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Original Research Article

Distribution of Hydrocarbon Degrading Fungi in Soil in Kukawa, Borno State, Nigeria

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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 fungi in the soil. Total fungi (TF) on Sabraoud dextrose agar (SDA) and hydrocarbon utilizing fungi (HUF) were enumerated on Oil agar (OA). The fungi were identified using macroscopic and microscopic examinations. It was observed that the fungi (TF, and HUF) were more densely populated at 10cm depth. (TF: 1.1×10^2 – 2.4×10^2 cfu/g, and HUF: 8.0×10^4 – 11.0×10^3 cfu/g than at 20 cm depth (TF: 5.0×10^2 – 13.0×10^2 cfu/g, HUF: 3.0×10^2 – 7.0×10^2 cfu/g). The HUF was genera of *Penicillium, Aspergillus,* and *Rhizopus. Aspergillus* species were represented by *Aspergillus niger, Aspergillus umigates,* and *Aspergillus oryzae* others were *Rhizopus oryzae* and *Penicillium notatum*. While the distribution of hydrocarbon utilizing fungi and their percentage of occurrence show that *Aspergillus niger, Aspergillus oryzae, Aspergillus, umigates* and *Penicillium notatum* occurred (100%), only *Rhizopus oryzae* occurred (80%). *Aspergillus niger* and *Penicillium notatum* were able to grow luxuriantly (+++) while *Aspergillus umigates*, *Aspergillus oryzae* and *Rhizopus oryzae* grow moderate (++). The weight loss of crude oil by Fungi ranged from 25.0% to 48.3% after 21 days. Of the fungi *Penicillium notatum* and *Aspergillus niger* were more potent in utilizing the crude oil and degraded 48.3% and 44.3% of the oil after 21 days respectively.

Keywords: Degradation, Distribution, Fungi, Hydrocarbon

INTRODUCTION

Soil is a rich source of microorganisms capable of degrading hydrocarbons and residual oil (Atlas and Bartha., 1999). 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). Although hydrocarbon degraders may be expected to be readily isolated from a petroleum-polluted environment, the same degree of expectation may be anticipated for microorganisms isolated from a total unrelated environment (Warhust and Fewson, 1994; Oboh *et al*., 2006; Chikere *et al*., 2009). The rate of crude oil biodegradation in the soil seems to be rapid. This may be due to the fact that the microorganisms in the soil have efficient ability in utilizing the residual crude oil as a source of carbon and energy. Indigenous and adapted microorganisms are more efficient for biodegradation of oil pollutant (Atlas, 1981). The adapted organisms degrade oil normally, but rate of this action is critically dependent on different factors including microbial composition, contaminant type, geology of polluted site and chemical conditions at the contaminated site (Sepahi *et al.,* 2008). Yeasts and filamentous fungi all appear to be important hydrocarbon degraders. Okoh (2006) reported various genera of fungi

