Physicochemical and Microbiological properties of soil in Ahoko: A Suspected Petroleum Bearing Site (SPBS)

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Abstract: Microbial surface prospecting is a vital process in the exploration of hydrocarbon suspected site, as a result the aim of this study was to assess the microbial and physicochemical properties of soil in a suspected petroleum bearing site at Ahoko, Kogi State, Nigeria, to ascertain the potent site for exploration using the presence of microbial anomalies. The four identified sites; A, B, C and D were mapped and gridded. Soil samples were collected from each of the sites and analysed for physicochemical properties, culturable heterotrophic aerobic bacteria, total fungi and distribution of specific hydrocarbon oxidizing microbes. Physicochemical properties showed that pH of the sites were within the range of 5.30 to 7.05. Site A had the highest total organic carbon (1.18%), conductivity (12.0 ± 3.50 µ∫/cm), available Phosphorus (12.06 ± 0.00 mg/kg), base saturation (66.03 ± 0.00%) and exchangeable acid (2.28 ± 0.58 cmol/kg) compared to other sites. The aerobic heterotrophic bacteria in the soil varied with respect to the month of sampling/site at the range of 0.38 x 10^6 CFU/g to 2.88 x 10^6 CFU/g. Total number of culturable fungi ranged from 1.27 x 10^3 CFU/g to 11.0 x 10^3 CFU/g. Site A had the highest number of methane and ethane oxidizing microbes. Site C showed the highest number of propane oxidizing microbes of 1.15 x 10^2 CFU/g while C had the least, 0.04 x 10^2 CFU/g. There was little or no growth of butane oxidizing microbes in all the sites. The study revealed the presence of high concentrations of hydrocarbon oxidizing bacteria in Ahoko area of Bida basin. Site A had higher population of ethane oxidizing bacteria than the rest of the sites and therefore, it could be a possible site for hydrocarbon in Bide basin.

KEYWORDS: Petroleum, Soil, Ahoko, Total count, Sites, Microbes

1.0 Introduction

Bida basin has been reported as one of the suspected petroleum bearing sites in Niger State, Nigeria, this Basin is, located in the North Central Region (Figure 1). According to Ladipo (1998) the Basins' sedimentary fill comprises post-orogenic molasses and thin unfolded marine sediments. The basin is a gently down-warped trough whose origin is closely connected with Santonian orogenic movements in South East Nigeria and the Benue valley. The basin trends perpendicular to the main axis of the Benue Trough and the Niger Delta Basin and is regarded as the North West extension of the Anambra Basin, both of which were major depocentres during the third major transgressive cycle in the Late Cretaceous (Obaje *et al.,* 2011). The areas surrounding Bida and South of Bida towards Pategi, Muregi, Baro, Agbaja, Ahoko, Abaji, GadaBiyu are the most prospective for hydrocarbon exploratory drilling campaigns (Obaje *et al.,* 2011; Usman, 2019)

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Petroleum forms by the breaking down of large molecules of fats, oils and waxes that contributed to the formation of kerogen (Mansoori *et al.*, 2016) and it is mostly been recovered by oil drilling which is carried out after studies of structural geology (at the reservoir scale), sedimentary basin analysis, and reservoir characterization have been completed (Guerriero *et al.*, 2012) and one of the methods used for hydrocarbon exploration, characterization and identification of microorganisms from hydrocarbon suspected site is commonly known as microbial prospecting and it is based on the fact that, gaseous hydrocarbons through effusion and diffusion migrate upward from subsurface petroleum accumulations, and are utilized by a variety of microorganisms present in the sub-soil ecosystem (Etiope, 2015; Hubert and Judd, 2020). The methane, ethane, propane, and butane-oxidizing bacteria exclusively use these gases as carbon source for their metabolic activities and growth. These microorganisms are mostly found enriched in the shallow soils and can differentiate between hydrocarbon prospective and non-prospective areas (Rasheed *et al.*, 2014; Laso-Perez *et al.*, 2019). With the increase in demand for petroleum products and diminishing indigenous production, it has become necessary to look out for probable potential zones with the aim of expanding the national exploration and production base and adding to the proven reserves. There is therefore the need for microbiological studies to prove that this Basin indeed can be explored for petroleum. Hence this study was directed to identify the presence of hydrocarbon gas oxidisers and anomalies as indicators to potent sites for exploration.

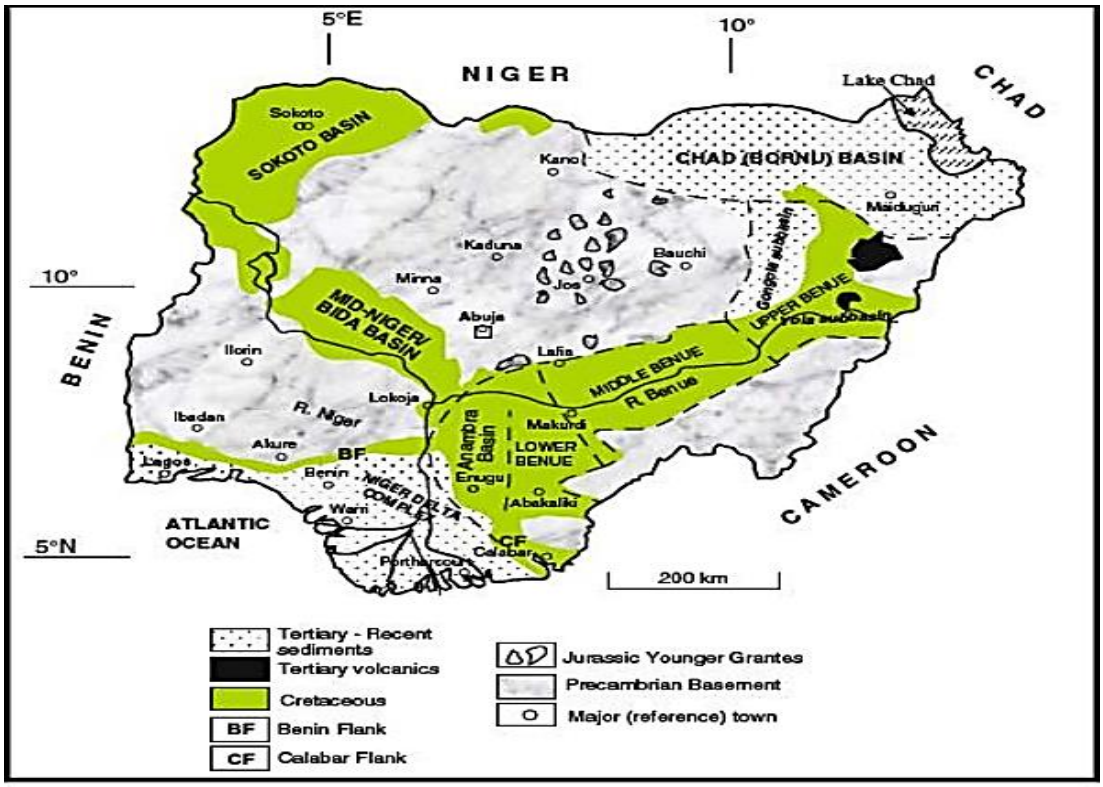

Figure 1: Geological map of Nigeria showing the position of Bida Basin (Obaje *et al.*, 2011)

