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Bioremediation potential of *Lentinus subnudus* in decontaminating crude oil polluted soil

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Abstract

The aim of this work was to study the efficacy of using a white-rot fungi *Lentinus subnudus* to treat crude oil polluted soil by employing its biodegradation and bioaccumulation potentials. The polluted soil was amended with plantain peels and treated with spawns of *Lentinus subnudus* for 180 days. The physicochemical parameters monitored during the study were Total Hydrocarbon Content(THC), Total Nitrogen, Total Organic Carbon, Ash content, Phosphate, Potassium, Nitrate, Organic matter, pH and heavy metals (Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni and V). The mushroom demonstrated ability to biodegrade hydrocarbon pollutants in the soil as evident by decreased levels of THC which ranged from 20.46% - 91.94%. Results also showed decreased concentrations of all heavy metals analysed. *Lentinus subnudus* was more efficient in decreasing the levels of Cd, Cr, Pb, Ni and V than Fe, Mn, Cu and Zn. Observed values of Mn and V were 33.5% and 98.16%, respectively, after 180 days of treatment. This study demonstrated that *Lentinus subnudus* could be used in bioremediation of crude oil polluted soil amended with plantain peels.

Keywords: Biodegradation, bioaccumulation, *Lentinus subnudus,* plantain peels.

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Introduction

Soil contamination emanating from oil spills is receiving global attention presently (Millioli *et al.,* 2009). The concern stems primarily from health risks posed from direct contact with the contaminated soil, vapors from the contaminants, and from secondary contamination of water supplies within and underlying the soil (Thapa *et al*., 2012). Leaks and accidental spills occur regularly during the exploration, production, refining, transport and storage of petroleum and petroleum products. The release of hydrocarbons in the environment whether accidentally or due to human activities is a main cause of water, air and soil pollution. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutation (Nwilo and Badejo, 2001).

Bioremediation has been globally accepted as a method for treating contaminated soil. This technology takes advantage of the biodegradation, biotransformation and bioaccumulation capabilities of bacteria and fungi to remove pollutants from the soil. The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation (Alexander, 1999). The great potential of fungi in bioremediation is by virtue of their aggressive growth, extensive hyphae reach in the environment and their degradative enzymes (Novotny *et al*., 1999; Ashoka *et al*., 2002).White-rot fungi are ubiquitous in nature. They are so called because their degradation processes result in a bleaching of wood substrates (Kirk *et al*., 1992). They have strong enzyme system that allows them digest lignin and cellulose in wood giving it a bleached appearance. The enzymes of the lignin degradation system of white-rot fungi are extracellular and have low substrate specificity, thus conferring on them the ability to degrade a wide range of pollutants, particularly those which have chemical bonds similar to lignin compounds (Barr and Aust, 1994). This unique capability has been exploited for amelioration of crude oil polluted soils on laboratory scale. Laboratory studies have shown that white-rot fungi can transform and degrade recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAHs) (Punnapayak *et al*., 2009), Polychlorinated biphenyl (PCB) (Moeder *et al*., 2005), Pesticides (Yang and Rinker, 2004) and herbicides (Kadian *et al*., 2008). They have also been found to be involved in bioaccumulation of heavy metals (Hitivani and Mecs, 2003).

The potential of using mushroom to remove many toxic pollutants released into the environment by human activities has drawn many researchers into finding suitable candidates and medium for mycoremediation. Many studies have reported the use of *Pleurotus* species in soil bioremediation (Isikhuemhen *et al*., 2003; Baldrian *et al*., 2006; Olusola and Ejiro, 2011; Okwujiako *et al.,* 2013). Adenipekun and Fasidi (2005) reported the ability of *Lentinus subnudus* to mineralize soil contaminated with various concentrations of crude oil. Various lignocellulosic substrates can support the vegetative growth of *Lentinus subnudus*. Plantain consumption is high in Bayelsa State, making plantain peels agro-wastes of interest in mycoremediation in this environment. The objective of this study was to evaluate the bioremediation potential of *Lentinus subnudus* in decontaminating crude oil polluted soil amended with plantain peels.

