Science World Journal Vol 9 (No 1) 2014
www.scienceworldjournal.org
ISSN 1597-6343

Full Length Research Article

A STUDY OF THE MICROBIOLOGY AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) COMPOSITIONAL PROFILE AND SOURCES IN DRILL CUTTINGS FROM OLOGBO OILFIELD WELLS AT EDO STATE, NIGERIA.

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ABSTRACT
A study was carried out on the drill cuttings from three different oil and gas wells located at Ologbo Community at Edo State with respect to their microbiology and polycyclic aromatic hydrocarbons (PAHs) compositional profile and sources. Isolation and enumeration of heterotrophic bacteria and fungi was carried out using pour plate techniques. pH and electrical conductivity were monitored using single electrode meters. Polycyclic Aromatic Hydrocarbon was determined using gas chromatography, oil and grease concentration was analyzed using spectrophotometric method. Results of total heterotrophic bacterial counts ranged between 5.4 x 105 cfu/g at well 2 to 7.23 x 105 cfu/g while the heterotrophic fungal counts ranged from 3.7 x 104 cfu/g to 3.0 x 105 cfu/g. Total heterotrophic anaerobic bacterial counts also ranged from 0.8 x 102 cfu/g to 1.7 x 102 cfu/g. The most predominant bacterial isolates were Clostridium spp. (16.6%) and Mycobacterium spp. (14.5%). Species of Aspergillus (16.6) and Penicillium (14.2) also had the highest frequency of occurrence among the fungi isolates. The pH ranged from 5.42 to 5.78. The highest electrical conductivity value was 383 µS/cm. The range for oil and grease was from 8352 to 9654.61 mg/kg. Of the heavy metals iron had the highest concentration (126 ppm) while least concentration was observed in zinc (61 ppm). The 3-ring PAHs had the highest percentage composition for samples from the three (3) different wells (53, 73 and 89 for well 1, 2 and 3 respectively). The source identification by isomer ratios of PAHs revealed that drill cuttings from Wells 1 - 2 were of pyrogenic origins, while cuttings from well 3 indicated both petrogenic and pyrogenic origin. It was quite clear that drill cuttings emanating from Ologbo oilfield wells were unsafe for disposal and therefore will require a strict adherence of the procedures and instructions described for oil drilling exploration and procedure (E and P) waste management in Nigeria.

Keywords: : drill-cuttings, muds, microbial qualities, PAHs, heavy metals.

INTRODUCTION
Drill cuttings are the solid waste which results from exploration, appraisal and production wells drilling activities and comprise small pieces of the strata through which the well is drilled. The first commercial quantity of oil produced in Nigeria was in Oloibiri community of Bayelsa State in 1958. Since then, Nigeria oil sector had continued to witness increasing number of exploration and production activities, with the Niger Delta region playing host to well over 85 % of these exploration and production activities (Ifeadi et al., 1985, Okpokwasili and Odokuma, 1996).
Drilling muds and their additives have been observed to greatly influence the biology and chemistry of the resulting drill cuttings. (Okparanma et al., 2009, Imarhiagbe and Atuanya, 2013). The two primary types of drilling muds are water based muds and non-aqueous based muds (Mairs et al., 1999). Water based muds consist of water mixed with bentonite clay and additives such as barium sulfate (barite). The non-aqueous drilling muds comprise all non-water and non-dispersible based muds and they include Oil Based Muds (OBM), Low Toxicity Mineral Based Muds (LTMBM), Enhanced Mineral Oil Based Muds (EMOBM) and Synthetic Based Muds (SBM) (Mairs et al., 1999). The volumes of cuttings generated during exploration depend on the type of mud used, the depth of the well and the size of the borehole USEPA (1993) cited by Gbadebo et al. (2010).
Prominent among the composition of the exploratory wastes are the aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH) and heavy metals (Gbadebo et al., 2010). Polycyclic aromatic hydrocarbons (PAHs) are the class of hydrocarbons containing two or more fused aromatic hydrocarbons (Ayotamuno et al., 2009). According to Ayotamuno et al. (2009), some of these compounds are potentially carcinogenic or mutagenic and sixteen of them are in the priority list of United States Environmental Protection Agency (USEPA) due to their toxic effect to mammals and aquatic organisms (Latimer and Zheng, 2003). Heavy metals and hydrocarbon contents of the onshore drill cuttings have been known to be capable of polluting the environment with the resultant effects on human health (Gbadebo et al., 2010). Disposal of drill muds and cuttings has remained a growing concern to both the government regulatory agencies and to the researchers around the world because of their negative effects on the health, safety and environments (Moseley, 1983). It is well known that due to the adverse effects experienced from oil based- cuttings, regulatory agencies of crude oil producing countries have out-lawed the use of oil based muds (Okparanma et al., 2010). This study therefore aimed at assessing the environmental effects of drill cuttings generated from Ologbo oilfield wells with respect to their microbiological characteristics and the poly cyclic aromatic hydrocarbons compositional profile and their possible sources.

