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REMEDIATION POTENTIALS OF *PLEUROTUS FLORIDA* ON SPENT LUBRICATING OIL CONTAMI NATED SOIL

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ABSTRACT: Soil contamination by spent lubricating oil (SLO) is a common occurrence in developing countries w here agriculture is the mainstay of rural inhabitants, especially in the oil producing ones. The white-rot fungus, *Pleur otus florida* was investigated for its ability to mineralize heavy metals, improve soil nutrients and reduce the Total Pe troleum Hydrocarbon (TPH) in spent lubricating oil contaminated soil after 2 months. Effect of different contaminati on levels on the growth of mycelia was also studied. 4 kg of top soil was thoroughly mixed with100 ml, 200 ml, 400 ml, 800 ml and 1600 ml of the SLO to give 2.5 %, 5 %, 10 %, 20 % and 40 % contamination levels respectively and a set of control was kept at 0 %. Ten grams of vigorously growing spawn of the fungus was added and observed at 1 and 2 months of incubation. The mycelia growth decreased as the level of contamination increased. At 2 months, the TPH reduced from 265.33 ml/kg to 232 ml/kg for 10 % contaminated soil and from 52.33 ml/kg to 35 ml/kg for 2.5 %. Nutrient contents were high at 1 month, but decreased after 2 months. Levels of heavy metals like Mn, Ni, Pb, Cr and Zn fluctuated at 1 month but were followed by significant decreases after 2 months. The levels of Mn and Ni red uced from 9.53 mg/kg to 8.81 mg/kg, and 9.51 mg/kg to 8.23 mg/kg respectively for 40 % contamination after 2 mon ths. The improvement of nutrient contents, bioaccumulation of heavy metals, and reduction of TPH across all concen tration tested through inocubation with *P. florida* is of importance for mycoremediation of spent oil polluted soils.
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Key words: Mycoremediation**,** Total Petroleum Hydrocarbons, pollution, *Pleurotus florida*, contaminated soil

Introduction

Soil and surface water contamination by used lubricating oil is a common occurrence in most developing countries. This is rapidly increasing due to global increase in the usage of petroleum products [1]. Nigeria is a major producer of crude oil in the world, and pollution of the environment due to oil spillage has steadily increased. In the Niger Delta Area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment [2]. Similarly, in Europe, it has been estimated that 3.5 million sites only in the European Union may be potentially contaminated, with 0.5 million sites being really contaminated and needing remediation [3].

There has been growing interest by researchers in the application of organisms and nutrients to contaminated soils for effective biodegradation of oil, probably owing to the problems associated with other methods (physical, mechanical and chemical). Bioremediation is being used or proposed as a treatment option at many hydrocarbon contaminated sites [4]. White rot fungi are increasingly investigated and used in remediation because of their ability to degrade an extremely diverse range of very persistent or toxic environmental pollutants [5]. White rot fungi are also responsible for the destruction and decay of polysaccharides, lignins and lignin-like substance [6]. Several studies have confirmed the remediation potentials of different white-rot fungi such as *Phanerochaete chrysosporium, Pleurotus ostreatus, P. pulmonarius, P. tuber-regium* and *L. squarrosuhus*. [7, 8, 9]. For instance, engine oil contaminated soil incubated with *Lentinus squarrosulus* resulted in an increase in nutrient contents and a high percentage degradation of total petroleum hydrocarbon after 90 days of incubation [10].

Although several studies have been conducted on mineralization or degradation of hydrocarbon by microorganism [11], and exotic mushrooms like the oysters, very little work has been done on indigenous

white-rot fungi. Hence this study is aimed at investigating the effect on the contaminant on the mushroom (mycelium), the ability of *P. florida* to reduce the total petroleum hydrocarbon, and mineralize soil contaminated with different concentrations of spent lubricating oil.

Materials and Methods

Top soil was collected from the crop garden at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria and sieved with 2 mm mesh size to remove large plant debris. Soil was then moistened with distilled water to achieve a soil moisture content of approximately 70 % (w/v). Four kilograms of top soil was thoroughly mixed with 100 ml, 200 ml, 400 ml, 800 ml and 1600 ml of the oil to give varying contamination levels (2.5 %, 5 %, 10 %, 20 % and 40 % w/w) respectively and a set of control was kept at 0 %. Spawns of *P. florida* were gotten from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Spent lubricating oil was collected at Daniels auto workshop, Ring road, Ibadan, Nigeria. The substrate, rice straw was collected at the mushroom unit, NIHORT and cut into 0.1 - 2 cm using a pair of scissors.