Materials and Methods

Study Area: The crude oil polluted soil was excavated from oil spill sites in Kalaba and Ayambele swamps, located in Yenagoa Local Government Area of Bayelsa State, Nigeria. These sites were selected due to high level of pollution as a result of perennial oil spillage from ruptured pipelines around Okordia manifold at Ikarama community.

Soil treatment: The test organism *Lentinus subnudus* was collected from tree stump in Yenagoa and taken to Mushroom Science Unit of the Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria for identification. Tissue culture of the freshly collected mushroom was prepared by cutting the tip of the stipe after removing the pileus and placed on Potato Dextrose Agar (PDA) medium. This was carried out under sterile condition. The pure cultures of isolate was maintained on potato dextrose agar (PDA) slants and subsequently transferred to PDA plates and incubated at 25°C to obtain a good mycelial growth. Two hundred grams of polluted soil samples were transferred into cleaned and dried 475 ml mayonnaise bottles. Twenty grams each of plantain peels and groundnut shell were used to overlay the soil samples. Afterwards, the bottles were tightly covered with aluminum foil and sterilized in an autoclave at 121ºC for 15 min according to the modified method of Adenipekun and Fasidi (2005).Each bottle was inoculated with 20g of vigorously growing mushroom spawn and incubated at room temperature for 180 days. Polluted soil without nutrient amendment and inoculation with mushroom was used as the control experiment. All experiments were in triplicate.

Physicochemical analysis: Total Hydrocarbon Content (THC), Total Nitrogen (TN), Total Organic Carbon (TOC), Ash content, Phosphate, Potassium, Nitrate and Organic matter of all the test samples were carried out using solvent extraction method in accordance with Standard Test Methods (ASTMD). Heavy metal (Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni and V) were determined by Atomic absorption spectrophotometer (AAS) according to the methods of APHA (1985).

Results

Physiochemical properties of soil and organic waste used for amendment: The physiochemical properties of the polluted soil and the plantain wastes used for soil amendment to sustain mushroom growth are shown in Table 1. The polluted soil had a pH of 8.12 and a low concentration of nitrogen, potassium and phosphorus of 0.14%, 180 (mg/100g) and 2.15 (mg/100g) respectively. The plantain peels had a pH of 7.53 and rich in nitrogen, phosphorus and potassium. The polluted soil under investigation can be described as heavily contaminated with hydrocarbon contaminants; with THC concentration of 39280 mg/kg. Heavy metals characteristic of the polluted soil is as given: Iron (780mg/l), manganese (17mg/l), Cupper (2.7mg/l), Zinc (23mg/l), Lead (0.03mg/l), Chromium (0.025mg/l), Cadmium (1.28mg/l), Nickel (2.3mg/l) and Vanadium (1.36).

Table 1: Physicochemical parameters of crude oil polluted soil and amendment additive

Physicochemical Parameter	Contaminated Soil	Plantain peel
pH	7.32	8.12
Total Kjeldahl Nitrogen (%)	0.14	0.79
Phosphate (mg/100g)	2.15	250 (mg/kg)
Potassium (mg/100g)	180	2230 (mg/kg)
Total Organic Carbon (%)	19.72	13.2
Total Organic Matter (%)	4.47	22.5
Ash Content (%)	50.25	30.10
THC (mg/kg)	29280	25
Fe(mg/kg)	780	150
Mn(mg/kg)	17	1 2.1
Cu(mg/kg)	2.7	5.16
Zn(mg/kg)	23	8.8
Cd(mg/kg)	1.28	-
Cr(mg/kg)	0.025	-
Pb(mg/kg)	0.03	-
Ni(mg/kg)	2.30	-
V(mg/kg)	1.36	-

Bioremediation of crude oil polluted soil with Lentinus subnudus: Lentinus subnudus was able to degrade hydrocarbon pollutants in the soil as evident by decrease in Total Hydrocarbon Content (THC) (Table 2). After 30 days of growth, the fungus effectively reduced the THC in the contaminated soil by 20.46%. This continued progressively till the 180th day of sampling. The percentage THC reduction observed in the study ranged from 20.46% - 1.94%. The pH values ranged from 7.24-7.85.The removal efficiency expressed in percentage loss with time is given in Figure 1.

