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Effects of Drilling Wastes on the Farmland Microbial Spectrum in Egbema, South-South Nigerian Community

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Abstract: The effects of drilling wastes on the farmland microbial spectrum in Egbema, a community in Niger Delta area of Nigeria was investigated. Bacterial species in 12 genera were isolated and include *Escherichia*, *Klebsiella*, *Pseudomonas*, *Xanthomonas*, *Alcaligenes*, *Bacillus* and *Staphylococcus*. Others are *Serratia*, *Flavobacterium*, *Micrococcus* and *Nitrobacter* species. All the organisms had their lowest prevalence nearest the waste pit (10 m), followed by 50 m away while there was no significant difference between the 100 m and control samples (p = 0.05) during the dry season. In rainy season, the prevalence rates were similar for 10 and 50 m but significantly different from 100 m and control points. The bioload of the various groups of organisms investigated followed the same pattern i.e., 10 m > 50 m > 100 m > control. While the least affected group was the sulphur reducing bacteria, the most affected were Nitrifying bacteria. Time and distance influenced soil pH as it increase with increase in distance (towards alkalinity) but decreased with time (toward acidity). The highest temperature was observed nearest the waste pit (10 m) and the lowest occurred further away (100 m) but increased with time. Change in pH and temperature indicated metabolism of the drilling wastes as observed in the screening of isolates for chrome-lignoso/fonate utilization. Turbidity, lowering of pH and increase in temperature were observed in chrome-lignoso/fonate utilizing bacterial species as seen in *Pseudomonas*, *Alcaligenes* and *Serratia* species. This was followed by *Micrococcus*, *Staphylococcus* and *Bacillus* species, while *Xanthomonas*, *Citrobacter*, *Klebsiella* and *Flavobacterium* species did not metabolize much of the substrate and showed no significant changes in the parameters mentioned. The drilling wastes were therefore concluded to have affected the soil microbial spectrum adversely but the affects waned very gradually with time and distance.

Key words: Drilling waste, bacteria, petroleum, soil, seasons

INTRODUCTION

Crude oil is located thousands of metres into the Earth crust and to have it brought to the surface for use requires extensive drilling operations (SPDC, 1998; NNPC, 1991). The drilling process therefore requires drilling fluid. A drilling fluid, which serves in the transportation of geopressure (Gray and Darley, 1979) must be carefully selected. Consequently the selection of a proper type is governed by the specific requirements of the geologic area in question and depends on the drilling-fluid's ability to perform the functions in the area (De Nevers, 1977; Gray and Darley, 1979; SPDC, 1998; Wills, 2000).

Modern drilling fluids regardless of whatever name is assigned to them usually contain clays, water soluble chemical (including salts), a pH control additive (hydroxyl source) and one or more organic polymer or hydrogel, surfactant and deflocculant (Ifeadi *et al*., 1985; Moore, 1974; Clark and Daniel, 1987). During routine drilling operations in the extraction of oil and gas, drilling mud and

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cuttings and infact other waste materials generated in the process must be properly disposed. The amount of wastes generated depends on hole size and days of operation. The type of disposal method is dependent on the ecological zone-land, swamp and off-shore. While land and swamp require burrows pits and containment, offshore requires seiving and see bottom disposal (Ifeadi *et al*., 1985; Carlton and Darby, 1979) at the well sites.

In Nigeria, information concerning the effects of these ill-treated and carelessly disposed drilling wastes remain very scanty. Much attention has been on the effects of petroleum pollution. This work therefore seeks to draw attention to the effects of the drilling wastes on farm lands, especially in Egbema.

MATERIALS AND METHODS

Egbema lies in the tropical rain forest zone of the Niger Delta area of Nigeria (NAOC, 1990). The people are mainly farmers and fishermen with a few engaged in oil production activities with the various oil companies operating in the area some of these drilling wastes are disposed in these farm lands causing pollution.

