Bioaccumulation of Heavy metals and Nutrient content Supplementation by two White rot fungi in Crude oil polluted soils.

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Abstract: Crude oil polluted soils of different concentrations (0%,1%,5%,10%,20%,30% and 40%) were inoculated and incubated with *P.pulmonarius* and *P.ostreatus* for 0,1 and 2 months to study and compare their effect in the bioremediation of crude oil and bioaccumulation of heavy metals from polluted soils. Nutrient contents of the soil were determined on monthly basis. Also, the heavy metals accumulation by the fungi from the polluted soil was monitored. Results showed that both white rot fungi were able to biodegrade and ameliorate the soils by increasing the nutrient contents as the concentration of the crude oil in the soils increases with increase in incubation period. Highest increase in concentration of these nutrient contents were obtained at 40% crude oil contaminated soils after 2 months incubation period. Organic matter was the highest nutrient content recorded followed by organic carbon, potassium, nitrogen, and phosphorus in decreasing order. *P.pulmonarius* had the highest nutrient content than *P.ostreatus* having 32.60% and 30.46% organic matter; 18.91% and 17.67% organic carbon, 1.96% and 1.85% nitrogen; 13.50mg/kg and 12.60mg/kg phosphorus and 1.91cmol/kg and 1.70cmol/kg potassium respectively. The pH values reduced for both white rot fungi after inoculation into crude oil contaminated soils. *P.pulmonarius* had the highest pH values of 6.50 and 6.40 at 0% crude oil polluted soil for 1 and 2 months and lowest pH values of 4.70 and 4.80 at 40% and 10% crude oil contaminated soils. While *P.ostreatus* had the highest value of 5.97 and 6.17 at 0% and lowest pH of 4.63 and 5.27 at 30% and 40% crude oil contaminated soils. Heavy metal accumulation increases as the concentration of crude oil increased and decreased as the incubation period increased from 1 to 2 months for both fungi. Iron (1.26mgkg^{-1} at 40% crude oil concentration) was the highest heavy metal accumulated by *P.pulmonarius* after one month while copper (2.28mgkg^{-1} at 40% crude oil concentration) was the highest heavy metal accumulated by *P.ostreatus* for the same period. Nickel was the least heavy metal accumulated. The result obtained showed the ability of *P.pulmonarius* and *P.ostreatus* to bioremediate a hydrocarbon and heavy metal polluted soil.

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Introduction

There is growing public concerns as a wide variety of toxic organic chemicals area being introduced in advertently or deliberately into the environment. Petroleum hydrocarbons are one common example of these chemicals which enter the environment frequently and in large volumes through numerous routes (Balba *et al.*, 1998). Most often, the crude oil, major petroleum hydrocarbon is released to the environment as a result of spillage which might be due to accident, exploration or sabotage. As a result of this, the petroleum industry is responsible for the generation of high amount of organic residue as well as for the pollution of soils, rivers and seas.

In Nigeria, the exploration practices and the breaking of oil pipes, led to incessant pollution especially in the Niger Delta area and southern part of Nigeria (Salu, 1999). These spills have the largest immediate and economic impact as they cause harmful effects to a large extent to the ecosystem more than just the isolated location (George-Okafor *et al.*, 2009). These spills also brought about heavy metal contamination of the soil. Presence of heavy metals in soil, air, water and living object is a severe public health concern and due to detrimental effect of them on man and environment, their removal is deemed important to the protection of environmental health (Das, 2005). Despite its toxicity to the environment, crude oil could be degraded and heavy metals could be bioaccumulated from the environment.

Different methods of breaking down and cleaning environment of oil spill abound, but the most preferred approach which is environment friendly is the biological method. Microbial degradation is the major mechanism for the elimination of spilled oil from the environment (Ibe and Ibe, 1984; Atlas, 1995). This method is known as bioremediation. Bioremediation offers a promising means to reclaims, such contaminated soil (Bartha, 1986; Bragg *et al*, 1994) as well as bioaccumulate heavy metals. By definition,

bioremediation is the use of living organisms, primarily microorganisms to degrade the environmental contaminant into less toxic form (Vidali, 2001). Bioremediation involves the transformation of complex or simple chemical compounds into non-hazardous forms by biological agents resulting in materials of higher nutritive value or simply reducing the final bulk of the product (Grady, 1985). It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment (Vidali, 2001). The recognition of oil as a complex but largely biodegradable mixture of hydrocarbons and the knowledge that hydrocarbon degraders can be enriched in many, if not most, types of environment (Atlas, 1981) have contributed greatly to the development of the oil bioremediation techniques (Margesin and Schinner, 1997). Of these microorganisms, fungi have been found to be better degraders of petroleum than traditional bioremediation techniques, including bacteria (Batelle, 2000).

