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Comparative Studies on Biodegradative Abilities of *Pleurotus ostreatus* and *P. pulmonarius* in Soils Contaminated with Crude and Used Engine Oils

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Abstract

Crude and used engine oil degrading ability of two white rot fungi *Pleurotus ostreatus* and *P. pulmonarius* were investigated for six months. One hundred grams of sterilized soil moistened with 75% distilled water (w/v) were weighed into 9 × 9 × 4 cm (350 cm^3) jam bottles and mixed thoroughly with bonny light crude oil and used engine oil at different concentrations (0%, 5%, 10%, 15%, 25% and 30%), separately. Each bottle was then inoculated with two agar plugs of a vigorously grown mycelium of *P. ostreatus* and *P. pulmonarius* using a sterile cork borer. The bottles were incubated at room temperature for 6 months. The mycelia-ramified waste was separated from soils and analysed for physicochemical parameters such as organic matter, carbon, nitrogen, phosphorus, potassium, pH and total hydrocarbon content, (THC) after drying. The organic carbon, nitrogen and phosphorus contents in contaminated and inoculated soils were increased after six months. However, decrease in potassium, pH and THC occurred in these soils after the period of investigation. *P. ostreatus* reduced the initial THC to 8% and 9% in soils contaminated with 20% of crude and engine oils, respectively, which was lower than that of *P. pulmonarius*. The two white rot fungi could be exploited in bioremediation of soils contaminated with bonny light crude and used engine oils.

Keywords

White Rot Fungi, Biodegradation, Crude Oil, Used Engine Oil

1. Introduction

Crude oil is a major contaminant of soil and water in oil producing countries as a result of extraction and proc-

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essing of the oil [1]. Crude oil spills from pipelines and refineries cause damage to the environment. Improper disposal and handling of oily sludge contaminate soil and may pose a serious threat to groundwater. The contamination changes the physicochemical and biological properties of the soil because oil may be toxic to some soil microorganisms and plants [2].

Augmentation of contaminated soils with appropriate [1] inocula of microorganisms is a promising technique to enhance the biodegradation of hydrocarbons [3] [4]. White rot fungi (WRF) are increasingly being investigated and used in bioremediation, because of their ability to degrade an extremely diverse range of very persistent or toxic environmental pollutants [3]. Adenipekun and Isikhuemhen [5] reported that engine oil contaminated soil incubated with *Lentinus squarrosulus* resulted in an increase in nutrient contents and a high percentage degradation of total petroleum hydrocarbon after 90 days of incubation. The use of fungi is expected to be relatively economical as they can be grown on a number of inexpensive agricultural or forest wastes such as corncobs and sawdust. Moreover, their utilization is a gentle and non-aggressive approach. The application of bioremediation capabilities of indigenous organisms to clean up pollutants is viable and has economic values [6]. The present investigation was carried out to determine the biodegrading potentials of *P. ostreatus* and *P. pulmonarius* on bonny light crude oil and used engine oil contaminated soils.

2. Materials and Methods

2.1. Collection of Materials

Mushroom spawns of *P. ostreatus* and *P. pulmonarius* were obtained from the Department of Microbiology, University of Ibadan, Nigeria while bonny light crude oil and used engine oil were collected from Port Harcourt and a mechanic workshop along Federal University of Technology, Akure (FUTA) respectively. Soil samples were obtained from FUTA research farm while rice bran was purchased at Oba market, Akure.

2.2. Cultivation of *P. ostreatus* and *P. pulmonarius*

Twenty grams of pretreated rice bran were laid on the contaminated soil in each bottle, covered with aluminum foil and autoclaved at 121˚C for 15 minutes. The culture conditions were according to the method of Baldrian *et al.* [7] and modified as follows. A 100 g of sterilized soil moistened with 75% distilled water (w/v) were weighed into 9 × 9 × 4 cm (350 cm^3) jam bottles and then mixed thoroughly with bonny light crude oil and used engine oil concentrations (1%, 5%, 10%, 15%, 20%, 25% and 30%) separately. Each bottle was then inoculated with two agar plugs of a vigorously growing mycelium of *P. ostreatus* and *P. pulmonarius* using a 7 mm sterile cork borer. The bottles were incubated at room temperature for 3 - 6 months. For the control experiment, crude oil and used engine oil were not added to the soils but inoculated with the fungi. At 3 and 6 months after incubation, the mycelia-ramified waste was separated from the soils and analyzed for physicochemical parameters after air-drying. A completely randomized design experiment was used for biodegradation studies and each replicated three times.

