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MYCOREMEDIATION OF PETROLEUM HYDROCARBON POLLUTED SOIL BY *Pleurotus pulmonarius*

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Abstract
Mycoremediation of petroleum hydrocarbon polluted soil was investigated using Pleurotus pulmonarius for a period of 62 days. Hydrocarbon (Petrol + diesel + spent petrol engine oil + spent diesel engine oil in ratio 1:1:1:1) polluted soil in 2.5%, 5%, 10% and 20% concentration were inoculated and incubated with pure culture of P. pulmonarius obtained from commercial mushroom laboratory of Federal Institute of Industrial Research Oshodi (F.I.I.R.O.) Lagos Nigeria. Inoculation was done by adding 20 g of vigorously growing spawn of P. pulmonarius. A set of control treatment was used where different concentrations of the petroleum hydrocarbon were added to all soils but no inoculation with the fungus. The results showed that the initial organic matter content of the soil increased with increase in the concentration of petroleum mixture added to the soil. The highest impact of P. pulmonarius on the organic matter content of the soil was noticed in soil with 10% concentration treatment (68.34%) and the least was in soil with 2.5% treatment concentration (22.12%). The initial nitrogen, potassium and phosphorus contents of the soils samples decreased with increase in the petroleum concentration. The difference was significant at 2.5%, 5%, 10% and 20% concentration of contamination for organic matter, nitrogen, potassium and phosphorus ($p<0.05$). A significant ($p<0.05$) reduction in concentration of the heavy metals (manganese, copper, and zinc) after 62 days of incubation with P. pulmonarius suggested that the mushroom is a good agent for heavy metal remediation. The highest reduction of Mn was at 10% concentration (24.00±0.04 to 1.73±0.10), Cu at 10% concentration (37.24± 0.02 to 0.00), and Zn was at 10% concentration (63.03±0.02 to 5.75±0.14). The percentage loss of the TPH due the growth of P. pulmonarius decreased with increase in the concentration of petroleum added to the soil. The percentage loss for 2.5%, 5%, 10% and 20% concentration are 52.60%, 38.71%, 27.20% and 8.31% respectively. Heptane, toluene, octane, M-p xylene, Alpha xylene, nonane, propylbenzene, decane, tridecane, tetradecane, anthracene and pentadecane had high reduction; however, the reduction is more significant ($p<0.05$) in soil inoculated with P. pulmonarius. Our results suggest that P. pulmonarius can be used to clean soils polluted with moderate level of petroleum products mixture

Key Word: *Mycoremediation, petrol, diesel, spent engine oil, P. Pulmonarius, TPH*

Introduction
One of the major environmental problems facing the world today is the contamination of soil, water, and air by toxic chemicals. Contamination of soil by hydrocarbons (mostly petroleum

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hydrocarbons) is becoming prevalent worldwide (Njoku *et al*., 2008). This is probably due to heavy dependence on petroleum as a major source of energy throughout the world, rapid industrialization, population growth and complete disregard for the environmental health. However, the disposal of spent engine oil (SEO) into gutters, water drains, open vacant plots and farms is a common practice in Nigeria especially by auto mechanics. This oil also called spent lubricant or waste engine oil is usually obtained during servicing and subsequently draining from automobile and generator engines (Anoliefo and Vwioko, 2001) and much of this oil is poured into the soil. There are relatively large amounts of hydrocarbons in the used oil, including the highly toxic polycyclic aromatic hydrocarbons, pentachlorophenols, polychlorinated biphenyls, 1,1,1-trichloro–2,2-bis (4-chlorophenyl) ethane, benzene, toluene, ethylbenzene xylene and trinitrotoluene (Wang *et al*., 2000). These hydrocarbon pollutants usually cause disruptions of natural equilibrium between the living species and their natural environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Das and Chandran, 2010).

