Applied Science and Biotechnology Journal for Advanced Research e-ISSN: 2583-553X
Peer Reviewed Journal
Volume-2, Issue-2 (March 2023), Page: 13-17 https://doi.org/10.31033/abjar.2.2.3

Effect of Crude Engine Oil and Aromatic Fractions of Pleurotus Pulmonarius Fries (Quelet)

I.O. Adenipekun[1] and C.O. Fasidi[2]
[1]Research Scholar, Department of Microbiologym, Federal University of Technology, Akure, NIGERIA
[2]Lecturer, Department of Microbiology, Federal University of Technology, Akure, NIGERIA

[1]Corresponding Author: adenipekun45@gmail.com

Received: 05-02-2023 Revised: 22-02-2023 Accepted: 17-03-2023

ABSTRACT
The ability of two white rot fungi, Pleurotus ostreatus and P. pulmonarius, to degrade crude and used engine oil was examined for six months. In 9 9 4 cm (350 cm3) jam bottles, 100 grams of sterilized soil were weighed and wet with 75% distilled water (w/v). They were then completely mixed with bonny light crude oil and used motor oil at various concentrations (0%, 5%, 10%, 15%, 25%, and 30%), individually. Then, using a sterile cork borer, two agar plugs of a strongly growing P. ostreatus and P. pulmonarius mycelium were inoculated into each bottle. For six months, the bottles were kept at room temperature. After drying, the mycelia-ramified waste was removed from the soil and examined for physicochemical characteristics such total hydrocarbon content (THC), organic matter, carbon, nitrogen, phosphorus, and potassium. After six months, both contaminated and inoculated soils had higher levels of organic carbon, nitrogen, and phosphorus. However, during the experiment period, these soils had a drop in THC, pH, and potassium. In soils contaminated with 20% of crude and engine oils, respectively, P. ostreatus lowered the initial THC to 8% and 9%, which was less than P. pulmonarius. The two white rot fungus may be used in the bioremediation of soils contaminated with old motor oil and bonny light crude.

Keywords: used engine oil, white rot fungi, and biodegradation

I. INTRODUCTION

As a result of the extraction and processing of the oil, crude oil is a significant pollutant of soil and water in nations that produce oil [1]. Environment harm results from crude oil leaks from refineries and pipelines. Oily sludge that is handled and disposed of improperly contaminates land and may seriously endanger groundwater. Because oil may be hazardous to some soil microorganisms and plants, pollution alters the soil's physicochemical and biological characteristics [2]. A possible method to speed up the biodegradation of hydrocarbons is to supplement polluted soils with the proper [1] inocula of microorganisms. Due to their capacity to breakdown an incredibly wide spectrum of extremely persistent or harmful environmental contaminants, white rot fungi (WRF) are being studied and utilized in bioremediation more and more [3]. After 90 days of incubation, Adenipekun and Isikhuemhen found that motor oil-contaminated soil incubated with Lentinus squarrosulus increased its nutritional content and significantly degraded the total petroleum hydrocarbon. Fungi may be grown on a variety of cheap agricultural or forest wastes, including sawdust and corncobs, therefore their use is anticipated to be quite economical. Additionally, using them is a mild and non-aggressive method. It is practical and beneficial economically to use native species' bioremediation ability to remove contaminants. The goal of the current experiment was to evaluate the biodegradation capacity of P. ostreatus and P. pulmonarius on soils polluted with bonny light crude oil and used motor oil.

II. RESOURCES AND PROCEDURES

2.1. Materials Gathering

The Department of Microbiology at the University of Ibadan in Nigeria provided the P. ostreatus and P. pulmonarius mushroom spawns, and Port Harcourt and a mechanic shop near the Federal University of Technology, Akure (FUTA) provided the bonny light crude oil and used engine oil, respectively. At the FUTA research farm, soil samples were collected, and rice bran was acquired at the Oba market in Akure.

