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OCCURRENCE OF HYDROCARBON DEGRADING BACTERIA IN SOIL IN KUKAWA, BORNO STATE

I.A. Allamin[1]*, U.J.J. Ijah[2], H.Y. Ismail[3] and M.L. Riskuwa[4]
[1,3]Department of Microbiology, University of Maiduguri P.M.B 1069
[2]Department of Microbiology, Federal University of Technology Minna, Niger State
[4]M.L. Riskuwa (Dept. of microbiology, Usmanu Danfodio University Sokoto)
*Corresponding author: ibnallamin@gmail.com

Abstract

Soil samples were collected from five sites covering petroleum exploration station in Kukawa, Kukawa Local Government Area of Borno State, Nigeria between October, 2012 and February, 2013 at two different depths (0-10cm and 10-20cm) to enumerate and identify hydrocarbon degrading bacteria in the soil. Total aerobic heterotrophic bacteria (TAHB) were enumerated on Nutrient agar (NA), and Hydrocarbon utilizing bacteria (HUB) enumerated on Oil agar (OA). The bacterial isolates were identified using morphological and biochemical tests. It was observed that the microorganisms (TAHB, and HUB) were more densely populated at 10cm depth. (TAHB: 5.3×10^8 - 11.4×10^8cfu/g, and HUB: 2.4×10^5 - 5.3×10^5 cfu/g, than at 20 cm depth (TAHB: 3.0×10^8 - 5.7×10^8 cfu/g, and HUB: 2.1×10^5 - 4.8×10^5 cfu/g). The HUB was identified as species of *Bacillus, Pseudomonas, Klebsiella, Lactobacillus, Micrococcus, Corynebacterium,* and *Actinomyces. Bacillus, and Pseudomonas* species were more constantly isolated than other isolates and they constitute 100% of total bacterial isolates. The potential of hydrocarbon utilizing bacteria isolated to degrade hydrocarbon was studied. Nineteen (19) bacterial species was screened, *Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus, Klebsiella pneumoniae, Micrococcus leteus,*and *Lactobacillus casei,* utilized and degrade crude oil at considerably high rates after 21 days of incubation. The degradation efficiency was confirmed by GC-MS analysis, which indicated that the bacterial isolates utilized most of the crude oil components particularly straight chain alkanes and cycloalkanes

Key words: Occurrence, Degradation, Bacteria and Soil

Introduction

Soil is a rich source of microorganisms capable of degrading hydrocarbons and residual oil (Atlas and Bartha, 1999). It has been found that soils receiving hydrocarbons like the area of oil sludge, oil fields etc. have significant higher population of hydrocarbon degrading microorganisms. But normal populations of hydrocarbon utilizing microorganisms account for 1% of the population but may reach 100% under selective pressure after a spill or prolonged chronic discharges, returning to background levels after the pollutant is removed (Van Hamme and Singh, 2003; Abioye *et al.,* 2012). However hydrocarbon degrading microorganisms are widely distributed in marine, freshwater, and soil ecosystems (Ogbonna, 2008).

The ability to degrade, hydrocarbon substrates is exhibited by a wide variety of microorganisms (Leahy and Colwell, 1990; Chikere *et al.,* 2009) that are widely distributed in oil-polluted as well as pristine soils (Cappello *et al.,* 2007; Chikere *et al*.,2009).Using culture dependent and independent isolation techniques, different bacterial genera have been characterized from hydrocarbon polluted soils. The hydrocarbon utilizing microorganisms include species of *Bacillus, Lactobacter, Arthrobacter, Pseudomonas, Micrococcus, Zoopage, Serratia, Corynebacterium and Articulosporium* (Ajayi *et al.,* 2008). In most cases *Bacillus* sp. predominated, especially in the crude oil polluted soil. This may be due to the ability of the organisms to produce spores, which may shield them from the toxic effects of the hydrocarbons (Latha and Kalaivani, 2012).