Evaluation of the effects of spent lubricating oil on the mycelia elongation *P. florida*

To evaluate the effects of spent lubricating oil on mycelia elongation of *P. florida*, we used the procedure previously described by [12], with modifications: 50 g of the 6 different soil treatments were poured into 6 clean test tubes labeled A to F. Five grams of rice straw which served as substrates for the oyster mushrooms was chopped into 2 cm and added to each treatment to a marked point before clean white cotton wool plugs were used to cork the test tubes. Top of the tubes were wrapped with aluminum foil and all the samples were put in different polyethylene bags according to their contamination level. There were four replications, with a total of 24 test tubes. Soil level was noted by marking with a red marker pen. The samples were sterilized in the autoclave at a temperature of 121ºC for 15 minutes to kill all microorganisms present or reduce the microbial load especially those of other soil dwelling fungi that may inhibit or suppress the growth of the host fungus to be added. The samples were allowed to cool before inoculating 5 g spawns of *P. florida* to the samples with an inoculating loop to prevent re-contamination. Labeled test tubes with samples were arranged in a modified fume cupboard using a completely randomized design (CRD). Mycelia colonization/elongation was measured every 5 days using a thread and a ruler and recorded.

Fungal cultivation and incubation

The procedures were according to according to the procedures of [8, 13], with modifications: 200 g each of the different treatments were weighed into transparent polyethylene bags. Plastic necks were used to support the plastic bags so that they could be corked with cotton wool and the necks wrapped in foil paper. Before adding the cotton wool (which served as air filter for the mushrooms), 5 g of rice straw was laid. The soil samples were sterilized in the autoclave at a temperature of 121ºC for 15 minutes. After cooling, an inoculating loop was used to add 10 g each of vigorously growing spawns of *P. florida* to the various treatments in an inoculating chamber, and kept at room temperature in a completely randomized design (CRD). At 1 and 2 months after inoculation, the mycelia-ramified waste separated from the spawns and rice straw were analysed for physico-chemical properties (nutrients), total petroleum hydrocarbon and heavy metal content after air-drying to compare the rate of bioremediation.

Determination of soil nutrients and heavy metals in the contaminated soil

The method of the Association of Official Analytical Chemists [14] was used analyse soil nutrients and heavy metals. 10ml of 1 N $k_2Cr_2O_7$ were added to a pre-weighed 5 g of ground soil sample; 20 ml of concentrated H_2SO_4 were subsequently added and shaken gently to cool. The suspension was made up to approximately 150 ml mark with distilled water in a conical flask. Ten drops of diphenylamine indicator were introduced and the colour turned black. Titration was done with 0.4 N Ferrous ammonium sulphate. Duplicate blank was also determined from the value of potassium reduced using the formula: % C = (10.0-0.04) x 0.004 x 100/Weight of soil taken.

Heavy metals contents determination was done by the Atomic Absorption Spectrophotometry after digestion with Aqua – regia (1: 3 of HCl – HNO_3) . This method rapidly determines Pb, Cu, Fe, Zn and Mn in soil samples using atomic absorption spectroscopy after double acid extraction. In this technique the soil samples are not completely digested. However, the labile fractions of the metals are leached into the extracts solution.

Determination of Total Petroleum Hydrocarbon in soil

This was determined according to the procedure of [15] with modifications. We weighed 10 g of the different concentrations of the contaminated soil into 50 ml flask and added 20 ml toluene. After shaking for 30 minutes on an orbital shaker (model TP4643D). The liquid phase of the extract was measured at 420nm using a UV/VIS spectrophotometer (Spectrumlab 752s). The total petroleum hydrocarbon (TPH) in the soil was estimated

with reference to standard curve derived from fresh used engine oil diluted with toluene.

Data and statistical analysis

The experiment was arranged in a completely randomized design with 3 replicates. Standard error values were calculated and data generated was subjected to one-way analysis of variance (ANOVA) as well as Duncan's Multiple Range Test [16]. The Statistical Analysis System (SAS 9.1) for windows was used for statistical analyses.