Table 2: Results of bioremediation of crude oil polluted soil with *Lentinus subnudus*

	Day 0 THC (mg/kg) pH	Day 30 THC (mg/kg) pH	Day 60 THC (mg/kg) pH	Day 90 THC (mg/kg) pH	Day 120 THC (mg/kg) pH	Day 150 THC (mg/kg) pH	Day 180 THC (mg/kg) pH
Treatment	39280 7.85	31243 7.84	26448 7.84	19246 7.70	10552 7.51	5228 7.33	3166 7.24
Control	39280 7.32	37759 7.50	36330 7.38	35982 7.51	33150 7.46	31705 7.22	28940 7.30

Bioremoval of heavy metals by Lentinus subnudus: Results showed decrease in concentrations of all heavy metals (Table 3). *Lentinus subnudus* was more efficient in removing Cd, Cr, Pb, Ni and V than Fe, Mn, Cu and Zn. Mn was the least removed at 33.5% and V was the most removed at 98.16% after 180 days of treatment. The removal efficiency expressed in percentage loss with time is given in Figure 1.

Table 3: Results of bioremoval of heavy metals by *Lentinus subnudus*

Heavy metal	Concentration (mg/kg)						
	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Fe	780	733.54	693.34	587.50	472.06	465.11	302.20
Mn	17	17	16.3	15.7	14.3	12.5	11.3
CU	2.7	2.46	2.02	1.97	1.72	1.55	1.31
Zn	23	16.3	14.9	14.3	13.5	10.2	8.59
Cd	1.28	0.83	0.79	0.72	0.61	0.34	0.12
Cr	0.025	0.0205	0.0183	0.0129	0.0085	0.0077	0.0023
Pb	0.03	0.018	0.017	0.010	0.0078	0.0041	0.0022
Ni	2.3	1.79	1.30	1.18	0.86	0.12	0.051
V	1.36	1.19	0.79	0.56	0.21	0.086	0.025