Presence of petroleum hydrocarbons has been reported to influence the biodiversity, distribution and population of microorganisms in an environment (Afuwale and Modi, 2012).The ability to isolate high numbers of certain oil-degrading microorganisms from an environment is commonly taken as evidence that those organisms are the active degraders in that environment; likewise hydrocarbon micro seepage is a widely distributed natural phenomenon in the geochemical carbon cycle (Etiope and Ciccioli, 2009). The problems of hydrocarbon contamination are known to be widespread in oil producing areas, and are of public health concern. Attempt to remediate hydrocarbon polluted sites using physiochemical methods had been in existence since (Okoh, 2003), but due to other implications their application were discouraged. Bioremediation using microorganisms capable of degrading hydrocarbon is now believed to more promising and effective. Kukawa area is a potential exploration site of petroleum. The NNPC has initiated the oil exploration activities in the area. The activities of petrochemical industry have been associated with damage to the environment, altering the microbiological and physicochemical properties of the environment (Chikere *et al*., 2009). Identifying the microbial species, their abundance and distribution would help in tackling environmental problems associated with such activities (Nkwelang *et al.,* 2008).Hence, the microbial species capable of utilizing petroleum hydrocarbon and their effectiveness in hydrocarbon biodegradation need to be investigated. The objective of the study was to assess the occurrence of hydrocarbon utilizing bacteria in soil in Kukawa, Borno State.

Materials and Methods

Study Area

The study area is Kukawa, in Kukawa local government area of Borno State, North Eastern Nigeria. The area is located close to Lake Chad with geographical coordinates $12^0$55'33'' North and $13^0$34'12'' East. It has an average elevation/altitude of 277 meters. Kukawa has a population of over 16,077 people (Anatol, 2010). Most of the inhabitants are engaged in farming, grazing, fishing, and salt mining as means of livelihood. The vegetation is Sahel savanna, with mainly grasses, shrubs and few trees. Kukawa has a long dry season (November-May), a short rainy season (June- September), and cold harmattan period (December- February). Annual rainfall ranges from 500mm to 1000 mm, with an average temperature range of 25-40^{o}C (Borno State Government, 2007). Kukawa is a crude oil prospecting area by the Nigerian National Petroleum Corporation (NNPC). The NNPC has demarcated the area into twelve phases comprising phases 1-5 (dry land) and 6-12 (lake water phase) for crude oil prospecting.

