mushrooms could be useful for crude oil degradation.

Introduction

The soil is a key component of natural ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem (Adriano et al. 1998). 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 (Anon. 2003, Achuba, 2015). Oil pollution prevents normal oxygen exchange between soil and atmosphere due to the hydrophobic properties of oil (Atlas 1977). It also inhibits seed germination and plant growth (Odjegba and Sadiq 2002). 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 (Onuoha et al. 2003, Kafada, 2012). Kafada (2012) reported that the exploration of oil contributed to Nigeria becoming one of the five most petroleum damaged ecosystems of the world.

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. This study investigated the degradation of crude oil by three indigenous fungi.

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 and polyaromatic 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 [17] 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. 1 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 Figures 1 and 2 (supplementary document). The three fungi degraded crude oil but at different rates. After a period of 15 weeks, crude oil degradation in substrates inoculated with PP was 87.4% for aliphatic hydrocarbon (AH) and 71.2% for polyaromatic hydrocarbon (PAH). Crude oil degradation in substrate inoculated with PT was 73.7% for AH and 51.9% for PAH. Degradation of crude oil in substrate inoculated by LS was 50.0% for AH and 57.0% for PAH. The AH contents are quantitatively higher than the PAH contents (Figure 1). TPHCWG (1998) observed that most petroleum mixtures contain very low concentrations of PAH. There was no significant degradation of crude oil observed in the control set-up to which no fungi had been added.

The different ways in which PP, PT and LS degraded crude oil for the 15-week period can be attributed mainly to enzyme production since other parameters such as substrate and pollutant are uniform. Although the three test fungi are recognized as white-rot fungi (Isikhuemhen and Nerud 1999; Shide et al. 2004; Buswell et al. 1996; Ghosh et al. 1998), their responses to crude oil degradation differ. 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 polyaromatic crude oil fractions. Nerud and Misurcova (1996) observed that white-rot fungi can be divided into groups depending on the type of lignolytic enzymes they produced. Buswell et al. (1996) 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 (Cohen et al. 2002) which were found to initially oxidise and further hydrate PAH compounds.

Isikhuemhen and Nerud (1999), reporting a preliminary work on enzymes produced by PT, observed 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. For LS, Wuyep et al. (2003) reported the production of ligninase by the fungus. 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 the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG 1998) that most petroleum hydrocarbon mixtures contain very low concentrations of PAHs. However, the International Programme on Chemical Safety (IPCS 1998) observed that the major concern regarding PAHs is the potential carcinogenicity of some

