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Environmental Evaluation of the Drill Cuttings at Ologbo Oilfield Wells, Edo State, Nigeria: A Case Study of its Microbiological and Heavy Metals Composition

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Abstract
The environmental evaluation with attention on the microbiological and heavy metals composition was carried out on drill cuttings from four different oil and gas wells located at Ologbo community in Edo State. Heterotrophic bacterial and fungal counts were enumerated using the standard plate count techniques. Heavy metals, oil and grease, pH and electrical conductivity were analyzed using standard analytical procedures. The heterotrophic microbial counts ranged from 7.9x102 ±1.0 cfu/g at 0-610 m to nil at 2745-3660 m for aerobic heterotrophic bacterial counts and 3.7x102 ±0.01 at 0-610 m to nil at 2745-3660 m for the fungal counts. The highest count of 5.2x103 ±1.5cfu/g was recorded for anaerobic heterotrophic bacterial counts. Gram positive bacteria had the highest percentage occurrence among the bacterial isolates. Among the fungal isolates Penicillium spp. and Aspergillus spp. were recorded to have the highest percentage frequency of occurrence. Results of heavy metals showed that copper had the least concentration while iron had the highest concentration. Oil and grease, pH, conductivity showed relative increase along the depth profile. Statistical analysis showed significant differences (P<0.05) for microbial counts and heavy metals. Based on the findings of this study, oil exploration and production companies operating in Ologbo community in Edo State, Nigeria are encouraged to adhere strictly to the procedures and instructions of waste management, so as to avoid unintended environmental pollution.
Keywords: Drill cutting, microbial, physico-chemical, heavy metals

Introduction
The mixture of rocks and particulates released from geological formulations in the drill holes made for crude oil drilling, that are usually coated with the drilling fluid are described as drill cuttings[1]. These cuttings have been found to be largely influenced by the chemical composition of drilling muds [2, 3]. The resultant spent oil based mud and drill cuttings (drilling wastes) consist of hydrocarbons, water, soils, heavy metals and water soluble salts such as chlorides and sulphates [4, 2]. Drilling wastes, which are toxic due to the presence of hydrocarbons, heavy metals and other chemical additives, if not properly treated before disposal, pose serious environmental hazards and risk to public health [5].
Heavy metals of the onshore drill cuttings have been known to be capable of polluting the environment with the resultant effects on human health [2]. 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. It is 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 [1, 6]. Increasing exploration and production (E & P) activities, coupled with improper waste disposal practices have encouraged widespread contamination of both the aquatic and terrestrial ecological systems in this area of E & P activities. Contaminants, such as used drilling mud's and drill cuttings are serious threats to the biota of natural ecosystems, especially at the location of the borehole [7]. In line with the fore-going, this work was designed to evaluate the effect of drill cuttings from Ologbo oil field wells with attention on the microbiological and heavy metal composition; as well as the possible depth variations among the resultant drill cuttings.

Materials and Method
Sources and collection of Samples: The drill cutting samples were collected in sterile clean plastic containers during drilling processes of the onshore wells at Ologbo community in Edo State for microbiological and

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physicochemical analyses. The samples 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 (well 2), E: 350017.979 m, N: 229465.442 m (well 3) and E: 350017.981m,N:229460.400m (well 4).

Isolation and enumeration of microorganisms:

Heterotrophic bacterial and fungal were enumerated using the standard plate count techniques [8]. Appropriate dilutions of samples were plated out in duplicates on nutrient agar and potato dextrose agar (incorporated with 2 µg/l of chloramphenicol) for bacterial and fungal isolates respectively. A set of nutrient agar plates were incubated at 30^0C for 48 hours (aerobic bacteria) while the other sets were incubated in an anaerobic jar with an oxygen removing system (oxoid gas pack) to create an anaerobic environment. The plates for fungal isolation were incubated at room temperature for 5 - 7 days. The means of triplicate 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 enumerated and isolated using mineral salt medium (MSM) [9]. The minerals salt medium had in litre, $MgS0_4.7H_20$, 0.42 g/l, KCl, 0.30 g/l, KH_2PO_4, 0.8 g/l, K_2HPO_2, 1.3 g/l, $NaNO_3$, 0.42 g/l, pH 7.4 and Agar 15 g/l. One mililitre (1 ml) of drilling mud was added. The triplicate plates were incubated at 29 ^{o}C 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 [10, 11].