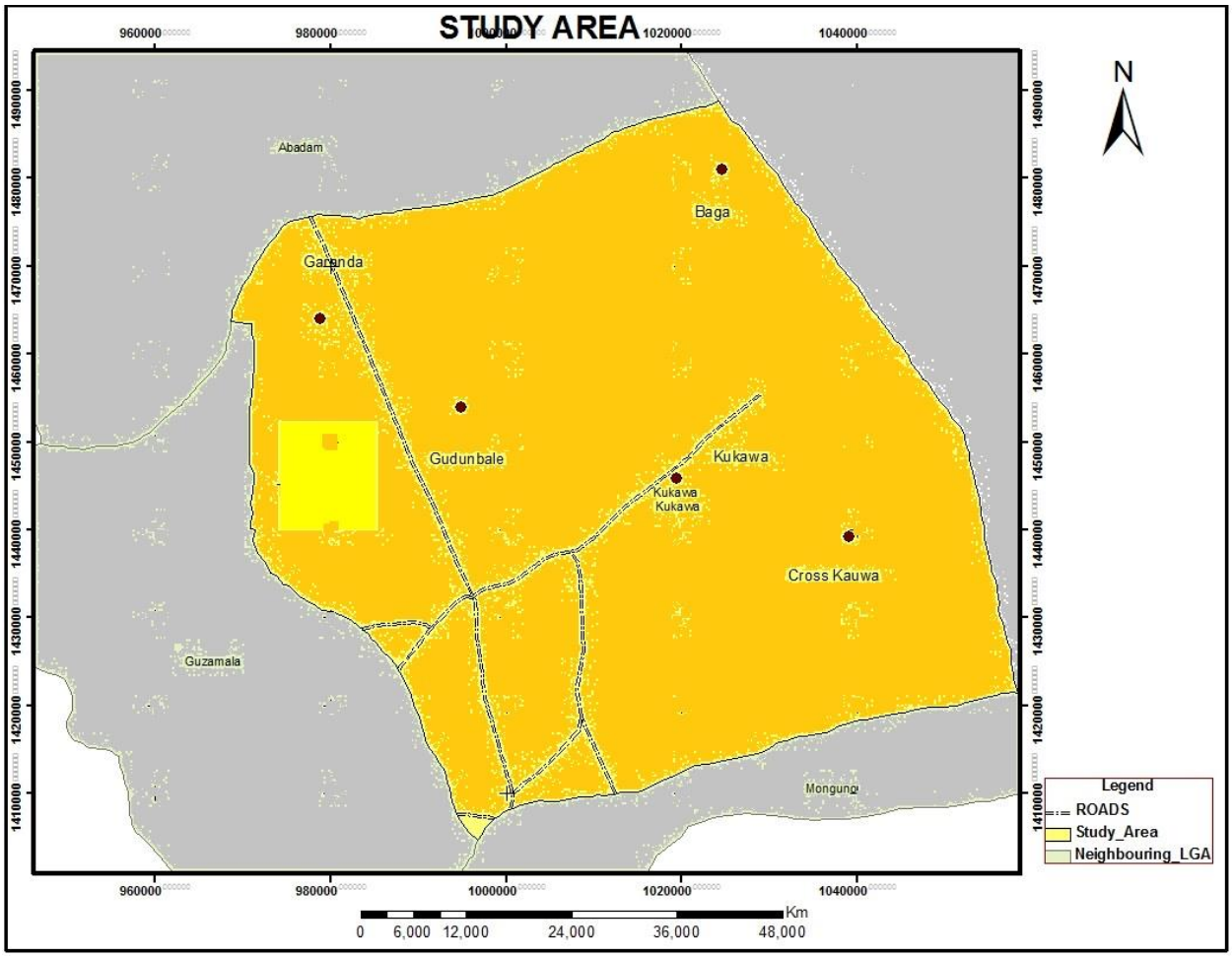

Figure 1.Map of Kukawa showing sampling sites (●)

Experimental Design and Sample Collection

A complete randomized design was used in a laboratory setting. The soils samples were collected over an area of approximately 5 km^2. Soil samples were collected from the proposed sites of crude oil exploration: Cross Kukawa (B1), Baga Area (B2), Kukawa (B3), Gudunbale shuwari (B4), and Garanda (B5). Soil samples were obtained using soil auger at two different depths of 10cm and 20cm (Onifade and Abubakar, 2007) Representative samples were obtained randomly and bulked and transported to the laboratory in polythene bags for analysis. The soil samples were collected 1 km interval and twice in all the holes in every month October, December, 2012 and February 2013. Escravos light crude oil was collected from Kaduna Refinery and Petrochemical Company Kaduna, Nigeria.

Enumeration of Total Aerobic Heterotrophic and Hydrocarbon Utilizing Bacteria

One gram each of the samples was weighed aseptically and placed in test tube containing 9ml of distilled water the test tube was shaken vigorously in order to dislodge the microorganisms that adhered to the soil particles. The content of the tube was serially diluted. Aliquot (0.1ml) from dilution (10^{-7}) was plated in triplicates on sterile Nutrient agar (NA) and (10^{-5}) on oil agar (OA) for the enumeration of total aerobic heterotrophic bacteria and crude oil utilizing bacteria respectively. The plates were incubated at room temperature (30 ± 2^0C) for 48 hours and 5 days for NA and OA, respectively. The colonies which developed on the plates were counted and recorded as colony forming units per gram (cfu/g) of soil. Pure cultures of the isolates were obtained by repeated sub-culturing on media used for primary isolation. The pure isolates were maintained on agar slants for further characterization and identification.

Characterization and Identification of Isolates

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell morphology. Other tests performed included spore staining, motility, oxidase and catalase production, citrate utilization, oxidative/fermentative (O/F) utilization of glucose, indole production, methyl red-Voges Proskaur reaction, urease and production of H_2S from triple sugar iron (TSI) agar and sugar fermentation. The tests were performed according to the methods described by Oyeleke and Manga (2008). Bacterial identification was performed using the keys provided in the Bergey's Manual of Determinative Bacteriology (Holt and Williams, 1994).