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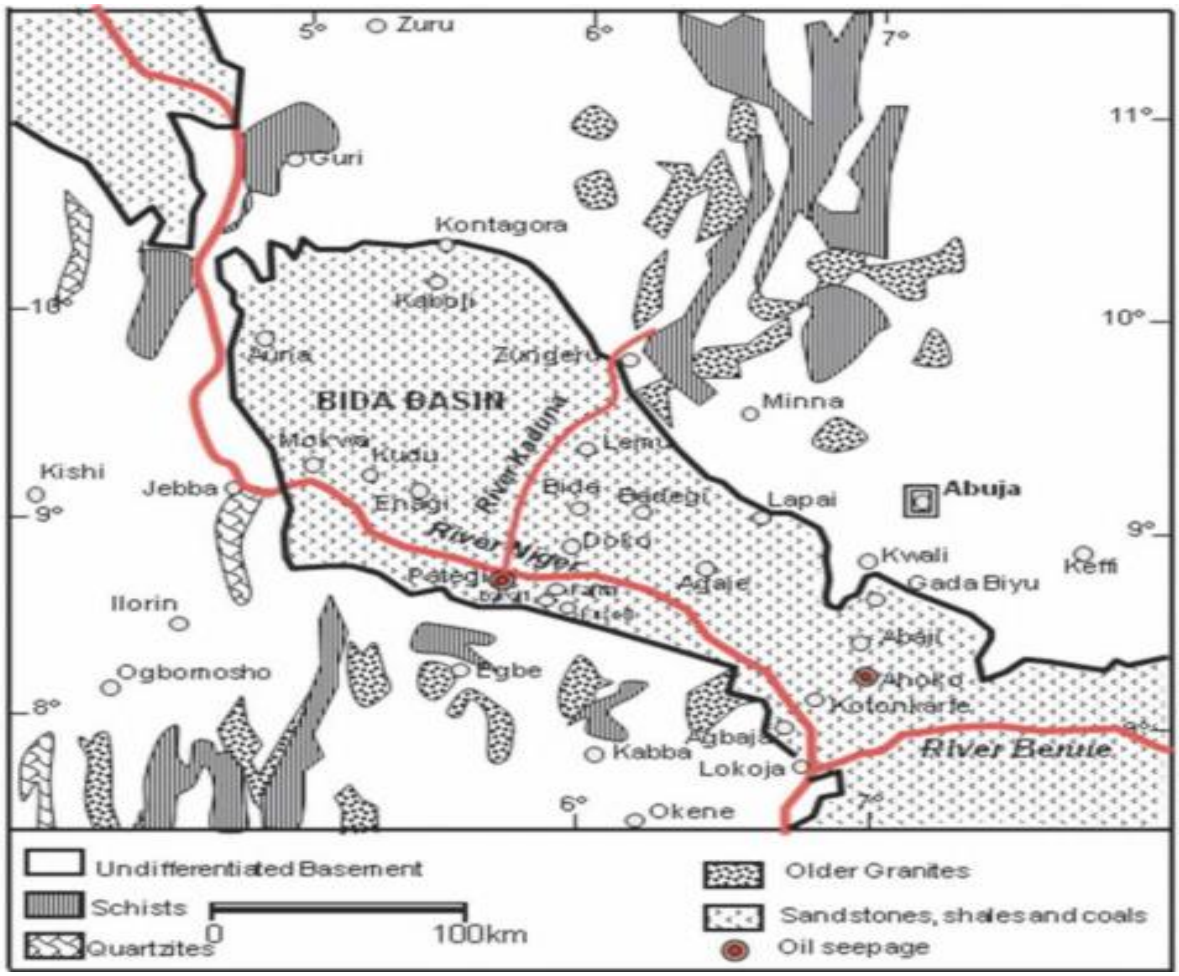

Figure 2: Geology and Location of Bida Basin and its environs, showing Ahoko the study site (Obaje *et al.*, 2011

2.0 MATERIALS AND METHODS

2.1 Identification and Mapping of the Study Area

The study area (Ahoko) is one of the four major areas (Ahoko, Petegi/Muregi, Enagi and Patitiabakolo) within the Bida basin.

Ahoko (Ebira, Gbagi and Idu) found along Abuja-Lokoja Express Way is a local government in lokoja, Kogi State, North central Nigeria. The area is characterized by two climatic seasons: dry season (November – March) and rainy season (April – October). The inhabitants of the area are predominantly Ebira, Gwagi and few Fulani whose major occupations are farming and Fishing. Its lies on longitude 8° 00″N and latitude 6° 00″E (Figure 3).

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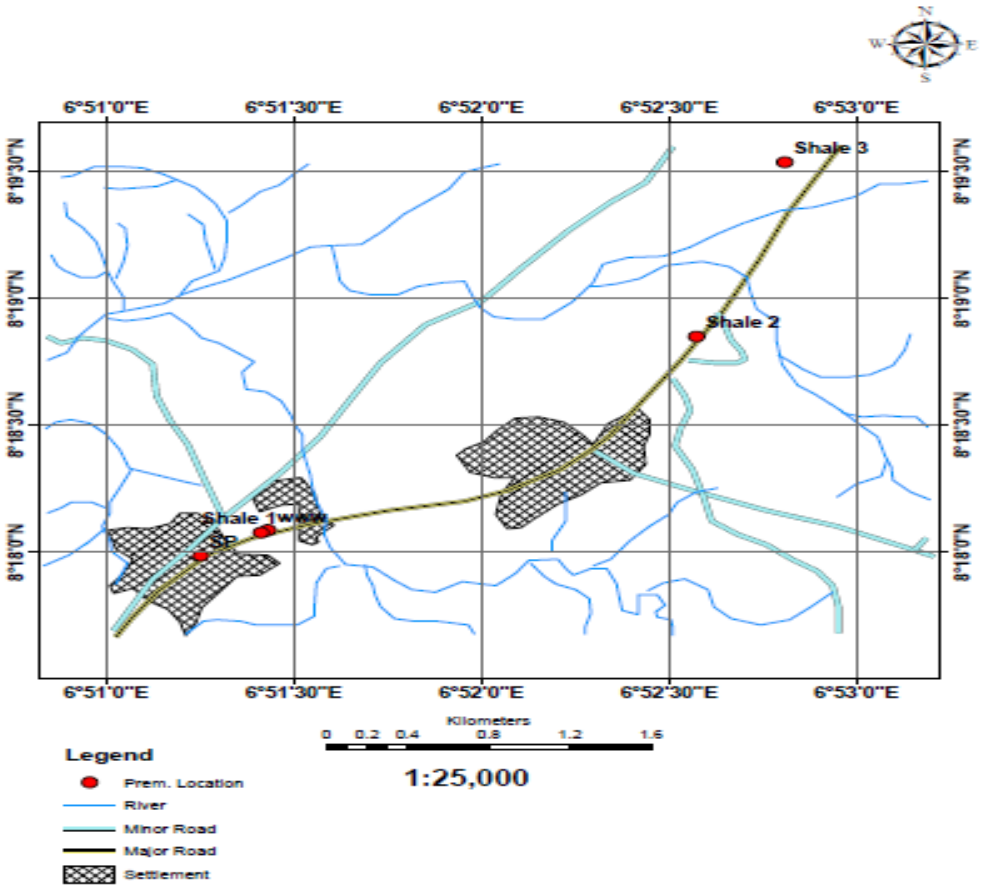