Results

Effect of spent lubricating oil on mycelia elongation of *P. florida* after 15 DAI

There was a general increase in elongation which later decreased as the concentration of the contaminant increased (Fig 1). At 5 days after incubation (5 DAI), the control (0 %) had the highest value which was not significantly different from other contamination levels except at 20 % and 40 %. At 10 days after incubation (10 DAI), there was also a decrease in elongation from 0 % to 40 %, however, 0 % and 2.5 % were significantly different from 5 % and 10 % and 20 %. The same trend was also observed at 15 days after incubation (15 DAI).

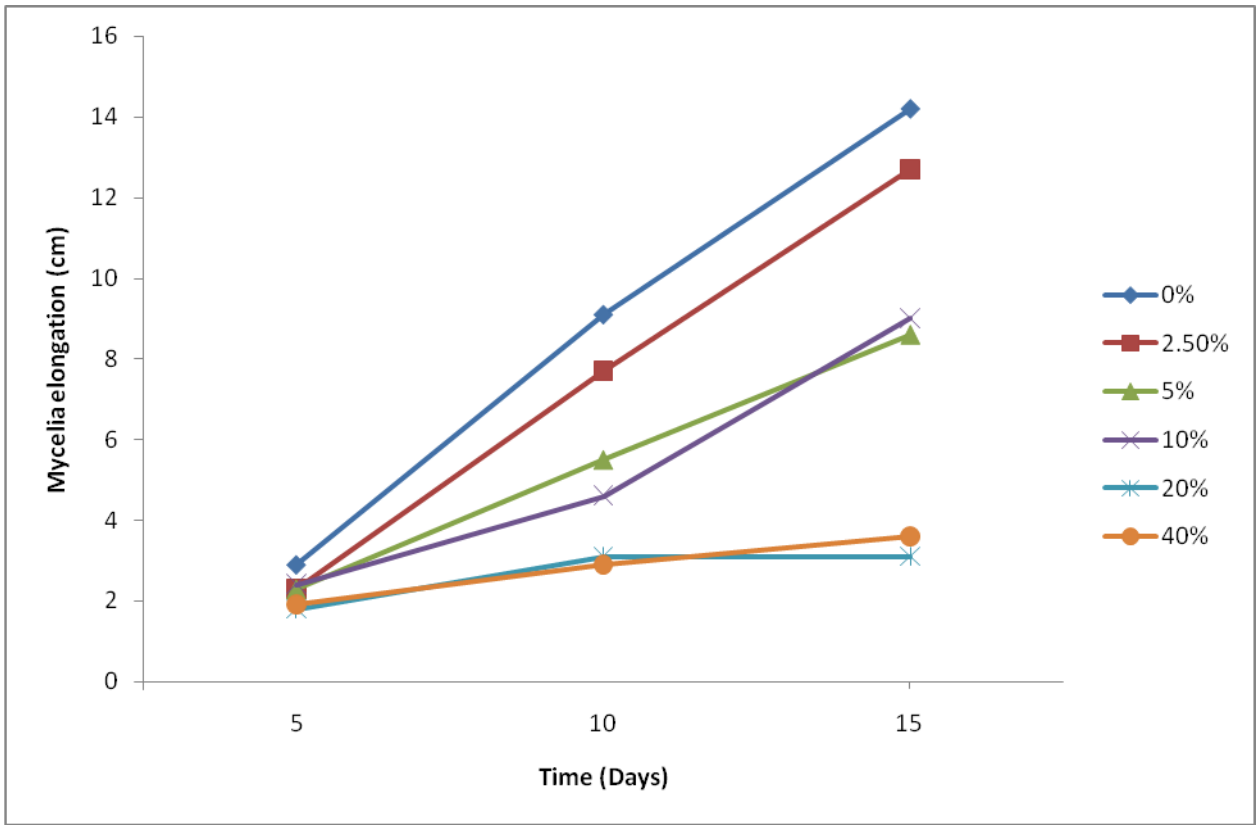

FIG. 1: Mycelia elongation of *P. florida* at different days after incubation

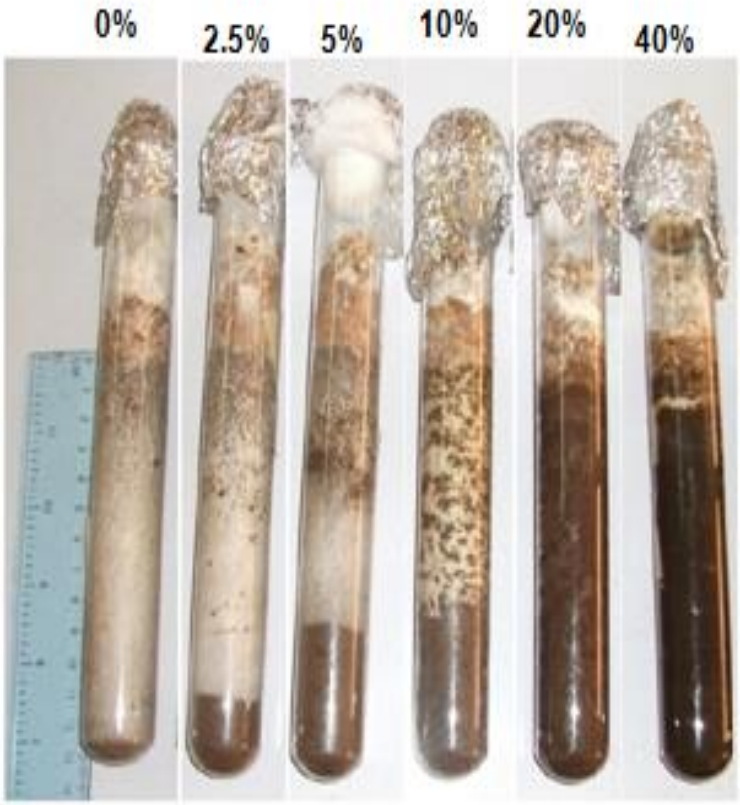

Plate 1. **Test tubes showing mycelia growth at different levels of SLO contaminated soil**

Total petroleum hydrocarbon of spent lubricating oil incubated with *P. florida* after 2 months

Total Petroleum Hydrocarbon increased as the concentrations increased for 0 months. After one month, significant reductions were observed across all the treatments. At two months after incubation with *P. florida*, significant reductions were also noticed for all the treatments. The 2.5 %, 5 % and 10 % having reduced from their initial values of 52.33 ml/kg, 141.33 ml/kg and 265.33 ml/kg to 35 ml/kg, 97.33 ml/kg and 232 ml/kg respectively (Table 1).

Table 1: Total petroleum hydrocarbon (ml/kg) of SLO contaminated soil incubated with *P. florida* at 0, 1 and 2 months

Treatments (Conc. Of SLO)	Time(Months)				
	0 Month	1 Month	% Reduction	2 Months	% Reduction
