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Full Length Research Paper

Mycoremediation of polycyclic aromatic hydrocarbons (PAH)-contaminated oil-based drill-cuttings

Reuben N. Okparanma*, Josiah M. Ayotamuno, Davidson D. Davis and Mary Allagoa

Department of Agricultural and Environmental Engineering, Rivers State University of Science and Technology, Port Harcourt, P.M.B 5080, Rivers State, Nigeria.

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Spent white-rot fungi (*Pleurotus ostreatus)* substrate has been used to biotreat Nigerian oil-based drill cuttings containing polycyclic aromatic hydrocarbons (PAHs) under laboratory conditions. The Latin square (LS) experimental design was adopted in which four options of different treatment levels were tested in 10 L plastic reactors containing fixed masses of the drill cuttings and fresh top-soil inoculated with varying masses of the spent *P. ostreatus* substrate. Each option was replicated three times and watered every 3 days under ambient conditions for a period of 56 days. Microcosm analysis with a series II model 5890 AGILENT Hp® GC-FID showed that, the PAHs in the drill cuttings were mainly composed of 2 to 5 fused rings with molecular-mass ranging from 128 to 278 g/mol, while the total initial PAHs concentration of the drill cuttings was 806.31 mg/kg. After 56 days of composting, the total amount of residual PAHs in the drill cuttings decreased to between 19.75 and 7.62%, while the overall degradation of PAHs increased to between 80.25 and 92.38% with increasing substrate addition. Individual PAH degradation ranged from 97.98% in acenaphthene to 100% in fluorene, phenanthrene and anthracene. Statistical analysis, using the 2-factor analysis of variance (ANOVA), showed that there were no significant differences ($p < 0.05$) in the biodegradation of the PAHs due to the substrate levels applied and remediation period, as well as a nonsignificant ($p < 0.05$) interaction between substrate levels applied and remediation period. These results showed that spent white-rot fungi *(P. ostreatus)* substrate may be suitable for biotreating PAH-contaminated Nigerian oil-based drill cuttings.

Key words: Drill-cuttings, polycyclic aromatic hydrocarbons *Pleurotus ostreatus,* mycoremediation, composting

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are the class of hydrocarbons containing two or more fused aromatic hydrocarbons. They can persist in the environment due to their low water solubility (Cerniglia, 1992) and some (> 5-rings) are highly recalcitrant to bioremediation (Allard and Neilson, 1997). PAHs are comparatively simple to detect, relatively abundant in the environment and toxic to mammals and aquatic organisms as they can be carcinogenic or mutagenic. Consequently, the United States Environmental Protection Agency (USEPA) has classified 16 non-substituted PAHs, which include naphthalene, acenaphthylene, acenaphthene, anthrax-cene, phenanthrene, fluorene, pyrene, benzo(a) anthrax-cene, fluoranthene, chrysene, dibenzo(a,h) anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, benzo(g,h,i) perylene and indeno(1,2,3-cd) pyrene, among priority pollutants (Latimer and Zheng, 2003).

Often, drilling mud (water-based, oil-based or synthetic) and mud-additives are used during crude oil drilling

*Corresponding author. E-mail: rokparanma@yahoo.com. Tel: +234 803 2626 169.

Abbreviations: PAHs, Polycyclic aromatic hydrocarbons; **OBM,** oil-based mud; **TDUs,** thermal desorption units; **RENA,** remediation by enhanced natural attenuation; **BDE,** biodegradation-efficiency; **OBDC,** oil-based drill cuttings; **SMS,** spent fungal (mushroom) substrate; LS, Latin square; C_0, C_t, PAH concentration at the initial and over a time period, t (mg/kg) respectively; K_1, pseudo-first order kinetic constant (day^{-1}); **t,** time (days); $T_{1/2}$, half-life (days).

