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Comparison of Two *Pleurotus* Species and Their Consortium to Evaluate Their Potential for Biodegradation of Spent Drilling Mud

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Authors' contributions

This work was carried out in collaboration between both authors. Author EAU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HOS managed the analyses of the study. Author EAU managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Biodegradation potentials of two white rot fungi (*Pleurotus ostreatus and Pleurotus pulmonarius*) in soil contaminated with spent oil based drilling mud were investigated. The study was conducted over 63 days, with periodic monitoring after every 21 days. Four treatment options were used for the experimental set-up: Treatment A had *P. ostreatus,* B had *P. pulmonarius,* AB had both *P. ostreatus and P. pulmonarius* and the Control (without fungal spawn). The parameters monitored were pH, conductivity, total organic carbon (TOC), total nitrogen, available phosphorus, chloride, total heterotrophic bacterial (THB) count, total fungal (TF) count, heavy metals (Zinc, lead, Copper, Cadmium and Barium), and Total Petroleum Hydrocarbon (TPH). Furthermore, a phytotoxicity test was carried out to determine the effect of the treatments on maize (*Zea mays var. indentata*) plant growth. The results showed an increase in microbial population. Treatment A recorded the highest THB count of 2.65×10^7 (cfu/g) and Treatment C the highest TF count of 1.07×10^5 (cfu/g). The

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percentage loss of TPH was in the order of: AB (98.08%)>A (94.79%)>B (87.02)>Control (6.12%) at the end of 63 days. The values for pH for the treatments ranged from 6.05-8.08; conductivity (µS/cm) ranged from 6580-14540; TOC (%) ranged from 2.15-5.19; total nitrogen (mg/kg) ranged from 696.36-742.77; available phosphorus (mg/kg) ranged from 402.71-642.38; chloride (mg/kg) ranged from 3200-5980; zinc (mg/kg) ranged from 43.66-128.47 respectively, the copper (mg/kg) ranged from 20.09-89.29; barium (mg/kg) 53.92-237.98; cadmium (mg/kg) 5.38-24.98 and lead (mg/kg) ranged from 4.41-22.27. The results revealed that treatment option (consortium) AB was more efficient in remediating the contaminated soil. The phytotoxicity results showed that the vegetative growth attributes of the maize plant were more prolific in the treated and uncontaminated soil than in untreated soil after 28 days of cultivation. Although, the study indicates that *P. ostreatus* and *P. pulmonarius* showed good levels of biodegradation potential, the consortium proved to be a better alternative in biodegradation compared to the individual species.

Keywords: Biodegradation; drilling mud; Pleurotus ostreatus; Pleurotus pulmonarius.

1. INTRODUCTION

Petroleum exploration and production activities are strongly associated with drilling operations, during which potentially harmful waste drilling muds, which often require conscientious handling and disposal, are generated [1]. These wastes are usually contaminated with oil, hydrocarbons, complex chemical compounds and metals of varying toxicity.

An important aspect of the environmental impact of offshore drilling operations is the discharge of oil based drilling mud. The treatment of this oil based drilling mud has become one of the major problems facing crude oil-production in developing countries like Nigeria. This is because, the officially recommended [2] treatment method, incineration, is prohibitively expensive [3] and exposes personnel and equipment to the resulting fugitive dusts. Consequently, it is important to adopt a cheaper and much more ecologically sound treatment technique for this type of petroleum waste.

There has been a large body of recent literature indicating bioremediation has high potential for restoring polluted media with very low negative impact on the environment, at relatively low cost. This technology has been used in bio-treating exploration and production (E&P) wastes [4]. Several researchers have demonstrated high bioremediation efficiency for oil polluted soils by adopting various strategies to aid bioremediation [5-10]. Available literatures suggest that *Pleurotus* species are able to remove and degrade heavy metals and hydrocarbon pollutants from impacted matrix [11-12].