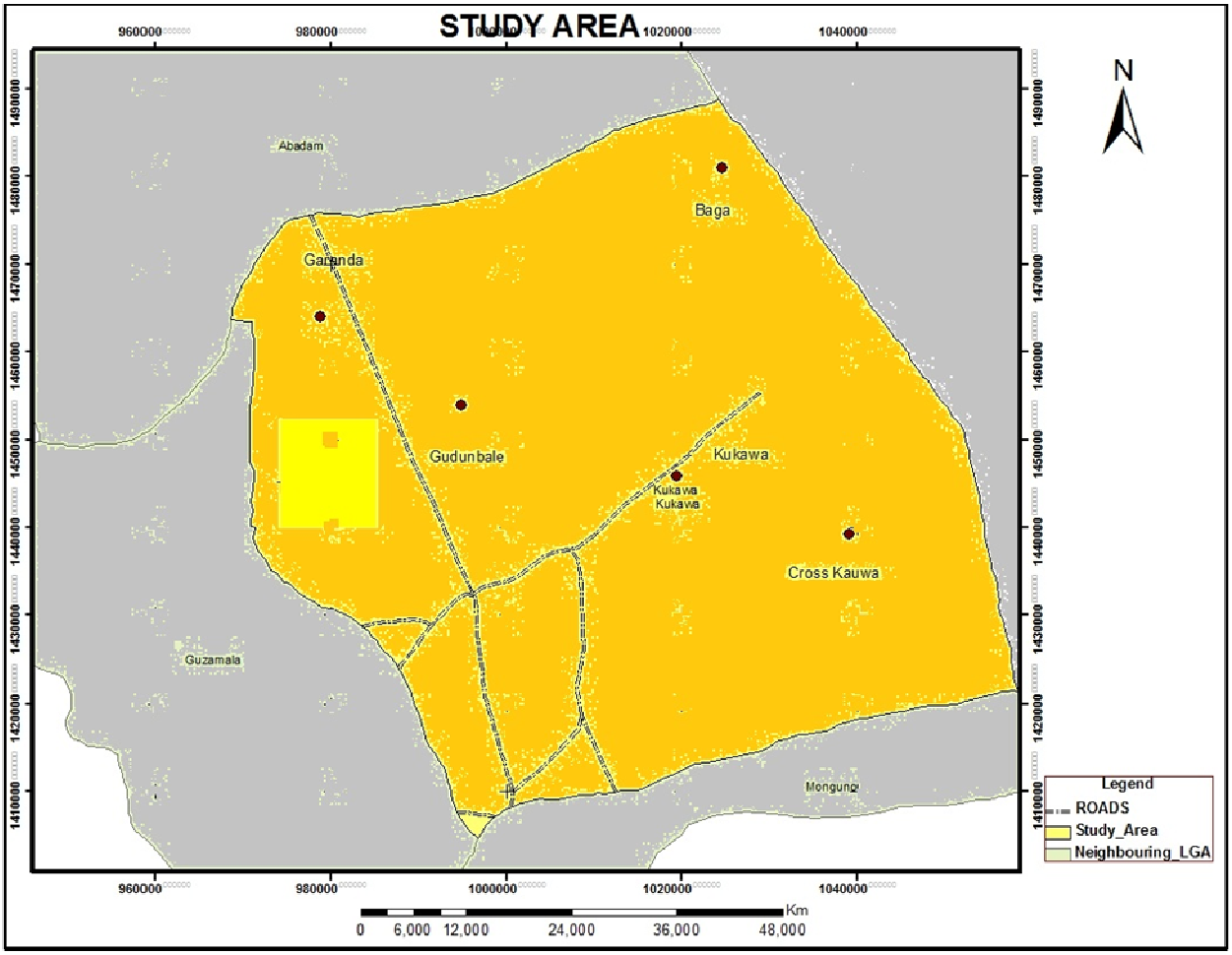

Figure 1. Map of Kukawa showing sampling site (●)

have ability to utilize hydrocarbon. They found that fungi played an important role in the hydrocarbon-oxidizing activities of the soil samples. The fungal genera most frequently isolated from soils were those producing abundant small conidia, e.g., *Penicillium* and *Verticillium* spp. *Aspergillus niger* and *Aspergillus terreus*. Saadoun *et al.* (2008) reported that the fungal species isolated were *Aspergillus umigates, Aspergillus oryzae, Aspergillus wentii, Aspergillus flavus, Aspergillus niger, Trichoderma* sp.*, Penicillum notatum, Rhizopus stolonifer and Rhodotorula* sp. Some species of fungi including, *Aspergillus* spp. And *Fusarium* sp. Capable of initiating the degradation of n-alkanes by sub terminal oxidation has been reported (Nkwelang *et al*., 2008).

Studies in Nigeria have reported the presence of hydrocarbon utilizing fungi in both crude oil contaminated and pristine environments (Nkwelang *et al.,* 2008). However, there is no literature report on the occurrence of fungi in Kukawa, Borno State. Kukawa is suspected by Nigerian National Petroleum Corporation to harbor petroleum deposits of economic value that can be explored in recent times. Hence, the fungal species capable of utilizing petroleum hydrocarbon and their effectiveness in hydrocarbon biodegradation need to be investigated.

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)

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 and Hydrocarbon Utilizing fungi

Aliquot (0.1 ml) from dilution (10^{-2}) was plated in triplicates on sterile Sabrouraud Dextrose Agar (SDA) and Oil Agar (OA) with streptomycin (50 mg/ml) to suppress bacterial growth. The SDA and OA were used for the enumeration of total fungi and crude oil utilizing fungi respectively. The plates were incubated at room temperature (30 ± 2^{0}C) for 5 days for both SDA and OA. The colonies which developed on the plates were counted and recorded as colony forming units per gram (cfu/g) of soil (American Public Health Association, 1995). Pure cultures were obtained by repeated sub culturing on fresh SDA. The pure cultures were maintained on agar slants for further characterization and identification.

Characterization and Identification of fungi

Fungal species were characterized and identified based colonial appearance and microscopic observation as described by Carlile *et al*. (2001) and Gadd *et al.* (2007).