MATERIALS AND METHODS
Sources and collection of Samples:
The drill cutting samples were collected in sterile clean plastic containers during drilling process of the onshore

wells at Ologbo community in Edo state, for microbiological and physicochemical analysis. They were transported to the laboratory in ice-cooled containers where they were immediately analyzed. The geographic position system (GPS) of the wells were E: 350017.978 m, N: 229469.956 m (well1), E: 350020.000 m, N: 229477.600 m (well2), E: 350017.979 m, N: 229465.442 m (well3).

Isolation and enumeration of microorganisms:
Heterotrophic bacteria and fungi were enumerated using the standard plate count technique (Cheesbrough, 2000). Appropriate dilutions of samples were plated out in duplicates on nutrient agar and potato dextrose agar (incorporated with 2 µg/l of chloramphenicol) for bacteria and fungi respectively. A set of nutrient agar plates were incubated at 300C for 48 hours (aerobic bacteria) while the other sets were incubated with the aid of an anaerobic jar with an oxygen removing system (oxoid gas pack), to create an anaerobic system. The plates for fungal isolation were incubated at room temperature for 5-7 days. The means of duplicate colony counts were calculated and used to compute the colony forming unit per gram (cfu/g).

Enumeration of drilling muds utilizing microorganisms:
Drilling mud utilizing microorganisms were isolated and counted using mineral salt medium according to (Okpokwasili and Okorie, 1988;. The composition of the mineral salt medium include: MgS04.7H20, 0.42 g/l, KCl, 0.30 g/l, KH2PO4, 0.8 g/l, K2HPO2, 1.3 g/l, NaNO3, 0.42 g/l and Agar 15 g/l. the pH of the medium was adjusted to 7.4. The plates were incubated at 29 oC for 6 days after which the growth of drilling mud degraders were observed and counted. For fungal plates, 2 µg/l of chloramphenicol was incorporated to inhibit the bacterial growth. The various isolates were further characterized and identified (Buchanan and Gibbons, 1974; Barnett and Hunter, 1975).

pH and Electrical conductivity:
The samples were prepared by homogenizing 25 g of the sample in 25 ml of water. The contents of the beaker were then thoroughly stirred and left to stand for at least 6 hours, with occasional stirring. Following standardization with pH distilled 7 and 4 buffers, the pH of the homogenate was determined in duplicate using a single electrode pH meter (Jen-way Patterson Scientific, London). Also from the above homogenate, the electrical conductivity was determined using a single electrode conductivity meter (Jen-way Pattern Scientific, London).

Determination of Heavy metal content of samples:
The Heavy metals such as iron, zinc and lead were determined after an initial digestion of drill cutting samples with concentrated nitric acid. The resultant sample filtrates were analyzed for the heavy metal content using the Atomic Absorption Spectrophotometer (AAS), Buck Scientific model 210 VGP.

Determination of oil and grease:
Five grams (5 g) of the drill cuttings sample was weighed into a 150 ml glass bottle for extraction. Twenty milliliters (20 ml) of tetrachloromethane was added into the bottle containing the weighed sample and extraction was done in a water bath for 3 hrs. The content in the bottle was allowed to settle and thereafter was filtered into a clean bottle and 5 g of anhydrous sodium sulphate was added to the filtrate. Absorbance was read at 420 nm to determine the concentration of oil and grease. Value in mg/kg was calculated as below:

Oil and grease (mg/kg) =

$$\frac{\text{Instrumental Reading. X Vol. X 103}}{\text{Wt. of sample X 104}}$$

Determination of polycyclic aromatic hydrocarbon (PAH):
Ten grams of drill cuttings were weighed into a solvent rinsed beaker and 50ml of 50:50 mixtures of acetone and dichloromethane (DCM) was added. The sample was spiked with 1ml of a surrogate mixture (orthoterphenyl-OTP) and placed in a sonicator for 15 min at 20 0C. Ten (10) grams of anhydrous sodium sulphate was added to the sample and allowed to stand until a clear extract developed and was decanted. One milliliter (1ml) of the extracted samples was dissolved in hexane (HPLC grade) in order to elute the aliphatic hydrocarbons. The eluted samples were concentrated using rotatory evaporator to about 3ml and was transferred into a teflone lined screwed vial and labeled for gas chromatography (GC) for PAH analysis.