operations and their chemical compositions largely influence the chemistry of the resulting drill cuttings. Drill cuttings are mixtures of rocks and particulates released from geologic formations in the drill holes made for crude oil drilling. Because of its peculiar drilling properties, the continued use of oil-based mud (OBM) (Okparanma and Ayotamuno, 2010) containing PAH-carrying petroleum fractions such as diesel invert in preference to other types of drilling mud when drilling at certain depths results in the high total hydrocarbon content (Okparanma and Ayotamuno, 2008) and PAH content (DPR, 2002) of the drill cuttings. Apparently, it becomes imperative to have these drill cuttings treated before final disposal to reduce their impact on the environment. Currently, drill cuttings are treated by physico-chemical methods such as thermal desorption with prohibitive cost implications (Shkidchenko et al., 2004) and environmentally threatening consequences (DWMIS, 2004) including high-level occupational hazard associated with the operations of thermal desorption units (TDUs). These challenges prompted the recent shift of emphasis to biological treatment technologies like bioremediation, which according to several literatures has high potentials for restoring polluted media with least negative impact on the environment at relatively low cost.

Bioremediation, the basis of which may date back to the work of Atlas and Bartha (1972), is the use of microorganisms (bacteria and fungi) to accelerate the natural decomposition of hydrocarbon-contaminated waste into nontoxic residues. The use of different strains of bacteria such as *Pseudomonas aeruginosa*, *Azotobacter* and *Bacillus subtilis* (among others) in the bioremediation of hydrocarbon-contaminated soil, oily sludge and drill cuttings has been widely reported (Antai, 1990; Onwurah, 1996; Cunningham and Philip, 2000; Chokshi and Nelson, 2003; Odokwuma and Dickson, 2003; Ouyang et al., 2005; Ayotamuno et al., 2007; Ayotamuno et al., 2009; Okparanma et al., 2009). Similarly, different species of fungi including *Lentinus subnudus, Lentinus squarrosulus* Mont*., Pleurotus ostreatus, Pleurotus tuber-regium* Fr*.* Singer*, Irpex lecteus* and *Phanerochaete chrysosporium* have been used in the bioremediation of engine-oil polluted soil, chemically polluted soil, crude-oil contaminated soil and wheat straw (Bezalel et al., 1996a,b; Adamovic et al., 1998; Marquez-Rocha et al., 2000; Bhatt et al., 2002; Eggen and Sasek, 2002; Adenipekun and Fasidi, 2005; Adenipekun, 2008; Adenipekun and Isikhuemhen, 2008; Bishnoi et al., 2008; Ogbo and Okhuoya, 2008). *P. ostreatus*, in particular, according to literature (Sack and Gunther, 1993; Vyas et al., 1994) have the potentials to degrade PAHs.

In the recent past, a few researches on the field-scale remediation of oil-field drill cuttings have been centered on the combined use of some agro-technical means and amendments to stimulate the activities of naturally occurring bacteria (remediation by enhanced natural attenuation – RENA) as typified in KMC Oiltools (2005), Al-Mahruki et al. (2006), Rastegarzadeh et al. (2006) and environmental bacterial isolates to augment the naturally occurring cells (remediation by bioaugmentation) as exemplified in Ayotamuno et al. (2009) and Okparanma et al. (2009). But field-scale treatment of drill cuttings by RENA whether in biopiles or windrows has serious limitations in terms of high groundwater pollution potentials and limited land availability due to recent upsurge of industrialization and urbanization, while the use of bioaugmentation has huge field-scale adaptability challenges. Consequently, efforts are now geared towards evolving potentially high biodegradation-efficiency (BDE) composting systems for the large-scale treatment of drill cuttings with particular emphasis on the composting substrate that may likely bring this about.

Mycoremediation, which is the use of mushroom mycelium (a fungal species) in the remediation of polluted media, as stated earlier, has been used in the treatment of various contaminated media with reported significantly high BDE but has yet to be applied in the treatment of oil-based drill cuttings (OBDC) containing PAHs. The present study therefore, aims to investigate the degradability of PAHs in Nigerian OBDC by and biodegradation efficiency of the white-rot fungi (*P. ostreatus*) in the reclamation of Nigerian OBDC under laboratory composting conditions.

MATERIALS AND METHODS

The drill-cuttings and composting materials

To ensure the integrity of the samples, sampling was done strictly in line with DPR (2002) standards. Using plastic containers, composite samples of the drill-cuttings were collected from a mud-pit close to a recently completed crude oil well in the Niger Delta region (5°19'N, 6°28'E), Nigeria at standard atmospheric pressure for different treatment measures and analyses. The fresh top-soil, which was obtained from within the Faculty of Agriculture Teaching and Research Campus of the Rivers State University of Science and Technology, Nkpolu, Port Harcourt, served as a bulking agent. The spent fungal (mushroom) substrate (SMS) was obtained from the waste stream from the NDDC/RSUST/DILOMAT Mushroom/Spawn Production and Research Centre of the Faculty of Agriculture Teaching and Research Campus, Rivers State University of Science and Technology, Nkpolu, Port Harcourt.