Screening of Isolates for Hydrocarbon Utilizing Ability

The hydrocarbon degrading ability of the bacterial isolates was tested using turbidity method as described by Oboh (2006). The isolates were inoculated in nutrient broth (NB) and incubated at room temperature (30 ± 2^0C) for 24 hours. One milliliter of NB grown culture ($\times 10^6$ cells) was inoculated into Mineral salt broth containing 0.5% of Escarvos light crude oil and incubated at 30^0C without shaking for 7 days. Turbidity of the medium was used as measure of bacterial growth.

Measurement of Rates of Crude Oil Biodegradation by Isolates, and Gravimetric analysis method

The rate of crude oil degradation by selected microbial isolates was determined using gravimetric analysis and gas chromatographic- mass spectrophotometric (GC-MS) analysis techniques (Ijah *et al*., 2008, Ibrahim, 2008; Abioye *et al.*, 2012).

One milliliter of Nutrient broth grown isolates was inoculated into 100ml of mineral salts medium (Ijah, 1998) in Erlenmeyer flask. Then 0.5ml of escravos crude oil was introduced to each Erlenmeyer flask. Control flask contained 100ml of mineral salts medium plus 0.5ml of Escravos crude oil but without added organism. The flasks were incubated at 30^0C with manual shaking at 100 strokes per minute (Ijah *et al.*, 2008) for 21 days. At 7 days interval, flasks per organism were removed and the amount of crude oil left was determined by extracting the residual crude oil with n-hexane and noting the its absorbance reading at 500nm wavelength. The amount of crude oil degraded was calculated using the formula:

$$\frac{\text{Weight of crude oil (control)} - \text{weight of crude oil (degraded)}}{\text{Weight of crude oil (control)}} \times 100$$

Gas chromatographic/ mass spectrophotometric analysis of oil extracts

The oil extracts were dried with 0.1 g anhydrous Na_2SO4 and filtered using Whatman No.1 filter paper. Then the n-haxane was allowed to evaporate off at room temperature (30 ± 2^0C) in a fume chamber. One microlitre (µl) of the extractable crude oil was diluted with 1 µl of n-pentane and analyzed on a gas chromatograph/ mass spectrophotometer (6890, Agilent Technologies, USA) equipped with a flame ionization detector (FID). Split injection was used with helium as carrier gas. The oven temperature was initially set at 45^0C for 3 minutes and increased at a rate of 10^0C per minute to 280^0C (Ijah, 1998; Ibrahim, 2008).

Statistical Analysis of Data

Statistical analysis of data was carried out using Analysis of Variance (ANOVA) with Analytical software Statistics version 8.0.to test the difference among the data at 95% probability level.

Results and Discussions

The results on the occurrence of hydrocarbon degrading bacteria in soil in Kukawa, Borno State showed that the total aerobic heterotrophic bacterial (TAHB) counts in the five sites sampled at different depths ranged from $8.9\pm5.2\times10^8$cfu/g - $11.4\pm3.0\times10^8$cfu/g at 10cm depth and from $4.4\pm2.3\times10^8$cfu/g - $5.7\pm1.4\times10^8$cfu/g at20cm depth in October. In December the counts were $6.3\pm3.3\times10^8$cfu/g - $10.6\pm3.6\times10^8$cfu/g and $3.3\pm4.8\times10^8$cfu/g - $5.1\pm3.7\times10^8$cfu/g at 10cm and 20cm depths respectively. In February the (TAHB) counts were $5.3\pm2.2\times10^8$cfu/g - $10.3\pm4.0\times10^8$cfu/g and $3.0\pm2.8\times10^8$cfu/g - $4.2\pm1.1\times10^8$cfu/g at 10cm and 20cm depths respectively. The results indicated that total heterotrophic bacterial (TAHB) counts were higher

at 10cm depth than that at 20cm and also October had higher counts than December and February (Table 1)

Table 2 shows the counts of hydrocarbon utilizing bacteria (HUB). The HUB counts in October at 10cm depth range from $3.9\pm3.1\times10^5$ - $5.3\pm4.9\times10^5$cfu/g and from $3.3\pm1.7\times10^5$- $4.8\pm4.1\times10^5$cfu/g at 20cm depth. In December the counts ranged from $3.0\pm2.3\times10^5$ - $4.5\pm5.3\times10^5$cfu/g and from $2.8\pm1.7\times10^5$- $3.6\pm4.4\times10^5$cfu/g at 10cm and 20cm depths respectively. In February the counts ranged from $2.4\pm2.9\times10^5$ - $3.1\pm2.7\times10^5$cfu/g and from $2.1\pm4.3\times10^5$to $2.6\pm4.3\times10^5$cfu/g at 10cm and 20cm depths respectively. The (HUB) counts in 10cm depth were higher than those of 20cm and the counts in October and December were higher than those of February.