Figure 3: Map of study site showing points of sample collection

2.2 Experimental Design and Sample Collection

The identified sites were mapped and gridded 1km by 1km interval within an area of 4km^2. Samples were collected using soil core sampler by physical hammering into a depth of 1m. Each kilometre was partitioned into 5points and samples were collected in each point and were clustered into one sample. The samples were labelled along with their coordinates (Latitude and Longitude) ascertained using a Global Positioning System (GPS), thereafter it was transported to Microbiology Laboratory (Alex Ekwueme Federal University Ndufu Alike (AE-FUNAI) in an ice box where samples were analysed.

2.3 Microbiological Analysis of the Soil samples

2.3.1 Enumeration of culturable heteroaerobic bacteria

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Freshly prepared Ringer's solution according to Anyanwu *et al.* (2016) was prepared and used to dislodge the microbial load in the sample. This was done by dissolving 10 g of soil sample into 90ml of the Ringer's solution; the mixtures were shaken for 5 minutes using magnetic stirrer at 30^{o}C. It was allowed to stand for 30 miuntes, then one millilitre of the stock was taken into 9ml of the ringer's solution dispensed into test tubes and then serial dilutions were made to obtain the dilution of 10^{-1} to 10^{-10}, 0.05 ml of the diluents of 10^{-4} to 10^{-10} was inoculated into 18ml each of freshly prepared sterile molten Nutrient Agar, the mixture was transferred by pour plate method into sterile Petri dishes which were swirled and allowed to cool at ambient temperature (26^{o}C± 2^{o}C) for 30 minutes on a flat surface. The inoculated culture plates were incubated at 37^{o}C for 18hours; after which the colonies that developed were counted using a colony counter. The results were recorded as colony forming units per gram (CFU/g) of soil.

2.3.2 Enumeration of total fungi

Total fungi count was carried out on Sabourand Dextrose Agar (SDA) (Hi-Media) supplemented with 10 mg/l of streptomycin. Triplicate plates were prepared, inoculated and incubated at 28^{o}C for 72 hours. The colonies that developed were counted and recorded, and then it was allowed to grow for two more days to examine if other growth will occur. This was done for all the samples across the months sampled (February 2017-January 2018). The colony counts were expressed as colony forming units per gram of soil (CFU/g)

2.4 Isolation of specific hydrocarbon utilizers (The check organisms for oil prospecting)

Specific isolation of microbes associated with hydrocarbon seepage was done using Light carbon gas of C_1-C4 (methane, ethane, propane, butane). Isolation and enumeration of microorganisms for each sample was carried out by Standard Plate Count (SPC) method as used by Rasheed *et al.* (2015).

Serially diluted soil suspension was inoculated into sterile molten Mineral Salts Medium (MSM) containing 1.0 g $MgSO_4.7H_20$, 0.7g K_2HPO_4, 0.54 g KH_2PO_4, 0.5 g NH_4Cl, 0.2 g of $CaCl_2.2H_2O$, 4.0 mg of $FeSO_4.7H_2O$, 0.3 mg of H_3BO_4, 0.2 mg of $CoCl_2.6H_2O$, 0.1 mg of $ZnSO_4.7H_2O$, 0.06 mg $Na_2MoO_4.2H_2O$, 0.03 mg $MnCl_2.4H_2O$, 0.02 mg of $NiCl_2.6H_2O$, and 0.01 mg of $CuCl_2.2H_2O$ in 1000 mL of distilled water, at pH 7.0. The plates were placed in a desiccator, loaded with potassium hydroxide pellet at the bottom and closed with greased lid and paraffin. Then it was connected to vacuum pump for 5minutes to remove air, after vacuum was created, the loaded desiccator was filled with the desired hydrocarbon gas (methane/ethane/propane with 99.99 % purity). The desiccators were then kept in incubators at 35 ± 2^{o}C for 10 days. After incubation, the colonies of methane, ethane, propane and butane oxidizing bacteria was manually counted and reported in colony forming units per gram (CFU/g) of soil sample.

2.5 Physicochemical Analysis of Soil

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The Physical and Chemical properties of soil samples collected from the study areas were analysed using methods according to the Association of Official Analytical Chemists (AOAC, 1990).

2.6 Distribution of Hydrocarbon Oxidizing Microorganisms (HOM)

The distribution of hydrocarbon oxidizing microbes was achieved from distribution maps that were prepared with Geographical Information System (GIS) to delineate the potential areas with high yields (Veena Prasanna *et al.,* 2013). The concentration distribution maps of methane, ethane, propane and butane oxidizing microbes enumerated from the soil samples was plotted on geological map of the study area. The higher populations of these hydrocarbon oxidizing microbes was observed and used to delineate the hydrocarbon potential zones

3.0 RESULTS

3.1 Physicochemical properties of Ahoko soil

The phsiochemical propersities of the soil as shown in Figure 4 revealed the pH values to be between 5.30-5.58, 5.95-6.79, 6.72-7.05 and 6.15-6.81 for Site A, B, C and D respectively, which indicates the soil are slightly acidic with conductivity and pore space values of 12 μ∫/cm (160%), 2.60 μ∫/cm (174%), 4.10 μ∫/cm (178%) and 3.30 μ∫/cm(192%) respectively (Figure 4a-4d).

3.2 Microbiological Qualities of the Soil

3.2.1 Total aerobic heterotrophic bacteria in soil

The counts of bacteria in the soil from Ahoko study area varied with respect to the months of sampling, the counts ranged from $1.17x10^6$ CFU/g (March) to 2.67×10^6cfu/g in April for site A, 0.95×10^6 CFU/g in August to 2.88×10^6 CFU/g (April) for site B, 0.55×10^6 CFU/g in August, 2.8×10^6 CFU/g in May for site C and $0.38x10^6$ CFU/g in February to $2.02\ x10^6$ CFU/g in October for site D (Table 1). It was observed that there was an increase in the number of bacterial count from April in all the sites (A-D) this maybe because it's a mouth of rainfall onset where the temperature of the soil changes to favour the growth of organisms.

The water current which is high during the rainy season may have been responsible in sweeping the bacterial away, although the decrease in number are not significant ($P>0.05$) which implies that the organisms may be indigenous in the soil.