2.2. P. Otreatus and P. Pulmonarius Cultivation

Each bottle included 20 grams of pretreatment rice bran, which was then coated in aluminum foil and autoclaved at 121 °C for 15 minutes. The following modifications were made to the culture conditions using the Baldrian et al. technique. 100 g of sterilized soil were weighed into jam bottles of 9 x 9 x 4 cm (350 cm3), moistened with 75% distilled water (w/v), and thoroughly combined with concentrations of bonny light crude oil and used engine oil (1%, 5%, 10%, 15%, 20%, 25%, and 30%, respectively). Then, using a 7 mm sterile cork borer, two agar plugs of a P. ostreatus and P. pulmonarius mycelium that was actively growing were inoculated into each bottle. For three to six months, the bottles were incubated at room temperature. In the control experiment, spent motor oil and crude oil were inoculated with fungi rather than being added to the soil. The mycelia-ramified waste was removed from the soils 3 and 6 months after incubation, and after air-drying, physicochemical properties were assessed. For the biodegradation studies, a completely randomized design experiment was employed, and each was reproduced three times.

2.3. Analyzing the Organic Carbon

The Association of Official Agricultural Chemists' technique was used to calculate the amounts of organic matter, organic carbon, percentage nitrogen, phosphorus, and potassium. Each soil sample was mixed 1:1 with water to determine the pH, which was then measured using a Jenway 3015 pH meter in accordance with Bates' method.

2.4. Counting the Total Amount of Petroleum

At the Global Environmental Consultants Laboratory in Warri, Nigeria, Fourier transform infrared spectroscopy was used to compare the total petroleum hydrocarbon content of the soil before and after deterioration.

III. RESULTS

Except at a concentration of 30% after three months, the organic matter of soil contaminated with crude oil and inoculated with P. ostreatus was generally higher (Figure 1(a)). Additionally, it was found that at six months, the organic matter of soil inoculated with P. ostreatus and contaminated with old motor oil at a concentration of 0% to 20% was greater. When P. pulmonarius was injected after six months, the organic matter gradually increased as well (Figure 1(b). In soils inoculated with P. ostreatus after 3 and 6 months, there was an increase in percentage carbon (% C) as the percentage of crude oil increased. After six months, soil exposed to engine oil contamination and P. pulmonarius vaccination saw an increase in the percentage of carbon. However, following 3 and 6 months of contamination with crude and engine oils, there was a modest decrease in% C of all the soils with P. pulmonarius when compared with P. ostreatus (Figures 2(a) and 2(b). After 3 months, it was discovered that the proportion of nitrogen (% N) in soils treated with P. ostreatus and P. pulmonarius generally decreased as the amount of crude oil applied increased. After 3 and 6 months, the percentage of N in soils treated with crude oil and inoculated with P. ostreatus was higher than that in soils inoculated with P. pulmonarius (Figure 3(a). When utilized engine oil concentration grew and the soil was inoculated with P. ostreatus, the nitrogen content of the soil gradually decreased, whereas P. pulmonarius increased after three months from concentration 5 to 20. Additionally, after six months, P. pulmonarius-inoculated soil had a higher nitrogen content than P. ostreatus (Figure 3(b).

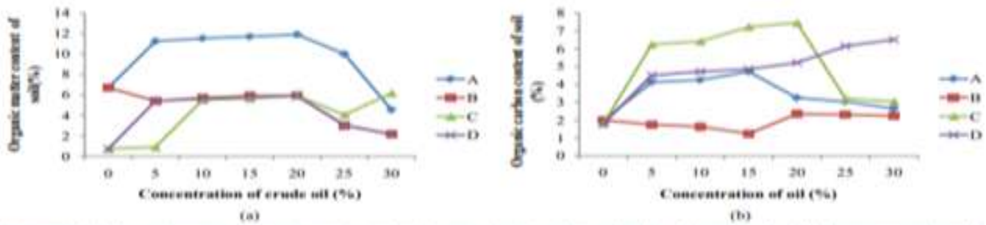

Figure 1. (a) Percentage organic matter content of soil contaminated with crude oil and inoculated with *Pleurotus* species; (b) Percentage organic matter content of soil contaminated with engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil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