Recently, one of the oil companies operating in Egbema successfully executed the drilling of a well. The well was drilled using water-based drilling fluid classified as Cl-cls mud whose principal ingredients are fresh water, bentonite, caustic soda, chrome-lignosultate surfactant with some other additives. The ensuring wastes were pretentiously disposed in burrow pits but without the accompanying embankment for containment. This study was carried out between February 2005 to September 2006.

Sample Collection

Soil samples for analyses were asceptically collected 3-5 cm below the surface in sterile specimen bottles with screw caps. These were analyzed within 1-2 h of collection. Samples were collected from different distances from the waste pit - 10, 50 and 100 m away and then a control away from the effects of the wastes. Three samples were collected at each distance and pooled together as one for that distance.

Sampling was done 20 times, 10 for each climatic season (Dry and Rainy seasons) and cultured to obtain prevalence of each organism observed. Organisms observed were characterized and identified according to Cheesbrough (1987), Cowan and Steel (1976) and Buchanam and Gibbons (1976).

To obtain the bioload of various groups of bacterial species, the soil samples were cultured on different specialized (specific) media using the spread plate technique after serial dilution as described by Cowan and Steel (1976) and Chessbrough (1987). The bioload was them obtained in cfu g^{-1} of soil.

Chrome-lignosulfate utilization by the isolated organisms was investigated using the mineral salt medium of Mills *et al*. (1978) as modified by Okpokwosili and Okorie (1988). To 9.9 mL of the medium was added 0.1 mL of chrome lignosulfate at the concentration used at the drilling boreholes -800 ppm. The chrome-lignosulfate used in this work was a high grade one obtained from Baker and Hughes Nigeria Limited, Port-Harcourt.

RESULTS

Twelve bacterial species were isolated and characterized in this research. There were *Escherichia*, *Klebsiello*, *Pseudomonas*, *Xanthomonas*, *Alcaligenes* and *Bacillus* species. Others were *Staphylococcus*, *Citrobacter*, *Serratia*, *Flavobacterium*, *Micrococcus* and *Nitrobacter* species. The prevalence of these organisms according to distance from the burrow waste pit and season of study are shown in Table 1.

Table 1: Prevalence of Microorganism in the various soil samples examined

	Dry season											
	10 m			50 m			100 m			Control		
Organisms	Ns	Ni	%	Ns	Ni	%	Ns	Ni	%	Ns	Ni	%
Echericha sp.	10	3	30	10	5	50	10	7	70	10	8	80
Klebsiella sp.	10	2	20	10	3	30	10	5	50	10	5	50
Pseudomonas sp.	10	4	40	10	6	60	10	7	70	10	7	70
Xanthomonas sp.	10	-	-	10	2	20	10	3	30	10	3	30
Akaligenes	10	3	30	10	4	40	10	6	60	10	6	60
Bacillus sp.	10	5	50	10	7	70	10	8	80	10	10	100
Staphylococcus sp.	10	2	20	10	4	80	10	5	50	10	6	60
Citrobacter sp.	10	-	-	10	2	20	10	3	30	10	4	40
Serratia sp.	10	2	20	10	5	50	10	6	60	10	5	50
Flavobacterium sp.	10	3	30	10	5	50	10	6	60	10	6	60
Micrococcus sp.	10	2	20	10	4	40	10	5	50	10	6	60
Nitrobacter sp.	10	-	-	10	2	20	10	5	50	10	6	60
	Rainy season											
	10 m			50 m			100 m			Control		
Organisms	Ns	Ni	%	Ns	Ni	%	Ns	Ni	%	Ns	Ni	%
Echericha sp.	10	2	20	10	3	30	10	5	50	10	6	60
Klebsiella sp.	10	-	-	10	2	20	10	4	40	10	5	50
Pseudomonas sp.	10	2	20	10	3	30	10	6	60	10	6	60
Xanthomonas sp.	10	-	-	10	2	20	10	3	30	10	3	30
Akaligenes	10	3	30	10	3	30	10	4	40	10	4	40
Bacillus sp.	10	4	40	10	4	40	10	6	60	10	10	100
Staphylococcus sp.	10	2	20	10	2	20	10	4	40	10	5	50
Citrobacter sp.	10	-	-	10	-	-	10	2	20	10	2	20
Serratia sp.	10	2	20	10	2	20	10	4	40	10	5	50
Flavobacterium sp.	10	-	-	10	2	20	10	4	40	10	5	50
Micrococcus sp.	10	3	30	10	3	30	10	5	50	10	5	50
Nitrobacter sp.	10	-	-	10	3	30	10	5	50	10	5	50

