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Crude Oil Degradation Using Spent Mushroom Compost (SMC) of *Pleurotus florida*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This paper investigated the effectiveness of spent mushroom compost (SMC) of *Pleurotus florida* in the biodegradation of crude oil contaminated soil for a period of 42 days. The crude oil contaminated soil was supplemented with different concentrations of the SMC of *P. florida* throughout the period of study. Microbiological and physicochemical parameters including Total Petroleum Hydrocarbon (TPH) content were monitored from the baseline to the 42nd day. Results showed significant decreases in the physicochemical parameters during the study period. The percentage loss of TPH at the end of the investigation was 90.09%. The hydrocarbon utilising bacterial isolates were *Bacillus* sp, *Pseudomonas* sp, *Flovobacterium* sp, *Micrococcus* sp and *Arthrobacter* sp. The hydrocarbon utilizing fungal isolates were *Penicillium* sp, *Fusarium* sp, *Sacchoromyces* sp, *Microsporum* sp, *Cryptococcus* sp *and Mucor* sp. This study showed that SMC of *Pleurotus florida* is an effective nutrient source for biodegradation.

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Keywords: Biodegradation; total petroleum hydrocarbon; spent mushroom compost; Pleurotus florida.

1. INTRODUCTION

Crude oil exploration has been on-going for about six decades in the oil-rich Niger Delta region of Nigeria. Several land sites close to oil industry facilities in this region have their soil contaminated beyond the Nigerian national standards, as set out in the Environmental Guidelines and Standards for the Petroleum Industries in Nigeria (EGASPIN). The UNEP [1] Environmental Assessment of Ogoniland report revealed that the pollution of soil by hydrocarbons in Ogoniland is extensive in land areas, sediment and swampland. Oil spills have had adverse effects on the region's ecosystem leading to adverse environmental pollution and economic hardship. Oil spill in an area usually results to an imbalance in the inorganic components of the affected soil [2]. Bioremediation, a process involving the use of microorganisms can be adopted to reduce, eliminate or transform contaminated soils, sediment, air and/or water [3]. The technology involves the manipulation of microbial metabolic processes and enzymatic actions to degrade compounds of concern in polluted sites. Due to the ubiquity of hydrocarbon-degradation microorganisms in the soil, bioremediation of crude oil contaminated soil is considered of greater economic value in terms to the cost and effectiveness.

The use of spent mushroom substrate has been proposed in bioremediation, as it helps to stimulate the population of indigenous microorganisms which breakdown hydrocarbon compounds [4-6]. Mushrooms make use of several agro-waste products as growth substrate leaving behind a nutrient-rich substance that can be used to promote the growth of crude oil degrading microorganisms in the soil. This paper investigated the effectiveness of bioremediation of crude oil contaminated soil using different concentrations of SMC of *P. florida*.

2. MATERIALS AND METHODS

2.1 Study Area

The soil used for this study was collected from K-Dere community in Gokana Local Government Area of Rivers State, Ogoniland in the Niger Delta region of Nigeria. Farming and fishing are the predominant occupation of the people. There have been various cases of crude oil pollution in this area as reported in the Ogoni environmental assessment report by United Nations Environmental Programme [1].

2.2 Collection of Samples

Soil samples from crude oil contaminated sites were collected at about 0-15cm depth, using soil augar at four different locations within the community. The four samples were lumped together in a plastic bag and transported to the Environmental Microbiology Laboratory of the University of Port Harcourt for bioremediation studies. The Spent mushroom compost of *P. florida* was collected from the University of Port Harcourt Demonstration Farm, Choba, Rivers State, Nigeria.

2.3 Experimental Design

Three treatment options with varying concentrations of *P. florida* SMC in polluted soil and a control were set up. Biodegradation under a controlled environmental condition was monitored for 42-days of study. The experimental design is as shown in Table 1.

2.4 Physicochemical Analysis of Soil

The pH of the soil sample was measured in 1:1 (soil: water) ratio using Winlab digital pH meter. Moisture content and total organic carbon content were determined following the methods of Walkey and Black [7]. Soil Nitrogen and phosphate were determined quantitatively following the methods of America Public Health Association [8]. Gas chromatographic analysis was conducted to determine the residual total petroleum hydrocarbon over a 42-day study period.