Experimental design

The Latin square (LS) experimental design was adopted in this investigation. Four options (consisting of three treatment options and one control, without treatment with the fungal substrate) of different treatment levels were tested in 10 L plastic reactors of very low thermal conductivities containing fixed masses of the drill cuttings (2000 g) and fresh top-soil (500 g) inoculated with varying masses of the fungal substrate (500 g for option 1 at 4:1:1, 1000 g for option 2 at 4:1:2, 2000 g for option 3 at 4:1:4 and option 4 was the control with no fungal substrate addition). Each option was replicated three times and the set-ups were watered every 3 days under ambient temperature of 30°C for a period of 56 days. The plastic reactors were sufficiently lagged with wood shavings to reduce conductive heat losses associated with small-scale reactors

(Vander-Gheynst, 1994). Temperature changes in the reactors were monitored throughout the remediation period using mercury-in-glass thermometers. Microcosms of the compost mixture in the treatment and control reactors were withdrawn every 28 days and analyzed for residual PAHs concentration.

Laboratory analysis

The samples were analyzed for PAHs according to the procedures of the USEPA (1996) method 8270B using an Agilent Hp® gas chromatogram model 5890 series II, equipped with a flame ionization detector (FID).

Theory

The biodegradation rates of persistent PAHs were evaluated by comparing the reaction rate constants of the pseudo-first-order kinetics. According to Lagergren (1898), the integrated and linearized pseudo-first-order kinetic expression is given as:

$$\log(C_o - C_t) = \log C_o - \frac{K_1}{2.303} t \quad (1)$$

The value of the reaction rate constant, K_1 was determined using regression analysis by fitting on a number of experimental data points, using the LINEST function in Microsoft® excel 2007. Then, the half-lives of these selected PAHs were evaluated using the half-life ($T_{1/2}$) expression below:

$$T_{\frac{1}{2}} = \frac{In\,2}{K_1} \quad (2)$$

Biodegradation efficiency (BDE) was determined using equation 3 below:

$$BDE(\%) = \frac{(C_o - C_t)}{C_o} \times 100 \quad (3)$$

Statistical evaluations

The mean and standard deviation (SD) using the AVERAGE and STDEV functions respectively in Microsoft® excel 2007, as well as simple percentages were calculated. Experimental data were analyzed using the two-factor analysis of variance (ANOVA). Differences were considered as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Initial PAH profile of the drill cuttings and composting materials

The PAH profile of the untreated drill cuttings, the sandy loam soil and the spent fungal substrate are presented in Table 1. The PAHs in these materials were mainly composed of 2 to 5 fused rings with molecular-mass ranging from 128 g/mol in naphthalene to 278g/mol in dibenzo (a,h)anthracene. The total initial PAH concentration of the drill cuttings and spent fungal substrate were 806.31 and 4.71 mg/kg, respectively. No PAH fraction was detected in the sandy-loam soil as they were well below the laboratory detection limit of the equipment. The most abundant PAH fraction in the drill cuttings was D(a,h)A (constituting 31.29%, dry weight, of the total PAHs) while the least abundant was Fluorene (constituting only 0.03%, dry weight, of the total PAHs). The predominant PAH ring-group in the drill cuttings was the 4-ring PAHs (representing 52.89% of the total PAHs).

Incidence of biodegradation of PAH fractions

Table 2 shows the extent of degradation of individual PAHs by the different levels of fungal substrate applied during the 56 days of bioremediation. The incidence of degradation of individual PAH fractions differed clearly both in terms of their properties such as molar mass and ring group and the level of fungal substrate applied. As may be observed in Table 2, the amount of PAHs remaining decreased with increasing levels of added fungal substrate as the degradation of PAHs increased with increasing fungal substrate level over time.