The filamentous fungi possess some attributes that enable them to act as good potential agents of degradation by ramifying the substratum and digesting it through the secretion of extracellular enzymes which are non specific. The branching filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil (Hamman, 2004). 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 (George-Okafor *et al*, 2009). Of all the fungi species, the white rot fungi have been used for bioaccumulation of heavy metals, biodegrading of toxic organic compounds and mineralizing or nitrifying of contaminated soils. The white rot fungi so far used for bioremediation include *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Pleurotus tuber-regium*, *Lentinus squariosulus* etc. The biodegradation of crude oil and other organic petroleum products by white rot fungi to improve the nutrient composition of the contaminated soil and bioaccumulation of heavy metals have been reported from different research findings (Adenipekun, 2008; Adenipekun and Omoruyi; 2008; Adenipekun and Fasidi, 2005; Isikhuemhen *et al*., 2003; Ogbo and Okhuoya, 2009; Ogbo, 2006). Much work has not been done to compare different species of white rot fungi or white rot fungi of the same genus but different species to know their capabilities of their level of heavy metal accumulation and nutrient supplementation on the breakdown of crude oil in the contaminated soil. Therefore, .the objective of the present work was to compare the bioaccumulation level of heavy metals and nutrient supplementation on crude oil contaminated soil by *Pleurotus pulmonarius* and *Pleurotus ostreatus*.

Materials and Methods

Sample Location and Collection

Soil: - Soil samples used for this experiment were collected from the Botanical Nursery of the Department of Botany and Microbiology, University of Ibadan at a depth of 1-8cm, air dried and sieved with a 2mm mesh. The soil was taken to the Plant Physiology Laboratory in polythene bags for use.

Fungi: The pure culture of *Pleurotus pulmonarius* was collected from Pathology Laboratory of Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan while the pure culture of *Pleurotus ostreatus* was collected from Plant Physiology Laboratory of Department of Botany and Microbiology, University of Ibadan. Pure spawns of the two fungi were prepared according to the methods of Jonathan and Fasidi (2001).

Rice Straw: Freshly harvested rice straw was collected from Africa Rice farm located at the International Institute of Tropical Agriculture (IITA) Ibadan; sun-dried to remove moisture content in the straw and to prevent decomposition. The straw was cut into 0.1-2cm size with a guillotine.

Wheat Bran: Wheat bran was obtained from Kara, Bodija Market, Ibadan.

Crude Oil: Forcados blend crude oil was collected from one of the flow stations around Warri creek in Delta state from Shell Petroleum Development Company (SPDC).

Preparation of Pure Isolate Spawn: The spawn of the fungi were prepared according to the method of Jonathan and Fasidi (2001). Rice straw was soaked in water for one hour and then squeezed through a muslin cloth until no more water oozed out. Wheat bran was added as additive which was mixed thoroughly and put into 13x8x8cm ($350cm^3$) bottles covered with aluminum foil, autoclaved at 121°C for 15 minutes. After cooling, the bottles were divided into two parts and labeled based on the pure culture of fungi inoculated into them. Bottles were inoculated with pure cultures of *Pleurotus pulmonarius* for first part and pure cultures of *Pleurotus ostreatus* for the remaining bottles. All bottles were incubated at $28^0C \pm 2^0C$ for 3 weeks until the substrate was completely ramified.

Experimental Set Up for Culture Conditions: The culture conditions were according to the method of Adenipekun and Fasidi (2005). Two hundred grammes (200g) of soil were weighted into 13x8x8cm ($350cm^3$) bottles and then mixed thoroughly with different concentration of crude oil (1,5,10,20,30 and 40%). Forty grammes (40g) of moistened clean rice straw were laid on the contaminated soil in each bottle

separated with wire gauze and covered with aluminum foil. The bottles were then autoclaved at 121°C for 20 minutes. Two replicates for each experiment based on percentages of contaminated crude oil on the soil were prepared and labeled for the species of fungi used.

There were two sets of experimental setup. One set of bottles was inoculated with 10g of vigorously growing spawns of *Pleurotus pulmonarius* while the other set was inoculated with 10g of vigorously growing spawn of *Pleurotus ostreatus*. The bottles were incubated at room temperature for 1 and 2 months in an incubator. In the first set of control treatment, crude oil was not added to the soils while in the second set, different percentages of oil were added to the soils but not inoculated with the fungus.

At the end of the period of incubation, the mycelial-ramified substrate was carefully separated from the soil layer ensuring that soil particles did not mix with it. All contaminated soil samples were analyzed for physio chemical parameters after drying in an oven at 80 ^{0}C.

Nutrient Content Analysis

To determine the soil pH, 20g of air-dried soil sample were weighed into a 50ml, beaker. 20ml of distilled water was then added and it was allowed to stand for 30 minutes after which it was stirred occasionally with a glass rod. The glass electrode pH meter (pH meter model) was used in taking the readings (Bates, 1954).