Statistical analysis:
The data obtained were subjected to descriptive statistical analysis such as mean, standard deviation and analysis of variance (Ogbeibu, 2005)

RESULTS
The mean total heterotrophic bacterial counts (cfu/g) of drill cuttings for the investigated wells ranged between 5.4 x 105 cfu/g at well 2 to 7.23 x 105 cfu/g (well 1) while the heterotrophic fungal counts ranged from 3.7 x 104 cfu/g (well 2) to 3.0 x 105 cfu/g (well 1). Total heterotrophic anaerobic bacterial counts also ranged from 0.8 x 102 cfu/g at well 3 to 1.7 x 102 cfu/g at well1. The highest recorded drill mud utilizing bacterial counts was 7.7 x 103 cfu/g for medium amended with water based mud while the least drill mud utilizing bacterial counts (3.1 x 102 cfu/g) was recorded for medium amended with non-aqueous drilling mud. For drill mud utilizing fungal counts, medium amended with water based mud also recorded the highest count (4.1 x 102 cfu/g). Bacterial and fungal isolates and their percentage frequencies are shown in Table 2. Micrococcus sp. (11.2%), Bacillus spp. (14.2%), Staphylococcus spp. (11.9%), Clostridium spp. (16.6%), Nocardia sp. (8.1%), and Mycobacterium spp. (14.5%) were more predominant than Gram-negative bacteria except Desulfotomaculum spp. (10.9%). Species of Aspergillus (16.6) and Penicillium (14.2) also had the highest frequency of occurrence among the fungi isolated in this work.

Table 3 shows the results for selected physicochemical parameters. The pH range from 5.42 to 5.78. Well 2 recorded the highest value of 5.78. The highest electrical conductivity value (383) was recorded at well 2. The amount of the oil and grease (mg/kg) ranged from 8352 to 9654.61. Results showed high heavy metals concentration. The heavy metal with the highest concentration was iron (126 ppm) while least concentration was observation in zinc (61 ppm). Results for the concentrations (mg/kg) and compositional distributions (%) of PAH fractions of composite drill cuttings are presented in Table 4. According to numbers of aromatic rings, the PAH compounds were divided into five (5) groups (2-6 fused rings) with molecular mass ranging from 128 g/mol (for naphthalene) to 278 g/mol (for dibenzo (a,h) anthracene). The 3-ring PAHs had the highest percentage composition for samples from the three (3) different wells (53, 73 and 89 for well 1, 2 and 3 respectively). The results evaluating the source identification by isomer ratios of PAHs (Table 5) revealed that the possible sources of the PAHs in the drill cuttings from Wells 1 - 2 might be pyrogenic origins, while cuttings from well 3 indicated both petrogenic and pyrogenic origin.

Table 1: Mean Total Heterotrophic Microorganisms Count (Cfu/G) Of Drill Cuttings.

	Total Heterotrophic Bacterial counts	Total Heterotrophic Fungal Counts	Total Heterotrophic Anaerobic Bacterial counts	Total Mud Utilizing Bacterial Count		Total Mud Utilizing Fungal Count	
				WBM	NABM	WBM	NABM
Well 1	$7.23x10^5$	$3.0 x10^5$	$1.7 x10^2$	$7.7 x10^3$	$5.2 x10^2$	$4.1 x10^2$	$1.3 x10^2$
Well 2	$5.4 x 10^5$	$3.7 x10^4$	$1.5 x10^2$	$6.5 x10^3$	$3.1 x10^2$	$3.2 x10^2$	$1.1x 10^2$
Well 3	$7.0 x 10^5$	$2.5 x 10^5$	$0.8 x10^2$	$6.3 x10^3$	$5.0 x10^2$	$3.1 x10^2$	$1.9 x10^2$

Over all mean values; WBM: water based mud; NABM: Non aqueous based mud.