Biodegradation rates of persistent PAHs in the drill cuttings

Table 4 shows that, the persistent PAHs had clearly different rates of degradation both in terms of their molar mass and ring-group. The values of the pseudo-first-order degradation rate constant for individual PAHs varied between 2.06×10^{-4} day^{-1} in chrysene and 4.52×10^{-3} day^{-1} in acenaphthene as half-lives ranged between 153 and 3356 days in chrysene and acenaphthene, respectively.

Composting starting materials and their PAHs compositional distribution

Although, in Table 1, no PAH fraction was detected in the sandy-loam soil as they were well below the laboratory detection limit of the equipment, the 4.71 mg/kg of PAHs found in the fungal substrate was considered negligible when compared with that of the entire sample mix and was estimated to constitute just 0.0001 to 0.00016% of the entire sample mix. The compositional distribution of PAHs in the drill cuttings, as may be observed in Table 1 were however, slightly at variance with our earlier report (Okparanma et al., 2009); suggesting that a different oil-producing company may have been involved in the drilling and may have used a different drilling mud in the drilling process. This observation was not unexpected as

Table 1. Initial PAH profile of the starting materials used for the composting.

PAHs	Molar mass (g/mol)	Ring group	LDL (mg/kg)	Starting materials (mg/kg)		
				Untreated drill cuttings	Composting materials	
					Sandy-loam soil	Spent white-rot fungi *(P. ostreatus)* substrate
Naphthalene	128	2-ring	<0.001	1.77 ± 0.02(0.22%)	nd	1.13 ± 0.02
Acenaphthylene	166	3-ring	<0.001	2.00 ± 0.07(0.24%)	nd	0.41 ± 0.01
Acenaphthene	166	‘’	<0.001	0.99 ± 0.08(0.12%)	nd	0.22 ± 0.04
Fluorene	166	‘’	<0.001	0.22 ± 0.01(0.03%)	nd	0.67 ± 0.04
Phenanthrene	178	‘’	<0.001	1.71 ± 0.02(0.21%)	nd	0.72 ± 0.06
Anthracene	178		<0.001	10.92 ± 0.02(1.35%)	nd	0.52 ± 0.03
Fluoranthene	202	4-ring	<0.001	92.20 ± 0.18(11.43%)	nd	0.35 ± 0.01
Benzo(a)anthracene	228	‘’	<0.001	205.72 ± 0.9(25.51%)	nd	0.11 ± 0.02
Chrysene	228	‘’	<0.001	128.62 ± 0.47(15.95%)	nd	0.13 ± 0.04
Benzo(b)Fluoranthene	252	5-ring	<0.001	53.04 ± 0.53(6.58%)	nd	0.21 ± 0.05
Benzo(a)pyrene	252	‘’	<0.001	56.82 ± 0.38(7.05%)	nd	0.11 ± 0.01
Dibenz(a,h)anthracene	278	‘’	<0.001	252.30 ± 1.29(31.29%)	nd	0.16 ± 0.02
Total				806.31	nd	4.74

Values are presented in mean ± standard deviation of three readings; nd, not detected; LDL , laboratory detection limit; PAH, polycyclic aromatic hydrocarbons.

different companies often go for different proprietary drilling mud of different formulations for specific purposes. Mud formulation, which dictates the chemistry of the resulting drill cuttings, has hitherto been a trade secret for drilling mud manufacturers. This also highlights the views in the literature (Wills, 2000) about the recently disturbing ‘semantic engineering’ being adopted by mud manufacturers and their apologists to conceal the fact that irrespective of the type of formulation, a major ingredient of drilling mud remains the seemingly indispensable mineral oil (organic-phase), which is largely responsible for the hydrocarbons usually found in drill cuttings.