Organic Carbon, Organic Matter, Percentage Nitrogen, Phosphorus and Potassium were determined using the methods of the Association of Analytical Chemists (A.O.A.C., 2003).

Heavy Metal Analysis of Soil Samples

After 1 and 2 months of incubation, the samples were dried in an oven at 35^{0}C for 48 hours to a constant weight, and then sieved through a 2mm sieve. Ten grammes of each soil sample were then weighed into an acid-washed 250ml polyethylene extraction bottle to which 100ml of extraction reagent (composition of the reagent) was added and shaken for 1 hour on a mechanical shaker, then filtered through Whatman No. 42 filter paper. Blanks were also prepared using the same procedures but without soil. Filtrates were then analyzed for each of the heavy metal (Pb, Mn, Ni, Fe, Cu and Zn) by flame atomic absorption spectrophotometer (Crosby, 1977).

Statistical Analysis:

A randomized factiorial experiment showing two white rot fungi, *Pleurotus pulmonarius* and *Pleurotus ostreatus*, contaminated soil samples with various percentages of crude oil were used while the incubated period were 0,1 and 2 months. An ANOVA table was prepared for each followed by the Duncan's multiple range test. The experiment was carried out in replicates of three.

Results

Table 1 shows the nutrient contents of crude oil contaminated soil incubated with *P.pulmonarius*. The organic matter, organic carbon, nitrogen, phosphorus and potassium increase as the concentration of the crude oil in the soil increases. The nutrient content also increases as the incubation period increases from 1 to 2 months. The nutrient contents were higher after 2 months of incubation than those incubated for 1 month. Organic matter, organic carbon, nitrogen, phosphorus and potassium were highest at 40% crude oil concentration in the soil (32.60%, 18.91%, 1.96%, 13.56mg/kg, 1.91cmol/kg) after 2 months incubation period. The nutrient content of the soil was lowest when there was no crude oil. From Table 1, nitrogen content reduced at 20% crude oil contaminated soil to 1.64% after 1 month incubation period. There were however, no changes in nitrogen content in the soil after 0 and 1 month incubation period for its control. The nutrient contents recorded after 2 months incubation period was higher than the one month incubation period. The pH values of the contaminated soil decreased from 6.50 and 6.40 in the control for both 1 and 2 months to 4.70 at 40% crude oil contaminated soil after 1 month incubation period and 4.80 at 10% crude oil contaminated soil after 2 months incubation . After 1 month incubation with *P.pulmonarius* the pH values decreased as the concentration of crude oil in the contaminated soil increases except at 10% crude oil contaminated soil where the pH increased to 5.47.

Table 2 shows the nutrient contents of crude oil contaminated soil incubated with *P.ostreatus*. The result of the nutrients content showed a similar trend with nutrient content of the soil incubated with *P.pulmonarius*. It showed that the nutrient contents of organic matter, organic carbon, nitrogen, phosphorus and potassium of the crude oil contaminated soil incubated with *P.pulmonarius* increased as the concentration of the crude oil in the soil increases. The nutrient content also increased as the incubation period increased. The organic matter, organic carbon, nitrogen, phosphorus and potassium had the highest value at 40% crude oil contamination (30.54%, 17.71%, 1.92%, 13.81mg/kg, 1.75cmol/kg) after 1 month incubation period except for nitrogen which had highest at 10% (1.92%) crude oil contamination. Highest value of the nutrient contents were also recorded after 2 months incubation. The lowest concentration of organic matter, organic carbon,

nitrogen, phosphorus and potassium were recorded at 0% (control) concentration (14.02%, 8.13%, 0.04%, 6.10mg/kg, and 0.55cmol/kg) after 2 months incubation period. The nutrient contents of the soil incubated with *P.ostreatus* was higher after 2 months incubation period except for all the nutrient contents of soil samples.

The pH values decreased from 5.97 at control to 4.63 at 30% crude oil contaminated soil after 1 month incubation period with *P.ostreatus*. The pH values also decreased from 6.17 at control to 5.27 at 40% crude oil contaminated soil after 2 months incubation period.

Comparing the nutrient contents of the crude oil contaminated soil incubated with *P.pulmonarius* and *P.ostreatus*, *P.pulmonarius* enhanced the nutrient content of the soil more in organic matter, organic carbon, nitrogen phosphorus and potassium than *P.ostreatus* after 1 and 2 months incubation. In terms of pH value, contaminated soil incubated with *P.pulmonarius* have higher pH values at 0% (6.54), 10% (5.47), 20% (4.85) and 30 (4.72) crude oil contamination than soils incubated with *P.ostreatus* after 1 month while *P.ostreatus* have higher pH values at 1% (5.47), 5% (5.30) and 40% (4.83) crude oil contamination.