Table 2: Percentage Frequency Of Occurrence Of Microbial Isolates

Microbial isolates	Percentage frequency of occurrence
Enterobacter sp.	4.9
Micrococcus sp.	11.2
Bacillus spp	14.2
Staphylococcus spp	11.9
Clostridium spp.	16.6
Mycobacterium spp.	14.5
Desulfotomacum spp.	10.9
Pseudomonas sp.	5.5
Citrobacter sp.	2.2
Nocardia sp.	8.1
Aspergillus spp.	16.6
Rhizopus spp	11.2
Penicillium spp.	14.2
Mucor sp.	11.9
Cladosporium sp.	4.9

Table 3: Physicochemical Parameters And Heavy Metals Of Drill Cuttings From Ologbo Drilled-Wells

Sources	pH	Electrical Conductivity (µS/cm)	Concentration of oil and Grease (mg/kg)	Concentration of Heavy Metals (ppm)		
				Fe	Zn	Pb
Well 1	5.42	302	8352.00	126	68	77
Well 2	5.78	383	9552.00	87	61	90
Well 3	5.47	262	9654.61	91	61	85

Over all mean values; WBM: water based mud; NABM: Non aqueous based mud.

Science World Journal Vol 9 (No 1) 2014
www.scienceworldjournal.org
ISSN 1597-6343

Table 4: Concentration And Compositional Distribution Of Pah Fractions Of The Studied Drill Cuttings.

POLYCYCLIC AROMATIC HYDROCARBON (PAH) COMPOUNDS	RING GROUP	MOLAR MASS	PAH CONCENTRATION (mg/kg) AT THE WELLS 1	2	3	COMPOSITION (%) 1	2	3
Naphthalene	2	128	122 ±0.15	nd	1.96±0.19	29	1	2
2-Methylnaphthalene		128	142±0.22	2.51±0.2	2.0±0.12			
Acenaphthylene		166	114±0.16	110±0.21	81±0.15			
Acenaphthene	3	166	56±0.19	51±0.15	61.5±0.15			
Fluorene		166	57±0.23	42±1.0	32±0.10			
Phenanthrene		178	13±0.21	13±0.3	18.1±0.12			
Anthracene		178	242±0.17	151±0.23	9.51±0.11	53	73	89
Fluoranthene		202	125±0.17	70±0.11	1.2±0.11			
Pyrene	4	202	11±0.21	17±0.21	2.50±0.17			
Benzo(a)anthracene		228	0.0	1.0±0.17	2.3±0.11			
Chrysene		228	0.0	1.0±0.17	2.9±0.21	15	17	4
Benzo(b)fluoranthene		252	17±0.17	21.1±0.15	2.4±0.13			
Benzo(k)fluoranthene	5	252	0.0	5.7±0.11	4.0±0.20			
Benzo(a)pyrene		252	13.0±0.23	14.2±0.12	1.8±0.11			
Dibenzo(a,h)anthracene		278	0.0	0.0	0.0	3	8	4
Benzo(g,h,i)perylene	6	276	0.0	2.1±0.10	2.0±0.19			
Indeno(1,2,3-cd)pyrene		276	0.0	0.0	0.0	0	0.5	0.9
TOTAL			912	501.61	226.72	100	100	100

PAH values are mean± standard deviation

Table 5: Source Identification By Isomer Ratio Of Pah

Diagnostic ratio	Molar mass	Concentration Ratio for the Wells 1	2	3	PAH Source Criteria* Pyrogenic source	Petrogenic source	Remark on PAH Source
ANT/(PHE + ANT)	178	0.9	0.9	0.3	>0.1	≤0.1	All Wells PAHs indicate pyrogenic source
Flu/(Flu + Pyr)	202	0.92	0.8	0.3	>0.50	<0.40	Wells (1-2) PAH are progenic while well(3) PAH indicates petrogenic

ANT:Anthracene; PHE: Phenanthrene; Flu: Fluoranthene; Pyr: Pyrene.