Several classes of chemicals have been targeted by the USEPA (2000) as priority pollutants due to their toxic effects on the environment and human health, they includes; Polycyclic aromatic hydrocarbons (PAH), Pentachlorophenol (PCP), Polychlorinated biphenyls (PCBs), Dichlorodiphenyltrichloroethane (DDT), Benzene-Toluene-Ethylbenzene-Xylenes (BTEX), and Trinitrotoluene (TNT) (Lau, 2003; Verdin *et al*., 2004). Also, most heavy metals such as vanadium, lead, aluminium, nickel and iron, which are usually below detection in unused lubricating oil, have been reported to give high values (ppm) in used oil (Whisman *et al*., 1974; Adongbede and Sanni, 2014). These heavy metals may be retained in soils in the form of oxides, hydroxides, carbonates, exchangeable cations, and/or bound to organic matter in the soil (Yong *et al*., 1992; Adesuyi *et al*., 2015). Nevertheless, this is dependent on the local environmental conditions and on the kind of soil constituents present in the soil-water system.

Various techniques have been employed to remediate soil environment contaminated by hydrocarbons, ranging from physical, to chemical and mechanical forms of treating or removing the contaminants (Abioye, 2011). Bioremediation offers a better technique for treatment and removal of these contaminants into an innocuous substance. Bioremediation is one of the most viable options for remediating soil contaminated by organic and inorganic compounds considered detrimental to environmental health. Bioremediation is a process that makes use of microorganisms/plants to detoxify or remove organic and inorganic xenobiotics from the environment. It is a remediation option that offers green technology solution to the problem of hydrocarbon and heavy metals contamination (Abioye, 2011).

Pleurotus pulmonarius is commonly known as the Indian Oyster, Italian Oyster, Phoenix Mushroom, or the Lung Oyster. It is similar to *Pleurotus ostreatus*, but with a few noticeable differences. *P. pulmonarius* is a wood decaying basidiomycetes which is capable of degrading not only lignin but also variable recalcitrant environmental pollutants due to its ability to secrete lignolytic enzymes such as lignin peroxidase, manganese peroxidase and laccases which aid in the degradation process (Ogbo *et al.*, 2006). Grey oyster mushroom (*Pleurotus ostreatus*), Shiitake mushroom (*Lentinula edodes*), Phoenix mushroom (*P. pulmonarius*), Tiny mushroom (*Schizophyllum commune*) and Turkey tail mushroom (*Coriolus versicolor*) are some mushrooms that have been shown to have the capability of degrading some recalcitrant materials (Jang *et al*., 2009; Olusola and Anslem, 2010; Rajput *et al*., 2011; Okparanma *et al*., 2011). Okparanma *et al*. (2011) stated that spent *P. ostreatus* substrate can be used to biotreat Nigerian oil-based drill cuttings containing polyaromatic hydrocarbons (PAH's) under laboratory conditions. Hence, the objective of this research work was to assess the effectiveness of *P. pulmonarius* in the remediation of soil contaminated with mixture of petroleum products.

Material and Methods

Source of Materials

Top soil sample used for this experiment was collected within 1 to 10 cm depth from the nursery site of the Botanical garden of the University of Lagos, Akoka, Lagos State, Nigeria. The soil was sieved to 2 mm to remove debris. Pure culture of *P. pulmonarius* was obtained from a commercial mushroom laboratory of Federal Institute of Industrial Research Oshodi (F.I.I.R.O.) Lagos, Nigeria. The spent engine oils were obtained from a petrol and diesel engine generator mechanic workshop while the petrol, diesel, and engine oil were obtained from Forte Oil filling station, University of Lagos, Akoka.

Contaminant Composition

The contaminant comprised 500 ml of petrol, 500 ml of diesel, 500 ml engine oil, 500 ml spent petrol generator engine oil and 500ml of spent diesel generator engine oil mixed together in a ratio 1:1:1:1:1. This was to simulate what happens in automechanic workshops where different types of petroleum products are spilled or discharged same time or at different times in same soil. The concentration was converted from the various percentages to millilitre using these formulae;

$$Conc. in\ ml = \frac{concentration\ in\ percentage\ \times weght\ of\ the\ soilin\ gramme}{100} --- (i)$$

Where; the weight of the soil used = 400g and Concentrations in percentage = 2.5%, 5%, 10% and 20% (v/w) respectively. This calculation was done to equivalent volumes of 10 ml, 20 ml, 40 ml, and 80 ml respectively.

