*Biotechnology Journal International*

***18(4): 1-10, 2017; Article no.BJI.34267***
***ISSN: 2456-7051***
*(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)*

# Enhancing the Performance of Three White-rot Fungi in the Mycoremediation of Crude Oil Contaminated Soil

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author MBA designed the study, supervised the work and wrote the first draft of the manuscript. Author OOO was involved in data collection and managed analyses of data. Both authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/BJI/2017/34267
*Editor(s):*
(1) R. Baskar, Department of Biotechnology, Kumaraguru College of Technology, Tamil Nadu, India.
*Reviewers:*
(1) Shimaa R. Hamed, National Research Centre, Egypt.
(2) Nwankwegu Amechi Sampson, Nnamdi Azikiwe University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/19992

***Original Research Article***

***Received 22nd May 2017***
***Accepted 22nd June 2017***
***Published 11th July 2017***

## ABSTRACT

Contamination of soils by petroleum hydrocarbon is on the increase, particularly in the oil producing areas of Nigeria. White-rot fungi have enzymes which are capable of turning these organic compounds into harmless substances. This work investigated the performance of three white-rot fungi (*Pleurotus tuber-regium, P. ostreatus* and *P. pulmonarius*) for the remediation of different concentrations (0, 1, 2.5, 5, 10 and 20% w/v) of crude oil contaminated soils with sawdust and poultry manure as bedding materials. Ten grammes of each of the white-rot fungi were separately inoculated in each bottle containing exhaustively cropped topsoil (200 g), rice straw (40 g) and wheat bran (20 g). Each treatment was conducted in three replicates and arranged in a 3 x 6 x 3 complete randomised design. The bottles (54) were incubated for zero, one and three months in a dark room, exposed to light, watered daily thereafter for twelve days for fruiting bodies to spring out and harvested. *P. ostreatus* had best agronomic performance and *P. tuber-regium* removed hydrocarbons and heavy metals more than either *P. ostreatus* or *P. pulmonarius* under similar experimental conditions. The yield and mycoremedial performance of the three tested white-rot fungi demonstrated potentials for cleaning-up petroleum hydrocarbon contaminated soil, but their performance reduced from 5.0% (w/v) crude oil substrate contamination.

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*Keywords: Crude oil contaminated soil; mycoremediation; poultry manure; sawdust; soil health; white-rot fungi.*

## 1. INTRODUCTION

Most *Pleurotus* species belong to the kingdom of macro-edible-fungi known as mushrooms with distinct fruiting bodies and filamentous with lignin-degrading ability [1,2]. These edible fungi play significant role in the environment and existence of humans as food, medicine, biodegrading agents, replenishment and remediation of polluted soils [3,4]. The unique ability of white-rot fungi to degrade lignin is largely attributable to the non-specific free radical mediated oxidizing reactions carried out by their extracellular lignin modifying enzymes they possess [5,6].

Enzymes provide white-rot fungi the unique ability to degrade a broad array of environmental pollutants such as dioxins, polychlorinated biphenyls, petroleum hydrocarbons, industrial dye effluents, herbicides and pesticides [7,8]. These enzymes are believed to enhance the ability of white-rot fungi in the breaking down of lignin in plant biomass and obtain a better access to the cellulose than by bacteria [7]. Therefore, white-rot fungi are good degraders of aromatic and polycyclic aromatic compounds in different environments, including soil [9-11]. For white-rot fungi to degrade these pollutants, it requires a substrate that will support its growth on such pollutants. *Pleurotus* species grow on most lignocellulosic materials such as rotten or rotting wood, wood residues and most of the agricultural wastes [12].

In Nigeria, petroleum hydrocarbons are common examples of these chemicals, which enter and pollute the environment frequently, both in small and large volumes through numerous routes [13,14]. Large volumes of these petroleum hydrocarbons enter the environment either through oil spills, tank leakages or oil pipe-line vandalisation [15]. As a result of the adverse effect of petroleum hydrocarbon on the environment, an environment-friendly method is required for the clean-up. One growing mechanism of decontamination that may fit these requirements is bioremediation. This technology stimulates natural processes that could lead to complete destruction of hazardous compounds into harmless products [16,17].