The hydrocarbon degrading bacteria isolated and identified belongs to the genera *Bacillus, Pseudomonas, Corynebacteria, Klebsiella, Micrococcus*, and *Lactobacillus.* And *Bacillus* was represented by the following species: *B. alvei, B. subtilis, Bacillus licheniformis and B. cereus* others were *Pseudomonas auriginosa, Klebsiella pneumoniae, Lactobacillus casei, Micrococcus luteus, Corynebacterium ovis, Proteus vulgaris, Yersinia rohdei, Actinomyces viscosus, and Morganella morganii* (Table 3). For screening test, however nineteen bacterial and five fungal species were tested for effectiveness, Out of 19 bacterial isolates screened, five (5) isolate namely, *Bacillus cereus, Pseudomonas auriginosa, Klebsiella pneumonia, Bacillus subtilis,* and *Micrococcus leteus* were able to grow luxuriantly (+++) on the hydrocarbons. Moderate (++) growth on the hydrocarbons was observed in the species of *Corynebacterium ovis, Bacillus licheniformis, Actinomyces viscosus, Bacillus alvei, Bacillus cereus, Lactobacillus casei, Proteus vulgaris, Corynebacteriumhotmanii,* and *Bacillus azotofomans.* Minimal growth (+) was recorded in species of *Yersinia rohdei* and *Morganella morganii* (Table 4). The results of the weight of crude oil due to microbial attack are presented in Figure 2. The weight loss of crude oil loss ranged from 37.6% to 50.6% after 21 days. Out of the five (5) crude oil degraders *Bacillus subtilis* (50.6%) was observed to be more potent in utilizing the crude oil than the rest of the isolates: *Pseudomonas auriginosa* (48.3%). *Klebsiella pneumoniae* (39.0%). *Bacillus badius* (38.3%) and *Micrococcus luteus* (37.6%). Statistical analysis of the data using Analysis of variance (ANOVA) indicated that there were significant variations ($P\geq0.05$) in the rates of crude oil degradation by the bacteria.

Table 1: Total aerobic heterotrophic bacterial counts (cfu/g)

	Bacterial counts ($\times10^8$cfu/g)					
	October		December		February	
	Depth(cm)		Depth(cm)		Depth(cm)	
Site	10	20	10	20	10	20
B1	11.4 ± 3.0^{a}	5.5 ± 2.8^{ab}	10.6 ± 3.6^{ab}	5.1 ± 3.7^{abc}	6.3 ± 3.3^{de}	3.6 ± 3.3^{bcd}
B2	10.3 ± 3.1^{ab}	5.3 ± 6.6^{ab}	9.0 ± 5.7^{bc}	4.8 ± 2.0^{abcd}	7.5 ± 2.8^{cd}	4.0 ± 5.0^{abcd}
B3	10.3 ± 4.2^{ab}	4.5 ± 2.9^{abcd}	6.6 ± 3.0^{de}	4.8 ± 4.6^{abcd}	5.3 ± 2.0^{e}	3.0 ± 2.8^{d}

B4	8.9±5.2^{bc}	5.7±1.4^{a}	7.6±3.3^{cd}	3.7±7.5^{abcd}	10.3±4.0^{ab}	4.2±1.1^{abcd}
B5	10.3±3.5^{ab}	4.4±2.3^{abcd}	6.3±3.3^{de}	3.3±4.8^{cd}	5.2±4.6^{e}	4.0±2.3^{abcd}

Key: B1=Cross Kukawa,B2=Baga,B3=Kukawa,B4=Shuwari,B5=Ngaranda, cfu/g=colony forming units per gram

In each column, means followed by different letter (s) are significantly different according to Turkey's HSD at $P < 0.05$.