Phytotoxicity assay using bio-indicator plants to show the inhibitive effects of industrial effluents on plant growth is a sensitive and cheap way of assessing the success of soil bioremediation [13-14]. The plant *Zea mays* is commonly used to monitor environmental quality by monitoring changes in its height, root length, stem girth, leaf width and leaf length [15,14,16]. These changes are often taken as indicative parameters of environmental pollution. The aim of this research was to maximize the biodegradation potentials of white rot fungi (mushroom), *Pleurotus* species, on soil contaminated with spent oil based drilling mud, both singly and in consortium.

2. MATERIALS AND METHODS

2.1 Sample Collection

Spent oil based drilling mud was collected from an offshore oil exploration site at Sapele, Nigeria. Garden soil of loamy texture used for this experiment was collected within 1 to 10 (cm) depth from an area within Abuja campus, University of Port Harcourt. Rice bran, sawdust and pure spawns of *Pleurotus pulmonarius* and *Pleurotus ostreatus* were obtained from Niger Delta Development Commission (NDDC)/ Rivers State University of Science and Technology (RSUST) Mushroom/Spawn Production and Research Centre, Rivers State University of Science and Technology, Nkpolu, Port Harcourt, Nigeria. Calcium carbonate and calcium sulphate were bought from a market in the city of Port Harcourt and used as nutrient supplement.

2.2 Substrate Preparation

The sawdust was composted for seven days. This was done by making a heap of 19 (kg) of sawdust mixed with urea (a source of nitrogen) and gypsum. Water was spread on the top as the heap was thoroughly mixed. After the composting period, the sawdust was mixed thoroughly with 3 (kg) of rice bran, 16 (g) of lime ($CaCO_3$) and 19 (g) of $CaSO_4$. Water was added to increase the moisture content. Each of the polythene bags used contained 200 (g) of this mixture (supplements) mixed with 800 (g) of soil. The bags were UV-sterilized before spiking with spent drilling mud and inoculating with spawn.

2.3 Experimental Design

The experimental design was set-up to include waste drilling fluid with or without mushroom spawn. Set-up A was seeded with *Pleurotus pulmonarius* spawn, B with *P. ostreatus* spawn, AB was the consortium and the control was unseeded.

2.4 Determination of Physicochemical Parameters of Soil Samples

The conductivity, total nitrogen, total organic carbon, available phosphorus and chloride were determined using the method described by the Association of Official Analytical Chemists [17]. The pH of the soil sample was measured using Pometer digital pH meter. Heavy metals were analyzed using the Atomic Absorption Spectrophotometer (Phoenix 986 AA spectrophotometer). Total Petroleum Hydrocarbon (TPH) was determined using gas Chromatograph, HP 5890.

2.5 Enumeration of Total Heterotrophic Bacterial and Total Fungal Counts

Total culturable heterotrophic bacterial and total fungal counts of the samples were determined at zero hour, 21st, 42nd and 63rd day of the experiment. Enumeration of total heterotrophic bacteria was carried out using the stated procedures reported by Chikere et al. and Nwachukwu et al. [18-19]. The media of choice were mineral salt agar ($MgSO_4.7H_2O$, 0.40 (g); KCl, 0.28 (g); KH_2PO_4, 0.80 (g); Na_2HPO_4, 1.20 (g); NH_4NO_3, 0.40 (g); NaCl, 15 (g); Agar, 20 (g) and distilled water 1000 (ml) for bacteria and the potato dextrose agar, (PDA) for fungi.

2.6 Phytotoxicity Assay

Ten seeds of the maize grains (*Zea mays var.indentata*) were planted into each nursery bags containing the treated soils, control soil and uncontaminated soil and the percentage seed germination potential were calculated after three days of planting. The analysis was carried out for four weeks. Plant height was determined by measuring the height of the plant from the border to the highest leaf. Stem girth was measured at a point 5 (cm) above the soil level using a measuring tape. Other growth measurements that were considered include; leaf-length, shoot length and leaf-width. These were all recorded regularly at weekly intervals.

2.7 Statistical Analysis

The data generated in this study were subjected to statistical analysis using two way analysis of variance (ANOVA) to determine levels of significance.