The use of fungi particularly mushrooms is relatively economical as they can be grown on a number of inexpensive agricultural and forest wastes such as corn cobs and sawdust [18-20]. White-rot fungi can withstand toxic levels of most organic pollutants [21,22]. Different species of *Pleurotus* have been used by different researchers to remediate different concentrations of crude oil contaminated soils. However, there is dearth of information on the inherent potential of these agro-industrial wastes (sawdust and poultry manure), despite their abundance in Nigeria, for enhanced agronomic performance of species of *Pleurotus*. Hence, this study seeks to compare the potentials of *P. tuber-regium, P. ostreatus and P. pulmonarius* for the remediation of different concentrations of crude oil contaminated soils when agro-industrial wastes such as sawdust and poultry manure are used as bedding materials.

## 2. MATERIALS AND METHODS

### 2.1 Soil Sampling and Fungi Spawn Preparation

Surface soil samples (0-15 cm) from exhaustively cropped farmland within the Obafemi Awolowo University, Ile-Ife, Nigeria was collected, air-dried for seven days and sieved with a 2 mm mesh to remove unwanted materials and stones. Harvested rice straw was collected from African Rice Section of the International Institute of Tropical Agriculture, Ibadan, sun-dried for seven days to remove the moisture content and cut to small sizes. Wheat bran was obtained from an open market in Ibadan, Nigeria and thereafter sun-dried. Jam empty bottles were bought from a local market in Ile-Ife, washed and sterilized.

Crude oil was collected from the Nigerian National Petroleum Corporation, Eleme, Nigeria and had the chemical compositions (total hydrocarbon 173.20 mg $l^{-1}$, Zn 20.33 mg $l^{-1}$, Fe 37. 03 mg $l^{-1}$, Pb 4.87 mg $l^{-1}$ and Cd 0.85 mg $l^{-1}$). Pure cultures of *P. tuber-regium P. ostreatus* and *P. pulmonarius* were collected from the Plant Physiology Laboratory, Department of Botany, University of Ibadan, Ibadan, Nigeria. The spawns of the three fungi were prepared according to the method of Jonathan and Fasidi [23]. Rice straws were soaked in distilled water for one hour and then squeezed through a muslin cloth until no more water oozed out. Wheat bran (20 g) was added as an additive which was mixed thoroughly and put into the empty bottles covered with aluminium foil, autoclaved at 121ºC temperature for 15 minutes.

## 2.2 Experimental Design and Layout

Two hundred grammes of the air-dried and sieved surface soil sample from an exhaustively cropped land collected was put into each bottle and thereafter mixed with 12 t $ha^{-1}$ of the agro-industrial manure (composted poultry: ashed sawdust) in equal proportions. Different concentrations (0, 1, 2.5, 5, 10 and 20% w/v) of the crude oil were used to contaminate the soil. Forty grammes of rice straw moistened with distilled water were introduced into the contaminated soil in each bottle separated with wire gauze and covered with aluminium foil. Ten grammes of actively growing spawn of *P. tuber-regium* (*Pt*), *P. ostreatus* (*Po*) and *P. pulmonarius* (*Pp*) were separately inoculated in each bottle. Each treatment was replicated thrice and arranged in a 3 x 6 x 3 complete randomised design to give a total of 54 bottles. The bottles were left for zero, one and three months period of incubation in a dark room; and thereafter exposed to light and watered daily for twelve days for fruiting bodies to spring out and then harvested.

## 2.3 Soil and Fungi Fruiting Bodies' Analyses

The properties of pre-cropping soil, ashed sawdust and poultry compost used in this study were determined using standard methods as described by Page et al. [24] (Table 1). The soil pH was determined in 1:1 soil-1 M KCl suspension using a glass electrode pH meter. Total nitrogen of the soil was determined by the macro-Kjeldahl method. Available phosphorus in the soil was extracted using Bray P1 method and P in the extractants was determined by colorimeter. The soil carbon was determined using Walkley-Black wet oxidation method. Calcium ion, $Mg^{2+}$ and $K^{+}$ concentrations in the soil were extracted using 1 M ammonium acetate buffered at pH 7.0 and their concentrations in the extracts were measured using Buck Scientific Model 200 (East Norwalk, Connecticut, USA) Atomic Absorption Spectrophotometer (AAS).