Experimental SetUp

The remediation experiment was conducted inside the mushroom Laboratory at Federal Institute of Industrial Research Oshodi Lagos. 400 g of soil was weighed into sterile bottles. The soil was experimentally contaminated with a mixture of the petroleum products 2.5, 5.0, 10.0, and 20.0 % (v/w), respectively, according to the method of Adenipekun and Kassim (2006). A mixture of 40 g of moistened clean rice straw with 40 g of saw dust and 10 g of $CaC0_3$ were mixed with the contaminated soil in each bottle separated with wire gauze and covered with aluminum foil. The bottles were then autoclaved at 15 psi pressure and 121°C for twenty minutes. After cooling, each bottle was inoculated with 20 g of vigorously growing spawn of *P. pulmonarius*. The bottles were then incubated at room temperature for two months (62 days). At the end of the incubation period, the contaminated soil samples were analysed for physiochemical parameters and total petroleum hydrocarbon (TPH) (Adenipekun and Fasidi, 2005).

Analysis of soil pH and Nutrient Content

The pH of the soil samples was determined using pH meter 3015 (Jenway, U.K.). The total organic matter, percentage nitrogen, phosphorus, potassium contents were determined using the method of Association of Official Analytical Chemists (A.O.A.C, 2003).

Heavy Metals Analysis

The digestion of samples for heavy metals analyses was done following the method described by Ritta *et al*. (2004). Heavy metals levels were determined using atomic absorption spectrophotometry.

Extraction and Determination of Total Petroleum Hydrocarbon

The extraction of Total Petroleum Hydrocarbon was carried out as was described by Chen *et al*. (2011). The level and types of total petroleum hydrocarbon in the soil samples were determined using gas chromatography –mass spectrometry.

Statistical Analysis

Statistical analysis of the data was by analysis of variance (ANOVA) and the different means of treatment compared using Duncan's Multiple Range Test ($p<0.05$). All analyses were carried out using the Graphpad 5.0 prism software.

Results and Discussion

The Nutrient Contents and the pH of the Soil Samples

The nutrient contents and the pH of the soil samples are shown in table 1. The initial organic matter content of the soil increased with increase in the concentration of petroleum mixture added to the soil. The highest impact of *P. pulmonarius* on the organic matter content of the soil was noticed in soil with 10% concentration treatment (68.34%) and the least was in soil with 2.5% treatment concentration (22.12%). The initial nitrogen, potassium and phosphorus contents of the soils samples decreased with increase in the petroleum concentration. The highest impacts of *P. pulmonarius* on the nitrogen, potassium and phosphorus contents of the soil samples were highest in samples from 10% treatment. These nutrients were lower in soil samples with *P. pulmonarius* than in samples without the mushroom. The same trend in percentage changes due to mushroom of these parameters was observed after two months for 2.5, 5.0, 10.0 and 20.0% of contamination with a sharp drop in 20%. At the end of the study the organic matter content decreased in soil samples with more reduction in the soils with *P. pulmonarius* compared to soil without the mushroom ($p<0.05$). The difference was significant at 2.5, 5.0, 10.0 and 20.0% concentration of contamination for organic matter, nitrogen, potassium and phosphorus. Their levels were found to be significantly different from the initial levels for all concentrations of contamination. As observed in this study, organic matter increased steadily as the concentration of petroleum hydrocarbon in the soil increases while nitrogen, phosphorus and potassium content in the soil decreased steadily as the concentration of petroleum hydrocarbon in the soil increases. This is similar to the work of Lehtomaki and Niemela (1975) where low values for nitrogen, potassium and phosphorus reserves in petroleum hydrocarbon contamination were reported. Benka-Coker and Ekundayo (1995) also reported low levels of nitrogen and phosphorus from a crude oil spill site in the Niger Delta of Nigeria. This observation might be due to the activities of the fungi.