0%	1.1± 0.15	0.9±0.05	18.18	0.99±0.05	10
2.5%	52.33± 0.33	48± 0.58	8.27	35± 0.58	33.12
5%	141.33± 0.67	137±1.53	3.06	97.33±0.88	31.13
10%	265.33± 0.88	236±0.57	11.05	232± 1.15	12.56
20%	410.67±1.20	400±0.33	2.60	378± 1.52	7.96
40%	809.33±0.67	805.33±0.88	0.50	772± 1.15	4.61

Each value is a mean of 3 replicates ± ***standard error***

Effect of *P. florida* incubation on nutrient contents of spent lubricating oil contaminated soil after 2 months.

At 1 month, soil pH increased for most of the contamination levels. The 0 % treatment having the highest mean value of 7.1 followed by 20 % with 7.0. The levels of Nitrogen increased progressively from 0 %

treatment except at 40 %. Organic Carbon and organic matter contents of the soils increased gradually. Potassium increased at 0 % and dropped significantly except at 10 % which had a mean value of 0.26 cmol/kg. The reduction was significant among the treatments except between 2.5 %, 20 % and 40 %. An increase was observed in all the treatments for Phosphorus (Table 2). At 2 months, low levels of pH were observed from 0 % to 5 %, this later increased at 10 % to 40 % contamination levels. A reduction in Nitrogen was observed with the 40 % contamination having the highest mean value of 1.85 g/kg. Organic carbon and organic matter both decreased from 0 % to 5 % and later increased significantly from 10 % to 40 % and different. The potassium levels increased significantly from 0 % to 40 % with the 40 % treatment having the highest value of 0.64 cmol/kg (Table 3).

Table 2. Nutrient content in soils contaminated with SLO and incubated with *P. florida* for 0 and 1 month.