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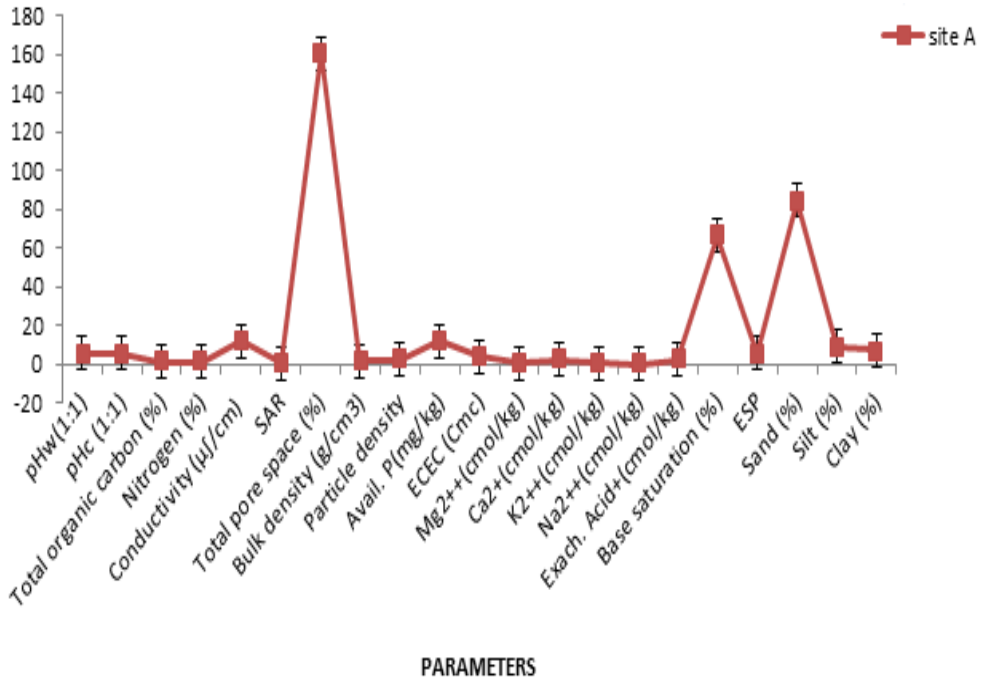

Figure 4a: Physicochemical properties of Ahoko soil, Site A

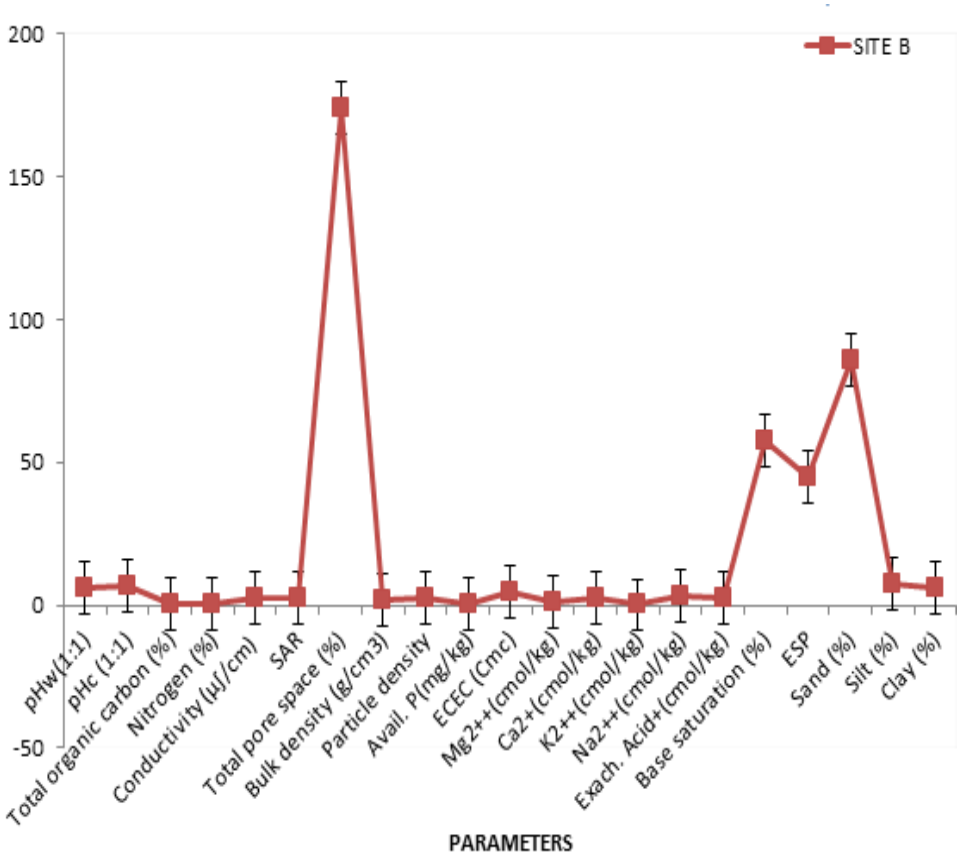

Figure 4b: Physicochemical properties of Ahoko soil, Site B

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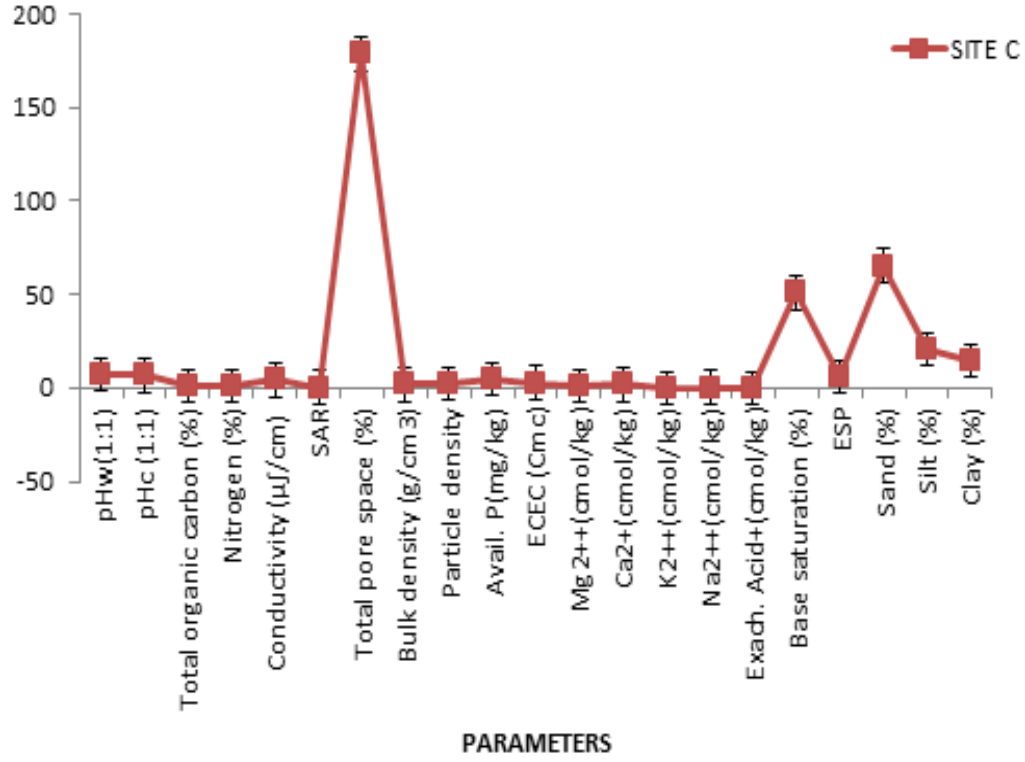