Ns: No. of samples, Ni: No. of isolates, %: Percentage occurrence

All the organisms had their lowest prevalence very close to the waste pit (10 m), followed by the 50 m distance. The difference between the control and 100 m distance was not significant. Correlation analysis show a significant effect of distance effect of distance from the source of pollutant (waste pit) in the dry season.

Table 1 also showed much similarity in the prevalence of organisms between 10 and 50 m from the pit, just like 100 m and control during the rainy season. While the dry season showed three zones, rainy season showed only two (Table 1).

Table 2 shows that all the groups of bacteria examined had their lowest bioload near the pit (10 m) during dry season, followed by 50 m samples. The difference between 100 and control was not significant ($p = 0.05$). Correlation analysis showed a significant increase in bioload with distance away from the waste pit (Table 2). The most affected bacterial species were the nitrifying bacteria (2.1×10^2 in 10 m to 3.7×10^7 in 100 m); followed by the coliform bacteria (2.3×10^4 - 8.1×10^4). The least affected was the sulfur reducing bacteria (Table 2) in the dry season.

Rainy season results showed a similar gradient in bioload. The data obtained for 10 and 50 m samples were quite similar and different from those of 100 m and control samples which were equally similar. This showed two major columns -10 and 50 m on one hand and control with 100 m on the other. The most affected bacterial group examined was the NB, followed by the CB (Table 2).

Table 2: Bioload of the various groups of bacterial species examined in the soil of the study area according to distance from the drilling waste pit

	Distance from the drilling waste pit			
Group of bacterial	10 m	50 m	100 m	Control
Dry season				
HUB	2.4×10^4	4.4×10^5	5.7×10^5	4.6×10^5
SRB	1.8×10^4	4.7×10^4	5.3×10^4	5.1×10^4
CB	2.3×10^4	4.1×10^4	6.1×10^4	3.3×10^5
NB	4.1×10^2	3.9×10^3	3.7×10^4	7.3×10^4
HB	2.8×10^5	4.3×10^5	2.7×10^6	3.1×10^6
Rainy season				
HUB	2.8×10^4	3.2×10^4	4.9×10^5	4.5×10^5
SRB	2.1×10^4	3.1×10^4	5.7×10^4	5.3×10^4
CB	2.5×10^4	3.2×10^4	5.8×10^4	3.6×10^5
NB	4.3×10^2	2.7×10^3	4.0×10^4	7.4×10^4
HB	3.0×10^5	3.8×10^5	3.0×10^6	3.2×10^6

Table 3: Changes in soil pH and temperature of the study site according to distance from waste pit

Condition		1st sampling	2nd sampling	3rd sampling	4th sampling
pH ranges					
	Control	7.32	7.14	7.23	7.36
Dry	100 m	7.34	7.29	7.31	7.32
Season	50 m	7.13	7.01	0.86	6.80
	10 m	6.91	6.87	6.81	0.00
Rainy	Control	7.33	7.31	7.40	7.33
Season	100 m	7.31	7.26	7.30	7.28
	50 m	7.03	6.89	6.92	6.81
	10 m	6.90	6.87	6.87	6.78
Temperature (°C)					
	Control	28.60	29.80	30.10	29.80
Dry	100 m	28.80	29.80	30.10	29.60
Season	50 m	29.10	29.80	30.80	30.30
	10 m	29.30	30.40	31.10	31.00
Rainy	Control	28.60	28.80	28.40	28.20
Season	100 m	28.90	28.90	29.00	29.00
	50 m	29.20	29.60	30.10	30.20
	10 m	29.90	30.70	30.90	31.20

Analysis of soil pH showed that pH increased slightly distances away from the waste pit but gradually decreased with time during both rainy and dry seasons. Ten meter soil samples gave 6.91, 50 m gave 7.13 while 100 m gave 7.34 and control 7.32 in first sampling. Three weeks later (2nd sampling) it gave 6.87 (10 m), 7.01 (50 m), 7.29 (100 m) and 7.41 (control). The patterned remained so in the 3rd and 4th sampling of 3 weeks intervals. Two way ANOVA, with time and distance as variables ($p = 0.05$) showed a significant influence of both variables. However, change towards acidity (decrease in pH) was much more gradual in the 10 m distance. A similar observation was noticed during the two seasons.