Table 1: Nutrient Contents of Soil Contaminated with Crude Oil and incubated with *Pleurotus pulmonarius* for 0, 1 and 2 months

Nutrient content	Treatment (concentration of crude oil in the soil and incubation period)																				
	Control (0%)			1%			5%			10%			20%			30%			40%		
	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month
Organic Matter (%)	16.01^{a}	16.55^{a}	14.02^{b}	21.63^{e}	23.22^{b}	25.34^{a}	23.31^{d}	26.95^{ab}	28.68^{a}	25.44^{d}	27.31^{b}	29.09^{a}	27.09^{d}	27.85^{c}	29.85^{a}	28.30^{c}	28.61^{bc}	32.13^{a}	30.49^{c}	31.48^{b}	32.60^{a}
Organic Carbon (%)	9.29^{a}	9.60^{a}	8.13^{b}	12.54^{e}	13.47^{b}	14.70^{a}	14.10^{d}	15.63^{b}	16.63^{a}	14.76^{d}	15.84^{b}	16.87^{a}	15.71^{d}	16.15^{c}	16.95^{a}	16.48^{b}	16.60^{b}	18.63^{a}	17.68^{c}	18.26^{b}	18.91^{a}
Nitrogen (%)	0.06^{a}	0.06^{a}	0.05^{b}	0.08^{d}	1.39^{b}	1.53^{a}	0.12^{c}	1.55^{b}	1.69^{a}	0.17^{c}	1.76^{b}	1.71^{b}	0.20^{e}	1.64^{c}	1.83^{a}	1.25^{d}	1.70^{c}	1.90^{a}	0.31^{d}	1.85^{b}	1.96^{a}
Phosphorus (mg/kg-1)	8.53^{a}	7.65^{a}	6.10^{c}	9.51^{d}	9.71^{c}	10.62^{a}	10.24^{c}	10.40^{bc}	11.36^{a}	11.30^{c}	12.43^{a}	11.70^{b}	11.67^{c}	12.83^{a}	11.85^{b}	12.73^{ab}	12.96^{a}	12.62^{b}	12.83^{c}	13.56^{ab}	13.50^{b}
Potassium (cmol/ kg)	0.61^{b}	0.67^{a}	0.61^{b}	0.77^{d}	0.92^{b}	1.52^{a}	0.86^{d}	0.93^{c}	1.64^{a}	0.92^{c}	1.23^{b}	1.71^{a}	0.96^{d}	1.30^{c}	1.74^{a}	1.53^{b}	1.40^{c}	1.79^{a}	1.73^{b}	1.86^{a}	1.91^{a}
pH	6.37^{a}	6.50^{a}	6.40^{a}	5.33^{ab}	5.43^{a}	5.10^{b}	5.13^{bc}	5.20^{bc}	4.98^{c}	5.23^{c}	5.47^{b}	4.80^{d}	4.90^{b}	4.85^{b}	4.94^{b}	4.93^{b}	4.72^{c}	4.96^{b}	4.73^{c}	4.70^{c}	5.03^{b}

Each value is a mean of three replicates. Values in the same row followed by different letters are significantly different according to Duncan's Multiple Range test (P0≤.05).

Table 2: Nutrient Contents of Soil Contaminated with Crude Oil and incubated with *Pleurotus ostreatus* for 0, 1 and 2 months

Nutrient content	Treatment (concentration of crude oil in the soil and incubation period)																				
	Control (0%)			1%			5%			10%			20%			30%			40%		
	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month
Organic Matter (%)	16.01^{a}	16.21^{a}	14.02^{b}	21.63^{e}	22.05^{d}	22.07^{c}	23.31^{d}	25.34^{b}	23.72^{cd}	25.44^{d}	25.73^{c}	27.24^{b}	27.09^{d}	27.26^{d}	28.29^{b}	28.30^{c}	28.68^{bc}	29.24^{b}	30.49^{c}	30.54^{c}	30.46^{c}
Organic Carbon (%)	9.29^{a}	9.40^{a}	8.13^{b}	12.54^{e}	12.79^{d}	13.17^{c}	14.10^{d}	14.70^{c}	13.76^{e}	14.76^{d}	14.92^{c}	15.80^{b}	15.71^{d}	15.81^{d}	16.41^{b}	16.48^{b}	16.63^{b}	16.96^{b}	17.68^{c}	17.71^{c}	17.67^{a}
Nitrogen (%)	0.06^{a}	0.06^{a}	0.04^{b}	0.08^{d}	1.28^{c}	1.32^{c}	0.12^{c}	1.74^{a}	1.67^{a}	0.17^{c}	1.92^{a}	1.70^{b}	0.20^{e}	1.55^{d}	1.74^{b}	0.25^{d}	1.68^{c}	1.79^{b}	0.31^{d}	1.76^{c}	1.85^{b}
Phosphorus (mg/kg)	8.53^{a}	8.35^{a}	6.10^{c}	9.51^{d}	8.81^{e}	10.09^{b}	10.24^{c}	11.14^{a}	10.59^{b}	11.30^{c}	12.63^{a}	10.74^{d}	11.67^{a}	12.72^{a}	11.53^{d}	12.73^{ab}	12.86^{ab}	11.86^{c}	12.83^{c}	13.81^{a}	12.60^{c}
Potassium (cmol/kg)	0.61^{b}	0.69^{a}	0.55^{c}	0.77^{d}	0.86^{c}	0.94^{b}	0.86^{d}	0.90^{cd}	0.99^{b}	0.92^{c}	0.95^{c}	1.25^{b}	0.96^{d}	0.99^{d}	1.43^{b}	1.53^{b}	1.57^{b}	1.57^{b}	1.73^{b}	1.75^{b}	1.70^{b}
pH	6.37^{a}	5.97^{c}	6.17^{b}	5.33^{ab}	5.47^{a}	5.53^{a}	5.13^{ba}	5.30^{b}	5.70^{a}	5.23^{c}	5.27^{c}	5.67^{a}	4.90^{a}	4.67^{c}	5.50^{a}	4.93^{b}	4.63^{d}	5.60^{a}	4.73^{c}	4.83^{c}	5.27^{a}