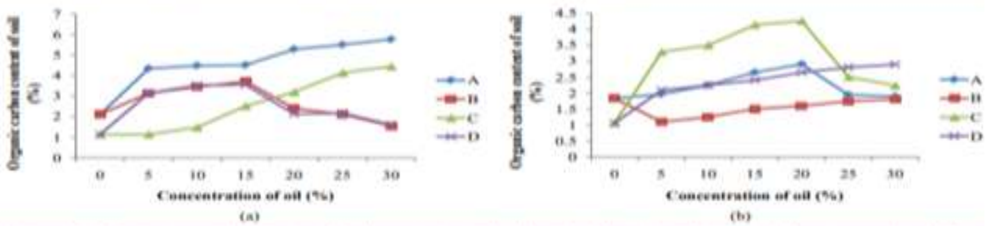

Figure 2. (a) Percentage carbon content of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Percentage carbon content of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated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

Image 1. (a) The percentage of organic matter in soil that has been inoculated with Pleurotus species and polluted with crude oil; (b) The percentage of organic matter in soil that has been inoculated with Pleurotus species and contaminated with engine oil. A: After three months, P. ostreatus was injected into the soil. B: After three months, P. pulmonarius is injected into the soil. C: After six months, P. ostreatus was injected into the soil. D: After six months, P. pulmonarius was injected into the soil.

e-ISSN: 2583-553X
Peer Reviewed Journal
https://doi.org/10.31033/abjar.2.2.3

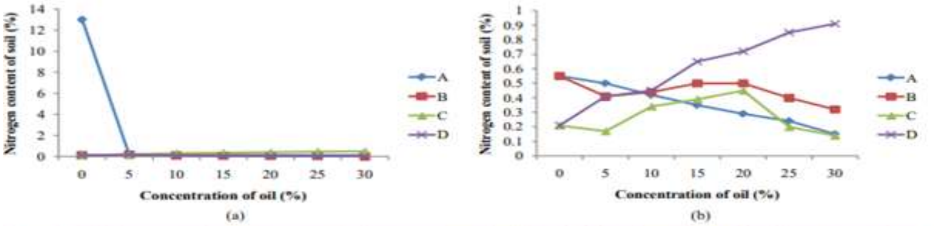

Figure 3. (a) Percentage nitrogen content of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Percentage nitrogen content of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated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

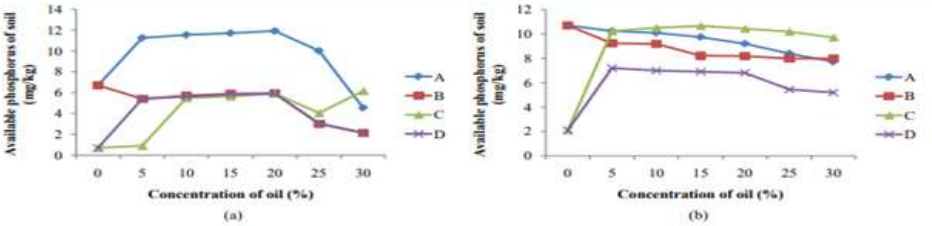

Figure 4. (a) Available phosphorus (mg/kg) of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Available phosphorus (mg/kg) of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil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

Image 2. (a) The percentage of carbon in soils that have been inoculated with Pleurotus species and are contaminated with crude oil; (b) the percentage of carbon in soils that have been inoculated with Pleurotus species and are polluted with used motor oil. A: After three months, P. ostreatus was injected into the soil. B: After three months, P. pulmonarius is injected into the soil. C: After six months, P. ostreatus was injected into the soil. D: After six months, P. pulmonarius was injected into the soil.