In temperature examination carried out, highest temperature was at 10 m spot in both seasons, followed by 50 m, the 100 m and control areas had similar temperature in the rainy season. First sampling gave values of 29.3°C (10 m), 29.1 (50 m) 28.8°C (100 m) and control, 28.6°C) for rainy season. This changed to 29.9°C (10 m), 29.2°C (50 m), 28.9°C (100 m) and 28.9°C (control) in dry season. The pattern remained similar in the 3rd and 4th sampling. This showed higher temperature with time and lower temperature distance away from the waste pit (Table 3). A similar observation was made during the dry season, though with slightly higher temperatures in all the sampling points (Table 3).

Screening of the isolates for chrome lignosulfate utilization showed very high growth in tubes containing *Pseudomonas*, *Alcaligenes* and *Serratia* species. This was followed by *Escherichia*, *Bacillus*, *Staphylococcus* and *Micrococcus* species. *Xanthomonas*, *Klebsiella*, *Citrobacter* and *Flavobacterium* species showed very scanty growth (Table 4).

Table 4: Utilization of Chrome-lignosofonate by the 180 isolated organisms pH changes

Organisms	Turbidity	Initial pH	Final
Eschericha sp.	+ +	7.80	6.78
Klebsiella sp.	+	7.80	6.62
Pseudomonas sp.	+ + +	7.80	0.09
Xanthomonas sp.	+	7.80	7.11
Alcaligenes sp.	+ + +	7.80	6.19
Bacillus sp.	+ +	7.80	6.45
Staphylococcus sp.	+ +	7.80	6.40
Citrobacter sp.	+	7.80	7.10
Serratia sp.	+ + +	7.80	6.17
Flavobacterium sp	+	7.80	6.80
Micrococcus sp.	+ +	7.80	6.32

+: Scanty growth; ++: Moderate growth; +++: High growth

A marked (significant) change in pH was observed in tubes with +++ growth from 7.8 to between 6.09-6.19. Tubes with ++ gave pH values of 6.34-6.78 while 6.90-7.11 was observed for tubes with +. Only tubes containing *Nitrobacter* species showed no growth and no change in pH. Statistical analysis showed a correlation between growth and pH change as more growth meant greater change in pH towards acidity (i.e., decrease in pH values).

DISCUSSION

In this analysis twelve bacterial species were observed. This could be expected as the soil has been described as home to many organisms (Prescot *et al*., 2002; Pelczar *et al*., 1993). However, all the organisms did not occur at the same rate which indicated some stress effects of the drilling wastes. Observation showed that *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Serratia*, *Alcaligenes* and *Micrococcus* species were more prevalent than *Xanthomonas*, *Klebsiella*, *Citrobacter* and *Nitribacter* species. This observation tallies with some other reports linking *Pseudomonas*, *Serratia*, *Alcaligenes* and *Micrucuccus* species as good degraders of Hydrocarbon including drilling fluid and lubricating oil (Okpokwasili and Okorie, 1988; Hill and Gennor, 1980; Atlas, 1981; Benker-Coker and Olumagan, 1976). It was also observed that gram positive organisms were more prevalent in the drilling wastes impacted soil which agrees with Nnubia and Okpokwasili (1993) and Okpokwasili and Okorie (1988). However, this slightly differs from Enechukwu and Okpokwasili (2000) who reported that *Xanthomonas* species showed high degradative index using experimentally contaminated soil with HBF 2000 fluid oil. Enechukwu and Okpokwasili (2000) also reported the presence of *Cryptococus* and *Saccharonyces* species which were absent in this study. This could be attributed to locality and type of drilling fluids involved as they worked on HBF 2000 drilling oil.