The highest initial soil pH was observed in soil sample treated with 5% petroleum (7.60) and lowest initial pH was observed in soil with 20% petroleum (6.84). The final pH of the soil samples was lower than the initial pH of the samples. The impact of *P. pulmonarius* on the soil pH was least in soil sample treated with 5% petroleum (0.29%) and highest in soil with 20% petroleum treatment (11.28%). The difference in pH was significant ($p<0.05$) at 2.5, 5.0, 10.0 and 20.0% concentrations of contamination. pH plays a vital role in the biodegradation process of crude oil contaminated soil. As observed in this study, there was reduction in the pH of the crude oil contaminated soil without mushroom and also further significant reduction in the crude oil contaminated soil inoculated with *P. pulmonarius*. A similar finding of Adenipekun and Omoruyi (2008) showed that the pH of cement contaminated soil incubated with *P. ostreatus* decreased from 7.55 in the control to 7.54 and 7.11 after 1 and 2 months; for diesel contaminated soil and for battery contaminated soils, the pH decreased from 5.90 at control to 4.68 after 1 month incubation. This observation maybe because of lignin degradation enzymes (laccase and other peroxidases) secreted by white rot fungi known to function best at low pH some times as low as pH 3.5 (Hossain and Anatharaman, 2006).

Table 1: Nutrient contents of Petroleum hydrocarbon polluted soil inoculated with *Pleurotus pulmonarius*

Concentration of Petroleum added		Organic matter	Nitrogen	Potassium	phosphorus	pH
2.5 %	Initial	66.05±0.51 [a]	69.27±0.15 [a]	43.02 ±0.06 [a]	35.71 ±0.13 [a]	7.10 ±0.04 [a]
	W/out mushroom	27.13±0.71 [b]	34. 65 ±1.15 [b]	20.81± 0.31 [b]	24.38 ±0.15 [b]	7.01± 0.01 [b]
	With mushroom	21.13c±0.17 [c]	23.83 ±0.39 [c]	17.72 ±0.36 [c]	13.85 ±0.37 [c]	6.63 ±0.20 [c]
	% change Due to Mushroom	22.12	31.23	14.85	43.19	5.42
5 %	Initial	67.68±0.29 [a]	68.69 ± 0.11 [a]	40.95 ±0.11 [a]	34.35± 0.15 [a]	7.60 ±0.02 [a]
	W/out mushroom	15.15±0.79 [b]	19.54 ±0.68 [b]	7.04 ±3.24 [b]	8.90 ±0.03 [b]	6.80 ±0.05 [b]
	With mushroom	7.93±0.23 [c]	10.80 ±0.39 [c]	2.89± 0.07 [c]	3.71 ±0.11 [c]	6.78 ±0.24 [c]
	% change Due to Mushroom	47.66	44.73	58.97	58.31	0.29
10 %	initial	68.97±0.05 [a]	67.50 ±0.36 [a]	39.74 ±0.09 [a]	32.87 ±0.09 [a]	7.13 ±0.04 [a]
	W/out mushroom	11.18±0.97 [b]	14.44± 0.63 [b]	7.57± 0.53 [b]	5.68 ±0.17 [b]	7.00± 0.48 [b]
	With mushroom	3.54±0.26 [c]	5.73± 0.35 [c]	0.00 [c]	0.53± 0.05 [c]	6.47± 0.17 [c]
	% change Due to Mushroom	68.34	60.32	100	90.66	7.58
20 %	initial	74.78±0.53 [a]	63.21± 0.28 [a]	37.12 ±0.13 [a]	32.13 ±0.03 [a]	6.84 ±0.33 [a]
	W/out mushroom	19.66±0.62 [b]	23.46 ±0.65 [b]	17.12 ±0.13 [b]	14.75 ±0.12 [b]	7.09 ±0.08 [b]
	With mushroom	13.33±0.51 [c]	14.59 ±0.29 [c]	8.00 ±0.28 [c]	10.66 ±0.14 [c]	6.29 ±0.20 [c]
	% change Due to Mushroom	32.20	37.81	53.27	27.73	11.28

Values in the same column followed by different letters are significantly different according to Duncan Multiple Range Test ($P<0.05$).