2.3. Determination of Organic Carbon, Nitrogen, Phosphorus, Potassium and pH Bonny Light Crude Oil and Engine Oil Contaminated Soils after 3 and 6 Months

Organic matter, organic carbon, percentage nitrogen, phosphorus and potassium contents were determined following the method of the Association of Official Agricultural Chemists [8]. The soil pH was determined by mixing each soil sample with water in ratio 1:1 and the pH value determined with Jenway 3015 pH meter according to the procedure of Bates [9].

2.4. Determination of Total Petroleum Hydrocarbon Content of the Soil

Total petroleum hydrocarbon content of the soil before and after degradation was determined using a Fourier transform infrared spectrometry at the Global Environmental Consultants Laboratory Warri, Nigeria.

3. Results

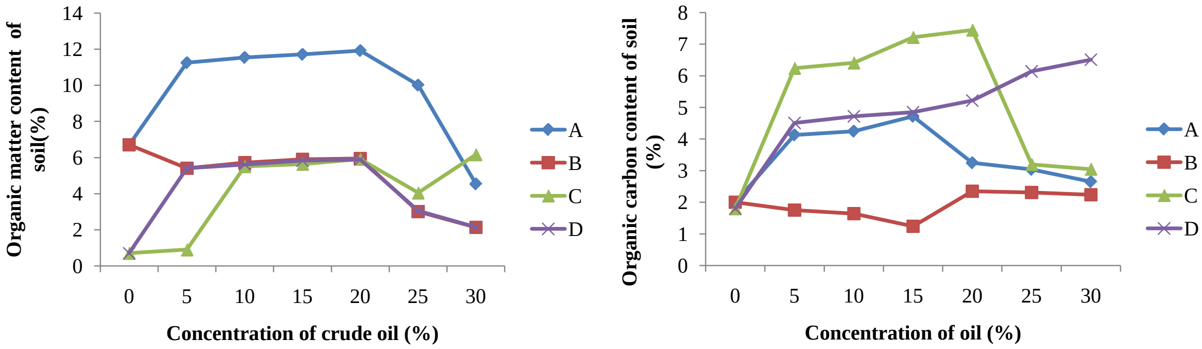
Effect of *Pleurotus ostreatus* and *P. pulmonarius* on Nutrient Analysis of Bonny Light Crude Oil and Engine Oil Contaminated Soils after 3 and 6 Months

Generally, the organic matter of soil contaminated with crude oil and inoculated with *P. ostreatus* was higher except at concentration 30% after three months (Figure 1(a)). It was also observed that the organic matter of soil contaminated with from zero to 20% concentration used engine oil and inoculated with *P. ostreatus* was higher at six months. Also, there was a progressive increase in the organic matter when *P. pulmonarius* was inoculated after six months (Figure 1(b)).

As the percentage of crude oil increased, there was increase in percentage carbon (% C) in soils inoculated with *P. ostreatus* after 3 and 6 months. Percentage carbon also increased in soil contaminated with engine oil and inoculated with *P. pulmonarius* after 6 months. However, there was slight decrease in % C of all the soils with *P. pulmonarius* when compared with *P. ostreatus* after 3 and 6 months of contamination with crude and engine oils (Figure 2(a) and Figure 2(b)).