Figure 4c: Physicochemical properties of Ahoko soil, Site C

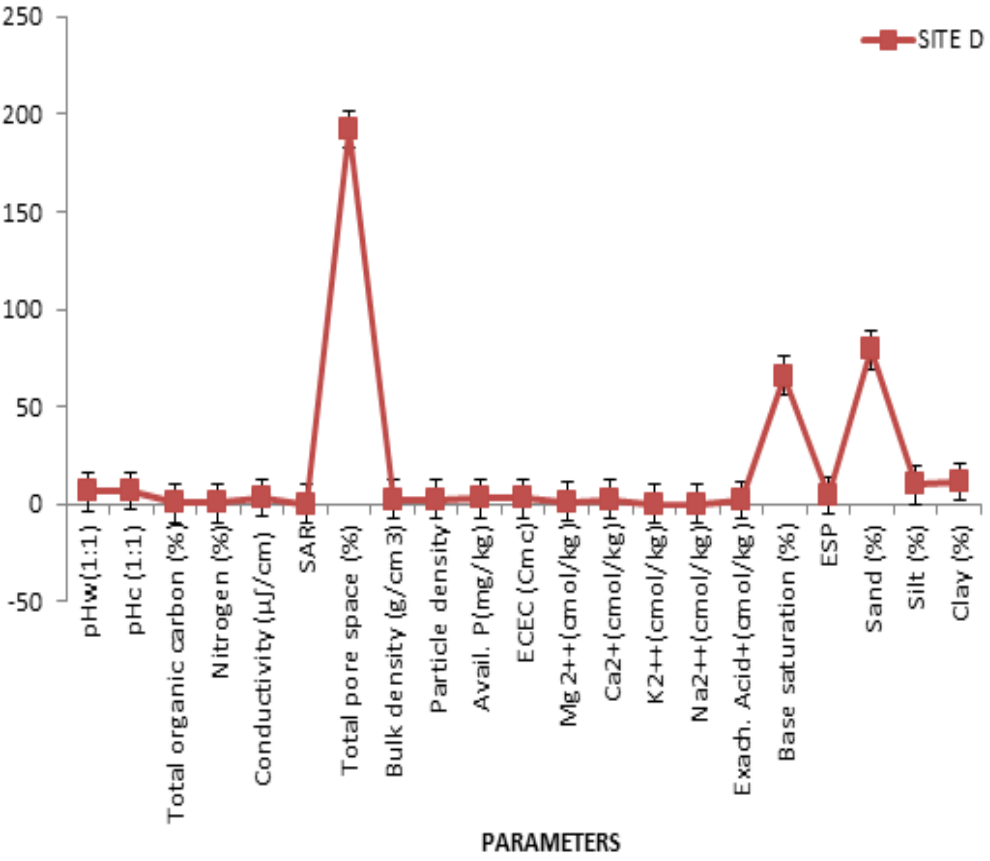

Figure 1d: Physicochemical properties of Ahoko soil, Site D

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3.2.2 Enumeration of Total fungi in Soil

The total number of culture-able fungi in the sampled soil ranged from 3.16×10^3 CFU/g to 6.60×10^3 CFU/g; 1.27×10^3 CFU/g to 4.51×10^3 CFU/g; 2.22×10^3 CFU/g to 11.0×10^3 CFU/g; 2.27×10^3 CFU/g to 6.6×10^3 CFU/g for Site A, Site B, Site C and Site D respectively. It was observed that the counts were not consistent following the month and season of the year, December, January and February had the least count across the study site, while May, June and July had higher counts of the fungi (Table 2). More so, there was an increase in the number of Fungi from $3.05x10^3$ CFU/g, $1.86x10^3$ CFU/g, $3.71x10^3$ CFU/g and $2.87x10^3$ CFU/g (March) to $3.51x10^3$ CFU/g, $2.19X10^3$ CFU/g $4.35x10^3$ CFU/g and $3.36x10^3$ CFU/g (April) within the sites A, B, C and D respectively this increase continued consistently till July and a slight decrease was observed in the month of August this maybe as a result in the break of rainfall popularly known as August break which lead to the decrease in the moisture content of the soil and thereby affect the growth of the fungi (Table 2).

Table 1: Bacterial (CFU/g $x10^6$) count in Ahoko soil

	Samples			
Months	Site A	Site B	Site C	Site D
February	1.4 ± 0.3^{ab}	1.05 ± 0.00^{a}	0.6 ± 0.025^{a}	0.38 ± 0.10^{a}
March	1.17 ± 0.05^{a}	0.99 ± 0.03^{a}	0.57 ± 0.029^{a}	0.67 ± 0.11^{a}
April	2.67 ± 0.2^{e}	2.88 ± 0.33^{e}	1.62 ± 0.075^{ab}	1.44 ± 0.2^{ab}
May	1.71 ± 0.25^{b}	0.97 ± 0.06^{a}	2.8 ± 0.05^{e}	1.5 ± 0.00^{b}
June	1.75 ± 0.38^{bc}	1.03 ± 0.06^{a}	0.8 ± 0.075^{a}	0.77 ± 0.025^{a}
July	1.18 ± 0.28^{a}	1.0 ± 0.05^{a}	0.58 ± 0.1^{a}	0.95 ± 0.05^{a}
August	1.20 ± 0.26^{a}	0.95 ± 0.00^{a}	0.55 ± 0.05^{a}	1.03 ± 0.025^{a}
September	1.63 ± 0.15^{a}	1.0 ± 0.05^{a}	1.09 ± 0.00^{a}	1.5 ± 0.1^{ab}
October	2.53 ± 0.1^{de}	2.27 ± 0.24^{abcd}	1.63 ± 0.3^{ab}	2.02 ± 0.3^{bc}
November	2.15 ± 0.21^{cd}	2.05 ± 0.1^{bcd}	1.90 ± 0.02^{bc}	1.60 ± 0.09^{ab}

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December	2.17±0.14^{cd}	1.52±0.06^{ab}	1.42±0.07^{ab}	1.52±0.1^{b}
January 2018	1.75±0.05^{b}	1.32±0.021^{a}	1.42±0.07^{ab}	1.20±0.19^{a}

Values are mean ± standard error of mean of triplicate determinations. Values with different alphabets are significantly different ($p<0.05$) between means along the column.