Heavy metals:

Two grams (2 g) drill cuttings were initially digested with concentrated nitric acid and filtered. The resultant filtrates were analyzed for the heavy metals content using the Atomic Absorption Spectrophotometer (AAS) (Buck Scientific Model 210 VGP). The heavy metals analyzed were iron, manganese, copper, lead, nickel and zinc.

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. Absorbance was read at 420 nm to determine the concentration of oil and grease. Value in mg/kg was calculated as below:

$$\textit{Oil and grease (mg/kg)} = \frac{\textit{Instrumental Reading X Vol. X } 10^3}{\textit{Wt. of sample X } 10^4}$$

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 triplicate 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).

Statistical analysis:

The data obtained were subjected to descriptive statistical analysis such as mean, standard deviation and analysis of variance [12].

Results

The results for the microbiological study of drill cuttings from different oil and gas wells are shown in Tables 1(A – C) with the frequencies of bacterial and fungal isolates being shown in Figures1 (A –B).

TABLE 1A: Mean total heterotrophic bacterial counts (cfu/g) of drill cuttings from drilling wells.

	WELL 1	WELL 2	WELL 3	WELL 4
0 - 610 m	$7.23x10^2 \pm 1.0$	$5.4x10^2 \pm 0.1$	$7.9x10^2 \pm 0.02$	$7.0x10^2 \pm 0.01$
915-1525 m	$3.0x10 \pm 0.1$	$3.3x10 \pm 0.03$	NG	NG
1830-2440 m	NG	NG	NG	NG
2745-3660 m	NG	NG	NG	NG

Overall mean value ± standard deviation, NG: No Growth.

TABLE 1B: Mean total heterotrophic fungal counts (cfu/g) of drill cuttings from drilling wells**.**

	WELL 1	WELL 2	WELL 3	WELL 3
0-305m	$3.0x10^2$ ±0.1	$3.7x10^2$ ±0.01	2.5x10 ±1.02	$2.1x10^5$ ±0.5
915 – 1525 m	8.0 x10 ± 0.01	3.0x10 ±0.1	NG	NG
1830 -2440 m	NG	NG	NG	NG
2745-3660 m	NG	NG	NG	NG

Overall mean value ± standard deviation, NG: No Growth.

TABLE 1C: Mean total heterotrophic anaerobic bacterial counts (cfu/g) of drill cuttings from drilling wells**.**

	WELL 1	WELL 2	WELL 3	WELL 4
0-305 m	$4.7x10^2$ ±0.03	$3.5x10^2$ ±0.05	$4.8x10^2$ ±1.0	$0.8x10^2$ ±0.1
915-1525 m	$5.2x10^3$ ±1.5	$3.1x10^3$ ±0.1	$4.0x10^3$ ±0.5	5.1x10 ±0.2
1830-2440 m	4.37x10 ±0.1	4.0x10 ±2.0	3.2x10 ±1.0	NG
2745-3660 m	NG	NG	NG	NG

Overall mean value ± standard deviation, NG: No Growth.

Figures 2 (A – F) shows the results of the heavy metals evaluation of the drill cuttings. The results revealed high counts of micro-organisms among the drill cuttings from the different wells. Aerobic heterotrophic bacterial counts (cfu/g) and fungal counts (cfu/g) were observed to range from $7.9x10^2$ ±1.0 cfu/g at 0 - 610 m to nil at 2745 - 3660 m for aerobic heterotrophic bacterial counts and $3.7x10^2$ ±0.01 at 0-610 m to nil at 2745 - 3660 m for fungal counts. While the highest count of $5.2x10^3$ ±1.5 cfu/g was observed for anaerobic heterotrophic bacterial counts at 915 – 1525 m. *Micrococcus* spp., *Staphylococcus* spp., *Bacillus* spp., *Desulfotomaculum* spp. and *Mycobacterium* spp. had the highest percentage occurrence among the bacterial isolates (Fig 1A). The fungal isolates were observed to be dominated by *Penicillium* spp. and *Aspergillus* spp. (Fig 1B).

