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Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in spent and fresh cutting fluids contaminated soils by *Pleurotus pulmonarius* (Fries). Quelet and *Pleurotus ostreatus* (Jacq.) Fr. P. Kumm

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The potential of *Pleurotus pulmonarius* and *Pleurotus ostreatus* on the degradation of PAHs in spent and fresh cutting fluids (SCF and FCF) contaminated soils was investigated. Different weights of soil samples were contaminated with varying composition (10, 20 or 30%) of spent and fresh cutting fluids separately then inoculated each sample with *P. pulmonarius* and *P. ostreatus* separately and incubated at 28 ± 2°C for two months. The samples were analyzed in triplicates for 16 priority polycyclic aromatic hydrocarbons (PAHs) by gas chromatography after extracting with hexane and dichloromethane (3:1). The initial PAHs in the cutting fluids were mainly composed of 2 to 6 fused benzene rings. Significant reductions in PAHs concentrations for SCF and FCF were observed after two months of incubation. The percentage total PAHs remaining in FCF soil ranged from 71.7 to 73.6% when inoculated with *P. pulmonarius* and 0.93 to 31.0% when inoculated with *P. ostreatus.* Similarly, the percentage total PAHs remaining in SCF soil ranged from 42.6 to 72.6% when inoculated with *P. pulmonarius* and 54.9 to 62.2% when inoculated with *P. ostreatus.* Overall range of PAHs degradation by *P. pulmonarius* inoculated on FCF contaminated soil was 17.3 to 27.3%, while for *P. ostreatus* inoculated soil was 69.0 to 99.07% at different contamination levels. In contrast, overall PAHs degradation for *P. pulmonarius* and *P. ostreatus* inoculated on SCF ranged from 27.4 to 57.4% and from 37.8 to 45.2%, respectively. Thus, *P. ostreatus* is found more effective as a biodegradation agent for PAHs in contaminated soils when compared with *P. pulmonarius.*

Key words: Biodegradation, polycyclic aromatic hydrocarbons (PAHs), cutting fluids, *Pleurotus ostreatus*, *Pleurotus pulmonarius.*

INTRODUCTION

Soil contamination with various organopollutants is now of great global concern. One major group among the soil contaminants is polycyclic aromatic hydrocarbons (PAHs) which are frequently found in threatening concentrations (Steffen et al., 2007). PAHs members are listed within the list of priority pollutants due to their toxic, mutagenic and carcinogenic effects. The concomitant low water solubility and bioavailability of these contaminants has made them recalcitrant to microbial attack (Petrucciolia et al., 2009). Indiscriminate disposal of lubricating oil by motor mechanics is a common source of soil hydrocarbon contamination in countries like

Nigeria where there is no stringent enforcement of environmental laws (Husaini et al., 2008). Cutting fluid which belongs to the general product family called lubricant is a complex mixture of hydrocarbons, fatty acids, emulsifiers, organo-corrosion inhibitors, amines and glycols (Gannon et al., 1981; Ejoh et al., 2012). However, with the new laws and stricter regula-tions currently in place for cleaning up PAHs contaminated sites, harsh methods such as incineration of soil, bio-augmentation and natural attenuation are employed. Physico-chemical remedial strategies are also adopted in cleaning up sites contaminated by these compounds. These methods have been reported as either not cost effective or adequate enough. The last decade had witnessed the use of fungi in the treatment of a wide variety of solid wastes and wastewaters. Their roles in the bioremediation of various hazardous and toxic compounds in soils had also been established (Leitao, 2009). Fungi have also demonstrated the ability to degrade, in some cases mineralize polycyclic aromatic compounds, polychlorinated biphenyls phenols and halogenated phenolic compounds (Singh, 2006). Mushroom forming fungi (mostly basidiomycetes) are among the nature's most powerful decomposer, secreting strong extracellular enzymes due to their aggressive growth and biomass production (Adenipekun and Lawal, 2012). Therefore, research on decontamina-tion is increasingly focused on biological methods for the degradation and elimination of these pollutants such as PAHs (Jain et al., 2005).

The objective of this study was to assess the ability of *Pleurotus ostreatus*, *Pleurotus pulmonarius* each to biodegrade the 16 priority PAHs in spent and fresh cutting fluids contaminated soils and biodegradation efficiency of the two *Pleurotus* species in spent and fresh cutting fluid contaminated soils were compared.

