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EFFECT OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) MYCELIA ON PETROLEUM HYDROCARBON CONTAMINATED SUBSTRATE

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ABSTRACT
A study on the mycoremediation effect of oyster mushroom (Pleurotus ostreatus) mycelia on petroleum-hydrocarbon-contaminated substrate was carried out in the mushroom unit of the Faculty of Agriculture Demonstration Farm, located in the University of Port Harcourt, Port Harcourt, Nigeria. The study aimed at determining the degree of reduction or breakdown of chains of hydrocarbon in a petroleum-hydrocarbon-contaminated substrate over time. Mushroom substrate comprised sawdust enriched with nutrients to allow for microbial proliferation was obtained from the demonstration farm, while crude oil obtained from the Nigerian National Petroleum Company (NNPC) was used as contaminant. 230g of fully ramified mushroom substrate was weighed out in triplicates and subjected to three treatment regimes (A, B and C). Treatments A, B and C were contaminated with 20ml, 40ml and 60ml of crude oil respectively, while their corresponding controls similarly contaminated with crude oil had no mushroom mycelia and were further sterilized to eliminate any microbial life. The experiment was monitored for four weeks within which total hydrocarbon concentrations in treatments A, B and C were reduced by 90%, 87% and 85% respectively over time while no change occurred in their corresponding control groups. It is recommended that the application of mushroom mycelium is a new and effective tool in the rapid remediation of sites contaminated with petroleum hydrocarbons over a short period of time as opposed to the usual conventional, time-consuming, and inefficient methods of waste management in the environment.

Keywords: Mycoremediation, Mycelia, Contaminated Soil, Oyster Mushroom

INTRODUCTION
Crude oil exploration and exploitation had been the major industry and mainstay of the Nigerian economy over the past decades. Besides its positive impacts such as job creation and economic development, the negative impacts arising from accidental oil spillages and gas flaring had led to environmental contamination and pollution and its concomitant degradation of natural resources such as land and water; all of which had threaten health and life of both humans and animals in the affected areas.

A major constituent found in the oil spills associated with contamination of land and water resources is petroleum hydrocarbon. According to Yadar and Reddy (2000), environmental contamination by petroleum hydrocarbon is caused by leakage from underground storage tanks and pipelines, accidental spills, improper waste disposal practices and leaching landfills. Leaking underground storage tanks is one of the largest sources of soil pollution, which also negatively affect adjacent ground water supplies (Zytner *et al* 2001). Owing to the sheer number of sites contaminated by petroleum hydrocarbon and their potential for causing a myriad of health problems in humans, they are among the most frequently treated contaminants by the United States Environmental Protection Agency (USEPA, 2007).

Bioremediation technologies which include on-site (*in-situ*) biological treatment are becoming an increasingly used alternative for the remediation of petroleum hydrocarbon-impacted sites because of its low cost and capacity for complete degradation of contaminants (Bryan, 2006). Bioremediation, which is the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes to minimize the risk to human health and environment, uses a wide range of living organisms such as microorganisms typically bacteria as well as fungi, plants and even macro-invertebrates (Gossy and Bush, 2002). Depending on the organisms in use, various terminologies have been employed to describe the bioremediation process such as bacterial bioremediation, referring to the use of bacteria in remediation, phytoremediation when plants are in use and that of fungi is mycoremediation.

Mycoremediaton employs the use of selected, cultured fungal mycelia to remove or degrade environmental contaminants. Mycoremediation has emerged as a favourable technology for the bioremediation of hydrocarbon contaminated soils due to its relatively low capital and operating costs, simple operation and design and relatively high treatment efficiencies (Namkong *et al*, 2002). Stamets (2005) noted that every ounce of soil hosts contain not just one species but literally thousands of species of fungi of the estimated 6,000,000 species of fungi in the world. Hence, the genetic diversity of fungi is vast by design and apparently crucial for life to continue (Stamets, 2005). Subsequently, fungi function in different ways in nature, such as their association with plants known as mycorrhiza which is beneficial to both the fungi and plant, saprophytes where they are responsible for the breaking down of all types of organic matter and in remediation where they are responsible for mycoremediation and mycofiltration ability to enhance environment, produce enzymes and secondary metabolites used in place of harmful chemicals. The mycelia of mushroom exude enzymes that breakdown complex molecules which otherwise would not have been digested by simple digestive enzymes of animals. Thus, lignin as well as petroleum hydrocarbon and other harmful pesticides are digested by enzymes exuded by mushroom (Schroder**,** 2004).