2.5 Microbiological Analysis

2.5.1 Enumeration of total heterotrophic bacteria and fungi

Ten-fold serial dilution of the samples was done; with one gram of the soil samples weighed out and dispensed into test tubes containing normal saline. Each mixture in the test-tube was shaken thoroughly for proper mixing. 1.0 ml of the aliquots was pipette into another test tube containing 9.0 ml of normal saline to give a dilution of 10^{-1}. The sample was diluted up to 10^{-5}. All test tubes were covered with cotton plug to

Table 1. Experimental design

Experimental set	Test Experiment
Set-up A	1000 g polluted soil + 250 g SMC of *P. florida*
Set-up B	1000 g polluted soil + 500 g SMC of *P. florida*
Set-up C	1000 g polluted soil + 1000 g SMC of *P. florida*
Set-up D (Control)	1000 g polluted soil

prevent further contamination. Aliquots of 0.1 ml serially diluted soil samples were plated in duplicates on nutrient agar and potatoes dextrose agar using spread plate technique. The plates were incubated at 35±2℃ for 24 hours for bacterial count and between 5-7days fungal count. Incubation was within 5-7days at 28±2℃

2.5.2 Enumeration of total hydrocarbon utilizing Bacteria and fungi

Vapour-phase method was adopted to estimate the population of total hydrocarbon utilizing bacteria (THUB), on a modified mineral salt agar (MSA) with the following composition: NaCl= 10.0 g, $MgSO_4$= 0.42 g, KH_2PO_4= 0.83 g, KCl= 0.29 g, $NaNO_3$= 0.42 g, K_2HPO_4= 1.25 g, agar = 15.0 g in one litre of distilled water as described by Chikere and Okpokwasili [9]. A sterile filter paper was saturated with sterile crude oil and placed inside the cover of each petri dish, kept in an inverted position, the plates (containing 0.1 ml of aliquots of serially dilute soil samples) were incubated at 35- 37℃ for 5-7 days. The crude oil served as the only source of carbon and energy for the growing culture. After incubation, the colonies were counted and the mean counts were recorded. The same procedure used for the enumeration of hydrocarbon utilizing bacteria was adopted for the enumeration of hydrocarbon utilizing fungi with addition of 1.0 ml lactic acid for the inhibition of the growth of hydrocarbon utilizing bacteria.

2.6 Purification of Isolates

Pure cultures were isolated from discrete colonies by sub-culturing on nutrient agar and incubated at 35±2℃ for 24 hours for bacteria and 5-7 days for fungi. The isolates were preserved in slants at 4℃ for identification.

3. RESULTS

Baseline microbiological and physicochemical properties of the crude oil contaminated soil and *P. florida* SMC are shown in Table 2. The baseline microbiological parameters were higher in the crude oil contaminated soil than in *P. florida* SMC except for hydrocarbon utilizing fungi. The baseline physicochemical parameters were higher in *P. florida* SMC than in the contaminated soil except for TPH which was only present in the contaminated soil and total organic carbon.

4. DISCUSSION

The amount of hydrocarbon utilizers in the SMC and the contaminated soil were considerably high and adequate for bioremediation. The pH of SMC has been documented by other researchers to fall within the range of 5.0 – 8.0 (Fig. 1). The weak acidic nature of the SMC used in this study was due to decomposition of organic matter present in the compost material.

Table 2. Baseline Properties of Contaminated Soil and Spent *P. florida* Compost

Parameter	Contaminated Soil	*P. florida* SMC
Total Heterotrophic Bacteria (cfu/g)	1.94×10^6	1.43×10^5
Total Heterotrophic Fungal (cfu/g)	1.23×10^6	7.3×10^5
Hydrocarbon Utilizing Bacteria (cfu/g)	8.2×10^5	6.6×10^5
Hydrocarbon Utilizing Fungal (cfu/g)	5.4×10^5	9.3×10^5
Total Petroleum Hydrocarbon (mg/kg)	13286.3	-
pH	5.6	6.7
Moisture (%)	11.4	56.7
Total Nitrogen (%)	4.0	21.0
Total Phosphorus (%)	0.71	4.45
Total Organic Carbon (%)	11.8	7.56