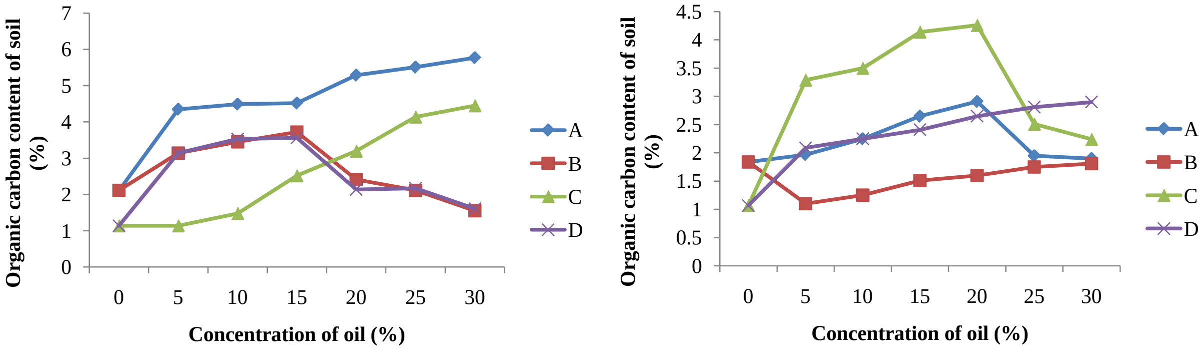
A general decrease was observed in percentage nitrogen (% N) as the concentration of crude oil applied increased in soils treated with *P. ostreatus* and *P. pulmonarius* after 3 months. The % N of soils treated with crude oil and inoculated with *P. ostreatus* was higher than that inoculated with *P. pulmonarius* after 3 and 6 months (Figure 3(a)). There was a progressive decrease in nitrogen content of soil with increase in concentration of used engine oil and inoculated with *P. ostreatus* whereas that of *P. pulmonarius* increased after three months from concentration 5 to 20. Also, the nitrogen content of soil inoculated with *P. pulmonarius* was higher than that of *P. ostreatus* after six months (Figure 3(b)).

Generally, there was increase in phosphorus content of soil samples contaminated with crude oil and inoculated with *P. ostreatus and P. pulmonarius* after the third and sixth months from concentrations 5% to 20% although the phosphorus content of *P. ostreatus* inoculated soil was higher (Figure 4(a)). Similar trend was ob-



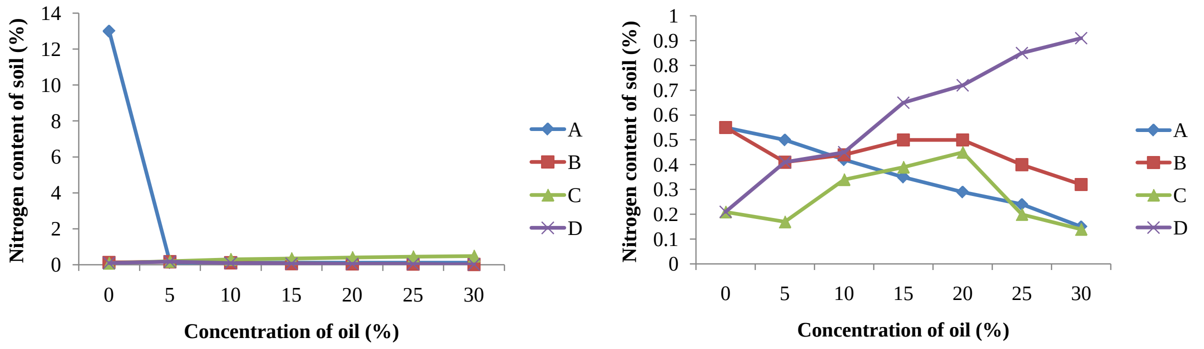
(a) (b)

Figure 1. (a) Percentage organic matter content of soil contaminated with crude oil and inoculated with *Pleurotus* species; (b) Percentage organic matter content of soil contaminated with engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated with *P. pulmonarius* after 3 months. C: Soil inoculated with *P. ostreatus* after 6 months. D: Soil inoculated with *P. pulmonarius* after 6 months.