MATERIALS AND METHODS

Sample location and collection

Soil

The soil used for this experiment was collected from the nursery site of the Department of Botany, University of Ibadan. The physiochemical parameters have been published by Adenipekun et al. (2013). Top soil was dug at 1 to 10 cm deep using stainless hand towel and sieved to 2 mm to remove debris.

Fungi

Pure cultures of *P. pulmonarius* and *P. ostreatus* were collected from Plant Physiology Laboratory of the Department of Botany, University of Ibadan.

Rice straw

Freshly harvested rice straw was collected from International Institute of Tropical Agriculture (IITA) in Ibadan, then taken to the Department of Botany, University of Ibadan and air –dried in a clear open space for seven days to reduce moisture content.

Wheat bran

Wheat bran was collected from the feed mill of Bodija Market, Ibadan.

Cutting fluid

Fresh and spent cutting fluid was obtained from the Physics Department workshop, University of Ibadan.

Substrate preparation

The pure spawn was prepared according to the method of Jonathan and Fasidi (2001). The substrate (rice straw) was cut into 0.50 cm using guillotine then soaked in water for 1 h to moisten the straw and later squeezed using a muslin cloth until no water oozed out. Small quantity of wheat bran (additive) was added to the moist straw which was then put into 350 ml sterile bottles, covered with aluminium foil and autoclaved at 151 lbs and 121°C for 15 min. The bottles were incubated at 28 ± 2°C for three weeks until the substrate was completely ramified to form a spawn.

Experimental set up for culture conditions

The culture condition was carried out according to the method of Adenipekun and Fasidi (2005) and modified as follows: 400 g of sterilized soil was weighed into sterile 350 mL bottles and then mixed thoroughly with varying concentrations of cutting fluid (0, 10, 20 and 30%). A mud balance in Petroleum Engineering Department, University of Ibadan was used to determine the fluid density from which the percent concentration was evaluated. 80 g of moistened rice straw were laid on the contaminated soil in each bottle separated with a wire gauze and covered with an aluminium foil. Three replicates for each experiment were prepared. The bottles were then autoclaved at a temperature of 121°C for 15 min. After cooling, each bottle was inoculated with 10 g of actively grown spawn of *P. pulmonarius* and *P. ostreatus* separately. The bottles were incubated at room temperature (28 ± 2°C) for two months. In the first set of control treatment, cutting fluids was not added to the soil but inoculated with fungi while in the second set different, level of oils were added to the soil but no fungal inoculation. At the end of the incubation period, the mycelia ramified substrate was carefully separated from the soil layer ensuring that soil particles did not mix with it. All contaminated soil samples were extracted and analyzed for PAHs according to the procedure of the USEPA (1996) using AP gas chromatogram model 6890 powered with HP chemstation Rev A. 09.01 (1206) software. The extraction of

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Abbreviations: FCF, Fresh cutting fluid; **SCF**, spent cutting fluid; **PAHs**, polycyclic aromatic hydrocarbons.

Table 1. Initial concentration of PAHs in spent cutting fluid contaminated soil.