Heavy Metals of the Soil Samples

The level of heavy metals in the petroleum hydrocarbon polluted soil inoculated with *P. pulmonarius* is shown in Table 2. The initial levels of the heavy metals in the soil samples increased with increase in the amount of petroleum added to the soil. Reduction of heavy metal levels were observed at the end of the study in the samples with *P. pulmonarius* than in samples without the mushroom at 2.5, 5.0, 10.0 and 20.0% concentration of contaminant in the soil. The reduction in metal levels was also noticed with the control sample for these metals. Except for Pb and Hg, the highest level of reduction of the heavy metals due to the growth of *P. pulmonarius* was noticed in soil samples treated with 10% petroleum (76.93% for Mn, 100% for Cu and 55.04% for Zn). Mercury was not detected in any of the soil samples. Lead was detected only in soil samples taken before the growth of the mushroom except in the soil samples with 20% treatment where it was detected in the sample without mushroom. There was 100% loss of Cu and Pb in the sample for 10% and 20% treatment respectively due to the growth of the mushroom. However, Duncan Multiple Range analysis showed that there was significant difference at 2.5%, 5.0%, 10% and 20.0% in percentage change due to mushroom activities ($p<0.05$).

Increase in heavy metals in the soil was as a result of crude oil contamination and the level of contamination. Results obtained indicated that more heavy metals were recorded after the introduction of the hydrocarbon and increased more as the concentration of the petroleum products mixture in the soil increased than before the addition of petroleum products mixture. This study showed that there was bioaccumulation of heavy metals by *P. pulmonarius*. The observation of Gabriel *et al* (1994) showed that wood rotting fungi accumulated cadmium, lead, aluminum and calcium from liquid medium, supplemented with appropriate amount of metal salt. This may suggest that the reduction of heavy metals in the soil sample could be due to bioaccumulation Kalac *et al.* (1996) and Gadd (1993) have also used fungi for the treatment of effluents containing heavy metals due to their ability to accumulate metals from their external environment. In this study it was observed that copper, lead, manganese and zinc are well accumulated after 2 months incubation. This present work suggests *P. pulmonarius* to be a good agent for remediation of manganese, zinc, copper and lead to an appreciable extent in petroleum products mixture contaminated soil.

Table 2: The level of heavy metals in the petroleum hydrocarbon polluted soil inoculated with *Pleurotus pulmonarius*

Concentration of Petroleum added to soil		Manganese	Copper	Zinc	Lead	mercury
2.5%	Initial	18.87±0.05 [a]	31.66±0.22 [a]	58.09±0.05 [a]	0.17± 0.04 [a]	0.00
	Without mushroom	23.55±0.08 [b]	19.17±0.39 [b]	34.11±0.29 [b]	0.00 [b]	0.00
	With mushroom	16.72±0.04 [c]	12.65±0.17 [c]	27.12±0.12 [c]	0.00 [b]	0.00
	% change Due to Mushroom	29.00*	34.01*	20.49*	0.00*	0.00
5 %	initial	20.84 ± 0.05 [a]	35.96 ± 0.08 [a]	59.15± 0.02 [a]	1.18 ±0.02 [a]	0.00
	Without mushroom	12.07± 0.13 [b]	8.75 ± 0.17 [b]	21.39 ±0.38 [b]	0.00 [b]	0.00
	With mushroom	6.00 ±0.14 [c]	2.59± 0.15 [c]	13.94± 0.18 [c]	0.00 [b]	0.00
	% change Due to Mushroom	50.29*	70.40*	34.83*	0.00*	0.00
10%	initial	24.00± 0.04 [a]	37.24± 0.02 [a]	63.03 ±0.02 [a]	3.18 ±0.04 [a]	0.00
	Without mushroom	7.50 ± 0.25 [b]	5.05 ±0.48 [b]	12.79± 0.62 [b]	0.00 [b]	0.00
	With mushroom	1.73 ±0.10 [c]	0.00 [c]	5.75± 0.14 [c]	0.00 [b]	0.00
	% change Due to Mushroom	76.93*	100*	55.04*	0.00*	0.00
20%	initial	29.22±0.25 [a]	40.41±0.04 [a]	6.49 ±0.03 [a]	6.19±0.33 [a]	0.00
	Without mushroom	18.04±0.13 [b]	14.95±0.33 [b]	27.44±0.15 [b]	6.30±0.55 [a]	0.00
	With mushroom	11.96±0.14 [c]	8.97±0.28 [c]	20.82±0.37 [c]	0.00 [b]	0.00
	% change Due to Mushroom	33.70*	40.00*	24.12*	100*	0.00