In general, the phosphorus content of soil samples contaminated with crude oil and inoculated with P. ostreatus and P. pulmonarius increased from concentrations of 5% to 20% after the third and sixth months (Figure 4(a). However, the phosphorus content of P. ostreatus inoculated soil was greater. A comparable pattern was observed in soil that had been exposed to motor oil and had been injected with P. ostreatus and P. pulmonarius six months later (Figure 4(b). After the third month, the potassium content of soils infected with P. pulmonarius and polluted with crude oil was 25% greater than that of P. ostreatus. Although there was a decrease in potassium content with an increase in crude oil concentration, the potassium content of the soil contaminated with crude oil was higher when P. ostreatus was inoculated after six months (Figure 5(a).

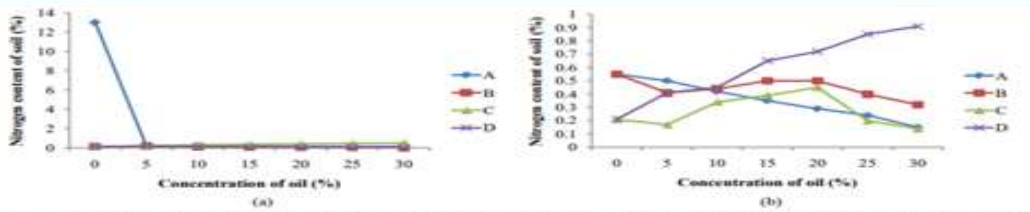

Figure 3. (a) Percentage nitrogen content of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Percentage nitrogen content of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil inoculated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

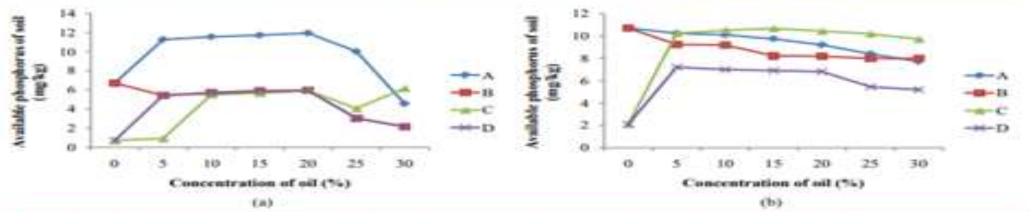

Figure 4. (a) Available phosphorus (mg/kg) of soils contaminated with crude oil and inoculated with *Pleurotus* species; (b) Available phosphorus (mg/kg) of soils contaminated with used engine oil and inoculated with *Pleurotus* species. A: Soil inoculated with *P. ostreatus* after 3 months. B: Soil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

Image 3. (a) The percentage of nitrogen in soils that have been inoculated with Pleurotus species and are contaminated with crude oil; (b) the percentage of nitrogen in soils that have been inoculated with Pleurotus species and are polluted with used motor oil. A: After three months, P. ostreatus was injected into the soil. B: After three months, P. pulmonarius is injected into the soil. C: After six months, P. ostreatus was injected into the soil. D: Six months after P. pulmonarius inoculation of the soil

After 3 months, P. ostreatus was inoculated into used motor oil-contaminated soil, and the soil's potassium content rose (Figure 5(b)). At three months after being infected with both Pleurotus species, the pH values of nearly all of the soils polluted with crude oil had somewhat increased, even though the pH values of the P. ostreatus inoculated soils were greater. However, after six months, the pH values of soils infected with P. pulmonarius were higher than those of P. ostreatus by 25% to 30% (Figure 6(a)). Figure 6 illustrates how pH values of soils inoculated with P. ostreatus were higher than those of soils inoculated with P. pulmonarius from 10% to 20% of used motor oil.(b). The total petroleum content (TPC) decreased from 165,724 to 14,581 in soils treated with 20% crude oil and injected with P. ostreatus throughout the course of 0 to 6 months. Similar TPC reduction (from 74,816 to 6972, or around 9% of initial TPC) was seen following treatment with 20% engine oil and P. ostreatus inoculation. (Table 1).