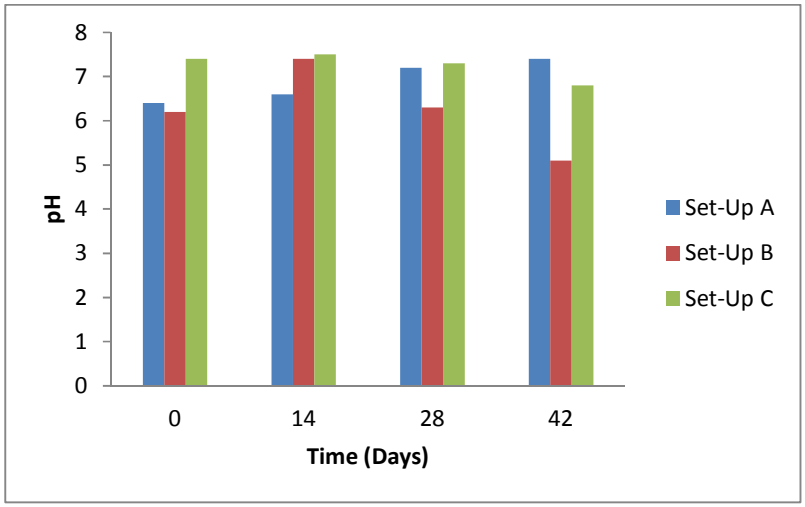

Fig. 1. Changes in pH values of the treatment options during remediation study

The pH value of the various treatment set up were slightly acidic and alkaline. This may be attributed to the enhancement or enrichment of the nutrient levels (by supplementing with various amounts of spent *P. florida* compost) of the soil. The total organic carbon (TOC) content of the contaminated soil prior to amendment was 11.80%. At the end of day 42, the TOC present in the sample was 7.40% for Set-up A, 7.40% for Set-up B, 8.61% for Set-up C experiments respectively as shown in Fig. 2.

There were slight decreases in total organic carbon (TOC) concentration in the various set up. The loss in TOC has been correlated with biomass increase in microbial systems. Ibiene et al. [10] and Adenipekun and Ogunjobi [11] however reported slight increases in TOC in their bioremediation studies. The total nitrogen content of the contaminated soil prior to amendment was 4.0% as shown in Table 2. After amendment of the experimental soil samples with various concentration of spent *P. florida* compost, the total available soil nitrogen at day 42 of the study period was 7.58% for Set-up A, 9.76% for Set-up B, 11.72% for Set-up C experiments respectively as shown in Fig. 3.

Also, the total phosphate content of the contaminated soil after amendment with various concentration of SMC for set-up A, B and C after 42 days of study increased to 3.96 mg/kg, 5.95 mg/kg and 9.156 mg/kg respectively (Fig. 4) when compared with the baseline of 0.71 mg/kg.

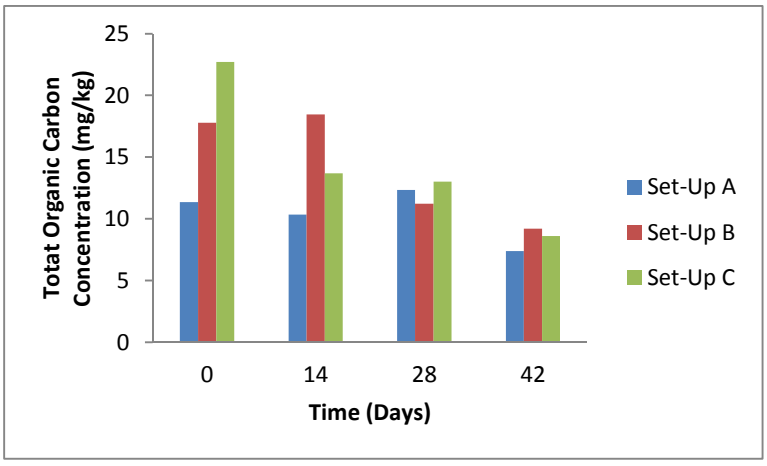

Fig. 2. Changes in Total Organic Carbon (TOC) concentration of the treatment options during remediation study

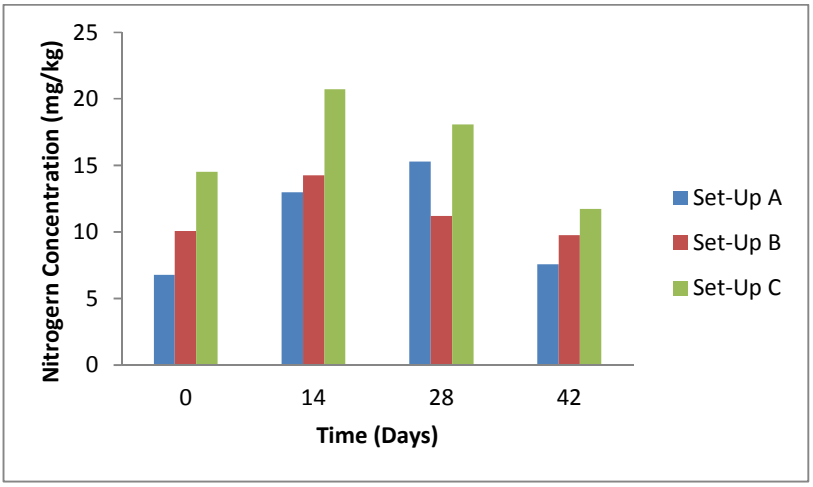

Fig. 3. Changes in nitrogen concentration of the treatment options during remediation study