PAHs fractions (µg/kg)	Number of benzene rings	Molecular weight (g mol^{-1})	IDL	Initial PAHs concentration (µg/kg)			PAHs fraction of the total PAHs (%)		
				10%	20%	30%	10%	20%	30%
Naphthalene	2	128.17	<0.001	0.575	0.825	1.113	1.34	1.42	1.28
Acenaphthylene	3	154.21	<0.001	0.341	0.468	0.575	0.79	0.81	0.66
Acenaphthene	3	152.2	<0.001	0.363	0.45	0.576	0.84	0.78	0.66
Fluorene	3	178.23	<0.001	0.792	1.035	1.391	1.84	1.79	1.60
Phenanthrene	3	178.23	<0.001	0.504	0.703	5.059	1.17	1.21	5.83
Anthracene	3	166.22	<0.001	3.477	3.658	5.519	8.09	6.31	6.36
Fluoranthene	3	202.26	<0.001	4.361	5.961	6.538	10.1	10.3	7.53
Pyrene	4	228.29	<0.001	2.658	3.442	4.216	6.18	5.94	4.86
Benzo(a)anthracene	4	228.29	<0.001	2.713	3.459	4.141	6.31	5.97	4.77
Chrysene	4	202.26	<0.001	3.828	4.559	5.923	8.90	7.87	6.82
Benzo(b)fluoranthene	5	252.32	<0.001	0.621	0.797	1.975	1.44	1.38	2.28
Benzo(k)Fluoranthene	5	252.32	<0.001	9.215	13.78	23.04	21.4	23.8	26.5
Benzo(a)pyrene	5	252.32	<0.001	11.56	15.71	18.24	26.9	27.1	21.0
Indeno(1,2,3,cd)pyrene	5	278.35	<0.001	0.949	1.127	1.3	2.21	1.94	1.50
Dibenzo(a,h)Anthracene	6	276.34	<0.001	0.537	0.628	0.73	1.25	1.08	0.84
Benzo(g,h,i)perylene	6	276.34	<0.001	0.493	1.356	6.467	1.15	2.34	7.45
Total PAHs remaining				42.99	57.96	86.8	100	100	100

IDL: Instrumental detection limit; PAHs fraction = (Initial PAHs concentration x 100)/Total PAHs.

PAHs was carried out by weighing 5.0 g of soil sample into 250 mL beaker of borosilicate material and 20 mL of double distilled hexane: dichloromethane (3: 1 v/v) was added. The beaker with its contents was placed on sonicator and refluxed for 2 h to extract the hydrocarbons. The organic layer was filtered into 250 mL beaker. The extract was dried with anhydrous sodium sulphate while filtering into a beaker and concentrated with a stream of nitrogen. The clean fractions were analysed for PAHs by a gas chromatography (GC) whose detector was flame ionization detector (FID). The column length, internal diameter and film size were 30 x 0.2 x 0.25 µm. Injection and detector temperatures were 250 and 320°C, respectively, while the detector temperature was 60°C. Mobile phase or carrier Nitrogen column flowed at a pressure of 30 psi. Hydrogen and compressed air pressures were 28 and 32 psi, respectively. Recovery study was carried out by spiking the soil sample, whose PAHs concentrations had previously been determined with an appropriate volume of known concentration of naphthalene standard. The spiked soil sample was homogenized and subjected to extraction procedure for PAHs determination. This spiking was replicated five times with five different soil samples and the average recovery was 94.1±2.3%.

Biodegradation efficiency [BDE]: This was calculated for each concentration of cutting fluid contamination using the ratio of the difference in PAHs concentration after incubation with the fungus to the initial PAHs concentration.

$$BDE\ (\%) = \frac{PAH_i - PAH_f \times 100}{PAH_i}$$

Where; PAHi is the PAHs concentration of PAHs in soil when there was no inoculation. PAH_f is the PAHs concentration of PAH in soil when incubated with white rot fungi *P. pulmonarius* and *P. ostreatus*.

Statistical analysis

The data were subjected to analysis of variance while the treatment means were carried out by Duncan multiple range test at 5% level of probability.

RESULTS AND DISCUSSION

Initial concentrations of PAHs in spent cutting fluid (SCF) and Fresh cutting fluid (FCF) contaminated soils

The initial concentrations of PAHs in SCF and FCF contaminated soil samples are shown in Tables 1 and 2. The PAHs were mainly composed of 2 to 6 fused benzene rings with their molecular-masses ranging from 128.17 g mol^{-1} for naphthalene to 278.35 g mol^{-1} in Indeno(1,2,3,cd) pyrene. The total initial PAHs concentration of SCF contaminated soil samples were 43.0, 58.0 and 86.8 µg/kg for 10, 20 and 30% contamination levels, respectively, (Table 1) while 43.5, 55.2 and 83.8 µg/kg were for 10, 20 and 30% contamination levels, respectively, in FCF contaminated soil samples (Table 2). The most abundant PAH compound in SCF contaminated soil was benzo(a)pyrene constituting 11.56 and 5.71 µg/kg at 10 and 20% contamination levels while at 30% contamination level, benzo (k) fluoranthene constituting 23.04 µg/kg was abundantly present (Table 1). Conversely, acenaphthylene was the least abundant PAH in SCF contaminated

Table 2. Initial concentration of PAHs in Fresh cutting fluid contaminated soil.