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^{0}C) for 24 hours. One milliliter of NB grown culture (×10^6 cells) was inoculated into Mineral salt broth containing 0.5% of Escarvos light crude oil and incubated at 30^{0}C without shaking for 7 days. Turbidity of the medium was used as measure of bacterial growth.

Statistical Analysis of Data

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

RESULTS AND DISCUSSIONS

It was observed that total fungi (TF) counts in the five sites sampled, October shows count ranged from 1.1±0.6 ×10^2 - 2.4±2.4×10^2 cfu/g and from 0.6±0.0×10^1 - 1.2±1.2×10^2 cfu/g at 10cm and 20cm depths respectively. In December the counts were 1.5±1.9 ×10^2 - 2.2±0.8×10^{2}cfu/g and from 0.8±1.1×10^1 - 1.3±2.0×10^{2}cfu/g in depth 10cm and 20cm respectively. In February the counts were 1.2±2.0 ×10^2 - 2.0±0.8×10^2 cfu/g and from 0.8±0.6×10^1 - 1.4±1.7×10^2 cfu/g in depth 10cm and 20cm respectively (Table 1).

Similarly hydrocarbon utilizing fungi (HUF) counts in October at 10cm depth ranged from 0.8±0.6×10^2 - 1.3±1.4×10^3 cfu/g and from 0.5±0.3×10^2 - 0.7±0.5×10^2 cfu/g at 20cm depth. In December the counts ranged from 1.1±0.5×10^2 - 1.3±1.4×10^2 cfu/g, and from 0.4±0.6×10^3 - 0.5±1.0×10^{3}cfu/g at 10cm and 20cm depths respectively. In February the counts were 0.5±0.3×10^3 - 1.0±0.5×10^2 cfu/g and from 0.3±0.6×10^3 - 0.4±0.8×10^3 cfu/g 10cm and 20cm depths respectively (Table 2). The hydrocarbon degrading fungi isolated and identified belongs to the genera *Aspergillus, Rhizopus,* and *Penicillium. Aspergillus* were represented by *Aspergillus niger, Aspergillus fumigatus,* and *Aspergillus oryzae* others were *Rhizopus oryzae* and *Penicillium notatum* as shown in table 3. While the distribution of hydrocarbon utilizing fungi in the five sampled sites and their percentage of occurrence show that *Aspergillus niger, Aspergillus oryzae, Aspergillus, fumigatus* and *Penicillium notatum* occurred (100%) Only *Rhizopus oryzae* occurred (80%) as shown in table 4. In the five fungal species *Aspergillus niger* and *Penicillium notatum* were able to grow luxuriantly (+++) while *Aspergillus fumigatus*, *Aspergillus oryzae* and *Rhizopus oryzae* grow moderate (++) (table 5).

Table 1. Total fungi counts (cfu/g) in soil samples sites at 10cm and 20cm depths.

	Fungal counts (×10² cfu/g)					
	October		December		February	
	Depth(cm)		Depth(cm)		Depth(cm)	
Site	10	20	10	20	10	20
B1	2.1±0.6^{ab}	1.1±2.4^{abc}	1.9±2.0^{ab}	1.3±2.0^{ab}	2.0±0.8^{ab}	1.4±1.7^{a}
B2	2.4±2.4^{a}	1.0±0.6^{abc}	2.2±0.8^{ab}	1.1±0.3^{abc}	1.9±2.0^{ab}	1.4±1.7^{a}
B3	1.1±0.6^{c}	0.6±0.0^{bc}	1.5±1.9^{bc}	0.8±1.1^{abc}	1.7±0.5^{bc}	0.8±0.6^{abc}
B4	1.2±1.2^{c}	1.2±1.2^{abc}	1.5±0.6^{bc}	0.9±0.6^{abc}	1.2±1.1^{c}	1.0±3.5^{abc}
B5	1.6±1.1^{bc}	0.9±0.8^{abc}	1.7±1.3^{bc}	0.9±0.8^{abc}	1.2±1.1^{c}	0.5±1.2^{c}

Key: B1=Cross Kukawa,B2=Baga,B3=Kukawa,B4=Shuwari,B5=Ngaranda, cfu/g= Colony forming units per gramme.
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) in soil samples at 10cm and 20cm depths