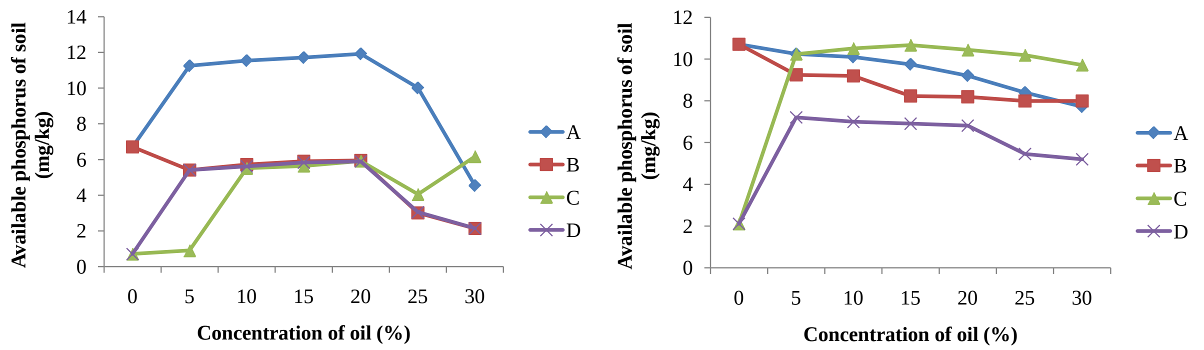
(a) (b)

Figure 2. (a) Percentage carbon content of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Percentage carbon content of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated with *P. pulmonarius* after 3 months. C: Soil inoculated with *P. ostreatus* after 6 months. D: Soil inoculated with *P. pulmonarius* after 6 months.



(a) (b)

Figure 3. (a) Percentage nitrogen content of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Percentage nitrogen content of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated with *P. pulmonarius* after 3 months. C: Soil inoculated with *P. ostreatus* after 6 months. D: Soil inoculated with *P. pulmonarius* after 6 months.



(a) (b)

Figure 4. (a) Available phosphorus (mg/kg) of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Available phosphorus (mg/kg) of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated with *P. pulmonarius* after 3 months. C: Soil inoculated with *P. ostreatus* after 6 months. D: Soil inoculated with *P. pulmonarius* after 6 months.

served in soil treated with engine oil and inoculated with *P. ostreatus* and *P. pulmonarius* after 6 months (**Figure 4(b)**).

The potassium content of soils contaminated with crude oil and inoculated with *P. pulmonarius* was higher than that of *P. ostreatus* up to concentration 25% after the third month. However, potassium content of crude oil contaminated soil was higher when *P. ostreatus* was inoculated after six months although there was decrease in potassium content with increase in concentration of crude oil (**Figure 5(a)**). The potassium content of soil contaminated with used engine oil increased when *P. ostreatus* was inoculated after 3 months (**Figure 5(b)**).

There was slight increase in pH values of almost all the soils contaminated with crude oil after inoculation with both *Pleurotus* species at three months although the pH values of *P. ostreatus* inoculated soils were higher. However, the pH values of *P. pulmonarius* inoculated soils were higher than that of *P. ostreatus* from 25% to 30% of crude oil after six months (**Figure 6(a)**). The pH values of *P. ostreatus* inoculated soils was higher than that of *P. pulmonarius* from 10% to 20% of used engine oil as shown in **Figure 6(b)**.

In soils treated with 20% crude oil and inoculated with *P. ostreatus*, the total petroleum content (TPC) reduced from 165,724 to 14,581 during 0 to 6 months. Similar reduction of TPC from 74,816 to 6972 (about 9% of initial TPC) was observed after treatment with 20% engine oil and inoculation with *P. ostreatus* (**Table 1**).