Each value is a mean of three replicates. Values in the same row followed by different letters are significantly different according to Duncan's Multiple Range test (P≤0.05).

Table 3 shows the heavy metals accumulation by the mycelia of *P.pulmonarius* after 0, 1 and 2 months incubation period on crude oil contaminated soil. There was an increase in accumulation of manganese, copper, lead, zinc, iron and nickel by the mycelia of *P.pulmonarius* as the concentration of crude oil in the contaminated soil increased. The accumulation level however decreased as the incubation period increased. The accumulation of manganese, copper, lead, zinc and iron by the mycelia of *P.pulmonarius* was highest at 40% (1.07mg/kg, 1.21mg/kg, 0.83mg/kg, 0,72mg/kg, 1.26mg/kg) crude oil contaminated soil after 1 month incubation period. Lead, zinc and iron were accumulated by the fungus better at 40% (0.61mg/kg, 0.65mg/kg, 0.92mg/kg) while manganese, copper and nickel were accumulated more at 10% (0.87mg/kg), 5% (0.73mg/kg) and 0% (0.25mg/kg) crude oil contaminated soil after 2 month incubation.

Iron was the highest heavy metal accumulated by the mycelia of *P.pulmonarius* after 1 month incubation followed by copper, manganese, lead, zinc and nickel (Fe>Cu>Mn>Pb>Zn>Ni). Iron was also the highest heavy metal accumulated by *P.pulmonarius* after 2 months incubation followed by copper, manganese, zinc, lead while nickel was the least accumulated (Fe>Cu>Mn>Zn>Pb>Ni) in both periods of incubation.

Table 4 shows the heavy metals contents accumulated by the mycelia of *P.ostreatus* after 0, 1 and 2 months incubation period on crude oil contaminated soil. The accumulation of these heavy metals by *P.ostreatus* is also similar to the accumulation level of *P.pulmonarius*. The heavy metals accumulation increased as the concentration of crude oil in the contamination soil increased. The accumulation of these heavy metals however, decreased as the incubation period increased. Manganese, copper, lead, zinc, iron and nickel were accumulated more at 40% crude oil contamination for both 1 month and 2 months incubation period. Minimum accumulation of these metals by the mycelia of *P.ostreatus* was at control for both 1 and 2 months incubation period except for copper and zinc which was lowest at 40%. There were a few fluctuations in metal accumulation as the concentration of crude oil in the soil increases. After 1 month incubation, there was no change in lead accumulation in 0% and 1% (0.16mg/kg) contamination while after 2 months incubation, there was a decrease in accumulation of manganese, and for zinc, there was a decrease at 1% (0.13mg/kg) contamination of soil. There was however no change in accumulation at 10% and 20% (0.62mg/kg) for copper and at 1% and 5% (0.06mg/kg) contamination.

Table 3: Heavy metal contents of Soil Contaminated with Crude Oil and incubated with *Pleurotus pulmonarius* for 0, 1 and 2 months