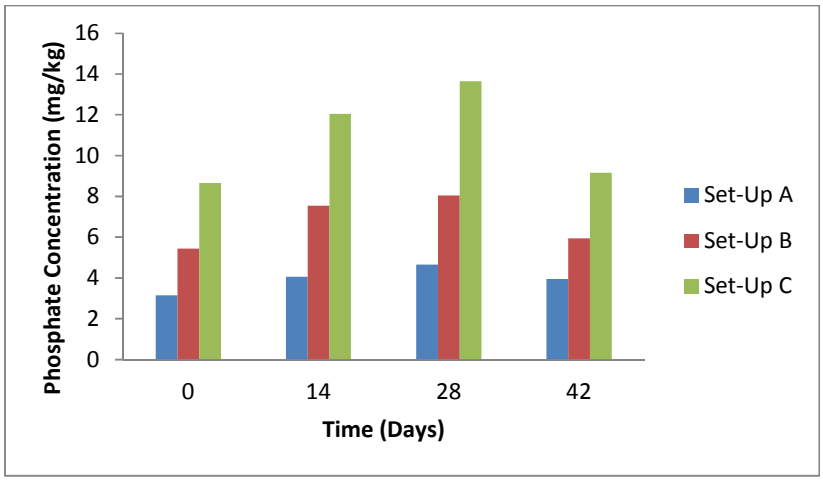

Fig. 4. Changes in Phosphorus concentration of the treatment options during remediation study

The result for total petroleum hydrocarbon (TPH) shown in Fig. 5 revealed a total percentage loss of 90.09% in Set-up C after the 42 days of study, a significant decrease with respect to time when compared to the baseline concentration. Decrease in TPH indicates the effectiveness of SMC of *P. florida* as a very useful bioremediating organic substance. Stanley et al. [12] and Adenipekun and Ogunjobi [11] in their study also reported a very high percentage loss of about 90% of TPH.

The hydrocarbon utilizing bacteria isolated were both Gram positive and negative bacteria. Okerentugba et al. [13] reported that Gram negative bacteria have a dominant population in crude oil contaminated soil. Bioremediation of crude oil by spent *P. florida* yielded favourable results when compared with mycoremediation. Spent *P. florida* compost provide great capacity to remediate polluted soil when compared to other members of *Pleurotus* family previously and commonly used in bioremediation.

Table 3. Microorganisms isolated from the contaminated soil and *P. florida* SMC

Bacterial isolate	Fungal isolate
Bacillus sp	*Penicillium* sp
Pseudomonas sp	*Fusarium* sp
Micrococcus sp	*Microsporum* sp
Flavobacterium sp	*Sacchoromyces* sp
Arthobacter sp	

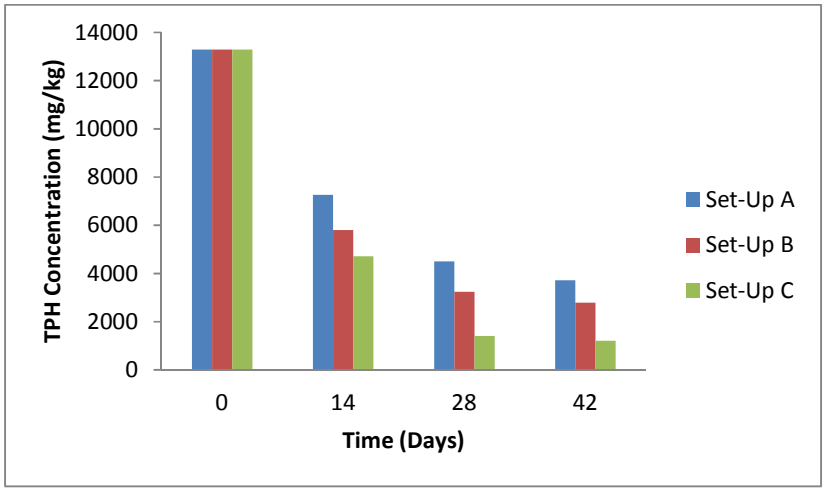

Fig. 5. Changes in Total Petroleum Hydrocarbon Carbon (TPH) concentration of the treatment options during remediation study

5. CONCLUSION

This research has shown the effectiveness of spent mushroom compost of *Pleurotus florida* in bioremediation. The result of the total petroleum hydrocarbon of 1212 mg/kg and a percentage loss of 92.09% after 42 days of study shows a significant decrease with time when compared with the initial TPH concentration of the contaminated soil of 13286.3 mg/kg. Therefore, future work should focus on the bioremediation of crude oil contaminated sites with optimum combination of spent *Pleurotus florida* and other organic wastes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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