The bioload of the various bacterial groups examined showed that the effects of the drilling wastes waned off as the distance from the waste pit increased as more organisms were observed in this regard. The least bioloads of the various groups of organisms were reported nearest the pit. The result agrees with Nwaugo *et al*. (2006b) involving the effects of petroleum produced water which increased in the same line. This could be attributed to the concentration of the wastes. Chinyere (2001) working on cassava Gyanide reported that wastes concentrations are highest nearest the source. Similar assertion had been made by Nwaugo *et al*. (2004), Benker-Coker and Olumagan (1976) and Nwaugo *et al*. (2003).

The most effected organisms were the nitifying bacterial species. Nwaugo *et al*. (2004, 2005) had earlier reported similar observation involving calcium carbide waste and Petroleum produced water. The some researchers stated that HUB and THB (Hydrocarbon utilizing bacteria and total heterotrophic bacteria) were the least affected in their study just as was observed in this research. The HUB could utilize the drilling fluid and waste contents as nutrients for growth. Organisms not capable of doing this will be adversely affected. However, this work differs from Nwaugo *et al*. (2006a) as SRB (Sulphur Reducing bacteria) observed in this work remained at a considerable high level. Again, this is attributable to the high sulfur content of the drilling fluid which contained sulfonate as an ingredient.

The sulfonate acted as a stimulant or nutrient to the sulfur reducing bacteria. The THB (could not be affected much as it is the sum total of all heteretrophic bacteria available in each sample.

The observation of slight pH and temperatures changes with time and distance could be attributed to the concentration and utilization of the drilling wastes. pH decreased gradually from control (7.36-7.50) to the waste pit (6.9-6.77) indicating that the waste was acidic or caused the production of acidic compounds which modified the soil pH to slightly acidic. On the other hand the pH decreased with time of sampling which indicated the action of micro-organisms on the waste. With time. Several researchers have stated that metabolism of Hydrocarbons often result in the production of organic acid intermediates (Okpokwaisli and Okorie, 1988; Obiukwu and Abu, 2003; Nwaugo *et al*., 2003, 2004). The various organisms observed in the soil might have degraded the drilling wastes to produce acidic intermediates which decreased the soil pH level to slightly acidic range.

Analysis of the data in this work clearly indicated seasonal influence. Higher bioloads and prevalence of the organisms was recorded during the rainy season than the dry season in all the distance examined. Nwaugo *et al*. (2004, 2006a) had attributed this to dilution effects. The rain water (run-offs) diluted the harsh ecological conditions in the dry season to a more conducive one in rainy season. In addition, organisms from other sources could equally have been brought in by the rain run-off water. The rains could equally have dispersed or spread the harsh dry season condition to other areas thereby reducing the boarden on the soil. This could be true as shown in the data obtained from the 10 and 50 m distance away from the waste pit in the rainy season showed. In bioload, organisms prevalence and even pH and temperature factors analyzed, there was no significant difference between 10 and 50 m distances during the rainy season but significance existed in the dry season. This observation agrees also with Nwachukwu and Otokunefor (2003) and Nwaugo *et al*. (2003).

The results obtained from the screening of isolates for chromelignosulfonate utilization buttressed some earlier results from the field. Those organisms which utilized much of the chrome-lignosulfonate for growth were more prevalent in the impacted soil. Those that could not utilize the compound showed low prevalence in the field. Similarly organisms which showed high pH changes in the screening were those most prevalent in the field and showed high growth too. This mean that change in pH was synonymous with bacterial growth (turbidity). This shows that without utilization of the chrome-lignosulfonate, there will be no change in pH which agrees with Okpokwasiri and Okorie (1988).

In conclusion therefore, the drilling wastes adversely affected soil microbial spectrum and caused pH modification. This calls for more stringent regulations and their enforcement if the enviroemnt most be protected. However, the results indicated self-remediation in cases of disposal in small quantities and over 100 m away from agricultural lands.

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