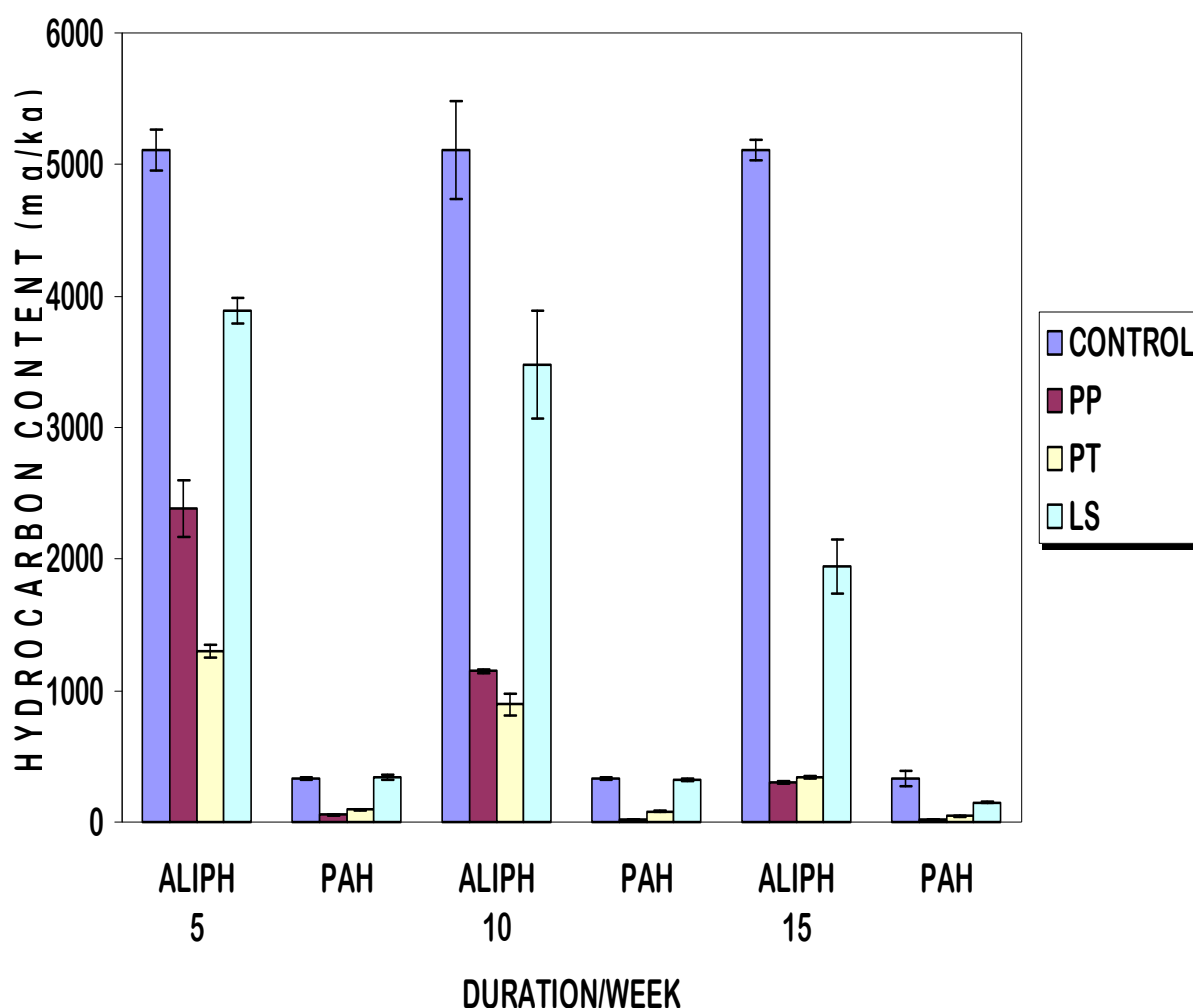


Figure 1. Crude-oil degradation by *Pleurotus pulmonarius* (PP), *Pleurotus tuber-regium* (PT) and *Lentinus squarrosulus* (LS) over a period of 15 weeks

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.

Disclaimer: - This manuscript was presented in the conference.

Conference name: "International Society for Mushroom Science"