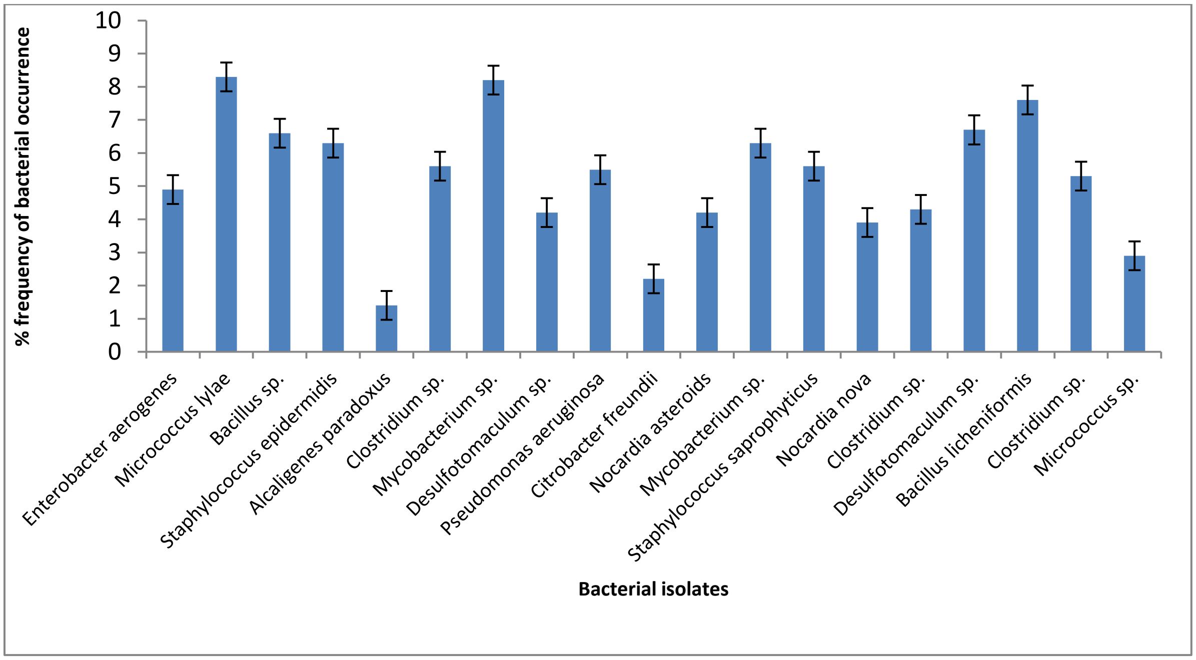

Figure 1: Percentage frequency of occurrence of bacterial isolates from these wells