Heavy metal content	Treatment (concentration of crude oil in the soil and incubation period)																				
	Control (0%)			1%			5%			10%			20%			30%			40%		
	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month
Manganese (mg/kg)	0.50^{a}	0.49^{ab}	0.46^{b}	0.60^{c}	0.70^{b}	0.77^{a}	0.68^{d}	0.76^{a}	0.81^{a}	0.77^{c}	0.83^{ab}	0.87^{a}	0.82^{c}	0.89^{a}	0.65^{d}	0.86^{b}	0.94^{a}	0.67^{c}	0.90^{b}	1.07^{a}	0.71^{c}
Copper (mg/kg)	0.60^{b}	0.56^{c}	0.48^{d}	0.68^{ab}	0.63^{b}	0.67^{ab}	0.76^{b}	0.57^{c}	0.73^{b}	0.86^{c}	0.94^{a}	0.62^{d}	0.93^{b}	1.03^{a}	0.60^{c}	1.65^{a}	1.16^{b}	0.57^{c}	2.18^{b}	1.21^{c}	0.49^{d}
Lead (mg/kg)	0.39^{a}	0.35^{b}	0.27^{c}	0.59^{a}	0.31^{c}	0.40^{b}	0.78^{a}	0.50^{bc}	0.57^{b}	0.79^{a}	0.63^{b}	0.57^{c}	1.41^{a}	0.68^{c}	0.53^{d}	1.52^{a}	0.75^{c}	0.56^{d}	1.68^{a}	0.83^{c}	0.61^{d}
Zinc (mg/kg)	0.17^{a}	0.16^{a}	0.11^{b}	0.21^{b}	0.19^{b}	0.25^{a}	0.26^{c}	0.53^{a}	0.30^{a}	0.36^{a}	0.57^{a}	0.40^{b}	0.43^{c}	0.63^{a}	0.54^{b}	0.48^{c}	0.66^{a}	0.60^{b}	0.62^{a}	0.72^{a}	0.65^{a}
Iron (mg/kg)	0.60^{a}	0.54^{c}	0.49^{d}	0.66^{b}	0.62^{bc}	0.71^{a}	1.20^{a}	0.65^{c}	0.77^{b}	1.33^{a}	0.90^{b}	0.78^{d}	1.41^{a}	0.96^{b}	0.81^{d}	1.43^{a}	1.10^{b}	0.87^{d}	1.53^{a}	1.26^{b}	0.92^{d}
Nickel (mg/kg)	0.10^{a}	0.10^{a}	0.25^{a}	0.15^{a}	0.06^{c}	0.03^{d}	0.16^{a}	0.04^{e}	0.47^{d}	0.22^{a}	0.05^{d}	0.04^{d}	0.31^{d}	0.07^{d}	0.03^{e}	0.37^{a}	0.06^{d}	0.02^{d}	0.42^{a}	0.09^{d}	0.03^{e}

Each value is a mean of three replicates. Values in the same row followed by different letters are significantly different according to Duncan's Multiple Range test (P≤0.05).

Table 4: Heavy metal contents of Soil Contaminated with Crude Oil and incubated with *Pleurotus ostreatus* for 0, 1 and 2 months

Heavy metal content	Treatment (concentration of crude oil in the soil and incubation period)																				
	Control (0%)			1%			5%			10%			20%			30%			40%		
	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month	0 month	1 month	2 month
Manganese (mg/kg)	0.52^{a}	0.54^{a}	0.38^{c}	0.60^{c}	0.64^{c}	0.42^{d}	0.68^{d}	0.71^{c}	0.46^{e}	0.77^{c}	0.80^{bc}	0.51^{d}	0.82^{c}	0.86^{b}	0.56^{e}	0.86^{c}	0.89^{b}	0.62^{b}	0.90^{d}	0.94^{b}	0.60^{d}
Copper (mg/kg)	0.60^{b}	0.64^{a}	0.48^{d}	0.68^{b}	0.69^{a}	0.51^{c}	0.76^{b}	0.83^{a}	0.55^{c}	0.86^{c}	0.90^{b}	0.62^{d}	0.93^{b}	0.97^{b}	0.62^{c}	1.65^{a}	1.70^{a}	0.53^{c}	2.18^{b}	2.28^{a}	0.42^{d}
Lead (mg/kg)	0.39^{a}	0.39^{a}	0.27^{c}	0.59^{a}	0.40^{b}	0.33^{c}	0.78^{a}	0.51^{bc}	0.41^{c}	0.79^{a}	0.61^{b}	0.45^{d}	1.41^{a}	0.73^{b}	0.49^{e}	1.52^{a}	0.81^{b}	0.54^{d}	1.68^{a}	0.92^{b}	0.56^{e}
Zinc (mg/kg)	0.17^{a}	0.16^{a}	0.15^{a}	0.21^{b}	0.16^{c}	0.13^{d}	0.26^{c}	0.20^{d}	0.19^{d}	0.36^{c}	0.27^{d}	0.26^{d}	0.43^{c}	0.32^{d}	0.31^{d}	0.48^{c}	0.37^{e}	0.41^{d}	0.62^{a}	0.41^{a}	0.58^{ab}
Iron (mg/kg)	0.60^{a}	0.56^{b}	0.42^{e}	0.66^{b}	0.57^{c}	0.57^{c}	1.20^{a}	0.80^{b}	0.67^{c}	1.33^{a}	0.85^{c}	0.73^{e}	1.41^{a}	0.92^{c}	0.76^{e}	1.43^{a}	0.95^{c}	0.86^{d}	1.53^{a}	1.99^{c}	0.90^{d}
Nickel (mg/kg)	0.10^{a}	0.06^{a}	0.04^{a}	0.15^{a}	0.08^{b}	0.06^{c}	0.16^{a}	0.10^{b}	0.06^{c}	0.22^{a}	0.15^{b}	0.08^{c}	0.31^{a}	0.25^{b}	0.10^{c}	0.37^{a}	0.30^{b}	0.14^{c}	0.42^{a}	0.36^{b}	0.19^{c}