Table 2: Hydrocarbon utilizing bacterial counts (cfu/g)

	Bacterial counts (×10^5 cfu/g)					
	October		December		February	
	Depth(cm)		Depth(cm)		Depth(cm)	
Site	10	20	10	20	10	20
B1	5.3±4.9^{a}	4.8±4.1^{a}	4.5±6.3^{abc}	3.6±4.4^{abc}	2.4±2.9^{e}	2.6±4.3^{bc}
B2	4.9±2.3^{ab}	4.4±5.5^{ab}	3.1±1.7^{cde}	2.6±1.7^{bc}	3.1±2.7^{cde}	2.2±6.6^{c}
B3	3.9±3.1^{abcde}	3.8±3.6^{abc}	3.0±2.3^{cde}	3.0±1.1^{abc}	2.8±1.1^{de}	2.5±0.6^{c}
B4	4.2±4.6^{abcd}	3.3±1.7^{abc}	3.2±1.8^{cde}	2.7±1.7^{bc}	3.0±2.4^{cde}	2.1±4.3^{c}
B5	5.2±3.0^{a}	4.5±1.7^{ab}	3.3±2.1^{bcde}	2.7±1.5^{bc}	2.6±1.3^{de}	2.3±2.8^{c}

Key: B1=Cross kukawa,B2=Baga,B3=Kukawa,B4=Shuwari,B5=Ngaranda, cfu/g= colony forming units per gram.

In each column, means followed by different letter (s) are significantly different according to Turkey's HSD at $P < 0.05$.

Table 3: Characterization and identification of hydrocarbon utilizing bacteria isolated from soil

Isolate code	Biochemical Characteristics																Bacteria
	GR	Shape	Spore	CAT	OXI	MR	VP	UR	IND	CIT	TSI						
											GLU	LAC	SUC	H_2S	GAS	MOT	
A11	+	Rods	-	+	-	+	-	+	-	+	+	-	+	-	+	-	*Corynebacterium ovis*
A12	+	Rods	+	+	-	-	+	-	+	+	+	-	-	-	-	+	*Bacillus licheniformis*
A25	-	Rods	-	-	-	-	-	+	-	+	+	+	+	-	-	+	*Yersinia rohdei*
A24	+	Rods	+	+	-	-	+	+	-	-	+	-	-	-	-	+	*Bacillus pumilis*
B13	+	Rods	+	+	+	-	-	-	-	+	+	-	-	-	-	+	*Bacillus coaglans*
B16	+	Rods	-	+	+	-	-	+	-	+	+	+	+	+	-	+	*Actinomyces viscosus*
B27	-	Rods	-	+	-	+	-	-	-	-	+	+	+	-	-	-	*Klebseilla pneumoniae*
B24	-	Rods	-	+	-	+	-	+	+	-	+	-	-	+	+	+	*Proteus vulgaris*
C19	-	Rods	-	+	+	+	-	-	+	-	+	-	-	+	-	+	*Pseudomonas auriginosa*
C110	+	Rods	+	+	-	-	+	+	+	+	+	-	-	-	-	+	*Bacillus cereus*

C25	+	Rods	+	+	+	+	-	-	+	+	+	+	+	-	-	+	*Bacillus subtilis*
C28	+	Rods	-	-	+	+	-	+	-	+	+	-	-	-	+	+	*Lactobacillus casei*
A23	+	Cocci	-	+	-	-	-	-	-	-	-	-	-	-	-	-	*Micrococcus luteus*
D11	+	Rods	+	+	-	-	+	+	-	-	+	-	-	-	-	+	*Bacillus alvei*
D14	+	Rods	-	-	-	-	-	+	+	+	-	-	-	-	+	-	*Corynebacterium hotmanii*
D29																	
E13	+	Rods	+	-	+	-	-	+	-	+	+	-	-	-	-	+	*Bacillus azotofomans*
E110	+	rods	+	+	-	-	+	+	+	-	+	-	-	-	-	+	*Bacillus lentus*
E11	+	Rods	-	+	-	+	-	+	-	-	+	-	-	-	-	+	*Morganella morganii*
E24	-	Rods	-	+	+	+	-	-	-	+	+	-	-	-	-	+	*Pseudomonas putida*