Table 2: Total fungi count (10^3 CFU/g) in Ahoko soil

	Sample			
	Site A	Site B	Site C	Site D
Months				
February(2017)	3.16±0.00^{b}	1.86±0.15^{a}	3.9±0.17^{bc}	2.98±0.7^{ab}
March	3.05±0.01^{b}	1.86±0.00^{a}	3.71±0.2^{bc}	2.87±0.05^{ab}
April	3.51±0.00^{bc}	2.19±0.1^{ab}	4.35±0.01^{c}	3.36±0.02^{bc}
May	6.6±0.1^{de}	2.34±0.06^{b}	11.0±4.72^{defg}	6.4±0.01^{de}
June	6.01±0.00^{d}	4.14±0.2^{c}	9.8±5.0^{f}	6.6±0.70^{e}
July	5.45±0.02^{cd}	4.51±0.06^{cd}	9.85±0.04^{fg}	6.6±0.70de
August	5.01±0.5^{bcd}	4.01±0.70^{c}	9.19±1.27^{f}	6.07±0.99^{e}
September	5.4±0.02^{cd}	3.47±0.27^{abc}	9.05±2.2^{f}	5.97±0.15^{de}
October	6.0±0.7^{abcd}	3.42±0.50^{ab}	9.05±1.0^{f}	6.16±0.1^{de}
November	5.4±0.33^{cd}	3.0±0.70^{b}	5.3±0.7^{d}	4.57±0.13^{abcd}
December	4.71±0.06^{d}	2.5±0.03^{b}	3.65±0.33^{c}	3.5±0.25^{c}
January(2018)	3.17±0.25^{b}	1.27±0.33^{a}	2.22±0.25^{ab}	2.27±0.25^{ab}

Values are mean ± standard error of mean of triplicate determinations. Values with different alphabets are significantly different ($p<0.05$) between means along the column

Values are mean of triplicate determinations.

Isolation of specific hydrocarbon utilizers (The check organisms for oil prospecting)

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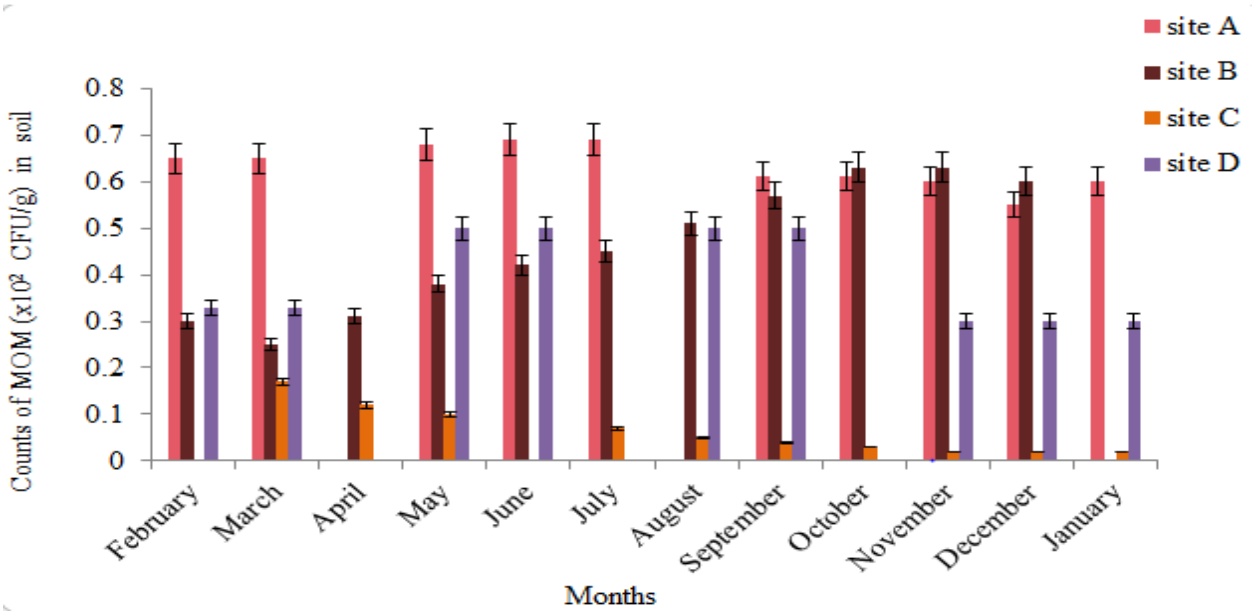
The result of methane oxidising microbes as shown in Figure 5 revealed that Site A had the highest number of count throughout the study period with no significant difference (P>0.05) in the months sampled (February to January). It was also observed that the count of MOM in site C consistently reduced from March ($0.1742x10^2$ CFU/g) to October ($0.03x10^2$ CFU/g) and reminded constant at $0.02x10^2$ CFU/g from November to January (Figure 5)



Key: Site A, B C and D are alphabetical codes used to represent the points where samples were collected (referring to samples)

Figure 5: Counts of methane oxidizing microbes (MOM) in soil

The result for the ethane oxidizing microbes ranges from $0.42x10^2$ CFU/g to $1.33 x10^2$ CFU/g for Site A, $0.3x10^2$ CFU/g to $0.92x10^2$ CFU/g, $0.05x10^2$ CFU/g to $0.67x10^2$ CFU/g, and 0.00 to $0.8x10^2$CFU/g for Site B, C and D respectively (Figure 6). The result reveals that the seasonal change within the period of study affect the microbial growth. For site A, the highest growth or microbial count was observed in the month of February ($1.33x10^2$ CFU/g), this is closely followed by $1.2x10^2$ CFU/g in March, $1.07x10^2$ CFU/g in November and $1.12x10^2$ CFU/g in January (Figure 6). This implies that the growths were favoured during the dry season rather than wet season as observed in Site C, this observation may be associated with the nature of the soil type