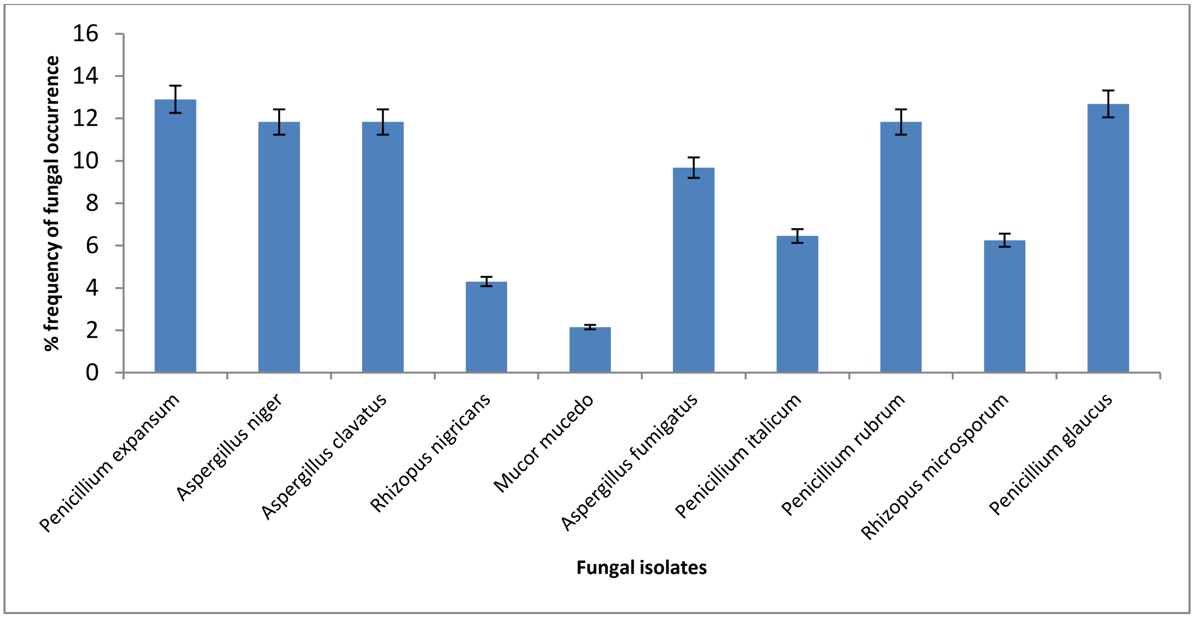

Figure 2: Percentage frequency of occurrence of fungal isolates from these wells

Findings from heavy metals analysis (Figs 2A - F) showed that there were variations in heavy metals composition among the drill cuttings with reference to the depth profile and the wells. Iron was observed to have the highest concentration with a range of 62 - 264 ppm while copper had the least concentration with a range of 0 - 0.06 ppm.

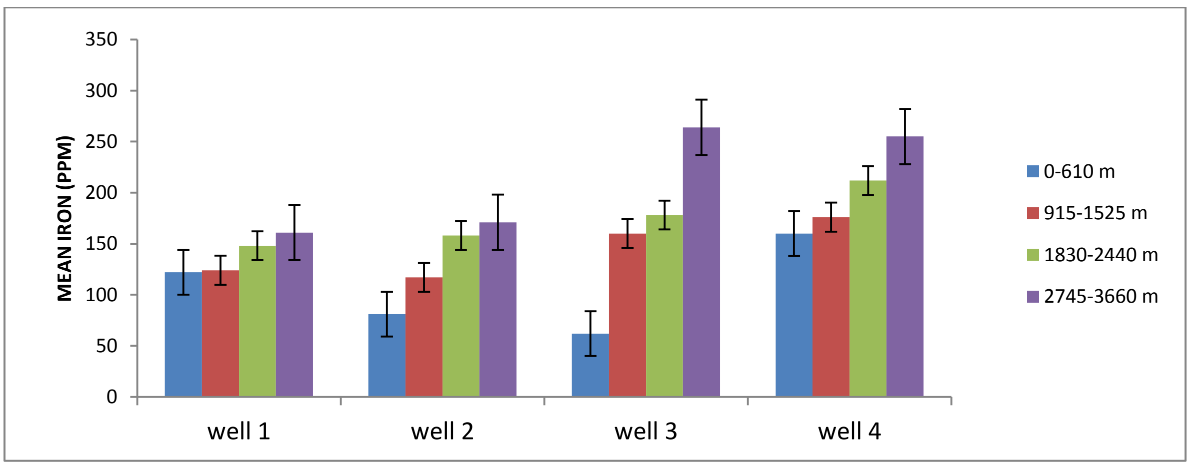

Figure 2 a: Mean iron (ppm) values of drill cuttings from drilling wells.