Keys: + =positive, - =negative, CAT= Catalase, OXI= Oxidase, MR= Methyl-red, VR=Voges Proskauer, UR= Urease, IND= Indole, CIT= Citrate, GLU=Glucose, LAC=Lactose, SUC=Sucrose, and MOT=Motility, TSI=Triple sugar ion test

Table 4: Growth of bacteria in crude oil medium

Bacterial isolates	Growth in crude oil medium after 7days
Corynebactrium ovis	++
Bacillus licheniformis	++
Yersinia rohdei	+
Bacillus pumilis	++
Bacillus cereus	+++
Actinomyces viscosus	++
Klebsiella pneumonia	+++
Proteus vulgaris	++
Pseudomonas aeruginosa	+++
Bacillus coaglans	++
Bacillus subtilis	+++
Lactobacillus casei	++
Micrococcus luteus	+++
Bacillus alvei	++
Corynebacterium hotmanii	++
Bacillus azotofomans	++
Bacillus lentus	++
Morganella morgani	+
Pseudomonas putida	++

+++: Maximum growth. ++: moderate growth. +: minimal growth

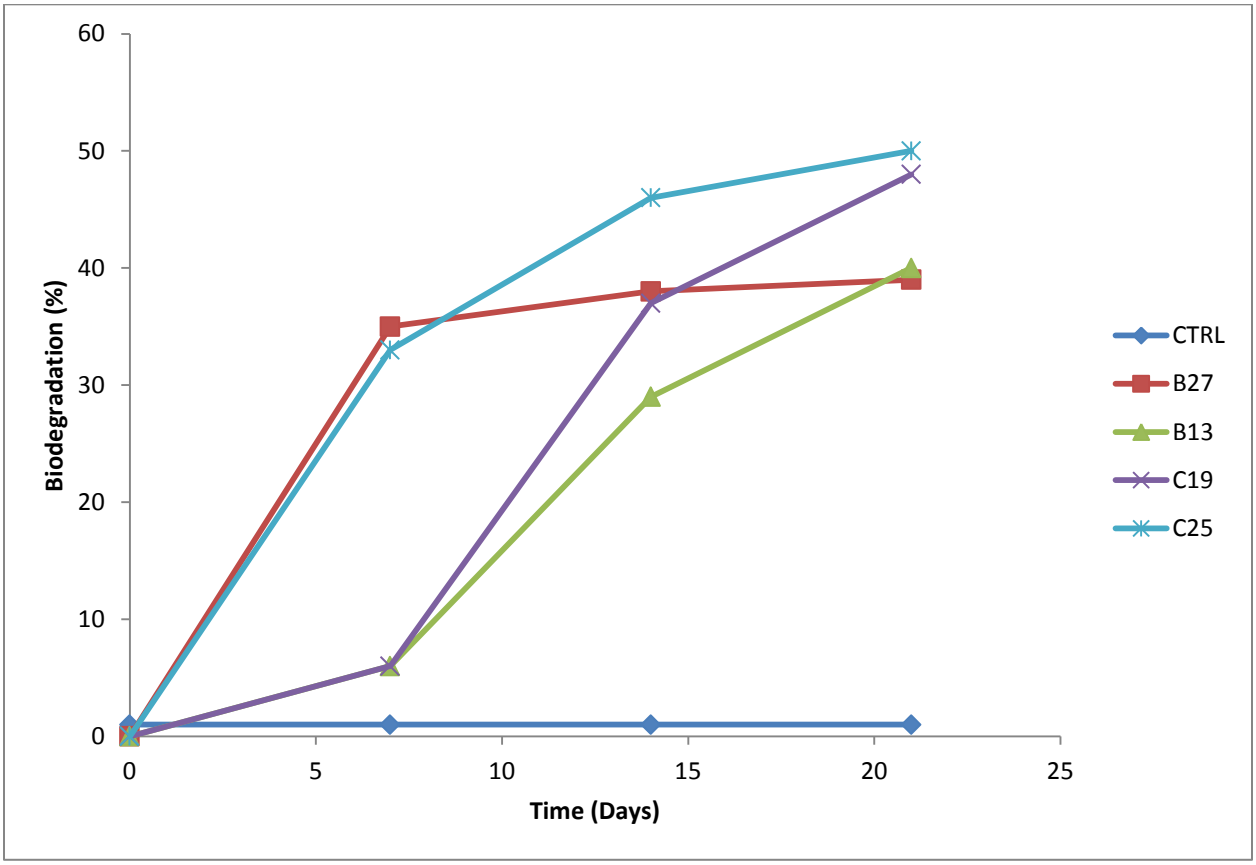

Figure 2: Biodegradation of crude oil by bacterial isolates

B27:*Klebsiella pneumoniae;* B13:*Bacillus cereus;* C19: *Pseudomonas auriginosa;* C25:*Bacillus subtilis;* A23: *Micrococcus luteus;* CTRL: Control