PAHs fractions (µg/kg)	Number of benzene rings	Molecular weight (g mol^{-1})	IDL	Initial PAHs concentration (µg/kg)			PAHs fraction of the total PAHs (%)		
				10%	20%	30%	10%	20%	30%
Naphthalene	2	128.17	<0.001	0.633	0.905	1.251	1.46	1.64	1.49
Acenaphthylene	3	154.21	<0.001	0.363	0.394	0.555	0.84	0.71	0.66
Acenaphthene	3	152.2	<0.001	0.383	0.562	0.73	0.88	1.02	0.87
Fluorene	3	178.23	<0.001	0.855	1.188	1.685	1.97	2.15	2.01
Phenanthrene	3	178.23	<0.001	0.692	0.483	0.474	1.59	0.87	0.57
Anthracene	3	166.22	<0.001	3.579	3.806	6.026	8.24	6.89	7.19
Fluoranthene	3	202.26	<0.001	3.900	6.017	10.71	8.98	10.9	12.8
Pyrene	4	228.29	<0.001	2.497	2.694	4.158	5.75	4.88	4.96
Benzo(a)anthracene	4	228.29	<0.001	1.805	2.994	4.744	4.15	5.42	5.66
Chrysene	4	202.26	<0.001	3.109	5.267	7.644	7.16	9.54	9.12
Benzo(b)fluoranthene	5	252.32	<0.001	0.569	0.824	1.142	1.31	1.49	1.36
Benzo(k)Fluoranthene	5	252.32	<0.001	9.598	9.232	16.46	22.1	16.7	19.6
Benzo(a)pyrene	5	252.32	<0.001	12.31	15.82	21.93	28.3	28.7	26.2
Indeno(1,2,3,cd)pyrene	5	278.35	<0.001	0.792	1.013	1.383	1.82	1.83	1.65
Dibenzo(a,h)Anthracene	6	276.34	<0.001	0.439	0.574	0.76	1.01	1.04	0.91
Benzo(g,h,i)perylene	6	276.34	<0.001	1.921	3.438	4.17	4.42	6.23	4.97
Total PAHs remaining				43.45	55.21	83.82	100	100	100

IDL: Instrumental detection limit; PAHs fraction = (Initial PAHs concentration x 100)/Total PAHs

soil with 0.341 and 0.575 µg/kg for 10 and 30% contamination levels while at 20% contamination level, acenaphthene (0.45 µg/kg) was the least abundant PAHs in SCF.

Furthermore, benzo(a)pyrene which ranged from 12.31 to 21.93 µg/kg at different contamination levels was the most abundant PAH compound in FCF soil while acenaphthylene which ranged from 0.363 to 0.555 µg/kg was the least abundant PAH in FCF soil (Table 2).

Reductions in the PAHs fraction residual concentrations by *P. pulmonarius* (*P.p*) and *P. ostreatus* (*P.o*) at two months of incubation period

The extents of degradation of individual PAHs fractions in FCF and SCF contaminated soils by *P. pulmonarius* (*P.p*) and *P. ostreatus* (*P.o*) were shown in Tables 3 and 4. Both *P.p* and *P.o* exhibited some substantial reductions ability after they were able to grow on spent and fresh cutting fluids contaminated soil containing PAHs fractions at varying concentrations. Bishnoi et al. (2008) similarly reported that *Phanerochaete chrysosporium,* a white rot fungus was able to degrade five PAHs fractions in soil from petroleum refinery in both sterile and unsterile conditions. *P.o* significantly reduced the PAHs fractions at all contamination levels compared to *P.p,* which slightly reduced the PAHs fractions in polluted soils except at 10% contamination level with SCF. At this contamination level, *P.p* was able to reduce the PAHs fractions more than *P.o* (Table 3). It is noteworthy that the PAHs fractions at 10 and 20% contamination levels for FCF contaminated soil inoculated with *P.o* were not significantly different (P≤0.05). Also, reduction at 20% contamination level for FCF soil inoculated with *P.o* was significantly high above other levels of contamination (10 and 30%) even when compared with *P.p* inoculated soil at similar levels of contamination. This rapid reduction indicates that 20% contamination level enhances degradation of any PAHs fractions most especially when inoculated with *P.o*. Some PAHs factions might have been used as carbon sources by degradation enzymes during the degradation process. Study by Clemente et al. (2001) explained further that when PAHs fractions such as naphthalene and phenanthrene were used as the carbon source for the growth of strains 837, 870 and 984 of ligninolytic fungi, an enzyme MnP exhibited the highest activity in the degradation of biodegradation of PAHs. The inability of *P.p* to reduced benzo(a)anthracene at 30% contamination level of FCF contaminated soil suggest that PAHs are toxic for the fungus to degrade. Also, an anomalous increase in pyrene from 4.216 to 4.444 µg/kg and benzo(g,h,i)perylene concentration from 6.467 to 7.250 µg/kg (control), respectively, at 30% contamination level for FCF contaminated soil by *P.p* showed that the increase may be due to conversion of one form of aromatics to another as fall-off in the metabolism process. Edema et al. (2011) further explain that magnification of PAHs fraction concentrations in the uncontaminated soil may be due to conversion of one