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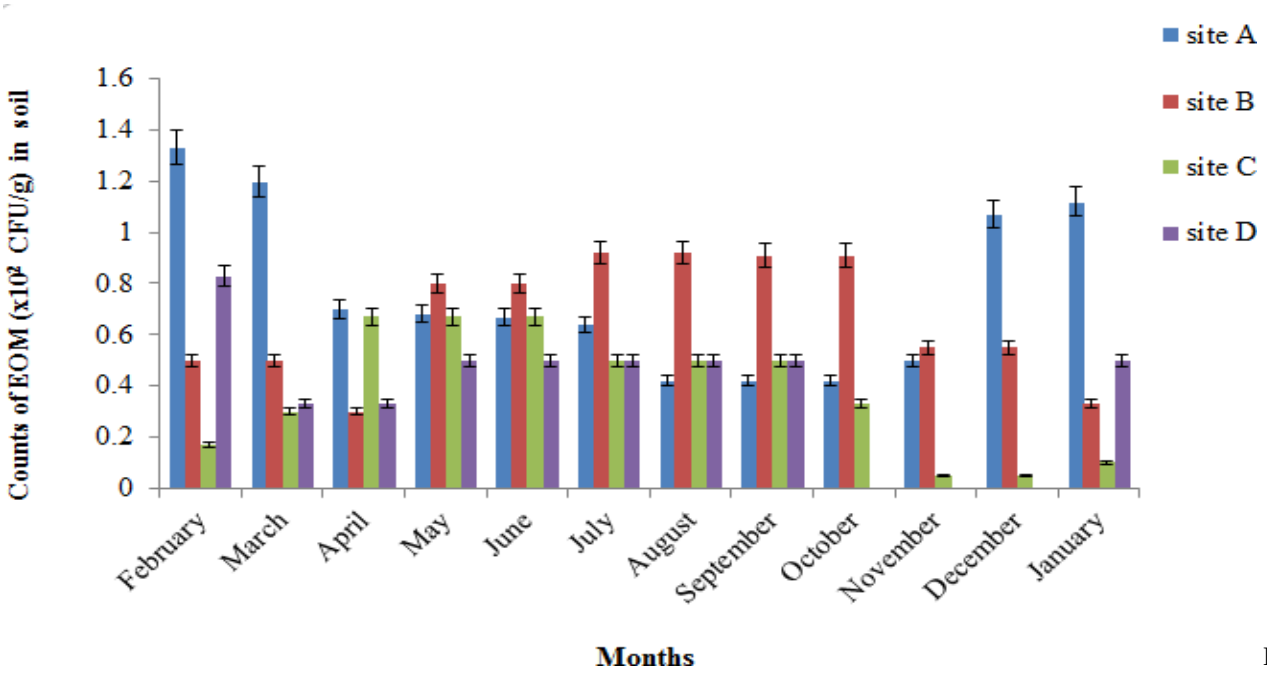
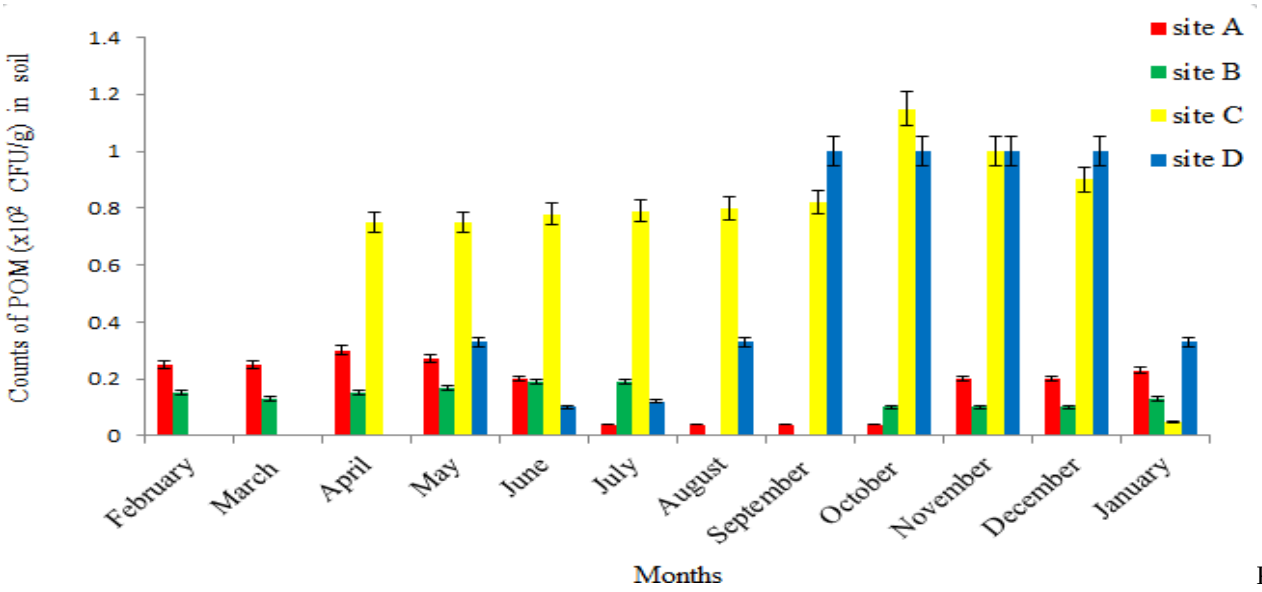


Key: Site A, B C and D are alphabetical codes used to represent the points where samples were collected (referring to samples)

Figure 6: Counts of Ethane oxidizing microbes (EOM) in soil

The number of culture-able propane oxidizing microbe was carried out from February 2017 to January 2018. It was observed that there were variation in number with respect to month and seasons of the year (Figure 7).



Key: Site A, B C and D are alphabetical codes used to represent the points where samples were collected (referring to samples)

Figure 7: Counts of Propane oxidizing microbes (POM) in soil

Table 3: Enumeration of Butane oxidizing microbes (BOM)@10^2 CFU/g

	Samples			
Months	Site A	Site B	Site C	Site D

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February	0.15±0.00^{ab}	0.15±0.001^{ab}	NG	NG
March	0.1±0.001a	0.13±0.002^{a}	NG	NG
April	NG	NG	NG	NG
May	NG	NG	NG	NG
June	NG	NG	NG	NG
July	NG	NG	NG	NG
August	0.04±0.00^{a}	NG	0.22±0.00^{a}	0.33±0.00^{b}
September	0.04±0.001^{a}	NG	0.32±0.00^{b}	0.1±0.00^{ab}
October	0.04±0.001a	0.1±0.00^{ab}	NG	0.01±0.00^{a}
November	0.2±0.00^{b}	0.1±0.0011^{ab}	0.01±0.00^{a}	0.01±0.00^{a}
December	0.2±0.00^{b}	0.1±0.003^{ab}	0.15±0.01^{ab}	0.1±0.011^{a}
January	NG	NG	NG	NG

Key: NG=No growth**.** Values are mean of triplicate determinations ± standard error of mean of triplicate determinations. Values with different alphabets are significantly different ($p<0.05$) between means along the column

The higher populations of these hydrocarbon oxidizing microbes were observed in Site A area as revealed by the concentration distribution maps of methane, ethane, propane and butane oxidizing microbes (Figure 8).These were used to delineate the hydrocarbon potential zones. The high potential zone as delineated by the map is site A (SA)