3. RESULTS AND DISCUSSION

3.1 Baseline Characteristics of Spent oil Based Drilling Mud, Supplements and Soil

The values of the baseline (before treatment) total heterotrophic bacterial counts, total heterotrophic fungal counts, physicochemical parameters (pH, conductivity, total nitrogen, total organic carbon, available phosphorus, potassium and chloride), heavy metals (copper, barium, cadmium, lead and zinc) and gas chromatographic analysis of total petroleum hydrocarbons (TPH), uncontaminated sand and (PAHS) in the spent drilling mud (SDM), supplements and soil are presented in Table 2. From the baseline characteristics, the type of inorganic constituents and hydrocarbons found in the drilling mud used in this study were consistent with the report of Gbadebo et al. [20] but with varying concentrations.

3.2 Microbial Counts

Microbial counts assessed on the untreated (control) and the treated (remediated) samples revealed that the contaminated oil based mud contained indigenous microorganisms of up to 2.85×10^4 (cfu/g) for total heterotrophic bacterial counts and 9.0×10^3 (cfu/g) for total fungal counts, as shown in Table 2.

During the nine-week degradation process, different trends were observed in the total heterotrophic bacterial counts and total fungi counts analyzed in the different treated and control samples (Figs. 1 & 2). There was a general increase for all treatments but treatment A recorded highest total heterotrophic bacterial count of $2.65x10^7$ (cfu/g). The THF counts in all the fungal treated samples, A, B, and AB increased from 10^3 (cfu/g) by day 0 to 10^5 (cfu/g) by day 63. The increase in microbial count can be attributed to substrate utilization for growth and metabolism.

Table 1. Design of experimental set-up

Set-up	Composition
A	200 (ml) drilling mud + 800 (g) sterilized soil + 200 (g) nutrients + 20 (g) spawn seeds (*Pleurotus ostreatus*)
B	200 (ml) drilling mud + 800 (g) sterilized soil + 200 (g) nutrients + 20 (g) spawn seeds (*Pleurotus pulmonarius*)
AB	200 (ml) drilling mud + 800 (g) sterilized soil + 200 (g) nutrients + 10 (g) spawn seeds (*Pleurotus ostreatus*) + 10 (g) spawn seeds (*Pleurotus pulmonarius*)
Control	200 (ml) drilling mud + 800 (g) sterilized soil + 200 (g) nutrients + no spawn seed

Table 2. Baseline characteristics of spent drilling mud, nutrient supplement and soil

Parameter	Value		
	SDM	Supplement	Soil
Total heterotrophic bacterial count (THBC) (cfu/g)	$2.85x10^4$	-	-
Total fungal count (TFC) (cfu/g)	$9.0x10^3$	-	-
pH	7.18	10.2	5.59
Conductivity (µS/cm)	15880	6899	80.25
Total Nitrogen (mg/kg)	41.21	2566	0.50
Available Phosphorus (mg/kg)	84.11	2110	20.2
Available Potassium, (mg/kg)	301.14	1650	17.3
Total organic carbon (TOC) (%)	3.20	11.2	0.56
Chloride (mg/kg)	6550	5.9	9.99
Zinc (mg/kg)	151.19	ND	12
Copper (mg/kg)	113.27	ND	3.2
Barium (mg/kg)	240.12	ND	ND
Cadmium (mg/kg)	29.04	ND	ND
Lead (mg/kg)	31.56	ND	ND
Total petroleum hydrocarbons (TPH) (mg/kg)	69400	ND	23
PAHS (mg/kg)	131.362	ND	ND