Degree of degradation of PAH fractions by *P. ostreatus*

From Table 2, it may be observed that naphthalene (2-ring and molar mass 128 g/mol) was short-lived as it was undetected shortly after treatment commenced up until the end of the remediation period. This rapid dis-appearance of Naphthalene may have much to do with its physical properties, such as high volatility, slight solubility in water (31 mg/l), high vapor pressure, possession of two number of rings and low molar-mass (128 g/mol), than with microbiological degradation. This is because, low molecular-mass PAHs are less likely sorbed onto solid matter hence, may be unavailable to PAH-degrading organisms (Yunker and MacDonald, 1995; Johnsen et al., 2005). A similar trend was observed in fluorene (3-ring and molar-mass 166 g/mol). However, a little consideration of Table 2 would show that phenanthrene (3-ring and molar mass 178 g/mol) in the first 28 days of treatment with 500 g of the fungal substrate was not within the laboratory detection limit but, after the 56th day increased to 0.01 mg/kg whereas, anthracene (3-ring and molar mass 178 g/mol) and bacteria (Ayotamuno et al., g/mol) were degraded below detection limits after 56 days of treatment with2000 g of the fungal substrate. This anomalous degradation pattern of the 3-ring PAHs by *P. ostreatus* was least complete biodegradation to harmless end-products. This degradation pattern was also observed in our previous study with some species benzo(a)pyrene (5-ring and molar mass 252 of 2009). In addition, the degradation of PAHs observed in this study compared favorably with the reports of other researchers as illustrated in Table 3, which demonstrated the potentials of spent white-rot fungi *(P. ostreatus)* substrate to remove PAHs from oil-based drill cuttings.

Half-lives of persistent PAHs

Clearly, some of the half-lives deduced and shown in Table 4 for the persistent PAHs were observed to have fallen out of the range earlier reported in the literature (Shuttleworth and Cerniglia, 1995) for these compounds. Okparanma and Ayotamuno (2010) observed a similar occurrence while composting with poultry droppings and suggest that these may be because unlike nuclear decay, the half-lives for decay of chemical toxins were affected by external conditions such as temperature, pressure or

Table 2. Incidence of PAHs degradation after days of composting key.

PAHs	LDL (mg/kg)	Untreated drill cuttings (mg/kg)	Conc. of residual PAHs after 28 days of composting (mg/kg)			Conc. of residual PAHs after 56 days of composting (mg/kg)		
			Option 1	Option 2	Option 3	Option 1	Option 2	Option 3
Naphthalene	<0.001	1.77±0.02	nd	nd	nd	nd	nd	nd
Acenaphthylene	<0.001	2.00±0.07	0.18 ±0.08	0.08 ±0.04	0.03 ±0.01	0.05 ±0.02	0.02 ±0.01	nd
Acenaphthene	<0.001	0.99±0.08	0.42 ±0.03	0.28 ±0.01	0.18 ±0.01	0.12 ±0.02	0.09 ±0.01	0.02 ±0.01
Fluorene	<0.001	0.22±0.01	nd	nd	nd	nd	nd	nd
Phenanthrene	<0.001	1.71±0.02	nd	0.08 ±0.02	0.03 ±0.01	0.01 ±0.01	nd	nd
Anthracene	<0.001	10.92±0.02	3.50 ±0.09	1.74 ±0.02	0.89 ±0.01	1.09 ±0.02	0.94 ±0.02	nd
Fluoranthene	<0.001	92.20±0.18	56.12 ±0.28	45.72 ±0.08	20.27 ±0.06	9.31 ±0.05	4.20 ±0.03	1.65 ±0.04
Benzo[a]anthracene	<0.001	205.72±0.9	140.98 ±1.22	113.82 ±0.28	93.45 ±0.48	60.63 ±0.04	41.56 ±0.03	25.41 ±0.03
Chrysene	<0.001	128.62±0.47	101.25 ±0.48	75.01 ±0.18	58.28 ±0.22	16.97 ±0.09	7.61 ±0.08	1.63 ±0.05
Benzo[b]Fluoranthene	<0.001	53.04±0.53	50.49 ±0.35	4.72 ±0.06	24.91 ±0.07	7.85 ±0.06	3.68 ±0.03	0.8 ±0.04
Benzo[a]pyrene	<0.001	56.82±0.38	15.10 ±0.18	8.15 ±0.17	4.46 ±0.09	0.64 ±0.03	0.23 ±0.04	nd
Dibenzo[a,h]anthracene	<0.001	252.30±1.29	121.93 ±0.42	105.71 ±0.48	92.81 ±0.16	62.61 ±0.07	40.29 ±0.01	30.21 ±0.02
Total PAHs remaining (mg/kg)		806.31	489.97	355.31	285.31	159.28	98.33	61.41
Total PAHs remaining (%)			60.8	44.07	35.38	19.75	12.20	7.62
BDE (%)			39.23	55.93	64.62	80.25	87.80	92.38