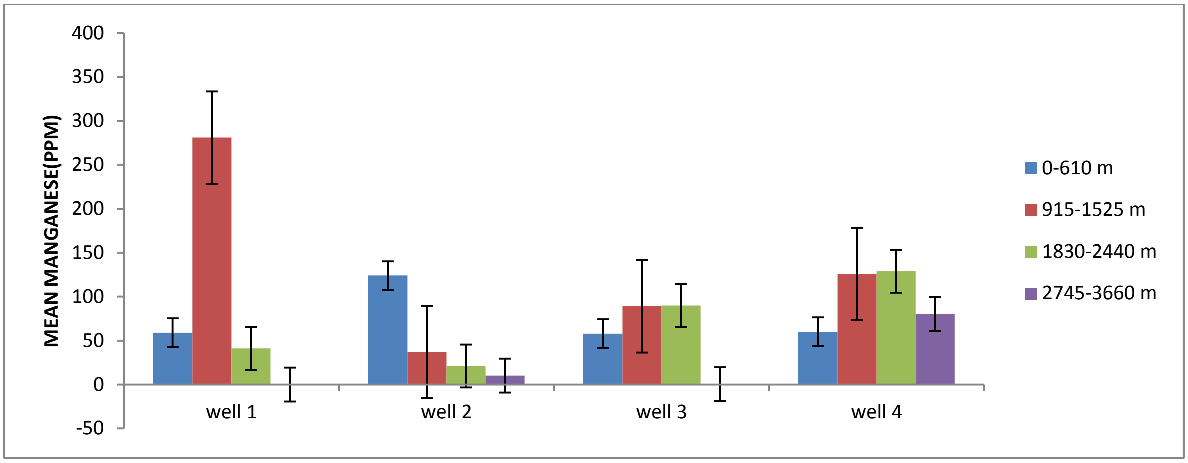

Figure 2 b: Mean manganese (ppm) values of drill cuttings from drilling wells.

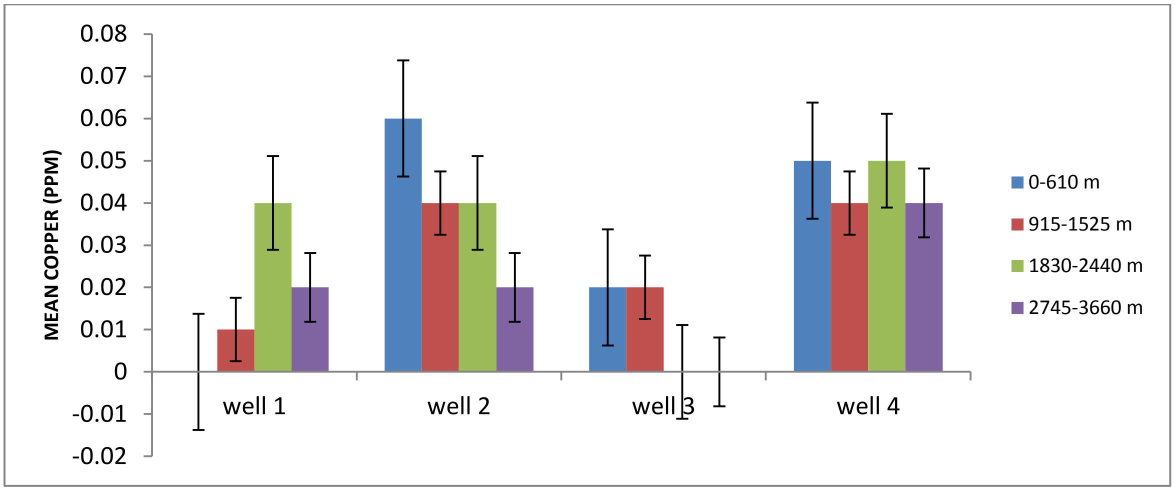

Figure 2 c: Mean copper (ppm) values of drill cuttings from drilling wells.