Each value is a mean of three replicates. Values in the same row followed by different letters are significantly different according to Duncan's Multiple Range test (P≤0.05).

Copper was the highest metal accumulated by *P.ostreatus* after 1 month incubation followed by iron, manganese, lead, zinc and nickel the least accumulated (Cu>Fe>Mn>Pb>Zn>Ni). Iron was the highest accumulated after 2 months incubation of the crude oil contaminated soil with *P.ostreatus*, followed by copper, manganese, zinc, lead and nickel (Fe>Cu>Mn>Zn>Pb>Ni).

In comparing Table 3 and Table 4, both white rot fungi accumulation of nickel was the least after 1 and 2 months while iron was better accumulated after 2 months of incubation by both fungi. *P.pulmonarius* accumulated more of the heavy metals after 2 months incubation period than *P.ostreatus* as the concentration of the crude oil increases. After 1 month incubation, *P.pulmonarius* accumulated more of manganese, zinc and iron than *P.ostreatus* as the concentration of the crude oil in the contaminated soil increased except in the control of manganese and nickel where *P.ostreatus* accumulated more.

P.ostreatus also accumulated more of copper, lead and nickel than *P.pulmonarius* at 1 month

incubation except for copper at 10% and 20% crude oil contamination; lead at 10% and nickel at control where *P.pulmonarius* accumulated more of these metals than *P.ostreatus*.

Discussion

The ability of these white rot fungi to grow on crude oil at different levels of contamination through their mycelia colonization is in conformity to Aust *et al*. (2003) who reported that white rot fungi can withstand toxic levels of most organo pollutants.

Oudot (1990) also demonstrated that fungi are able to grow optimally in the presence of harmful contaminants and are able to detoxify such contaminants. In this study, it was observed that organic matter, organic carbon, nitrogen, phosphorus and potassium increased as the concentration of crude oil in the contaminated soil increased in both 1 month and 2 months incubation with *P.pulmonarius* and *P.ostreatus*. It was also observed that organic matter, organic carbon, nitrogen, phosphorus and potassium also increased as the incubation period increases from 0 month to 2 months. This was evident from the result obtained. This increase in nutrient contents as the concentration of crude oil in the contaminated soil increased is in line with the observation of Atlas and Bartha (1972) who reported that the addition of crude oil to an ecosystem will enrich primarily the microorganisms capable of utilizing the hydrocarbons and secondary microorganisms capable of utilizing metabolites produced by the oil utilizing microorganisms. Both *P.pulmonarius* and *P.ostreatus* were able to utilize the crude oil found in the soil for their metabolic activities and growth. It also indicated that bioremediation has taken place and this was confirmed by the work of Grady (1985) who reported that bioremediation involves transformation of complex or simple chemical compound into non-hazardous forms by biological agents resulting in materials of higher nutritive value or simply reducing the final bulk of the product. The increase in all the nutrient contents in the soil as the concentration of crude increased when incubated with *P.pulmonarius* and *P.ostreatus* is similar to the work of Adenipekun and Omoruyi (2008) who reported that organic matter, potassium and organic carbon increased after 2 months of incubation with *P.ostreatus* on cement-contaminated soils and diesel-contaminated soil. Adenipekun and Fasidi (2005) also stated that *Lentinus subnudus* an indigenous white rot fungus in Nigeria inoculated on crude 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. From this work nitrogen, phosphorus and potassium content in the soil increased steadily as the concentration of crude oil in the soil increases with increase in incubation period for both white rot fungi. This could be attributed to the composition of crude oil which might be rich in phosphorus and potassium, strains of the fungi and conducive environmental factors in which the experiment was carried out. This is contrary to the work of Lehtomaki and Niemela (1975) where they reported low values for nitrogen, potassium and phosphorus reserves in petroleum hydrocarbon contamination. Benka-Coker and Ekundayo (1995) also reported low levels of nitrogen and phosphorus from a crude oil spill site in the Niger Delta of Nigeria. This implies that the activities of the fungi might be responsible for the observed high nutrient content of the contaminated soil.