Table 3. Comparison of the biodegradation of PAHs in spent cutting fluid (SCF) contaminated soil by white rot fungi.

PAHs (µg/kg)	10%			20%			30%		
	Initial PAHs concentration	PAHS Concentration after treatment		Initial PAHs concentration	PAHS Concentration after treatment		Initial PAHs concentration	PAHS Concentration after treatment	
		P.p	*P.o*		*P.p*	*P.o*		*P.p*	*P.o*
Naphthalene	0.575^{d}	0.390^{e}	0.090^{g}	0.825^{c}	0.611^{d}	0.095^{g}	1.113^{a}	0.987^{b}	0.305^{f}
Acenaphthylene	0.341^{d}	0.265^{e}	0.023^{f}	0.468^{c}	0.384^{b}	0.024^{f}	0.575^{a}	0.482^{b}	0.272^{e}
Acenaphthene	0.363^{c}	0.226^{d}	0.030^{e}	0.450^{b}	0.372^{c}	0.031^{e}	0.576^{a}	0.545^{a}	0.287^{d}
Fluorene	0.792^{c}	0.757^{c}	0.021^{d}	1.035^{b}	0.868^{c}	0.021^{d}	1.391^{a}	1.240^{a}	0.189^{d}
Phenanthrene	0.504^{d}	0.313^{e}	0.031^{f}	0.703^{b}	0.587^{c}	0.034^{f}	5.059^{a}	0.681^{b}	0.303^{e}
Anthracene	3.477^{b}	2.892^{b}	0.016^{c}	3.658^{b}	3.656^{b}	0.017^{c}	5.519^{a}	5.443^{a}	1.015^{c}
Fluoranthene	4.361^{b}	4.133^{b}	0.089^{e}	5.961^{a}	3.035^{c}	0.095^{e}	6.538^{a}	5.800^{a}	1.502^{d}
Pyrene	2.658^{c}	2.163^{d}	0.020^{e}	3.442^{b}	2.272^{d}	0.017^{e}	4.216^{a}	4.444^{a}	2.704^{c}
Benzo(a)anthracene	2.713^{c}	2.317^{cd}	0.017^{e}	3.459^{b}	3.195^{b}	0.017^{e}	4.141^{a}	4.141^{a}	1.973^{d}
Chrysene	3.828^{d}	2.645^{e}	0.036^{f}	4.559^{c}	4.051^{cd}	0.038^{f}	5.923^{a}	5.186^{b}	2.438^{e}
Benzo(b)fluoranthene	0.621^{bc}	0.400^{cb}	0.013^{c}	0.797^{cb}	0.577^{cb}	0.014^{c}	1.975^{a}	0.966^{b}	0.444^{cb}
Benzo(k)Fluoranthene	9.215^{e}	6.416^{g}	0.033^{h}	13.78^{c}	11.81^{d}	0.033^{h}	23.04^{a}	20.20^{b}	6.550^{f}
Benzo(a)pyrene	11.56^{c}	6.868^{d}	0.028^{e}	15.71^{b}	12.16^{c}	0.030^{e}	18.24^{a}	12.66^{c}	7.832^{d}
Indeno(1,2,3,cd)pyrene	0.949^{c}	0.708^{d}	0.034^{f}	1.127^{abc}	1.176^{ab}	0.036^{f}	1.300^{a}	1.014^{bc}	0.474^{e}
Dibenzo(a,h)Anthracene	0.537^{cb}	0.435^{c}	0.016^{e}	0.628^{ab}	0.503^{c}	0.016^{e}	0.730^{a}	0.728^{a}	0.306^{d}
Benzo(g,h,i)perylene	0.493^{b}	0.304^{b}	0.019^{b}	1.356^{b}	0.458^{b}	0.023^{b}	6.467^{a}	7.250^{a}	0.310^{b}
Total PAHs remaining	43.0	31.2	0.516	58.0	42.7	0.541	86.8	71.8	26.9
Total PAHs remaining (%)		72.7	1.2		73.6	0.93		82.7	31
BDE (%)		27.3	98.8		26.4	99.1		17.3	69