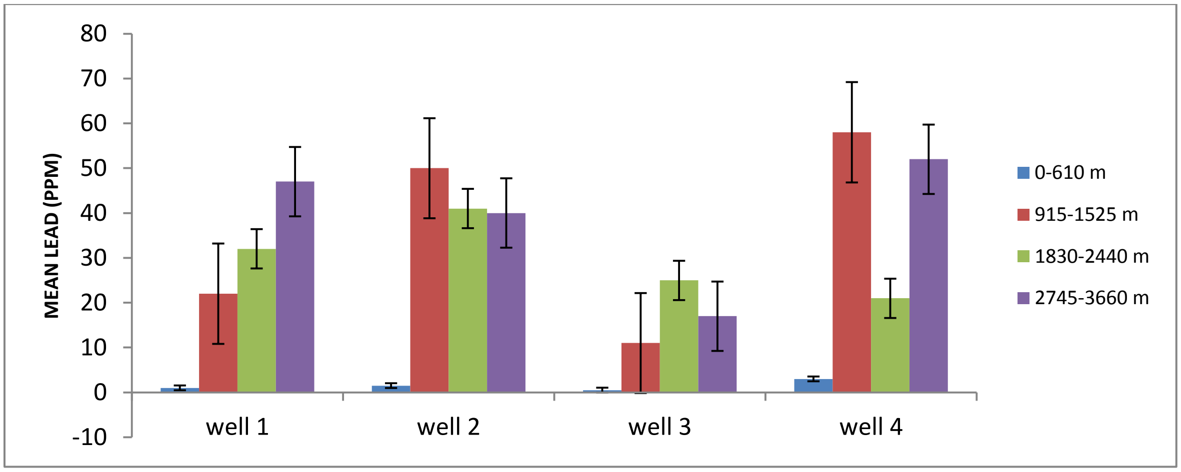

Figure 2 d: Mean lead (ppm) values of drill cuttings from drilling wells.

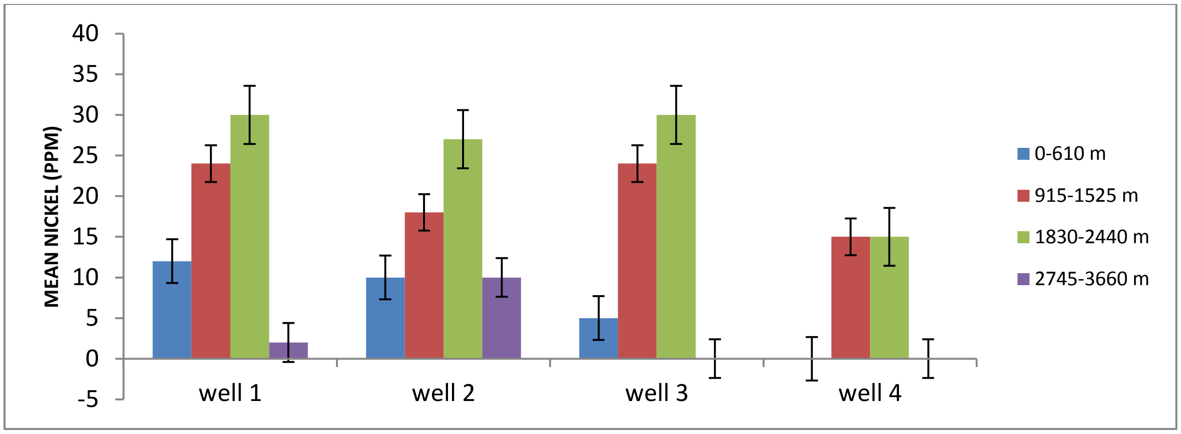

Figure 2 e: Mean nickel (ppm) values of drill cuttings from drilling wells.