116 Conference link is
 117 "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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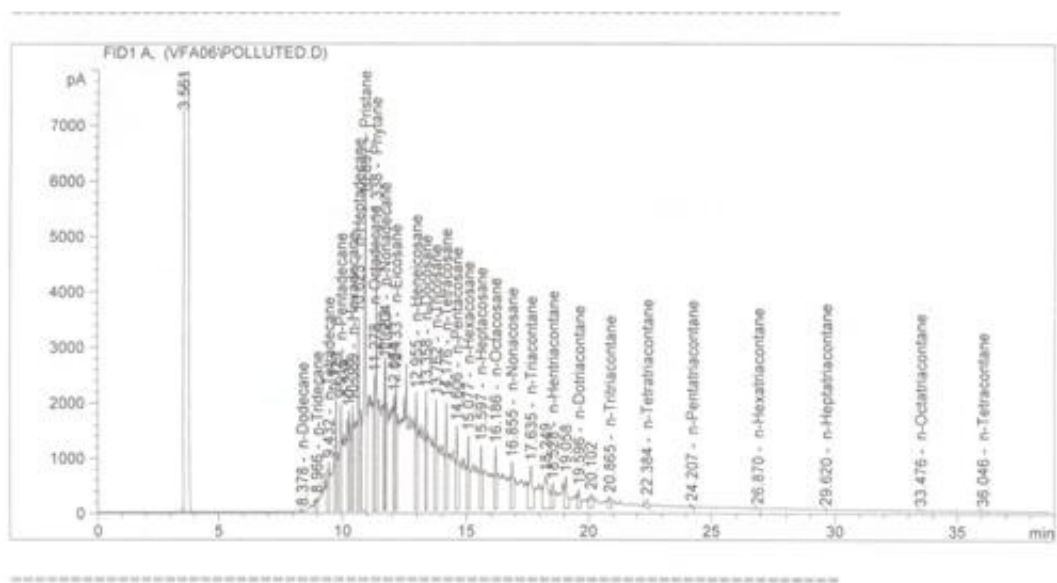
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161 Figure 2a. Chromatogram of control at 15 weeks: polyaromatic hydrocarbon (PAH), upper plot;
162 aliphatic hydrocarbon (AH), lower plot