Option 1: Compost containing 2000 g of drill cuttings + 500 g top-soil + 500g SMS at 4:1:1; Option 2: compost containing 2000 g of drill cuttings + 500 g top-soil + 1000 g SMS at 4:1:2; Option 3: compost containing 2000 g of drill cuttings + 500 g top-soil + 2000 g SMS at 4:1:4; values represent mean ± standard deviation of four replicates; LDL = laboratory detection limit; nd = not detected; BDE = biodegradation efficiency; Control is not shown.

state of chemical combination since chemical toxins, unlike radioactive atoms, could be rendered harmless by chemical reactions or by any other practical treatment, which might be brought about by bioremediation.

Table 5 shows the results of the 2-factor ANOVA performed on the results of the biodegradation of the PAHs. The results showed that the row, column and the three factorial components of the treatment (remediation period, fungal substrate levels and interaction between them) sources of variation were not significant at the 0.05 and the 0.01 probability levels, which implied that, there were no significant differences in the biodegradation of the PAHs due to the fungal substrate levels applied and remediation period and that different patterns of differences were not observed across the different levels of fungal substrate applied after 28 and 56 days of remediation. In order words, the fungal substrate levels applied resulted in nonsignificant differences in the biodegradation of the PAHs of the drill cuttings over time. The nonsignificant interaction between fungal substrate levels applied and remediation period indicated that, the differences observed over time was not significantly affected by the fungal substrate levels applied and that the fungal substrate effect on the biodegradation of the

Table 3. Comparison of PAHs degradation by different fungal species.

PAHs	Ring-group	BDE (%)	Fungal species	Medium	DAR (d)	Reference
Acenaphthene	3-ring	83.8	*P. chrysosporium*	Sterilized soil	42	Bishnoi et al. (2008)
		40.25	*P. chrysosporium*	Unsterilized soil	42	Bishnoi et al. (2008)
		50 - 99	*P. ostreatus*	Polluted soil	84	Eggen and Sasek (2002)
		97.98	*P. ostreatus*	Drill cuttings	56	This study
Fluorene	‘’	87 - 97	*P. ostreatus*	Polluted soil	84	Eggen and Sasek (2002)
		26 - 35	*P. ostreatus*	Polluted soil	98	Bhatt et al. (2002)
		41 - 67	*I. lacteus*	Polluted soil	98	Bhatt et al. (2002)
		100	*P. ostreatus*	Drill cuttings	56	This study
Phenanthrene	‘’	100	*P. chrysosporium*	Sterilized soil	42	Bishnoi et al. (2008)
		62.89	*P. chrysosporium*	Unsterilized soil	42	Bishnoi et al. (2008)
		87 – 97	*P. ostreatus*	Polluted soil	84	Eggen and Sasek (2002)
		20 – 56	*I. lacteus*	Polluted soil	98	Bhatt et al. (2002)
		0 – 20	*P. ostreatus*	Polluted soil	98	Bhatt et al. (2002)
		100	*P. ostreatus*	Drill cuttings	56	This study
Anthracene	‘’	92.6	*P. chrysosporium*	Sterilized soil	42	Bishnoi et al. (2008)
		44.02	*P. chrysosporium*	Unsterilized soil	42	Bishnoi et al. (2008)
		50 – 87	*P. ostreatus*	Polluted soil	84	Eggen and Sasek (2002)
		29 – 49	*I. lacteus*	Polluted soil	98	Bhatt et al. (2002)
		19 – 53	*P. ostreatus*	Polluted soil	98	Bhatt et al. (2002)
		100	*P. ostreatus*	Drill cuttings	56	This study
Fluoranthene	4-ring	79.8	*P. chrysosporium*	Sterilized soil	42	Bishnoi et al. (2008)
		38.94	*P. chrysosporium*	Unsterilized soil	42	Bishnoi et al. (2008)
		87 – 99	*P. ostreatus*	Polluted soil	84	Eggen and Sasek (2002)
	‘’	29 – 57	*I. lacteus*	Polluted soil	98	Bhatt et al. (2002)
		29 – 31	*P. ostreatus*	Polluted soil	98	Bhatt et al. (2002)
		98.21	*P. ostreatus*	Drill cuttings	56	This study
Benzo(a)anthracene	4-ring	13 – 20	*I. lacteus*	Polluted soil	98	Bhatt et al. (2002)
		0 – 13	*P. ostreatus*	Polluted soil	98	Bhatt et al. (2002)
		87.65	*P. ostreatus*	Drill cuttings	56	This study
Chrysene	‘’	16 – 32	*I. lacteus*	Polluted soil	98	Bhatt et al. (2002)
		0 – 42	*P. ostreatus*	Polluted soil	98	Bhatt et al. (2002)
		98.73	*P. ostreatus*	Drill cuttings	56	This study