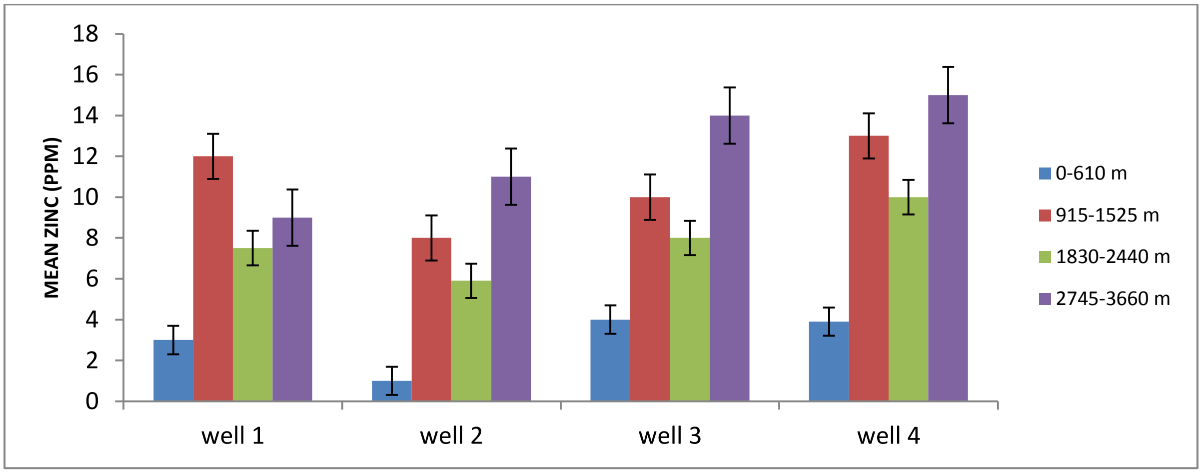

Figure 2 f: Mean zinc (ppm) values of drill cuttings from drilling wells.

Results for other physicochemical parameters generally revealed that cuttings from these oil and gas wells were heavily coated with oil and grease, with concentration ranging from 15.67 to 23243.66 mg/kg (Table 2). The pH ranged from 4.21 to 6.83, while electrical conductivity measurement was recorded to range 246 - 564 µS/cm.

Table 2: Mean oil and grease (mg/kg), pH, electrical conductivity (µs/cm) values of drill cuttings from drilling wells.

	0- 610 m			915 - 1525 m			1830 - 2440 m			2745 - 3660 m		
	Oil& grease	pH	Electrical conductivity	Oil& grease	pH	Electrical conductivity	Oil& grease	pH	Electrical conductivity	Oil& grease	pH	Electrical conductivity
Well 1	43.10	5.34	252	125.2	5.11	397	5121.39	5.12	401	12243.56	4.21	564
Well 2	15.67	5.98	256	105.02	5.47	300	6021.39	5.00	383	23243.66	4.41	552
Well 3	40.00	5.47	246	175.04	5.22	301	6287.52	5.14	388	14158.61	4.64	498
Well 4	48.00	6.83	246	93.22	6.50	301	3021.39	6.22	377	11241.76	4.45	563

Overall mean value

Discussion

The assessment of microbial density of the different wells revealed high aerobic heterotrophic bacterial and fungal isolates especially on surface drill cuttings ($7.9 \times 10^2 \pm 1.0$ cfu/g at 0 - 610 m for bacterial counts and $3.7 \times 10^2 \pm 0.01$ at 0 - 610 m for fungal counts). However, these results were contrary to the anaerobic heterotrophic bacterial counts, where the highest count was recorded at lower depths $5.2 \times 10^3 \pm 1.5$cfu/g). The predominance of Gram positive bacteria such as *Clostridium* spp., *Mycobacterium* spp. and *Bacillus* spp over Gram negative bacteria as observed in this study may be due to the high concentration of peptidoglycan in the cell wall of Gram-positive bacteria. High concentration of peptidoglycan layer had been reported to confer resistance to environmental stress [9, 13]. Results from this study showed species of *Aspergillus* and *Penicillium* to have the highest frequency of occurrence among the fungal isolates. The spore forming ability of the fungal and bacterial isolates 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 [14, 13]. The observed variations can be anchored on factors which may include available nutrients, oxygen availability and depletion, the chemical composition of the drill cuttings with reference to the drill mud used and other physico-chemical factors of the drilling formation [3, 1,]. According to [15, 16], microbes in the deep horizons can also be influence by the original environmental deposits which have persisted with in the geological formation for extended periods of time.