4. Discussion

Bioremediation is a cheap and easy method to reduce oily sludge contamination. This approach does not require the soil to be moved from its site, thus reducing any chance of secondary contamination. Fungi show tremend-

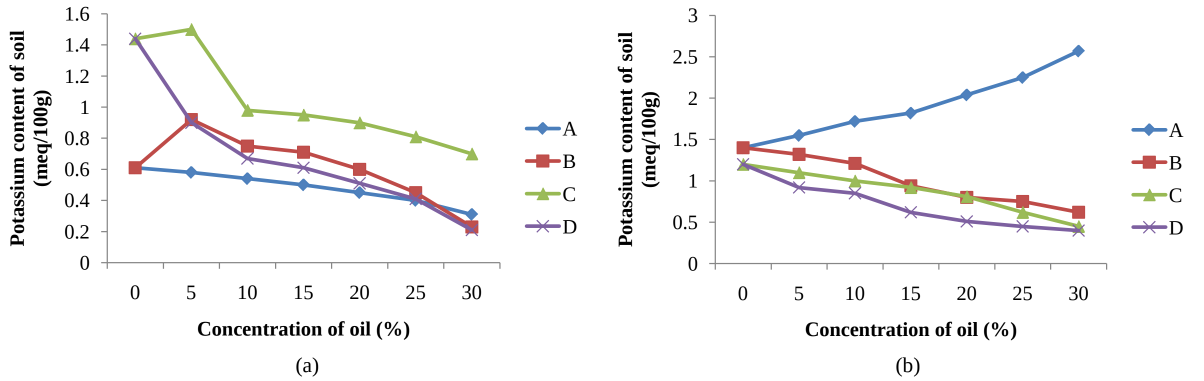


Figure 5. (a) Potassium content (meq 100 g) of soils contaminated with oils and inoculated with *Pleurotus* species; (b) Potassium content (meq 100 g) of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated with *P. pulmonarius* after 3 months. C: Soil inoculated with *P. ostreatus* after 6 months. D: Soil inoculated with *P. pulmonarius* after 6 months.

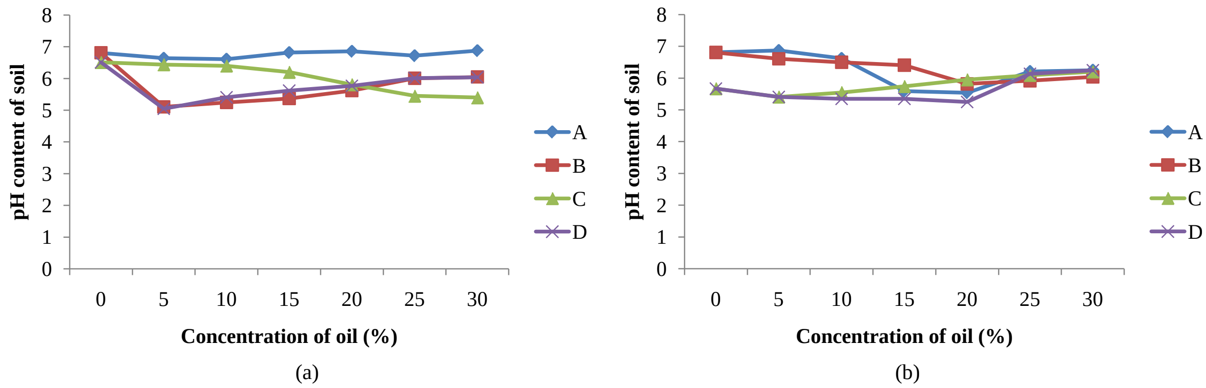


Figure 6. (a) Hydrogen ion concentration (pH) of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Hydrogen ion concentration (pH) of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated with *P. pulmonarius* after 3 months. C: Soil inoculated with *P. ostreatus* after 6 months. D: Soil inoculated with *P. pulmonarius* after 6 months.

Table 1. Total petroleum contents of soils contaminated with oils and inoculated with *Pleurotus* species.