Lead, Cd, Fe and Zn concentrations in the samples of soil and fungi fruiting bodies were determined using 5 ml of the acid mixture of conc. $HNO_3$ and $HClO_4$ in the ratio 2:1 and 5 ml of conc. $H_2SO_4$ to digest 0.5 g each of soil and fungi fruiting bodies for 2 h at 150℃. The digests were allowed to cool and each was made up to 25 ml with distilled water. Concentrations of Pb, Cd, Fe and Zn in the extracts were read on AAS. Total petroleum hydrocarbon in the soil and fungi fruiting bodies were extracted using 20 ml of xylene as extracting solvent on 10 g each of the samples using Greenberg et al. [25] and their concentration read on AAS.

## 2.4 Data Collection and Statistical Analysis

After the incubation periods and twelve days of exposure to light, the growth performance of the three white-rot fungi from different crude oil contaminated substrates were determined in terms of their height and girth of the stipe, pileus diameter and the fresh weight of the harvested fruiting bodies (Figs. 1 and 2). The data obtained from soil and fungi fruiting bodies analyses for the three species were subjected to descriptive analysis. The percentage removal of TPH was obtained using the following equation:

$$\text{Percentage removal of TPH} = \frac{mi-mf}{mi} \times 100\% \quad (1)$$

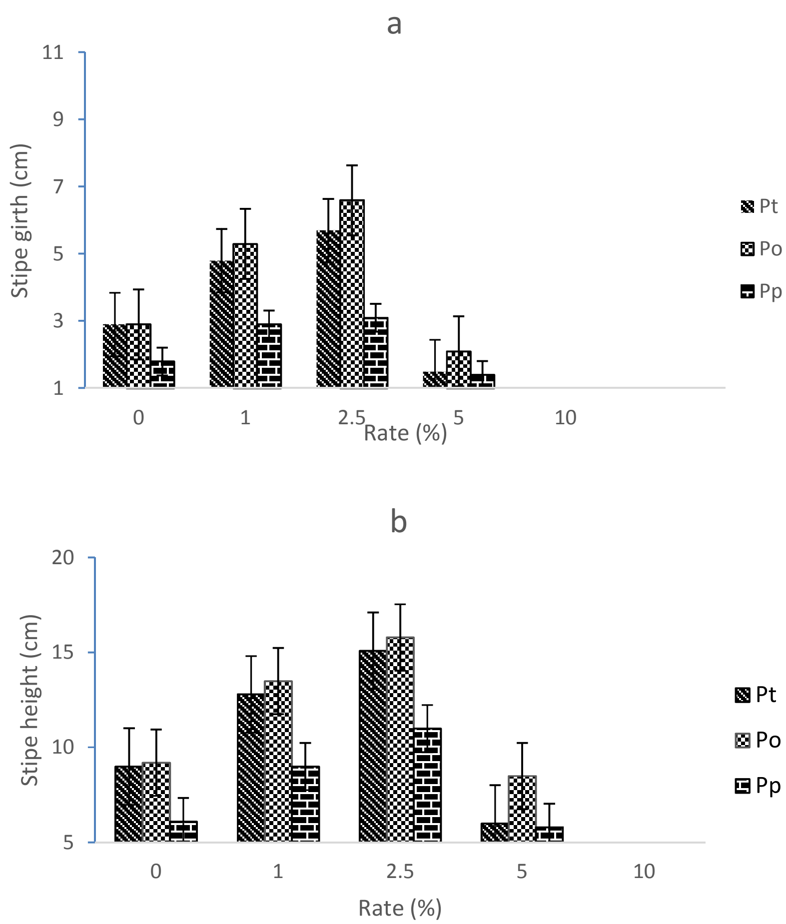
Where $mi$ = initial concentration of petroleum hydrocarbon at zero month of substrate inoculation, while $mf$ = final concentration of petroleum hydrocarbon at other periods of substrate inoculation. Data collected were subjected to analysis of variance and Bonferroni's Multiple Comparison post-tests for their treatment effects evaluation. The experimental precision achieved was reported by standard error at the probability level of 95%. Pearson correlation was used to test the relationship among the treatment means of TPH and selected heavy metals removal by white-rot fungus with best performance at 95% level of probability.