Each value is a mean of three replicates. Values in the same row with different letters as superscripts are significantly different by Duncan multiple range test (p≤0.05). *P.p: Pleurotus pulmonarius; P.o*: *Pleurotus ostreatus*. BDE: Biodegradation efficiency.

form of aromatics to another as fall-off in the metabolism process.

The overall percentage biodegradation efficiency (BDE) of *P.p* was decreasing with increase in contamination levels for both FCF and SCF soils. BDE of 27.3, 26.4 and 17.3% for 10, 20, and 30% contamination levels for SCF contaminated soil inoculated with *P.p*. In comparison, thehighest BDE of 99.1 at 20% contamination level for *P.o* was obtained (Table 3). For FCF contaminated soil, the highest BDE of 57.4% was obtained at 10% contamination level when the soil was inoculated with *P.p* whereas the highest BDE of 45.2% was obtained at 30% contamination level when the soil was inoculated with *P.o* (Table 4). The reduction in BDE for SCF contaminated soil inoculated with *P.p* could be attributed to high toxicity of contaminants which could have inhibited the fungus degrading enzymes capability retarding the fungus growth and consequently reducing its colonization. The increase in the BDE (%) of *P.o* inoculated on SCF can be attributed to the presence of high organic carbon content of the cutting fluid which is a major factor for sorption of organic pollutants such as PAHs. This is in consonant with the reports of Opuene et al. (2007); Fagbote and Olanipekun (2013) that abundance of total carbon is a significant factor which controls concentration of PAHs in soils.

The comparison of percentage biological degradation efficiency (BDE%) of *Pleurotus pulmonarius* (*P.p*) and *P. ostreatus (P.o*) for PAHs degradation (Table 5)

The range of BDE of *P.o* for FCF contaminated soil were 84.3 to 99.5%, 88.5 to 99.8 % and 35.9 to 94.0% for 10, 20 and 30% contamination levels, respectively, while that of *P.p* were 4.4 to 40.6%, 0.1 to 66.2%, 0 to 86.5%, respectively. Similarly, for SCF contaminated soil, BDE of *P.p* ranged from 25.3 to 85.8%, 5.3 to 88.2% and 10.2 to 88.2% for 10, 20 and 30% contamination levels, respectively, while BDE of *P.o* inoculated soil ranged from 18.4 to 82.8%, 2.2 to 89.6%, 2.7 to 64.5% for corresponding contamination levels. This is similar to the report of Marquez-Rocha et al. (2000) on the degradation of PAHs adsorbed by the white rot fungus, *P ostreatus*, after 21 days. In which case, 50% of pyrene, 68% of anthracene and 63% of phenanthrene were mineralized and their respective biodegradation percentages increased to 75, 80 and 75%, respectively, when 0.18% of Tween 40 was added. This is also similar to the findings of Eggen and Svenm (1999) who observed that the white rot fungus *P. ostreatus* had an overall positive effect on PAH degradation in aged creosote contaminated soil. It is noteworthy that, most PAH higher than naphthalene in molecular weight were almost completely

Table 4. Comparison of the biodegradation of PAHs in fresh cutting fluid (FCF) contaminated soil by white rot fungi.