De Vriels (2009) reported that increased fungal biomass can reduce nitrogen losses to the environment. Fungi are used to remove metal and aromatic hydrocarbon from contaminated soil (Eleke and Busuioc, 2010) as well as to remove metals from wastes (Prince *et al*, 2001). Thus, the bioremediation property of fungi is attributable to the network of hyphae strands known as mycelia which decompose organic compound and return nutrient to the soil while carbon dioxide (CO_2) is released for plants use. Owing to the network of mycelium which ramify the substrate, enzymes are released which breakdown long polymer chains of hydrocarbon into simple organic substances which are then absorbed by the hyphae. As a contribution to existing knowledge on mycoremedicaiton this work aimed at determining the ability of mushroom to serve as an effective bioremediation tool and the degree at which it can reduce petroleum hydrocarbon in contaminated substrate over a short period of time.

MATERIALS AND METHOD

Study area and preparation of mushroom substrate for ramification

The spawn of mushroom (*Pleurotus ostreatus)* used for this study was obtained from the Mushroom Unit of the Faculty of Agriculture Demonstration Farm of the University of Port Harcourt, Port Harcourt, Nigeria. The materials utilized included the following: Fully ramified mushroom substrate, sawdust, crude oil, weighing scale, gas chromatography, pressure cooker, masking tape and foil paper. The crude oil was obtained from the Nigerian National Petroleum company (NNPC) Port Harcourt. Mushroom substrate made up of

sawdust enriched with nutrients notably wheat bran and lime were mixed in the proportion of 90, 9.5 and 0.5% respectively. Water was added to the mixture to a moisture level of 65% in a polythene bag.

The spawn of *Pleurotus ostreatus* was used to inoculate the bag at the rate of 5% wet weight of the bags. The bags were incubated to ramify with mushroom mycelia. After 20 days of full ramification, the content of the bags were mixed thoroughly together and exposed to three different treatment regimes as in the experimental design below.

Experimental Design

Two hundred and thirty grams (230g) of well ramified mushroom substrate was weighted out in triplicates and given three different treatments labeled A, B and C. Treatment A (in triplicates) was contaminated with 20ml of crude oil, while treatments B and C also in triplicates were contaminated with 40ml and 60ml of crude oil respectively. Each treatment regime had a corresponding control which was not inoculated with mushroom mycelium. The controls were further sterilized in a pressure cooker at 15psi for one and a half hour to destroy any microbes which may be present to degrade the substrate.

Sample collection and data analysis

Following the contamination of ramified substrates with crude oil in treatments A, B and C, samples were taken out of each treatment to the laboratory for analysis of total presence of hydrocarbon (TPH) using the Gas chromatography (GC) method. In this method an ultra trace Gas chromatography (GC) was equipped with the ultrafast module (UFM) option. The system also features programmable temperature vaporizing (PTV), inlet flame ionization detector (FID) configuration. Injections were performed with triples auto-sampler. The UFM option contains a column module that includes a capillary column. Standard mixtures for evaluating injection performance were prepared by diluting n-alkanes ranging from n-C7 to n-C40 in n- Pentane at parts per million (ppm) levels. A gas oil reference mixture diluted in n-pentane was analyzed at different concentrations to test for linearity repeatability and sensitivity of the method. Extracts from the substrates were now analyzed for total presence of hydrocarbon. Statistical analysis was performed using percentage analysis to express the difference between the treatment regimes and the control, and also the influence of mycelial degradation on TPH over time.