% of oil applied	Treated soils with crude oil						Treated soils with engine oil					
	A	B	C	D	E	F	A	B	C	D	E	F
5	45,721	34,254	21,257	102,275	6724	4211	20,376	17,750	14,534	8712	3251	1648
10	91,254	72,325	56,871	40,825	9841	6524	35,813	20,256	21,964	10,282	5654	2941
15	120,522	90,521	104,624	62,524	12,628	9504	55,704	28,372	38,842	14,164	6263	3541
20	16,572	10,4256	121,257	84,625	14,581	11,871	74,816	35,862	46,420	20,724	6972	5821
25	102,549	95,271	115,282	60,561	11,251	9684	48,753	19,328	35,568	15,821	4826	3456
30	74,724	40,582	91,861	31,784	8214	7252	12,462	10,156	14,822	8244	2481	1828

A: Soil inoculated with *P. ostreatus* at 0 month. B: Soil inoculated with *P. pulmonarius* at 0 month. C: Soil inoculated with *P. ostreatus* after 3 months. D: Soil inoculated with *P. pulmonarius* after 3 months. E: Soil inoculated with *P. ostreatus* after 6 months. F: Soil inoculated with *P. pulmonarius* after 6 months.

ous diversity and adaptability in utilization of different organic molecule as a carbon source; however their abilities to degrade a specific hydrocarbon as a source of energy and/or biomass may differ [4]. Many studies have reported the use of *Pleurotus* species in bioremediation exercises [7]. Ishikuehmen *et al*. [3] showed that bioremediation of soil contaminated with crude oil was possible, especially when the fungus had been allowed to establish and fully colonize the substrate mixed with the soil.

In this study *P. ostreatus* inoculated on crude oil and used engine oil contaminated soil showed higher nutrient distribution of organic matter, carbon and phosphorus compared to the control with increase in crude oil concentration after 3 and 6 months. A lower value of phosphorus and potassium was observed with increase in crude oil concentration. Aislabie *et al*. [10] reported a low value of nitrogen, potassium, phosphorus reserves in petroleum hydrocarbon contamination. From this study, a reduction in nutrient contents of contaminated soils after introduction of the white-rot fungi was observed at higher levels of 10% - 20% crude oil concentration compared to lower levels of contaminated oil. This is similar to the findings of Isikhuemhen *et al*. [3]. Leahy and Colwell [11] also reported that very high concentrations of hydrocarbon will inhibit biodegradation by nutrient or oxygen limitation. Adenipekun and Fasidi [12] also reported the ability of *Lentinus subnudus* to mineralize soil contaminated with various concentrations of crude oil. They found that nutrient contents were generating higher after 6 months of incubation except potassium levels which were not significantly different from the control. Leahy and Colwell [11] stated that pH is a predominant factor in determining biodegradation in soil. In this work the pH of contaminated soils reduced after the introduction of the white-rot fungi from 6.81 to 6.01 and finally to 5.05 after 3 and 6 months of incubation, respectively. This is similar to the findings of [13] where they observed a decrease from 7.55 to 7.11 for cement polluted soil after 2 months of incubation. This is also in agreement with the report of Benneth *et al*. [14].

This study revealed that the total petroleum hydrocarbon values were higher on contaminated soils compared to the control suggesting the presence of more petroleum hydrocarbon. Ogbo and Okhuoya [15] reported similar effect in soil in mangrove and rain forest soils polluted with crude oil. The best rate of biodegradation of crude oil by *P. ostreatus* was after 3 months as similar to the findings of Pointing [16] who reported that incubation for biodegradation takes several months. A decrease in TPH was recorded from 3 to 6 months and this agrees with the observation of Sorkoh *et al*. [17]. The high rate of hydrocarbon degradation by *Pleurotus* species could have emanated from their massive growth and enzyme production responses during their growth phases as observed by George-Okafor *et al*. [18] while working with filamentous fungi. This could also be supported by the reports of Bogan and Lamar [19] which showed that extracellular lignolytic enzymes of white rot fungi are produced in response to their growth phases.

5. Conclusion and Recommendation

Pleurotus ostreatus and *P. pulmonarius* can be employed in decontaminating environment polluted with crude oil and used engine oil. Further research is, however, advocated in investigating the toxicity of the *Pleurotus ostreatus* and *P. pulmonarius* cultivated on bonnylight crude and engine oil contaminated soils before consumption.

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