SDM: Spent drilling mud; ND: Not detected

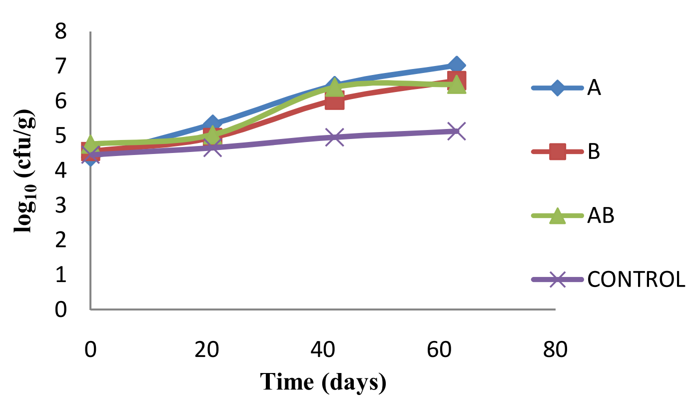


Fig. 1. Total Heterotrophic Bacterial (THB) counts obtained from different treatments during the biodegradation process

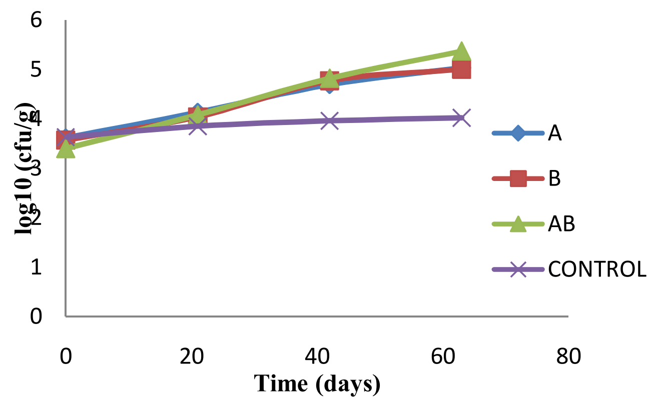


Fig. 2. Total fungi (THF) counts obtained from different treatments during the biodegradation process

3.3 Heavy Metals Levels

Fig. 3 shows changes in concentration of zinc, copper, barium, cadmium and lead respectively in the contaminated soil during the study. Bioaccumulation of metals, such as cadmium, cesium and zinc by several fungi has been reported [21]. Soil contaminated with spent oil based drilling mud for 63 days, in this study showed a reduction in zinc, copper cadmium, barium and lead. Increase in heavy metals in the soil was as a result of spent drilling mud contamination. There was a significant difference ($p<0.05$) between the different treatments and the control. Results obtained indicated that more heavy metals were recorded after oil based mud was added to the soil. From this study, the increase in heavy metals accumulation by the two white rot fungi as the incubation increased from day 0 to 63 is similar to the findings of Adenipekun et al. [22] 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. pulmonarius.*

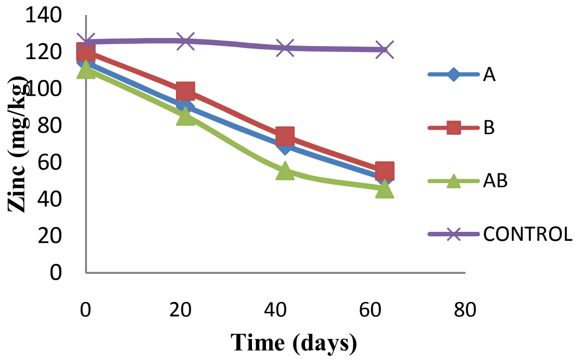
3.4 Percentage Total Petroleum Hydrocarbon Losses and Residual TPH Content

Fig. 4 shows the residual hydrocarbon content in different treatments during the period of the experiment. There was a greater decrease in TPH content in treatment AB and least in the control. The TPH loss of 98.08% by the consortium when compared with 86.25% loss by *P. ostreatus* and 81.92% loss by *P. pulmonarius*, showed the consortium is more effective than the individual species. The ANOVA indicated that there was significant difference ($p<0.05$) between the different treatments. It therefore showed that these selected species have potential applications in the bioremediation of soils polluted with oil based mud. This is in conformity to Aust et al. [23] who reported that white rot fungi can withstand toxic levels of most organopollutants. Thouand [24] also demonstrated that fungi are able to grow optimally in the presence of harmful contaminants and are able to detoxify such contaminants.