IV. DISCUSSION

Oily sludge contamination can be reduced easily and inexpensively through bioremediation. With this strategy, there is less danger of secondary contamination because the soil does not need to be removed from its location. Fungi are incredibly diverse and adaptable when it comes to using different organic molecules as carbon sources, yet their capacity to break down a given hydrocarbon into energy and/or biomass may vary [4]. The utilization of Pleurotus species in bioremediation processes has been documented in numerous research [7]. The bioremediation of crude oil-contaminated soil was demonstrated by Ishikuehmen et al. [3], particularly when the fungus had been given time to establish and completely colonize the substrate mixed with the soil. After three and six months, P. ostreatus inoculated on soil polluted with crude oil and used engine oil displayed higher nutritional distribution of organic matter, carbon, and phosphorus compared to the control with rising crude oil concentration. Potassium and phosphorus values decreased as crude oil concentration increased. Low levels of nitrogen, potassium, and phosphorus reserves were found in petroleum hydrocarbon pollution, according to Aislabie et al. [10]. According to this study, larger concentrations of contaminated oil—between 10% and 20%—were associated with reduced nutritional contents in contaminated soils when the white-rot fungus were introduced. This is in line with the conclusions reached by Isikhuemhen et al. [3]. Additionally, Leahy and Colwell [11] observed that very high hydrocarbon concentrations will prevent biodegradation caused by nutritional or oxygen deficiency. The ability of Lentinus subnudus to mineralize soil contaminated with varied quantities of crude oil was previously documented by Adenipekun and Fasidi [12]. Except for potassium levels, which were not statistically different from the control after 6 months of incubation, they discovered that nutritional contents were increasing. According to Leahy and Colwell [11], pH is a key element in regulating biodegradation in soil. In this study, the pH of polluted soils decreased from 6.81 to 6.01 and eventually to 5.05 after the white-rot fungi were introduced, respectively, after 3 and 6 months of incubation. This is comparable to the findings of [13], who found that after two months of incubation, cement-polluted soil decreased from 7.55 to 7.11. This is also consistent with the findings of Benneth et al.'s paper [14].

Table 1: Total petroleum content of oil-contaminated and Pleurotus

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. pul-*

According to this study, the total petroleum hydrocarbon values on contaminated soils were higher than they were on the controls, indicating the presence of more petroleum hydrocarbon. Similar effects on soil in mangrove and rain forest soils polluted with crude oil were found by Ogbo and Okhuoya [15]. Similar to the findings of Pointing [16], who stated that incubation for biodegradation takes several months, P. ostreatus showed the greatest rate of biodegradation of crude oil after 3 months. TPH decreased between three and six months, which is consistent with the finding made by Sorkoh et al. According to George-Okafor et al. who studied filamentous fungus, the rapid rate of hydrocarbon breakdown by Pleurotus species may have resulted from their huge growth and enzyme production responses throughout their growth phases. The studies of Bogan and Lamar, which demonstrated that extracellular lignolytic enzymes of white rot fungi are produced in response to their growth stages, may further lend credence to this.

V. CONCLUSION

It is possible to use Pleurotus ostreatus and P. pulmonarius to clean up environments that have been contaminated by spent motor oil and crude oil. However, more study is encouraged to determine the toxicity of Pleurotus ostreatus and P. pulmonarius grown on motor oil- and bonnylight-contaminated soils before ingestion.

.

REFERENCES

1. Adenipekun, C.O, & Lawal, Y. (2011). Mycoremediation of crude oil and palm kernel contaminated soils by pleurotus pulmonarius fries (Quelet). *Nature and Science*, *9*, 125-131.
2. Minai-Tehrani, D, & Herfatmanesh. A. (2007). Biodegradation of aliphatic and aromatic fractions of heavy crude oil-contaminated soil: A pilot study. *Bioremediation Journal*, *11*, 71-76.
3. Bates, R.A. (1954). *Electrometric determination*. New York: John Wiley Sons, Inc.
4. Leahy, J.G, & Colwell, R.R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews*, *54*, 305-315.
5. Bennet, J.W., Wunch, K.G, & Faison, B.D. (2002). Use of fungi in biodegradation. In: *Hurst, C.J., Ed., Manual of Environmental Microbiology*. Washington DC: ASM Press, pp. 960-971.