# 3. RESULTS

The proportions of sand, silt and clay of the soil used were: 846.0, 102.0 and 52.0 g $kg^{-1}$ respectively, indicating a loamy sand soil texture (Table 1). The soil had a pH of 6.1 in 1:1 soil- 1 M KCl medium, indicating an acidic soil condition. Other properties of the soil included: organic carbon 9.3 g $kg^{-1}$, total nitrogen 0.11 g $kg^{-1}$, available phosphorus 10.65 mg $kg^{-1}$, cation exchangeable capacity 1.40 cmol $kg^{-1}$ and total petroleum hydrocarbon 0.12 mg $l^{-1}$. The effects of crude oil contamination of the substrate on the growth performance of the three white-rot fungi at one month inoculation period are presented in Fig. 1. Optimum performance was however obtained at 2.5% rate of crude oil substrate contamination. No growth was recorded from 10% rate of the contamination. *P. ostreatus* recorded best performance, while *P. pulmonarius* had least. The results obtained at three months of inoculation period were higher and comparable with one-month inoculation (Fig. 2).

**Table 1. Pre-cropping soil, sawdust and poultry compost properties**

| Property | Soil | Sawdust | Poultry compost |
|---|---|---|---|
| Soil pH (1:1 soil:1 M KCl) | 6.10 | - | - |
| Organic carbon g $kg^{-1}$ | 9.60 | 198.40 | 33.40 |
| Total N g $kg^{-1}$ | 0.14 | 0.90 | 4.76 |
| Available P mg $kg^{-1}$ | 10.65 | 3.91 | 4.72 |
| Sand g $kg^{-1}$ | 840.60 | - | - |
| Silt g $kg^{-1}$ | 100.20 | - | - |
| Clay g $kg^{-1}$ | 59.20 | - | - |
| Soil texture | Loamy sand | - | - |
| Pb mg $kg^{-1}$ | 1.11 | - | - |
| Cd mg $kg^{-1}$ | 0.56 | - | - |
| Fe mg $kg^{-1}$ | 85.12 | - | - |
| Zn mg $kg^{-1}$ | 0.67 | - | - |
| TPH mg $kg^{-1}$ | 0.12 | - | - |



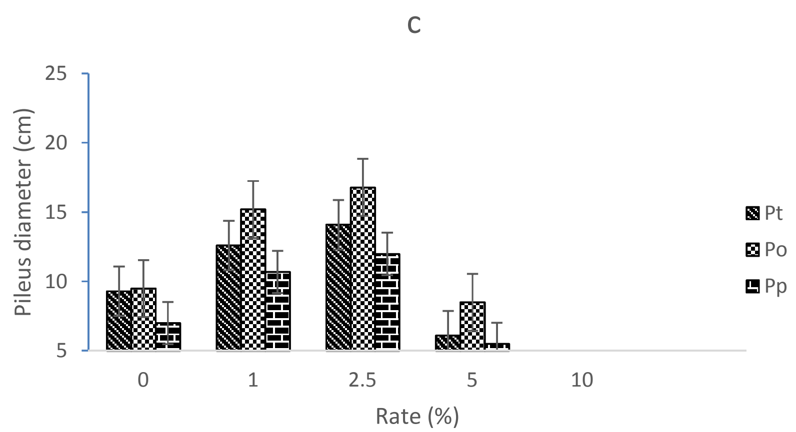
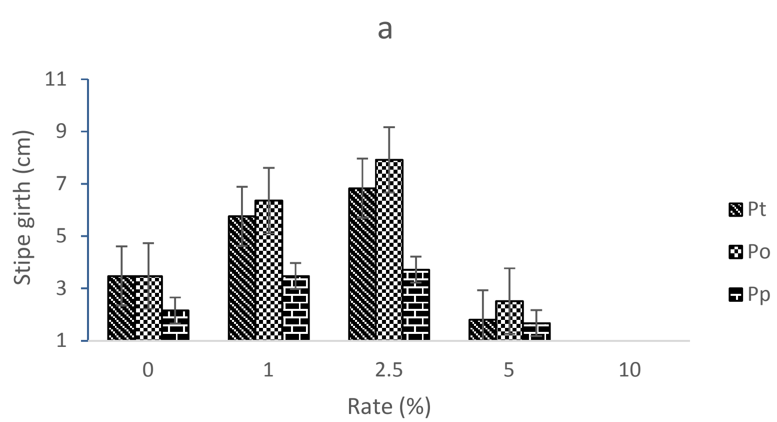
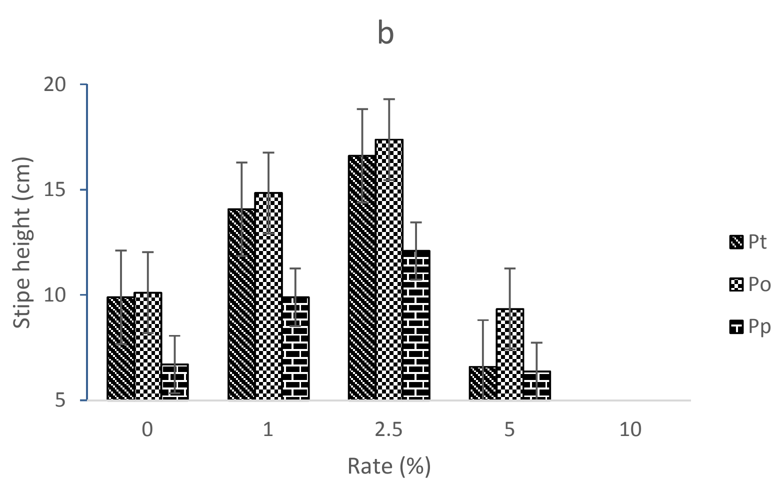