RESULT

Tables 1-3 summarized the individual and mean values of hydrocarbon concentrations of ramified mushroom substrates exposed to different levels of treatments (A, B and C) with crude oil (contaminant). Treatment A, mushroom substrate exposed to 20ml contaminant over a period of four weeks showed an initially high hydrocarbon concentration for the individual triplicates with a range from 2032.63021mg/l to 2846.01946 mg/l and a mean of 2385.28371mg/l at the start of the experiment (week o) (Table 1). However, by weeks two and four, sharp declines in individual values of the hydrocarbon concentration for each triplicate occurred with mean values of 967.57032mg/l (week 2) and 236.80320mg/l (week 4) respectively. A reduction value of 90% contaminant was recorded at week four compared with the individual values of the control set which remained unchanged over the period of four weeks (Table 1).

Treatment B, contaminated with 40ml crude oil showed, similar trends as in treatment A in which hydrocarbon concentrations ranged from 3154.62101 to 3762.97622mg/l with mean value of 3490.07675mg/l (in week 0). This reduced drastically by week 4 to a mean value of 437.49551 mg/l (ranged 398.45682-511.53691 mg/l); showing a reduction of 87% of

the contaminant, compared with the control group which remained unchanged (Table 2). Similarly, for treatment C contaminated with 60ml of crude oil, initial hydrocarbon concentration ranged from 5862.06324-8257.08489mg/l with a mean of 7447.73078mg/l in week 0. In subsequent four ? a drastic decline ranging between 1012.05982 -1232.42222mg/l with a mean value of 1109.59046 mg/l occurred, and 85 % reduction was recorded (Table 3). An overall comparison of the mean hydrocarbon concentration of the treatments and control sets showed an unchanged nature of control groups whereas treatments A, B and C recorded marked declines in total hydrocarbon concentrations over time regardless of treatment regime (Fig.1).

Table 1: Total hydrocarbon concentration of mushroom substrate mixed with 20ml of crude oil as contaminant (treatment A)

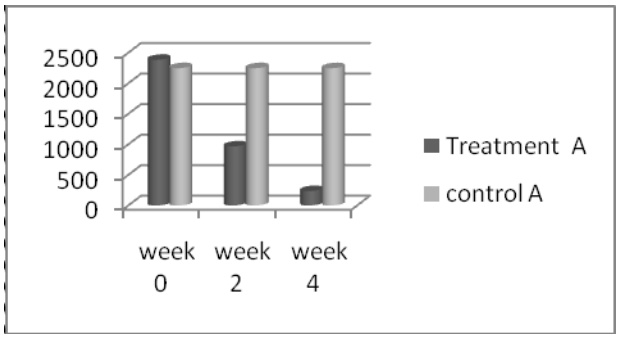
Treatment A (20ml) (wks)	control	Rep.1	Rep. 2	Rep. 3	Mean (Rep.1-3)	% reduction
Week 0	2248.10324	2277.20146	2846.01946	2032.63021	2385.28371	
Week 2	2248.10346	1438.08231	832.38201	632.2466	967.57032	59.44
Week 4	2247.10342	342.08862	262.0868	106.2341	236.80382	90.07

Table 2: Total hydrocarbon concentration of mushroom substrate mixed with 40ml of crude oil as contaminant (treatment B)

Treatment B (20ml) (wks)	Control	Rep.1	Rep. 2	Rep. 3	Mean (Rep.1-3)	% reduction
Week 0	3248.40184	3154.62101	3552.63302	3762.97622	3490.07675	
Week 2	3246.40182	1824.04021	1432.63302	2060.03242	1772.23522	49.22
Week 4	3248.40178	398.45682	402.49280	511.53691	437.49551	87.46

Table 3: total hydrocarbon concentration of mushroom substrate mixed with 60ml of crude oil as contaminant (treatment C)

Treatment C (60ml) (wks)	control	Rep.1	Rep. 2	Rep. 3	Mean (Rep.1-3) (X)	% reduction
Week 0	5036.97731	8257.08489	8224.04421	5862.06324	7447.73078	
Week 2	5035.97730	4066.08642	6440.62042	2486.20421	3730.97029	49.90
Week 4	5036.97730	1232.42222	1084.28934	1012.05982	1109.59046	85.10