Values in the same column with different letters are significantly different (P<0.05), *denotes %change not significant across concentration

The Total Petroleum Hydrocarbon Contents of the Soil Samples

The total petroleum hydrocarbon contents of the soil were higher in day 0 (initial level) than in the final day (day 62). The initial level of TPH in the soil samples increased with increase in the level of petroleum added to the soil (Table 3). Similar trend was observed in the final levels of the TPH in soil with *P. pulmonarius* and soil without *P. pulmonarius*. Lower TPH levels were observed in soils with the mushroom compared with the soils without the mushroom. The percentage loss of the TPH due the growth of *P. pulmonarius* decreased with increase in the concentration of petroleum added to the soil. However, for the soil without mushroom (loss due to natural attenuation) the percentage loss of TPH increased as the concentration increased from 2.5% to 10% but decreased as the concentration increased to 20%. The loss across all the different concentrations for both natural attenuation and mushroom inoculation were all significant ($p<0.05$). It was observed that the ability of *P. pulmonarius* in TPH reduction was adversely affected with increase in the concentration of the petroleum hydrocarbon in the soil. The highest level of reduction was at 2.5% contamination and the least was at 20% contamination. Isikhuemhen *et al*. (2003) recorded a 96.46% in the total petroleum hydrocarbon removal from engine oil contaminated soil at 1% level of contamination incubated with *Lentinus squarrosulus*. Adenipekun *et al.* (2011) also observed that *P. tuber-regium* another white rot fungus was able to degrade the total petroleum hydrocarbon in soil contaminated with cutting fluids.

Table 3: The Total petroleum hydrocarbon (mg/kg) in the petroleum hydrocarbon polluted soil inoculated with *Pleurotus pulmonarius*

Concentration of Petroleum added	Initial (day 0)	Final (day 62) level in soil without mushroom	% Loss due to natural attenuation	Final (day 62) level in soil with mushroom	% Loss due to mushroom
2.5 %	944.44	299.67*	68.27	142.04*	52.60
5 %	1037.75	251.34*	75.78	154.04*	38.71
10 %	1389.73	255.92*	86.59	186.31*	27.20
20 %	2278.23	671.12*	70.54	615.34*	8.311

*significant different (P<0.05) between soil inoculated with mushroom and soil without.

The different petroleum hydrocarbons at the beginning (initial) and the end of the end of the study are shown in table 4. Heptane, toluene, octane, M-p xylene, Alpha xylene, nonane, propylbenzene, decane, tridecane, tetradecane, anthracene and pentadecane had high reduction; however, the reduction is more significant ($p<0.05$) in soil inoculated with *P. pulmonarius*. Ethane, propane, isobutene and cyclohexane were absent in the soil samples treated with 2.5%, 5% and 10% petroleum at the beginning of the study. However after 62 days, Ethane, propane, isobutene and cyclohexane were present in the soils with *P. pulmonarius* and soils without the mushroom though higher in soils without the mushroom than in soils with the mushroom ($p<0.05$). Pristane, octadecane and phytane were only present in the soil with 20% contamination. Most of the hydrocarbons were higher in the soils at the beginning of the study (day 0) than at the end of the study (day 62), and it was generally higher in soil without mushroom than in soil with mushroom. There was 100 percent loss of some hydrocarbons in *P. pulmonarius* inoculated soils; toluene in 2.5% and 5% concentration, octane in 10%, pentadecane in 20% while hexadecane in 2.5% and 20% concentrations.