PAHs (µg/kg)	10%			20%			30%		
	Initial PAHs concentration	PAHS Concentration after treatment		Initial PAHs concentration	PAHS Concentration after treatment		Initial PAHs concentration	PAHS Concentration after treatment	
		P.p	*P.o*		*P.p*	*P.o*		*P.p*	*P.o*
Naphthalene	0.633^{cd}	0.280^{g}	0.365^{f}	0.905^{b}	0.561^{ed}	0.498^{e}	1.251^{a}	0.692^{c}	0.597^{d}
Acenaphthylene	0.363^{b}	0.137^{d}	0.224^{cd}	0.394^{b}	0.217^{cd}	0.356^{b}	0.555^{a}	0.319^{cb}	0.381^{b}
Acenaphthene	0.383^{c}	0.198^{d}	0.211^{d}	0.562^{b}	0.427^{c}	0.341^{c}	0.730^{a}	0.525^{b}	0.334^{c}
Fluorene	0.855^{cd}	0.391^{ef}	0.207^{f}	1.188^{b}	0.662^{ed}	0.218^{f}	1.685^{a}	1.092^{cb}	0.624^{ed}
Phenanthrene	0.692^{a}	0.129^{e}	0.325^{cd}	0.483^{b}	0.268^{d}	0.434^{cb}	0.474^{b}	0.392^{cb}	0.461^{b}
Anthracene	3.579^{cb}	0.685^{e}	2.403^{cd}	3.806^{b}	2.178^{d}	2.099^{d}	6.026^{a}	4.023^{b}	3.542^{cb}
Fluoranthene	3.900^{d}	2.912^{de}	2.090^{e}	6.017^{c}	5.697^{c}	2.152^{e}	10.71^{a}	8.915^{b}	3.804^{d}
Pyrene	2.497^{c}	1.148^{d}	1.887^{cd}	2.694^{cb}	2.046^{c}	2.636^{cb}	4.158^{a}	3.387^{ba}	2.312^{c}
Benzo(a)anthracene	1.805^{ed}	1.125^{e}	1.921^{ed}	2.994^{cb}	2.227^{cd}	2.488^{cd}	4.744^{a}	3.888^{ba}	2.543^{cd}
Chrysene	3.109^{d}	0.643^{f}	2.293^{e}	5.267^{c}	2.991^{d}	3.036^{d}	7.644^{a}	6.865^{b}	2.896^{d}
Benzo(b)fluoranthene	0.569^{cd}	0.372^{ef}	0.341^{f}	0.824^{b}	0.598^{cd}	0.470^{def}	1.142^{a}	0.715^{cb}	0.558^{cde}
Benzo(k)Fluoranthene	9.598^{bc}	3.703^{d}	5.334^{cd}	9.232^{bc}	6.983^{cd}	8.180^{bcd}	16.46^{a}	9.558^{bc}	12.08^{ab}
Benzo(a)pyrene	12.31^{c}	5.864^{e}	8.165^{d}	15.82^{b}	13.71^{c}	9.819^{d}	21.93^{a}	17.30^{b}	12.75^{c}
Indeno(1,2,3,cd)pyrene	0.792^{bcd}	0.393^{e}	0.646^{d}	1.013^{b}	0.686^{cd}	0.637^{d}	1.383^{a}	0.907^{b}	0.715^{cd}
Dibenzo(a,h)Anthracene	0.439^{cb}	0.273^{c}	0.291^{c}	0.574^{ab}	0.445^{cb}	0.415^{cb}	0.760^{a}	0.589^{ab}	0.457^{cb}
Benzo(g,h,i)perylene	1.921^{ab}	0.273^{b}	0.331^{b}	3.438^{a}	0.406^{b}	0.358^{b}	4.170^{a}	0.500^{b}	1.920^{ab}
Total PAHs remaining	43.445	18.526	27.034	55.211	40.102	34.137	83.822	59.667	45.974
Total PAHs remaining (%)		42.64	62.23		72.63	61.83		71.18	54.85
BDE (%)		57.4	37.8		27.4	38.2		28.8	45.2

Each value is a mean of three replicates. Values in the same row with different letters as superscripts are significantly different by Duncan multiple range test ($p \leq 0.05$). *P.p: Pleurotus pulmonarius; P.o*: *Pleurotus ostreatus.* BDE: Biodegradation efficiency.