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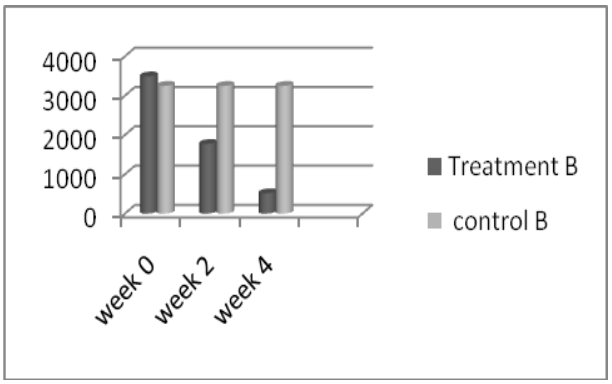

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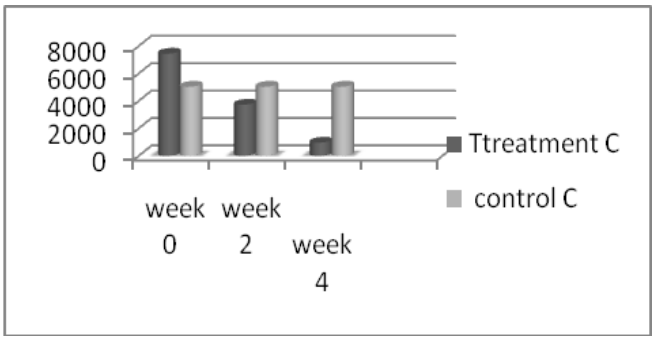

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Fig.1.Mean values of different treatment regimes (A,B,C) compared with their respective controls

DISCUSSION

The results of this study indicate that the mycelium of mushroom can be used to remediate hydrocarbon contaminated substrates. Tables 1-3 showed that there is a remarkable difference between the initial analyses (week 0) and the subsequent two to four weeks (i.e. weeks 2-4) of treatment of substrates containing ramified mushroom mycelia with petroleum hydrocarbon (crude oil). At the end of the two weeks, treatments A, B and C had mean percentage reductions of 59.44, 49.22 and 49.90 respectively, while in four weeks the percentage reductions of treatments A, B and C were 90.07, 87.46 and 85.10 respectively. By comparison there was no appreciable reduction of contaminants in either two or four weeks for the respective controls of the treatment groups in Tables 1-3. The results of this work therefore agree with the findings of Stamets (2003) who noted that most fossil fuels contain hydrocarbons which serve as energy source for mushroom mycelia.

Thus mushrooms have been used to significantly decrease the toxicity of wastes, degrade mineral contaminants such as arsenic, barium, manganese and many polynuclear aromatic hydrocarbons (PAHs) in contaminants (Stamets, 2003). He also demonstrated that spent mushroom composts could be used to amend a diesel contaminated site as they showed a rapid decrease in total presence of hydrocarbon (TPH) over time. It can be asserted from the foregoing that mycoremediation of crude oil polluted sites particularly soils contaminated with oil spills is a new environmental biotechnology tool which, if well developed, particularly in developing countries prone to incessant oil spillages in coastal waters and on land, will be useful in environmental clean-ups. This is because this study had demonstrated that the mycelia of oyster mushroom (*Pleurotus ostreatus*) can breakdown chains of hydrocarbon over a short period of time with the rapid reduction of TPH in substrates. Mushroom mycelium is of great importance in the remediation of oil spill sites both locally in

the oil producing Niger Delta region of Nigeria which is faced with incessant but occasional oil-spills as well as globally where oil exploration and exploitation occurs in the world.
The relatively constant values of the control groups which were not exposed to ramified mushroom mycelia and were further subjected to sterilization to eliminate microbial life showed that hydrocarbon can remain in a hydrocarbon polluted substrate over a long time and the substrate may become non-biodegradable. This observation agrees with the finding of Van *et al*. (2003) who noted that most living organisms present in the environments use contaminants as their energy source thereby breaking down the contaminants hence their absence in the environment may lead to a very serious problem.

CONCLUSION

Since this study had clearly shown that the mycelia of oyster mushroom (*Pleurotus ostreatus*) can breakdown chains of hydrocarbon over a short period of time with the rapid reduction of TPH in substrate, it then follows that mushroom mycelium will be of great importance and of universal applicability in the remediation of oil spill sites in the near future.

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