Microbial degradation involves breakdown breakdown of organic compounds through biotransformation into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) (Haritash and Kaushik, 2009). Fungi can also degrade *n*-alkanes such as tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane (C13-C18) and crude oil (Hadibarata *et al*., 2007; Elshafie *et al*., 2007; Pinedo-Rivilla *et al*., 2009). As observed in the analyses of the hydrocarbon polluted soil, the absence of Ethane, propane, isobutene and cyclohexane in the initial TPH at all treatment level and their presence after 62 days suggest they are by-products of degradation of hydrocarbon. There is possibility of fungus degrading TPH at a level of contamination as low as 1% engine oil concentration if the incubation period is elongated (Adenipekun and Isikhuemhen, 2008).

Table 4: Different petroleum hydrocarbon in the petroleum hydrocarbon polluted soil inoculated with *Pleurotus pulmonarius*

Compounds	Initial TPH Level (mg/kg)				Final without Mushroom (mg/kg)				Final with Mushroom (mg/kg)			
	2.5%	5%	10%	20%	2.5%	5%	10%	20%	2.5%	5%	10%	20%
Ethane	-	-	-	-	3.01	10.23	10.55	-	-	2.66	-	-
Propane	-	-	-	-	2.57	2.90	2.75	-	2.57	2.57	-	-
Isobutane	-	-	-	-	9.83	8.95	9.90	-	9.66	6.05	5.53	-
Benzene	-	-	-	2.98	9.35	9.74	9.74	-	8.04	7.88	3.95	-
Cyclohexane	-	-	-	-	7.36	7.63	7.63	-	4.87	4.59	3.35	-
Heptane	4.65	4.64	3.77	8.04	22.15	23.04	23.04	10.17	11.07	9.88	3.23	10.10
Toluene	13.94	6.52	10.62	15.87	8.72	9.15	9.15	-	-	-	12.27	19.35
Octane	33.87	41.40	62.64	119.04	36.73	12.69	38.19	52.05	13.30	8.89	-	44.12
M-p xylene	77.61	86.10	109.89	196.19	15.29	31.45	31.46	49.12	14.84	15.31	20.06	41.23
Alpha xylene	176.48	264.24	229.56	169.84	10.25	10.55	10.55	67.76	3.65	2.88	42.24	60.70
Nonane	131.34	144.84	182.85	300.74	26.93	28.13	28.14	149.12	23.36	25.83	20.96	133.86
Propylbenzen	33.24	-	47.58	66.04	19.56	20.89	20.90	19.31	14.20	17.54	14.62	16.03
Decane	131.38	143.69	178.40	25.67	10.05	11.69	11.70	69.51	10.10	5.70	5.64	57.90
Tridecane	88.50	100.04	132.54	21.93	12.51	14.63	14.65	48.24	12.51	12.51	4.19	36.73
Tetradecane	138.88	153.10	193.20	314.23	13.78	15.40	15.41	77.48	3.85	7.19	8.19	65.85
Anthracene	100.12	115.69	159.57	281.58	3.42	5.02	5.03	53.06	2.49	8.06	12.43	31.96
Pentadecane	7.03	8.95	32.67	122.55	4.69	2.70	7.00	5.91	5.80	12.18	7.00	-
Hexadecane	2.87	-	-	54.53	-	-	-	23.66		-	-	-
Pyrene	3.45	5.45	46.34	92.16	-	-	-	6.42	-	-	-	13.60
Pristane	-	-	-	33.84		-	-	9.57	-	-	-	-
Octadecane	-	-	-	14.49	-	-	-	8.31	=	-	-	4.21
Phytane	-	-	-	5.56	-	-	-	-	-	-	-	2.53

Conclusion

Remediation of hydrocarbon contaminated soil is a necessity in order to have a safe and healthy environment that will in turn results in healthy lifestyle across the globe. Biological remediation of hydrocarbon and metal contaminated soil offers a better and more environmentally friendly technique that if properly and thoroughly explored can bring our environment into a better place for both plant and animals well being due to its enormous advantages over other treatment methods. However, despite these enormous advantages of biological treatment method, its potential is yet to be fully utilized in restoration of contaminated soil. The fungus *P. pulmonarius* has been found from this study to be good bioremediating and bioaccumulating agents after being incubated in different level of hydrocarbon concentration in soil.

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