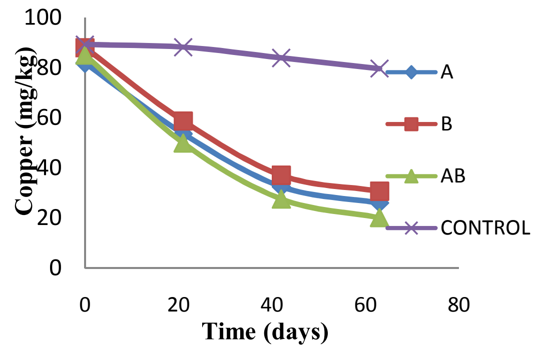
3.5 Phytotoxicity Assay

Zea mays var.indentata is a food crop commonly used for phytotoxity assay [14,25,16]. After three days of planting the maize seeds, for the untreated soil, out of the ten seedlings that was sown, only three of the seeds germinated, giving 30% effect on seed germination potential. On the contrary, for the remediated soil, A, B and AB, all ten seeds germinated, resulting in 100% effect on seed germination potential, indicating that the remediated soils exhibited 0% toxicity to seed germination. The uncontaminated soil gave a seed potential of 70%. This is can be as a result of the improved conditions in the treated compared to the untreated soil. Comparative evaluation of the plant height, root length, stem girth, leaf width and leaf length, are presented in Table 3. The average plant height of *Zea mays* grown in the untreated soil was 28cm while the average highest height of the treated soil was 59.25 (cm). Results showed that the vegetative growth attributes of the maize plant were more prolific in the treated and uncontaminated soil than in untreated soil after

28 days of cultivation. This clearly indicates that soil pollution by spent oil based drilling mud can impede the growth and metabolism of maize plants. The results of the study clearly show that fungi can transform soil contaminated with spent oil based drilling mud to arable soil; capable of supporting seed germination and plant growth and this surpasses the performance of the control (untreated soil). This therefore indicates that the use of *P. ostreatus* and *P. pulmonarius* as a good bioremediation agent for hydrocarbon degradation in drilling waste converts these waste materials into non-toxic end products.



(A)



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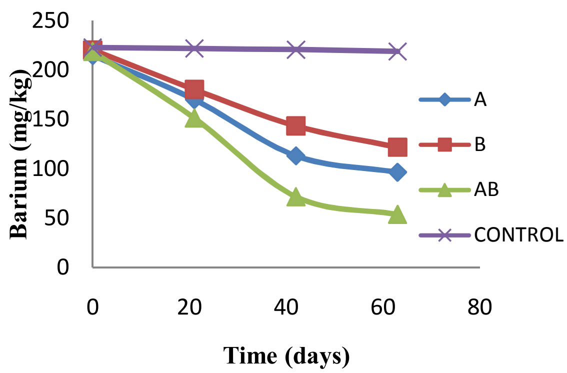

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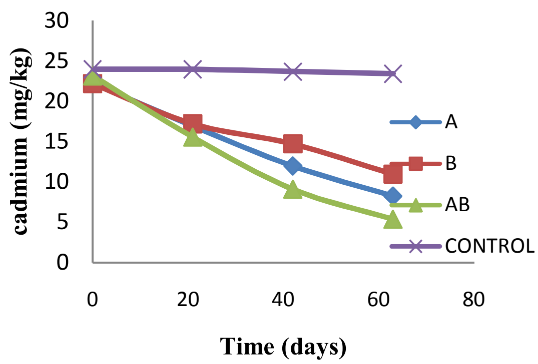
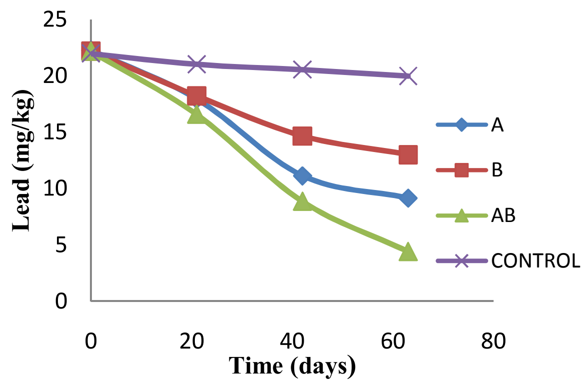