Table 5. Comparison of percentage biological degradation efficiency of *Pleurotus pulmonarius* (*P.p*) and *P. ostreatus* (*P.o*) for PAHs fractions degradation.

PAHs fractions (%)	Number of benzene rings	Molecular weight (g mol^{-1})	Fresh cutting fluid contaminated soil						Spent cutting fluid contaminated soil					
			10%		20%		30%		10%		20%		30%	
			P.p	*P.o*	*P.p*	*P.o*	*P.p*	*P.o*	*P.p*	*P.o*	*P.p*	*P.o*	*P.p*	*P.o*
Naphthalene	2	128.17	32.2	84.3	25.9	88.5	11.3	72.6	55.8	42.3	38	45.0	44.7	52.3
Acenaphthylene	3	154.21	22.3	93.3	17.9	94.9	16.2	52.7	62.3	38.3	44.9	9.6	42.5	31.4
Acenaphthene	3	152.2	37.7	91.7	17.3	93.1	5.4	50.2	48.3	44.9	24	39.3	28.1	54.2
Fluorene	3	178.23	4.4	97.3	16.1	98.0	10.9	86.4	54.3	75.8	44.3	81.6	35.2	63.0
Phenanthrene	3	178.23	37.9	93.8	16.5	95.2	86.5	94.0	81.4	53	44.5	10.1	17.3	2.7
Anthracene	3	166.22	16.8	99.5	0.1	99.5	1.4	81.6	80.9	32.9	42.8	44.9	33.2	41.2
Fluoranthene	3	202.26	5.2	98.0	49.1	98.4	11.3	77.0	25.3	46.4	5.3	64.2	16.8	64.5
Pyrene	4	228.29	18.6	99.2	34.0	99.5	*5.4	35.9	54	24.4	24.1	2.2	18.5	44.4
Benzo(a)anthracene	4	228.29	14.6	99.4	7.6	99.5	0.0	52.4	37.7	*6.43	25.6	16.9	18.0	46.4
Chrysene	4	202.26	30.9	99.1	11.1	99.2	12.4	58.8	79.3	26.3	43.2	42.4	10.2	62.1
Benzo(b)fluoranthene	5	252.32	35.6	97.9	27.6	98.2	51.1	77.5	34.6	40.1	27.4	43.0	37.4	51.1
Benzo(k)Fluoranthene	5	252.32	30.4	99.6	14.3	99.8	12.3	71.6	61.4	44.4	24.4	11.4	41.9	26.6
Benzo(a)pyrene	5	252.32	40.6	99.8	22.6	99.8	30.6	57.1	52.4	33.7	13.3	37.9	21.1	41.9
Indeno(1,2,3,cd)pyrene	5	278.35	25.4	96.4	*4.3	96.8	22.0	63.5	50.4	18.4	32.3	37.1	34.4	48.3
Dibenzo(a,h)Anthracene	6	276.34	19.0	97.0	19.9	97.4	0.3	58.1	37.8	33.7	22.5	27.7	22.5	39.9
Benzo(g,h,i)perylene	6	276.34	38.3	96.1	66.2	98.3	*12.1	95.2	85.8	82.8	88.2	89.6	88.0	54.0

*Higher than initial soil PAHs level.

removed by *P.o*. This agrees with the report of Edema et al. (2011) where mushrooms were able to completely removed PAHs higher than naphthalene in molecular mass. Also, there was an anomalous increase in some PAHs concentrations after 2 months of incubation with the fungi. The anomalous increase implies that degradation truly occurred. Similarly, Okparanma et al. (2011) reported an anomalous increase in phenanthrene (3-ring) which was not within the laboratory detection limit but, after the fifty sixth day of incubation with *P. ostreatus*, it increased to 0.01 mg/kg.

Conclusion

In the present study, the effectiveness of *P. pulmonarius* and *P. ostreatus* in biodegradation of PAHs fractions in both spent and fresh cutting fluids contaminated soil at different concentration has being elucidated. In fresh cutting fluid contaminated soil *P. ostreatus* degraded almost all the PAHs fractions more than naphthalene while *P. pulmonarius* degradation was slight one. Such a biodegraded soils can provide a cheap and sustainable means of reverting such soils after damage by cutting fluids and can subsequently be used for agricultural purpose.

Conflict of interests

The authors did not declare any conflict of interest.

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