**Fig. 1. Effect of different rates of crude oil contamination of the substrate on the growth components (a) stipe girth (b) stipe height and (c) pileus diameter of three white-rot fungi at one month of incubation. Vertical bars represent the SE**

*Legend: Pt = Pleurotus tuber-regium, Po = Pleurotus ostreatus and Pp = Pleurotus pulmonarius*



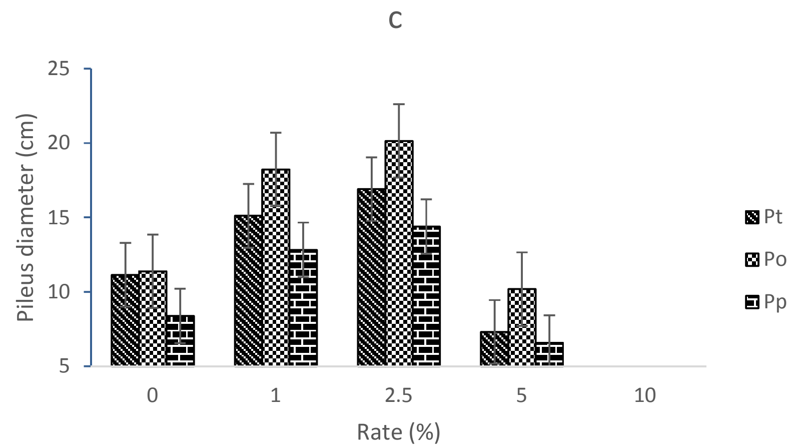



**Fig. 2. Effect of different rates of crude oil contamination of the substrate on the growth components (a) stipe girth (b) stipe height and (c) pileus diameter of three white-rot fungi at three months of incubation. Vertical bars represent the SE**

*Legend: Pt = Pleurotus tuber-regium, Po = Pleurotus ostreatus and Pp = Pleurotus pulmonarius*

**Table 2. Fresh weight (± SE in g) of the three white-rot fungi at different rates of crude oil contamination of the substrate and inoculation periods**

| Rate(%) | One month | | | Three months | | |
|---|---|---|---|---|---|---|
| | Pt | Po | Pp | Pt | Po | Pp |
| 0 | 133.5 ± 3.7b | 207.1 ± 4.0c | 109.5 ± 3.2a | 162.4 ± 6.3c | 212.3 ± 4.7c | 137.0 ± 2.5a |
| 1.0 | 195.7 ± 5.2a | 230.0 ± 5.5b | 111.0 ± 5.2a | 209.8 ± 3.4b | 255.5 ± 5.1b | 141.6 ± 3.4a |
| 2.5 | 205.7 ± 3.0a | 287.3 ± 4.7a | 118.2 ± 3.3a | 241.9 ± 3.1a | 313.1 ± 2.5a | 143.0 ± 2.5a |
| 5.0 | 40.3 ± 2.8c | 78.1 ± 2.3d | 37.8 ± 2.1b | 65.3 ± 2.5d | 92. 3 ± 2.8b | 55.8 ± 2.0b |
| 10.0 | - | - | - | - | - | - |
| 20.0 | - | - | - | - | - | - |