(D)



(E)

Fig. 3. Changes in concentration of (A) zinc, (B) copper, (C) barium, (D) cadmium and (E) lead in the contaminated soil during the remediation process

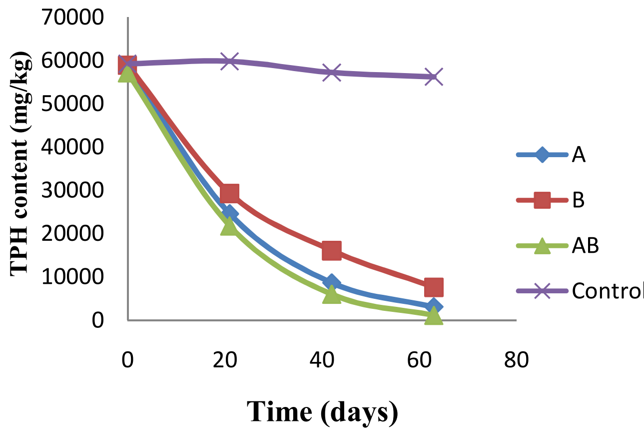


Fig. 4. Residual hydrocarbon content in different treatments during the period of the experiment

Table 3. Measurements of plant's vegetative part during phytotoxicity assay

Week	Treatment	Plant height (cm)	Shoot length (cm)	Stem Girth (cm)	Leaf length (cm)	Leaf width (cm)
1	Treated soil A	17.50	7.00	1.35	12.95	1.45
	Treated soil B	22.35	5.70	1.30	14.45	1.30
	Treated soil AB	20.20	6.15	1.45	12.45	1.50
	Uncontaminated soil	28.40	6.45	1.31	15.40	1.25
	contaminated soil	7.85	4.55	0.95	9.55	1.11
2	Treated soil A	38.80	10.20	1.55	28.10	1.35
	Treated soil B	42.52	12.70	1.50	32.15	1.50
	Treated soil AB	45.65	12.50	1.65	30.25	1.65
	Uncontaminated soil	43.10	10.98	1.45	31.65	1.33
	contaminated soil	9.15	6.80	1.15	21.00	1.20
3	Treated soil A	41.75	12.80	1.65	33.15	1.45
	Treated soil B	47.83	13.08	1.58	36.98	1.72
	Treated soil AB	55.35	15.20	1.69	34.80	1.68
	Uncontaminated soil	47.82	12.75	1.71	36.03	1.41
	contaminated soil	20.30	7.22	1.21	22.73	1.25
4	Treated soil A	45.25	14.60	1.72	38.20	1.55
	Treated soil B	50.26	13.40	1.64	41.80	1.90
	Treated soil AB	59.25	17.65	1.70	39.35	1.70
	Uncontaminated soil	48.90	13.28	1.73	40.40	1.47
	contaminated soil	28.90	8.25	1.25	24.45	1.30

***A:** Pleurotus ostreatus; **B:** Pleurotus pulmonarius; **AB:** Pleurotus ostreatus + Pleurotus pulmonarius (consortium)*

4. CONCLUSION

The results from the study revealed that the two white rot fungi, *Pleurotus ostreatus* and *Pleurotus pulmonarius* were able to degrade the petroleum hydrocarbon contaminant in the soil and take-up the heavy metals present in them. It is evident from this study that fungi consortia have more efficient biodegradative potential to degrade hydrocarbon contaminants than the individual species, since it has been reported that consortia systems provide advantages over individual cultures as they involve the combined and inductive effects of the individual species. Phytotoxity results revealed that the fungi were able to enhance the soil for better crop productivity. In the quest, for economical and ecologically sound methods for environmental remediation and cost effective waste management, the use of mushrooms is a very good approach and solution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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