Treatments	Period (Months)	N (g/kg)	pH (g/kg)	C (g/kg	OM (cmol/kg)	K (mg/kg)	P
0%	0	1.13c	6.49e	10.17d	17.53d	0.24c	9.44f
	1	3.15b	7.1a	20.25a	34.19a	0.28a	16.00a
2.5%	0	1.15c	6.57d	10.53c	18.15c	0.25b	12.35d
	1	2.94d	6.82b	19.3ab	33.28ab	0.24c	16.01a
5%	0	1.10d	6.90c	9.00e	15.49e	0.26ab	10.12e
	1	3.04c	6.95ab	18.10bc	31.21bc	0.21d	14.79bc
10%	0	2.04b	6.98b	12.78ab	22.03ab	0.24c	13.40a
	1	3.07c	6.93ab	17.90bc	30.86bc	0.26b	15.40b
20%	0	2.04b	7.10a	12.51b	21.57b	0.26ab	12.58c
	1	3.23a	7.0a	19.10ab	32.84ab	0.24c	15.10b
40%	0	2.12a	7.08a	12.91a	22.25a	0.27a	12.85b
	1	2.8e	6.93ab	17.00c	29.31c	0.23c	14.35c

Each value is a mean of 3 replicates. Values in the same column followed by the same letters are not significantly different according to Duncan's multiple range test $p < 0.05$. C: Carbon; OM: Organic matter; K: Potassium; Mg: Magnesium; Ca: Calcium; P: Phosphorus

Table 3. Nutrient content in soils contaminated with SLO and incubated with *Pleurotus florida* for 0 and 2 months.

Treatments	**Period (Months)**	**N (g/kg)**	**pH (g/kg)**	**C (g/kg**	**OM (cmol/kg)**	**K (mg/kg)**	**P**
0%	0	1.13c	6.49e	10.17d	17.53d	0.24c	9.44f
	2	0.5b	6.62b	4.00c	7.04c	0.31f	4.87c
2.5%	0	1.15c	6.57d	10.53c	18.15c	0.25b	12.35d
	2	0.80b	6.40c	4.05c	7.01c	0.36e	4.88c
5%	0	1.10d	6.90c	9.00e	15.49e	0.26ab	10.12e
	2	0.35b	6.31c	4.80c	8.28c	0.41d	4.94c
10%	0	2.04b	6.98b	12.78ab	22.03ab	0.24c	13.40a
	2	1.70a	7.34a	30.60b	52.76b	0.53c	5.98b
20%	0	2.04b	7.10a	12.51b	21.57b	0.26ab	12.58c
	2	1.80a	7.47a	33.15a	57.15a	0.59b	6.43a
40%	0	2.12a	7.08a	12.91a	22.25a	0.27a	12.85b
	2	1.85a	7.44a	35.10a	60.52a	0.63a	6.58a

Each value is the mean of 3 replicates. Values in the same column followed by the same letters are not significantly different according to Duncan's multiple range test $P < 0.05$
C: Carbon; OM: Organic matter; K: Potassium; Mg: Magnesium; Ca: Calcium; P: Phosphorus

Effect of *P. florida* on heavy metal contents of SLO contaminated soil after 2 months

Mn at 20 % concentration of the treatment had the highest mean of 8.86 mg/kg and significantly different from concentrations of 0 %, 2.5 %, and 5% but was not significantly from the concentrations at 10 % and 40 %. Other heavy metals followed the same trend with lower means for concentrations from 0 % to 5 % and higher means for concentrations from 10 % to 40 %. There was however a significant decrease compared to the 0 month (Table 4).

Table 4: Heavy metal contents (mg/kg) of spent lubricating oil contaminated soil after 2 months of incubation with *P. florida.*

Treatments	Period (Months)	Manganese	Lead	Chromium	Nickel	Zinc
0%	0	7.53f	1.82d	3.14d	6.18d	2.64c
	2	6.70b	1.77b	2.54b	5.85b	1.65c
2.5%	0	7.47e	2.20c	3.15d	6.24cd	3.18b
	2	6.70b	1.82b	2.40b	5.51b	1.65c
5%	0	7.55d	2.24c	3.32c	6.33c	3.38b
	2	6.80b	1.90b	2.40b	5.60b	1.62c
10%	0	9.35c	3.25b	5.26b	9.05b	5.28a
	2	8.54a	2.63a	3.57a	7.98a	1.89b
20%	0	9.46b	3.58a	5.42a	9.16b	5.36a
	2	8.86a	2.74a	3.64a	8.11a	1.92b
40%	0	9.53a	3.66a	5.45a	9.51a	5.46a
	2	8.81a	2.90a	3.55a	8.23a	2.01a

Each value is the mean of 3 replicates. Values in the same column followed by the same letters are not significantly different according to Duncan's multiple range test $p < 0.05$.