DISCUSSION
The monitoring of the microbial population density of the different wells revealed high aerobic heterotrophic bacteria and fungi of the surface drill cuttings (7.23 x 105 cfu/g, 3.0 x 105 cfu/g for well 1 aerobic heterotrophic bacteria and fungi) . However, this observed trend was contrary to anaerobic heterotrophic bacterial counts (1.7 x 102 cfu/g for well 1 anaerobic bacterial count). Gram positive bacteria such as Clostridium spp. (16.6%), Mycobacterium spp. (14.5%) and Bacillus spp. (14.2%) were more predominant than Gram-negative bacteria. This finding may be attributed to the high concentration of peptidoglycan in the cell wall of Gram-positive bacteria, which confer its resistance to environmental stress posed by drilling muds (Okpokwasili and Okorie, 1988; Prescott et al., 2002). Result from this study showed species of Aspergillus (16.6) and Penicillium (14.2) to have the highest frequency of occurrence among the fungi isolated in this work. This observation is in agreement with previous works of Nnubia and Okpokwasili 1993; Odokuma and Ikpe 2003and Okoro, 2011, who had earlier reported the predominance of Gram positive bacterial and fungal isolates. The spore forming ability of bacteria such as Bacillus spp., Clostridium spp., Desulfotomaculum spp. and Nocardia sp., and the fungal isolates also conferred in these microorganisms an additional advantage for their predominance (Nester et al., 1998, Prescott et al., 2005). Statistical analysis revealed no significant differences ($P > 0.05$) for counts of total heterotrophic bacteria, anaerobic bacteria and heterotrophic fungi.

Bacterial growths have been observed to be at optimum pH range of 6.0 - 8.0; while fungi have also been observed to grow at relative low pH concentration. The differences in the pH values in this study are a claim of differentiation in the characteristic and properties of the drill cutting profiles of oil and gas wells. These features could predict the proportional abundances of micro-organisms. The evaluation for the oil and grease concentrations on the drill cutting samples revealed that drill cutting wastes at the different sites were coated with high amount of oil and grease. Drill cuttings have been found to be heavily laden with oil and grease, especially when oil based or synthetic based mud is used. At high concentrations, it could seriously threaten the life of edaphic systems as well as people who depend on local ground water if it permeates through the soil and also as run-off into water bodies (Odokuma and Ikpe, 2003). It has been asserted that the effects of electrical conductivity is also been regarded as one of the major concerns in the disposal of drill cuttings (Gbadebo et al., 2010). The results of electrical conductivity indicated a high concentration of solutes impaired on the cuttings. The presence of heavy metals in the aquatic ecosystem has been observed to be of far-reaching implications to the biota and man (Gbadebo et al., 2010). The results showed that iron was relatively higher in concentration than others (Table 3). The values were observed to be generally higher when compared with the established standard for iron in inland fresh surface water FEPA (1991) cited by Gbadebo et al. (2010). From all indications, the release of these drilling wastes into an aquatic body will definitely lead to increase in the concentration of these heavy metals, beyond their recommended threshold levels.

According to Okparanma et al. (2010), knowledge of the origin of the PAHs present in the waste stream from drilling operations, such as drill-cuttings may provide an insight into whether or not environmentally harmful drilling mud composed of PAH-carrying petroleum fractions was employed in the drilling activity. The compositions and relative abundance of individual PAHs in this study were quite similar. The compositional pattern shows the predominance of 3-ring PAHs, with acenaphthylene and acenaphthene having the highest concentration. Okparanma et al. (2010) had earlier stated as established by Yunker and MacDonald (1995) that in considering the number of source-specific PAHs in a sample, the predominance of combustion-specific 3-ring PAHs (acenaphthylene and acenaphthene) in a drill cuttings, suggests that the PAHs in the drill cuttings are from a combustion source (pyrogenic). The source identification of isomer ratio of PAH (Okoro and Ikolo, 2007; Okparanma et al., 2010) confirmed the source of PAHs from well 1 and 2 to be pyrogenic while PAHs from well 3 showed pyrogenic and petrogenic (Table 5). The presence of pyrogenic PAHs may be attributed to the possible combustion of the petroleum fraction due to the heat generated in the drill-bit during drilling. However, the presence of PAHs of petrogenic origin, according to Okparanma et al. (2010) suggests that a drill mud containing petroleum fraction may have been used during drilling. In conclusion, it was quite clear that drill cuttings emanating from Ologbo oilfield wells were unsafe for disposal and therefore will require a strict adherence of the procedures and instructions described for oil drilling exploration and procedure (E and P) waste management in Nigeria. It is also worthy to state here that the formulation of the drill mud used in well 3 may have contradicted the claimed compliance to the ban of the use of oil-based mud by oil exploration companies Nigeria.

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