From the distribution profiles of the heavy metal contents of the drilling cuttings, it was observed that cuttings contain appreciable contents of toxic heavy metals. The results generally showed high heavy metals content in the drill cuttings. From this investigation, there were variations in concentration levels among the heavy metals that were analyzed. The levels of Iron (0 – 3660 m), nickel (915 – 2440 m) and zinc(0 – 3660 m) were observed to increase with depths among wells, contrary to the findings in manganese, copper and lead that had varied concentrations along the depths of the drilled wells. The deeper horizons that were observed for Iron, nickel and zinc and the varied levels of manganese, copper and lead may have been derived from the original deposits of the environment or due to type of drilling muds that were employed by the drilling company [15, 16].

The presence of heavy metals in the aquatic ecosystem has been observed to be of far-reaching implications to the biota and man [2]. The observed values were high when compared with the established environmental standards [22]. The continuous entry of these metals into the surrounding environment can result in serious contamination. [17, 18, 2] had earlier reported high concentrations of heavy metals in drill cutting samples. Heavy metals such as lead, zinc, copper etc have been incriminated with the potentials to accumulate in soil and plants. According to [19],excess heavy metal accumulation in soils and water bodies are toxic to humans and other animals; exposure to heavy metals is normally chronic (exposure over a longer period of time), due to food chain transfer. Acute (immediate) poisoning from heavy metals is rare through ingestion or dermal contact. Chronic problems associated with long-term heavy metal exposures include mental lapse, organ failures (kidney, liver and gastrointestinal tract) and skin poisoning [19]. However, some heavy metal ions may play important roles as “trace elements” in sophisticated biochemical reactions, other metals (e.g., silver, aluminium, cadmium, gold, lead and mercury), have no biological role and are nonessential and potentially toxic to microorganisms [20]. At higher concentrations these heavy metal ions form unspecific complex compounds within the cell, which leads to toxic effects, making them too dangerous for any physiological function. These metals may inhibit pollutant biodegradation through the interaction with microbial enzymes that may be directly involved in pollutant biodegradation or those involved in general metabolism [20]. Hence, preventing heavy metal pollution is critical, considering the fact that cleaning contaminated environment is extremely expensive and difficult. Also, Prevention is the best method to protect the environment from contamination by heavy metals [19].

The pH values in this study revealed the differentiation in the characteristic and properties of the drill cutting of oil and gas wells. The evaluation for the oil and grease concentrations on the samples revealed that drill cutting wastes at the different sites of this study were coated with high amount of oil and grease that were above established threshold levels (residual oil content less than 10g/kg, that is, one per cent of oil-on-cuttings) before discharge [6].This is however, not out of place owing to the fact that cuttings are pieces of formation rock produced by the drill bit that are returned up the annulus of the hole suspended in the drill fluid [21]. Drill cuttings have been found to be heavily laden with oil and grease, especially when non-aqueous based mud is used (Table 2). At high concentrations, it could seriously threaten the life of edaphic systems as well as people who depend on local ground-water, if it precipitates through the soil and also as run-off into water bodies. It has been asserted that electrical conductivity has also been regarded as one of the major concerns in the disposal of drill cuttings [2]. The high electrical conductivity was an indication of free ions of oil and grease laden drill cuttings. Statistical analysis revealed no significant differences ($P > 0.05$) for heavy metal concentration among the drill cuttings from the investigated wells.

Considering these observations therefore, oil and gas companies operating in Ologbo community in Edo State, should be encouraged to adhere strictly to the procedures and instructions of exploration and production (E and P) waste management in Nigeria (6) and globally. This will ensure that the environment is not negatively degraded in the course of their operations and also to avoid untended environmental pollution.

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