Discussion

It was clear from the results that all sites harbored some hydrocarbon degrading bacteria. As the depths of soil increased, the number of total aerobic heterotrophic bacteria (TAHB), total fungi (TF), hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) decreased significantly than when it was the surface of soil. The depth of the soil directly affected the number of microorganism. Results revealed that the bacterial count at a depth of 20cm declined to 55-65% when compared to a depth of 10cm. It may be due to the accumulation of aerobic microorganism to the surface soil as reported by Yousseff *et al.*, (2010) and Ogbonna, (2010). Ojo *et al.*, (2006) reported that hydrocarbon utilizers were more at depth 0 to 10cm depth than 10 to 20cm. Similarly Yousseff *et al.*, (2010) stated that oil degraders are mostly aerobes and will be more abundant in surface soil than in sub-surface sample. Microorganisms capable of hydrocarbon utilization are widely distributed in nature and have been found in areas not directly contaminated with hydrocarbon (Atlas, 1981; Yousseff *et al*., 2010). TAHB abundance patterns

were similar in sites B1 and B2, and B3, and B4 to B5, while TF abundance patterns were similar in sites B1 and B2, and B3, B4, to B5 only. It is clear that all sites shows high load of bacterial counts with B1 and B2 having higher counts. This may be due to the difference in sites receiving domestic effluents and agricultural runoff. This coincide with Syvonkiene and Micheniene, (2004) they reported that almost all natural ecosystems contain population of microorganism even if those system have not ever been exposed to oil or oil products. The results of soil sample shows that B1 and B2 had the highest average density of hydrocarbon degraders followed by B3 while the lowest average density was found in B4 and B5 (Youssef *et al*., 2010). Analysis of variance for the heterotrophic bacteria revealed that the counts between sample sites were significantly different ($P \leq 0.05$). And site B1 was observed to have the higher counts followed by B2 and B3, while B4 and B5 have the lower counts, probably due to the difference in farming and grazing activities in the sites (Ogbonna, 2010). The bacterial isolates obtained from the different sites (B1, B2, B3, B4 and B5) were members of the following genera: *Bacillus, Micrococcus, Klebsiella, Pseudomonas, Corynebacterium, Yersinia, Actinomyces, Proteus, Lactobacillus, Morganella morganii*. The Gram positive bacteria belonging to the genus *Bacillus* were mostly isolated followed by Gram negative *Pseudomonas,* which are also ubiquitous as reported by Chikere *et al*., (2009), which will be attributed to competent hydrocarbon degrading enzymes systems of the organism, its ability to form spores and emulsify crude oil. (Ijah *et al*., 2008; Chikere *et al*., 2009). The crude utilizing bacteria identified in this study have been isolated and implicated in crude oil biodegradation by several investigators (Antai, 1990; Ijah 1998; Ijah and Antai, 2003; Ajayi *et al.*, 2008). The high ability of *Bacillus* species isolated from Nigerian soil in degrading crude oil has consistently been observed in both contaminated and uncontaminated soil (Antai 1990; Ijah, 1998).

Conclusion

The result of this study revealed that hydrocarbon degrading microorganisms are abundant and widely distributed in Kukawa where crude oil exploration is underway, the diverse species of microorganisms encountered were mostly gram positive (*Bacillus subtilis*, *Micrococcus*, and *Lactobacillus*). The study also revealed that higher counts of crude oil degrading bacteria were encountered in surface layer of the soil of (0-10 cm), than the sub-surface (10-20 cm). This investigation provides information that would lead to selection of bacterial species for oil spill bioremediation of environments polluted with petroleum and petroleum products.

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