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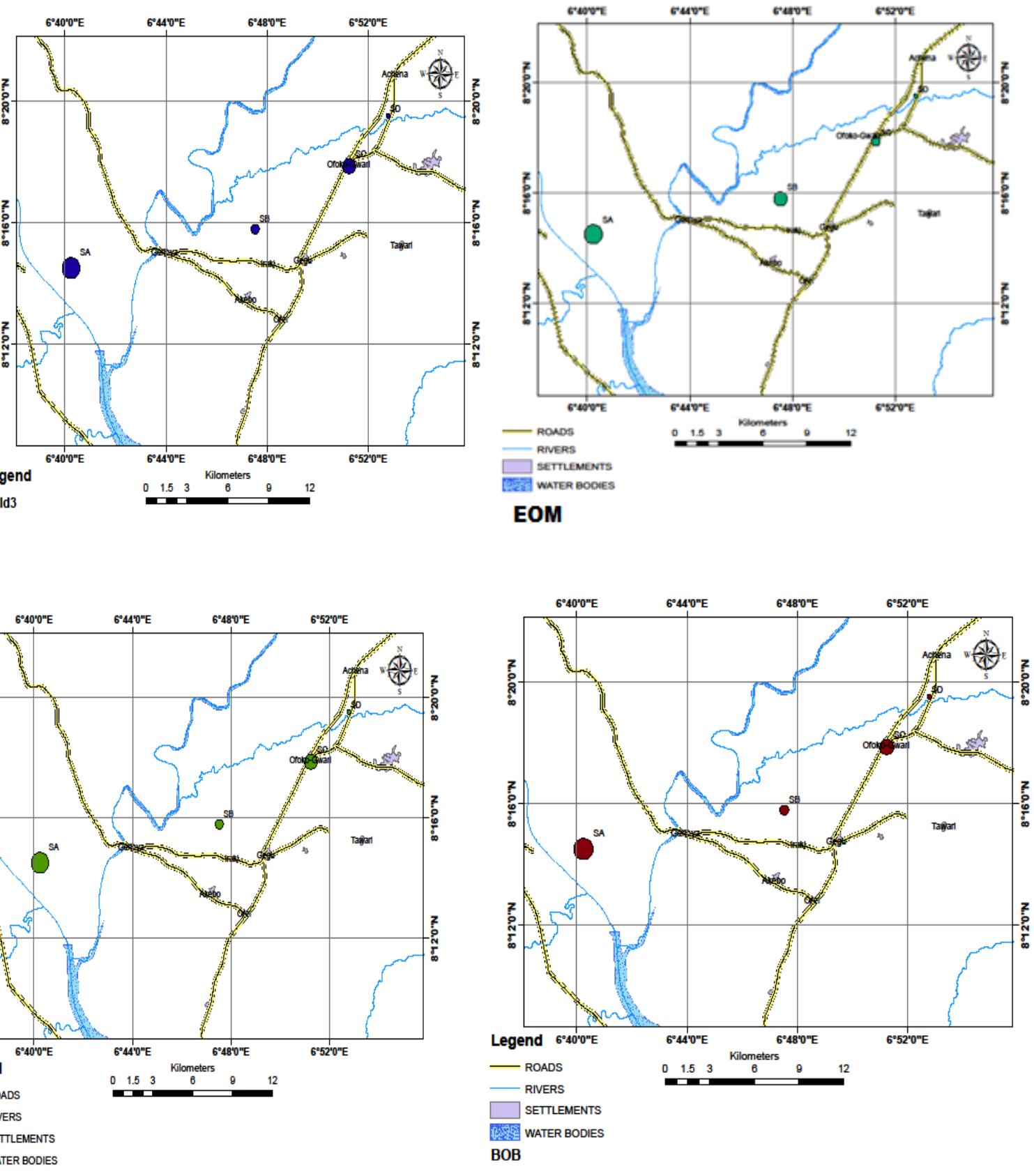

Figure 8: Concentration distribution maps of Methane, Ethane, Propane and Butane Oxidizing microbes

DISCUSSION

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It was clear from the results that all sites harboured hydrocarbon oxidizing bacteria and fungi, although there was variations in counts, The mean total bacterial and fungal counts showed that more counts of TB were observed in the month of April and October (Table 1), while Total fungal (TF) were observed in the month of May, July and October than the months of December and February (Table 2) this will be connected to water content of the soil because in October the soil was wetter than December and February. This is in accordance to the work of Eze and Okpokwasili, (2010), Allamin *et al*. (2014); and Abioye *et al*.(2014) who stated that seasonal differences with higher microbial counts in wet than in dry season months may be attributed to increased water content of the soil. TB and TF abundance patterns were similar in all the sites A to D with differences in counts; the difference in count of the sites could be due to the difference in sites receiving domestic effluents, grazing activity and agricultural runoff which is similar to the findings of Eze and Okpokwasili, (2010), Allamin *et al*.(2014); and Abioye *et al*.(2014); who reported that variation in counts may be due to the industrial and domestic discharges to the sites.

It was observed that BOM has it least count of $1.0x10^3$ CFU/g in the month of April (Table 3) while MOM, EOM and POM have their least count of $3.0x10^3$, $2.0x10^3$ and $2.0x10^3$ CFU/g respectively, in the mouth of March (Figure 5-7). This could be that the onset of the rain in April created a sudden shock on the organisms and this retarded their growth rate and affected their number. MOM recorded its highest count ($4.8x10^2$) in the month of April, EOM has its highest count ($4.6x10^2$) in April and December, and POM has its peak count in November, while BOM recorded a peak count of $3.0x10^2$ CFU/g in October. This implies that the numbers of these organisms are influenced by the seasons of the year which is tied to the temperature (Table 1-3). This is in agreement with Clark *et al.* (2000); Margesin *et al. (*2003); Head *et al.* (2006); Hamamura *et al*. (2006) and Kinnaman *et al.* (2010) whom reported a count of $2x10^{10}$ gyear^{-1} (methane), $1.9x10^9$ CFU/g (ethane), and $1.4x10^9$ (propane) from an established oil point seep field off shore of Santa Barbara, reported that the variations due to high dynamic environments is as a result of variable gas influx levels and seasonal changes in deposition.

The result for the ethane oxidizing microbes ranges from $0.42x10^2$ to $1.33x10^2$ for Site A, $0.3x10^2$ to $0.92x10^2$, $0.05\ x10^2$ to $0.67\ x10^2$, and 0.00 to $0.8x10^2$ CFU/g for Site B, C and D respectively (Figure 6). The result reveals that the seasonal change within the period of study affect the microbial growth. For site A, the highest growth or microbial count was observed in the month of February ($1.33x10^2$), this is closely followed by $1.2x10^2$ in March, $1.07x10^2$ in November and $1.12x10^2$ in January (Figure 6). This implies that the growths were favoured during the dry season rather than wet season as observed in Site C; this observation may be associated with the nature of the soil type, variable gas influx levels and seasonal changes in deposition (Head *et al.*, 2006; Hamamura *et al*., 2006 and Kinnaman *et al.*, 2010).

The presence of MOM is a sparing pointer to a potent site, but the high relative abundance and wide distribution of EOM (which are oxidizers of ethane gas C_2) and POM (oxidizers of propane gas C_3) especially in site A confirms the potency of this sites for oil exploration (Shennan, 2006; Kotani *et al.*, 2006), however, the production may not be in large commercial quantity because the total counts were less than 10000ppm as affirmed by Rasheed *et al*.(2018).

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CONCLUSION

This study attempts to explore the oil and gas prospects of the Bida Basin using the surface geochemical prospecting methods. Microbiological, and total organic and inorganic (physiocochemical properties) studies was applied to evaluate its hydrocarbon prospects. The microbial results indicate the presence of high bacterial anomalies for methane, ethane, and propane, and butane oxidizers near Akoko site indicating the area is prospective for hydrocarbons. The presence of propane and butane oxidising microbes is an indication that site A is a more potent site for oil exploration, although the quantity of oil may not be large to a commercial quantity because the number of organism were less than 1000ppm.

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