From this study, the pH played a vital role in the biodegradation process of crude oil contaminated soil. It was observed that the pH of the crude oil contaminated soil reduced after the introduction of *P.pulmonarius* and *P.ostreatus*. The pH values decreased as the concentration of crude oil in the soil increases while an increase was observed with increase in incubation period. Reduction in pH indicated that microbial activity took place in the soil after introduction of the white rot fungi. Adenipekun and Fasidi (2005) observed a decrease of pH value from 6.90 to 6.62 and finally to 6.25 after 3 and 6 months of incubation respectively with *Lentinus subnudus*, a Nigeria white rot fungus. A similar finding of Adenipekun and Omoruyi (2008) showed that the pH of cement contaminated soil incubated with *P.ostreatus* decreased from 7.55 in the control to 7.54 and 7.11 after 1 and 2 months; for diesel contaminated soil and for battery contaminated soils, the pH decreased from 5.90 at control to 4.68 after 1 month incubation. The pH range of 4.70 to 6.50 used by these white rot fungi *P.pulmonarius* and *P.ostreatus* favoured their biodegradation. This is supported by Hossain and Anatharaman (2006) who reported that the lignin degradation enzymes (laccase and other peroxidases) secreted by white rot fungi are known to function best at low pH some times as low as pH 3.5.

In this study, it was observed that heavy metals accumulation increased as the concentration of crude oil in the contaminated soil increased for both *P.pulmonarius* and *P.ostreatus* after one and two months incubation period. This indicates that bioaccumulation of heavy metals by these white rot fungi has taken place. This finding is similar to the observation of Gabriel *et al* (1994) that wood rotting fungi accumulated cadmium, lead, aluminum and calcium from liquid medium, supplemented with appropriate amount of metal salt. Siegel *et al*. (1990), Kalac *et al*. (1996) and Gadd (1993) have also used fungi for the treatment of heavy metals containing

effluent due to their ability to accumulate metals from their external environment. Increase in heavy metals in the soil was as a result of crude oil contamination and the level of contamination. This was observed from the result obtained from the analysis of the soil for heavy metal before and after crude oil was used to contaminate the soil. Results obtained indicated that more heavy metals were recorded after crude oil was added to the soil and increased more as the concentration of the crude oil in the soil increased than before the addition of crude oil. There was a decrease in heavy metals accumulation by the mycelia of *P.ostreatus* at 2 months incubation as the concentration of crude oil in the contaminated soil increased. This was similar to the report of Adenipekun and Isikhuemhen (2008) that *Lentinus squarrosulus* accumulated iron, zinc, copper and nickel from soils contaminated with engine oil after 90days of incubation with a steady increase from 0% to 30% at highest and then decreased at 40% concentration of engine oil in the soil. This shows the limitation in which the white rot fungi can accumulate these heavy metals in relation to concentration of the contaminant. From this study, the decrease in heavy metals accumulation by the two white rot fungi as the incubation period increased from 1 months to 2 months is similar to the findings of Adenipekun and Omoruyi (2008) that in cement polluted soil, battery waste polluted soil and black oil polluted soil, an increase in incubation period resulted in a general decrease in all the heavy metal content when incubated with *P.ostreatus*. This is an indication that the white-rot fungi reduced the heavy metals in the soil. Increase in the heavy metal accumulation of manganese, copper, lead, zinc and iron by *P.pulmonarius* after increase in incubation period at 1% and 5% crude oil concentrations is similar to the findings of Adenipekun and Omoruyi (2008) that there was a decrease in the lead, iron and nickel values after 1 month incubation with *P.ostreatus*.

In this study it was observed that iron was the highest metal accumulated by *P.pulmonarius* after 1 and 2 months incubation at 40% crude oil contamination. This suggests that iron was a major metal contaminant of the crude oil contaminated soil. This was followed by copper, manganese, lead, zinc and nickel. Nickel was the least metal accumulated by both white rot fungi. This indicates that nickel was not a major metal contaminant in soil. In the same trend *P.ostreatus* accumulated heavy metals as observed in *P.pulmonarius* for both 1 and 2 months.

Conclusion

The experiment carried out reaffirmed the ability of the two white rot fungi (*P.pulmonarius* and *P.ostreatus)* as good bioremediating and bioaccumulating agents after being incubated on different level of crude oil concentrations on contaminated soil at different incubation periods. This included improving the nutrient contents of the contaminated soil by increasing the organic matter, organic carbon, nitrogen, phosphorus and potassium as the concentration of the crude oil increased. Their accumulation of heavy metals such as manganese, copper, iron, zinc, lead and nickel proved to us that both *P.pulmonarius* and *P.ostreatus* are good bioaccumulation agents. The changes in pH as the incubation period increased also indicated their activities in the degrading of the contaminant.

Most importantly, the comparison of these two white rot fungi, based on the above parameters indicated the superiority of *P.pulmonarius* over *P.ostreatus* as a bioremediating and bioaccumulating agent for any polluted sites contaminated with crude oil or any petroleum product.

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