Discussion

This study showed that the mycelia elongation increased progressively up to the 15 days after incubation. However, this increase stopped at 5 % contamination level and was followed by a decrease from 10 % to 40 % contamination. The reduction in mycelial elongation as concentration of pollutants increased could be due to the toxicity of pollutants. This ability of the fungus to grow at all levels of contamination agrees with the work of Stamets [17] , who stated that fungi can grow under environmentally-stressed conditions such as low pH and poor nutrient status whereas bacteria growth might be limited. Degradation of the contaminant in soils was observed to have undergone a gradual but steady decrease in the total petroleum hydrocarbon content. This finding agrees with the report of Adenipekun and Isikhuemhen, [10] where they observed a percentage loss in TPH at 1 % engine oil concentration (94.46 %), which decreased to 64.05 % at 40 % concentration. The reason for the low percentage of oil degradation within the first month may be attributed to the toxicity of the oil on the microbial flora of the soil. This initial trend of low biodegradation due to high oil concentration has been reported by Ijah and Antai [18], who argued that high concentration of hydrocarbon can be inhibitory at the initial stage to the indigenous microorganisms in the soil. The decrease in total petroleum hydrocabon values noticed at all contamination levels suggests that bioremediation has occurred. The increase in organic carbon and organic matter as observed is similar to the findings of Adenipekun and Fasidi [13] in engine oil polluted soil, where higher organic carbon was reported compared to the control treatments. There was significant increase ($p>$ 0.05) in the phosphorus content compared to the control at one month of incubation. This agrees with previous findings that reported increase in phosphorus content in soil contaminated with engine oil [10]. The initial increase and subsequent decrease of Nitrogen and other nutrients after the second month may be due to the short life cycle of the mushroom used for this experiment (approximately between 28-30 days). Hence by the end of the second month, the fungi might have utilized the nutrients for growth. Adenipekun and Isikhuemhen [10], also reported that engine oil contaminated soil, incubated with *Lentinus squarrosulus* resulted in an initial increase in nutrient contents. The subsequent reduction in Nitrogen had been reported and it was concluded that the mushroom requires Nitrogen for its growth process [14]. Hence we can infer that *P. florida* used up the Nitrogen for its development. Reductions in the heavy metal contents of the contaminated soil samples after 2 months, were also observed from this study. This indicates that the fungus has accumulated the heavy metals. This is similar to what Gabriel *et al* [19] observed that wood rotting fungi accumulated Cadmium, Lead, Aluminium and Calcium from liquid medium, supplemented with appropriate amount of metal salt.

Kalac *et al* [20], also used fungi for the treatment of heavy metals containing effluents from contaminated site. The initial fluctuations in heavy metal levels after first month could be due to the effect of heat on polythene as a result of autoclaving. It has been reported that when polythene is boiled, the chemicals used to produce it can leach into the substance being prepared due to high temperature. Toxic fumes from ink, glue, petroleum products and recycled materials could leach due to the materials used in making them [21].

Conclusion

The application of *P. florida* in bioremediation is expected to be relatively economical because the fungus can be grown on a number of inexpensive agricultural or forest wastes such as rice straw, corn cobs and sawdust. The ability of these mushrooms to tolerate the pollutants and grow on them, suggests they could be employed as bioremediation agents on sites contaminated by these pollutants. Therefore, one can conclude from this study, that *P. florida* can behave as efficient microorganism in bioremediation. It may also be successfully utilized in processes related to the removal of heavy metals and total petroleum hydrocarbon, which play important roles in environmental pollution. Mycoremediated areas will provide the framework to jumpstart the ecological restoration process. However, further studies should be carried out to determine the enzymes (in the mycelia) produced by this mushroom that aids the remediation process. The challenges faced in the field application such as contamination by other fungi especially *Penicillium* sp., *Aspergillus* spp. needs to be also looked into and solutions recommended.

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