*Mean with the same letter in the column are not significantly different by Bonferroni's Multiple Comparison Test at p = 0.05*

*Legend: Pt = Pleurotus tuber-regium, Po = Pleurotus ostreatus, Pp = Pleurotus pulmonarius*

*- = No white-rot fungus fruit*

Effect of substrate contamination by crude oil on the fresh yield weight of the three white-rot fungi at different inoculation periods are presented in Table 2. The yield performance for the three white-rot fungi was in the order: *Pp* < *Pt* < *Po*. The fresh weight of the fungi increased with increase in the added crude oil as contaminant. There was however no yield from 10% rate of crude oil addition as contaminant. Order of increase of the dry weight of the three white-rot fungi was comparable with the wet weight (Table 3). After one month of inoculation period, *P. ostreatus* had the highest fresh weight, 287.5 ± 4.7 g at 2.5%.

Crude oil contamination and was significantly (p = 0.05) higher than 207.1 ± 4.0 g fresh weight at zero percent crude oil contamination. The three species of *Pleurotus* performed optimally at 2.5% crude oil contamination of the substrate. However, only 37.8 ± 2.1 g, the yield of *P. pulmonarius* at 5.0% crude oil contamination was significantly (p = 0.05) lower than others at lower substrate contamination.

Percentage of petroleum hydrocarbon removed by the three white-rot fungi at different substrate concentrations and inoculation periods are presented in Table 4. With one month of substrate inoculation, highest removal of 19.8, 18.8 and 18.9% of petroleum hydrocarbon by *P. tuber-regium* at substrate contamination levels 1.0, 2.5 and 5.0% respectively were obtained. Also, with three months substrate inoculation, highest removal of 39.8, 38.9 and 37.5% of petroleum hydrocarbon, also by *P. tuber-regium*

**Table 3. Dry weight (± SE in g) of the three white-rot fungi at different rates of crude oil contamination of the substrate and inoculation periods**

| Rate (%) | One month | | | Three months | | |
|---|---|---|---|---|---|---|
| | Pt | Po | Pp | Pt | Po | Pp |
| 0 | 26.6 ± 2.1b | 41.7 ± 2.8c | 24.8 ± 2.7a | 32.4 ± 3.3c | 42.4 ± 2.8c | 30.4 ± 2.0a |
| 1.0 | 44.3 ± 3.7a | 57.5 ± 3.1b | 27.8 ± 3.1a | 52.2 ± 1.8b | 68 .5 ± 2.3b | 35.2 ± 2.6a |
| 2.5 | 51.3 ± 2.6a | 85.5 ± 1.9a | 28.1 ± 2.8a | 71.3 ± 1.7a | 96. 9 ± 2.1a | 35.9 ± 2.1a |
| 5.0 | 10.9 ± 2.3c | 15.7 ± 1.5d | 8.7 ± 2.3b | 13.4 ± 2.3d | 16.5 ± 3.0d | 9.1 ± 1.8b |
| 10.0 | - | - | - | - | - | - |
| 20.0 | - | - | - | - | - | - |

*Mean with the same letter in the column are not significantly different by Bonferroni's Multiple Comparison Test at p = 0.05*
*Legend: Pt = Pleurotus tuber-regium, Po = Pleurotus ostreatus, Pp = Pleurotus pulmonarius*
*- = No white-rot fungus fruit*

**Table 4. Percentage of petroleum hydrocarbon removed by the three white-rot fungi at different substrate concentrations and inoculation periods**

| Rate(%) | One month | | | Three month | | |
|---|---|---|---|---|---|---|
| | Pt | Po | Pp | Pt | Po | Pp |
| 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1.0 | 19.8 | 10.7 | 15.6 | 39.8 | 35.9 | 34.3 |
| 2.5 | 18.8 | 13.9 | 12.9 | 38.9 | 36.9 | 35.1 |
| 5.0 | 18.9 | 15.3 | 12.4 | 37.5 | 33.7 | 32.5 |
| 10.0 | - | - | - | - | - | - |
| 20.0 | - | - | - | - | - | - |