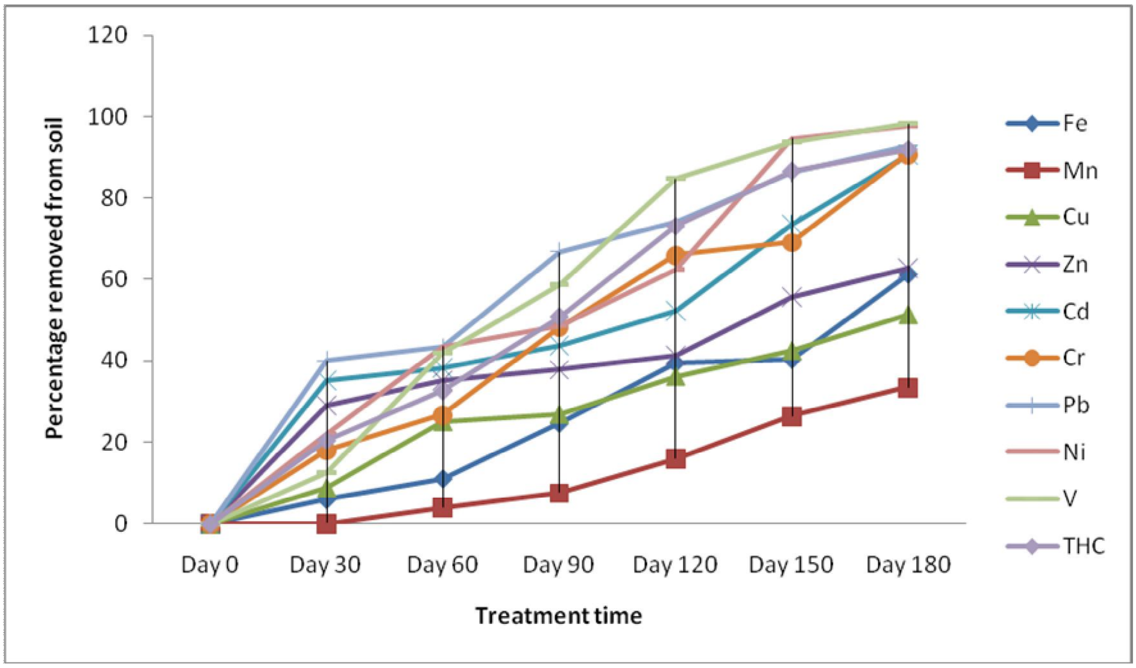

Figure 1: Percentage removal of THC and heavy metals by *Lentinus subnudus*

Discussion

The decontamination of crude oil polluted soil was monitored with respect to the degradation of Total Hydrocarbon Content (THC) and removal of heavy metals. The pH which is not only essential for determining the availability of many soil nutrients but also in determining the fate of many soil pollutants, their breakdown and possible movement through the soil was also monitored. The study period was 180 days, which is long enough to determine the bioremediation potential of any slow growing mushroom.

The pH values observed in this study ranged from 7.24-7.85. The pH of both the treatment and control experiment remained alkaline within the 180 days of study (Table 2). The pH of the contaminated soil slightly increased with addition of plantain peels. This is in agreement with Atlas and Bartha (1992) who reported that addition of nutrient to soil increases the pH of the soil mixture. pH is an important factor in determining biodegradation of contaminants in soil bywhite-rot fungi as reported by Adenipekun *et al*. (2011). The pH of the polluted soil decreased after inoculation with *Lentinus subnudus* from 7.85 to 7.24. This finding is consistent with the report of Adenipekun and Fasidi (2005a) where they observed a decrease in pH after 6 months of incubation of crude oil polluted soil with *Lentinus subnudus.*

It was observed during the study that THC concentration decreased over time after treatment with *Lentinus subnudus* (Table 2). The percentage increase inrate of bioremediation of THC was significant ($p<0.05$) throughout the period of experiment. Significant difference in levels of THC was also observed between the treated soil and the control. The percentage decrease of THC in the treated soil stood at 91.94% at the end of the study period, as against 26.32% in the control.Adenipekun and Fasidii (2005a) similarly reported degradation of crude oil in polluted soil by monitoring changes in Total Petroleum Hydrocarbon (TPH) levels in crude oil contaminated soils at varying concentration after 6 months of incubation with the white-rot fungi *Lentinus subnudus*. The advantage of using white-rot fungi like *Lentinus subnudus* over bacteria in bioremediation lies in their ability to degrade highly polluted soil. Udeh *et al*. (2013) reported that bacterial bioremediation is not effective at THC concentration above 5% but mushroom have been reported to bioremediate polluted soil with a very high THC concentrations (Adenipekun and Fasidi, 2005a).

The metal removal efficiency of *Lentinus subnudus* varies with the element but a general decreases in amounts of the heavy metals in the polluted soil were observed (Table 3). *Lentinus subnudus* was more efficient at removing Cd, Cr, Pb, Ni and V than Fe, Mn, Cu and Zn. Mn was the least removed at 33.5% and V was the most removed at 98.16% after 180 days of treatment. Hitivani and Mecs (2003) reported the bioaccumulation of Cd, Hg, Cu, Pb and Zn by *Lentinusedodes*. Adenipekun and Fasidi (2005b) reported the bioaccumulation of Fe, Cu, Zn and Ni by *Lentinus squarrosulus.* Fungi have excellent biosorption capability due to their ability to grow under extreme conditions of pH, temperature, minimal nutrient as well as high levels of metal concentration. The composition of the fungal cell wall and the filamentous morphology of fungi could have increased the ability of the mushroom to bioadsorb the heavy metals.

Conclusion

The application of white-rot fungi in bioremediation is gaining prominence. *Lentinus subnudus* presents itself as a good candidate for bioremediation of crude oil contaminated soil, as it showed potential to degrade hydrocarbon pollutants and remove heavy metals. It can suitably grow on inexpensive agricultural waste, plantain peel, which is in high supply in Bayelsa state.

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