Table 3. Contd.

DAR, Days after remediation; d = days.

PAHs did not differ significantly with time.

Conclusions

The study observed that the PAH profile of the untreated drill cuttings, were mainly composed of 2 to 5 fused rings with molecular-mass ranging from 128 to 278 g/mol. The total initial PAH concentration of the drill cuttings was 806.31 mg/kg. No PAH fraction was detected in the sandy-loam soil used as a bulking agent. The incidence of degradation of individual PAH fractions differed clearly

Table 4. The degradation rates of some persistent PAHs in the drill cuttings treated with 2000g of SMS.

PAHs	Ring-group	Molar mass (g/mol)	K_1 (day^{-1})	$T_{1/2}$ at the end of 56th day (days)	$T_{1/2}$ in literature* (days)
Acenaphthene	3-ring	166	4.52×10^{-3}	153	16 – 123
Fluoranthene	4-ring	202	3.29×10^{-4}	2107	-
Benzo(a)anthracene	‘’	228	2.34×10^{-3}	296	-
Chrysene	‘’	228	2.06×10^{-4}	3365	-
Benzo(b)Fluoranthene	5-ring	252	2.88×10^{-4}	2407	229 - >1400
Dibenzo(a,h)anthracene	‘’	278	2.26×10^{-3}	307	‘’

PAH, Polycyclic aromatic hydrocarbons. *Shuttleworth and Cerniglia (1995).

Table 5. Two-factor ANOVA on the result of the degradation of PAHs.

Source of variation	d.f.	S.S.	M.S.	*F* ratios
Row	2	243224.76	121612.38	-7.5^{ns}
Column	1	12.71	12.71	-0.0008^{ns}
Treatment	5	742195.4	148439.08	-9.2^{ns}
Remediation period	(1)	(560825.05)	560825.05	-34.59^{ns}
Fungal substrate level	(2)	(117219.3)	58609.65	-3.62^{ns}
Interaction	(2)	(64151.05)	32075.53	-1.98^{ns}
Error	15	-243185.27	-16212.35	-
Total	23	742247.6	-	-

d.f. = Degree of freedom; S.S. = sum of squares; M.S. = mean squares; ns = not significant at $p < 0.05$.

both in terms of their properties (molar mass and ring group) and the level of fungal substrate applied. After 56 days of composting, the total amount of PAHs remaining in the drill cuttings decreased to between 19.75 and 7.62%, while the overall degradation of PAHs increased to between 80.25 and 92.38% with increasing fungal substrate addition over time. Individual PAH degradation ranged from 97.98% in acenaphthene (3-ring) to 100% in fluorene (3-ring), phenanthrene (3-ring) and anthracene (3-ring), which demonstrated the PAH-removal capacity of spent white-rot fungi *(P. ostreatus)* substrate. The values of the pseudo-first-order degradation rate constant for individual PAHs varied between 2.06×10^{-4} and 4.52×10^{-3} day^{-1}, while the half-lives of these compounds ranged between 153 and 3356 days.

Recommendations

This study therefore recommends that:

- Drill cuttings may be bio-treated, to bring down the PAHs level to acceptable level before final disposal to reduce the level of inherent environmental pollution.
- Spent white-rot Fungi (*P. ostreatus*) substrate may be utilized in the reclamation of oil-based drill cuttings in composting systems as this may serve the dual purpose of reducing the bulk volume of the spent fungal substrate as a waste and the incidence of occurrence of PAHs in the environment.
- The findings of this study may be adopted by exploration and production (E&P) waste management companies in treating oil-based drill cuttings to reduce the cost, energy and pollution associated with thermal treatment methods.

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