*Legend:Pt = Pleurotus tuber-regium, Po = Pleurotus ostreatus, Pp = Pleurotus pulmonarius*
*- = No white-rot fungus fruit*

**Table 5. Pearson correlation between total petroleum hydrocarbon and selected heavy metals in *Pleurotus tuber-regium* post-cropped soil samples**

| Property | TPH | Pb | Cd | Fe | Zn |
|---|---|---|---|---|---|
| TPH | - | | | | |
| Pb | 0.67* | - | | | |
| Cd | 0.21 | 0.37 | - | | |
| Fe | 0.71* | 0.63* | 0.48 | - | |
| Zn | 0.15 | 0.21 | 0.32 | 0.54 | - |

** = Correlation was significant at 95% level of probability*
*Legend: TPH = Total petroleum hydrocarbon*

at the same substrate contamination levels were obtained. *P. pulmonarius* petroleum hydrocarbon removal performance was the least for the two periods of inoculation. Pearson correlation between total petroleum hydrocarbon and selected heavy metals removed by *P. tuber-regium*, the fungus that had the best performance is presented in Table 5. There was a significant (p = 0.05) positive correlation among TPH, Pb and Fe values compared. However, the positive correlation among TPH, Cd and Zn values was not significant.

## 4. DISCUSSION

After one month of inoculation period, the stipe girth, height and pilei diameter of the three white-rot fungi increased with addition of crude oil to the substrates (soil, rice straw and wheat bran). There was significant (p = 0.05) increase of the growth components of the white-rot fungi when compared together. Similar lower growth components of *Coprinus comatus*, another type of mushroom were reported by Dulay et al. [19] with increase in graded metal-contaminated substrates. Adenipekun [26] also reported the growth of *P. tuber-regium* at 40% engine-oil contaminated soil after six months of incubation. The variation in our results could be due to variations in the Chemistry of the contaminants and shorter period of incubation of the substrates we used. The harvested fresh and dry weight of the three fungi (*P. tuber-regium, P. ostreatus and P. pulmonarius*) followed the same order of increase. The implication of this was that the varietal differences in the fungi played major role in their mycoremedial potential vis-a-vis their moisture contents [6].

Increase in the period of substrate inoculation also increased the yield of the three white-rot fungi [27]. The fungi yield obtained at three months of inoculation period were higher and comparable with one month inoculation. Odu [28] affirmed that incubation for biodegradation of crude oil polluted soils took several months. In this study, our findings agreed with those of Adenipekun [26], and Adenipekun and Fasidi [29] who reported that application of supplements such as organic and inorganic fertilizers at higher inoculation periods enhanced the yield of white-rot fungi. In addition, Okafor and Nwankwegu [30] observed that woodchips, a type of organic bedding was a potential source of nutrients for microbial activity and enhanced crude oil soil remediation.

Reynante et al. [2] observed different remediating potentials of different *Pleurotus* in heavy metal pollution environment. As earlier pointed out, different fungi remediate differently. *P. tuber-regium*, *P. ostreatus and P. pulmonarius* exhibited these traits, particularly in different organic contaminants media. This agrees with the findings of Adenipekun et al. [31] who reported that the efficacy of *P. pulmonarius* for the degradation of petroleum hydrocarbon decreased with increase in the concentration of spent diesel oil in the soil. We however differ with an inverse relationship the Adenipekun et al. [31] obtained between the substrate inoculation period and percent petroleum hydrocarbon degraded. Variations in the chemical composition of the contaminants used (spent diesel oil and crude oil) could cause this.

## 5. CONCLUSION

*P. ostreatus* had best agronomic performance and highest yield among the three white-rot fungi compared when cultivated on crude oil contaminated soil. Also, *P. tuber-regium* removed hydrocarbons and heavy metals more than either *P. ostreatus* or *P. pulmonarius* from equal mixture of sawdust and poultry manure as bedding materials for mushrooms cultivation on soils contaminated with crude oil. However, the yield and mycoremedial performance of the three tested white-rot fungi reduced from 5.0% (w/v) crude oil substrate contamination. It was evident from this study that introduction of white-rot fungi could help decontaminate crude oil polluted soils and that their performance were species dependent.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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