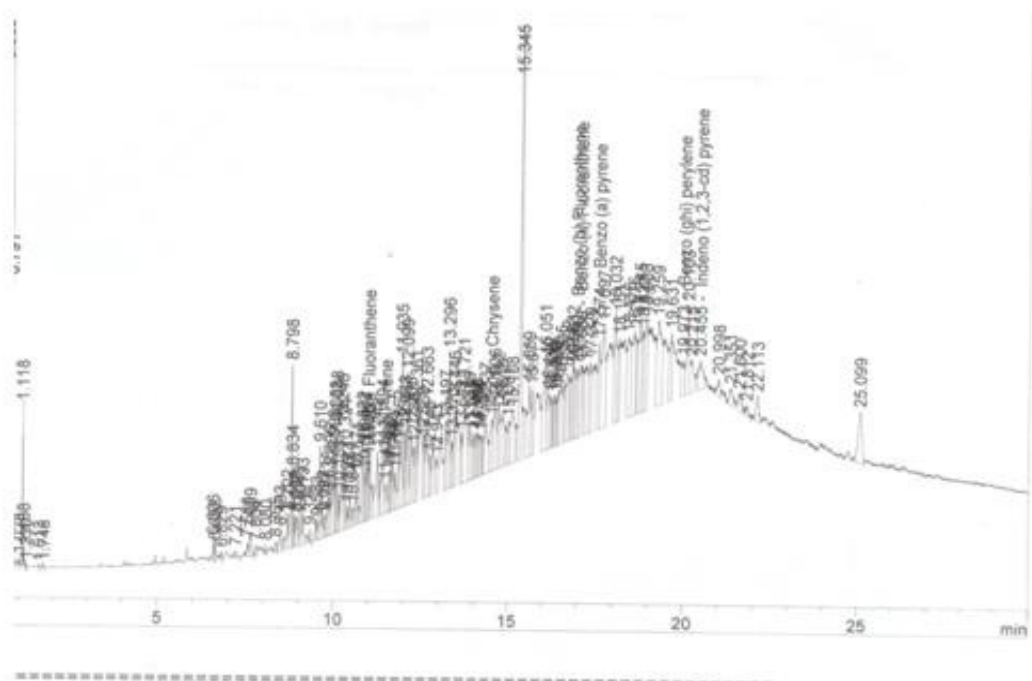
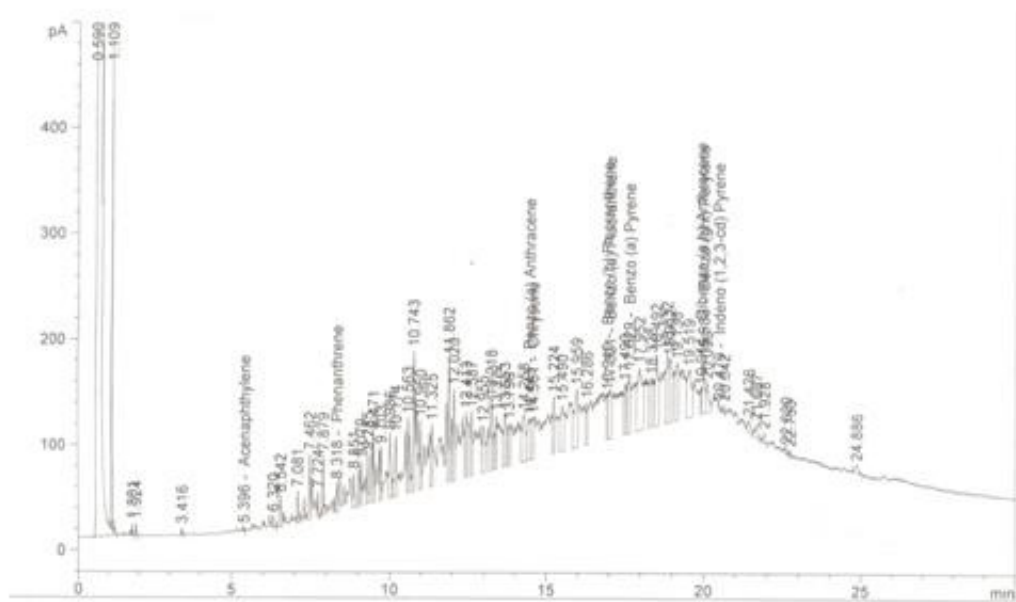
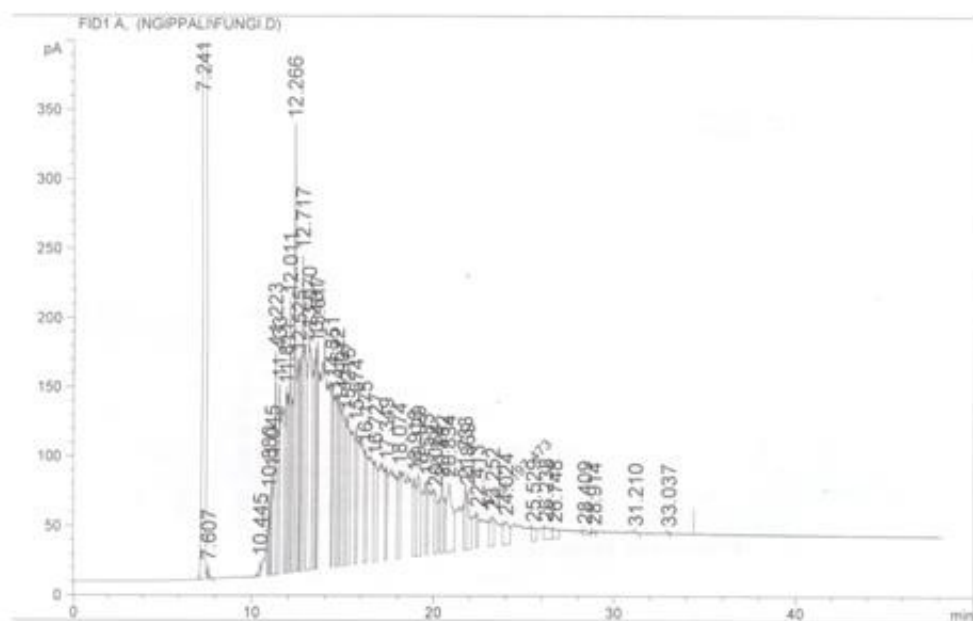


Figure 2b. Chromatogram of *Pleurotus tuber-regium* at 15 weeks: aliphatic hydrocarbon, upper plot; polyaromatic hydrocarbon, lower plot



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