	Bacterial counts (×10² cfu/g)					
	October		December		February	
			Depth(cm)		Depth(cm)	
Site	Depth(cm) 10	20	10	20	10	20
B1	1.1±0.3^{abc}	7±0.5^{a}	1.3±1.4^{ab}	5±1.0^{a}	1.0±0.5^{abcde}	4±0.8^{a}
B2	1.3±1.4^{a}	7±0.5^{a}	1.0±1.4^{abcde}	5±1.0^{a}	7±1.2^{cde}	4±0.6^{a}
B3	1.0±0.5^{abcde}	5±0.3^{a}	1.2±1.1^{abc}	4±0.6^{a}	7±0.8^{cde}	4±0.6^{a}
B4	0.8±0.6^{abcde}	6±0.5^{a}	1.1±0.5^{abcd}	5±0.8^{a}	6±0.6^{de}	3±0.6^{a}
B5	0.8±1.1^{bcde}	6±0.5^{a}	1.1±1.7^{abcd}	4±0.6^{a}	5±0.3^{e}	4±0.8^{a}

Key: B1=Cross kukawa,B2=Baga,B3=Kukawa,B4=Shuwari,B5=Ngaranda, cfu/g= Colony forming units per gramme.
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 fungi in the soil

Isolate code	Macroscopic Description	Microscopic Characteristics	Fungi
B33	Black and powdery colonies	Conidiophores terminates in vesicles, smooth walled, colourless with brownish shade	*Aspergillus niger*
B31	Bluish-green colonies	Conidiophores intermixed with aerial hyphae bearing Conidiosphores	*Aspergillus fumigatus*
B13	Black grayish colonies or Whitsh-grey brown	Sporangiosphores in group from node directly Above rhizods, colonies are fast growing	*Rhizopus oryzae*
B22	Dark green and powdery colonies	Smooth walled conidiophores Chain of single celled (ameroconidia)	*Penicillium notatum*
B11	Pale greenish yellow, powdery colonies	Long conidiophores often intermixed with aerial mycelium, Conidial radiate, pale Greenish yellow and later dull brown.	*Aspergillus oryzae*

Table 4. Distribution of hydrocarbon utilizing fungi in the sample sites and their percentage of occurrence

Isolates	Sites					
	B1	B2	B3	B4	B5	% occurrence of isolates at each site
Aspergillus fumigatus	+	+	+	+	+	100
Aspergillus niger	+	+	+	+	+	100
Aspergillus oryzae	+	+	+	+	+	100
Penicillium notatum	+	+	+	+	+	100
Rhizopus oryzae	+	+	+	+	+	80

+= Present. **-** = Absent
B1= Cross Kukawa, **B2**= Baga, **B3**= Kukawa, **B4**= Shuwari, **B5**= Ngaranda

Table 5. Growth of fungi in the crude oil medium

Fungi	Growth in crude oil medium after 7days
Aspergillus niger	+++
Aspergillus fumigatus	++
Rhizopus oryzae	++
Penicillium notatum	+++
Aspergillus oryzae	++

+++= maximum growth, ++= moderate growth, += minimum growth

DISCUSSIONS

It was clear from the results that all sites harbored hydrocarbon degrading fung, although there was variations in counts. As the depths of soil increased, the number total fungi (TF), 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 fungi. The mean total fungal counts shows that more counts of TF were observed in the month of October than the months of December and February (Table 1) Likewise HUF counts were observed to be higher in the month of 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) 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. TF abundance patterns were similar in sites B1 and B2, and B3, B4, to B5 only, the difference in count of the sites may be due to the difference in sites receiving domestic effluents and agricultural runoff which is similar to the finding of Eze and Okpokwasili, (2010) who reported that variation in counts in sites will be due to the industrial and domestic discharges to the sites.

Analysis of variance for the total fungi, and hydrocarbon utilizing fungi revealed that the counts between sample sites were significantly different ($P \leq 0.05$).

The fungi isolates were mainly *Aspergillus*, *Penicillium*, and *Rhizopus species*. These organisms have been previously implicated with petroleum product degradation (Ijah, 1998; Zhang *et al*., 2006; Kayode-isola *et al*., 2008; Abioye *et al*., 2012).

Out of the five fungi isolated (*Aspergillus niger, Aspergillus fumigatus, Penicillium notatum. Aspergillus oryzae,* and *Rhizopus oryzae*). (*Aspergillus niger*, and *Penicillium notatum*.) utilize hydrocarbon luxuriantly , while (*Aspergillus fumigatus, Aspergillus oryzae, and Rhizopus oryzae*) exhibited moderate ability in degrading the oil. This means that; these micro-organisms use the hydrocarbon as a source of carbon and energy. The variation in the capacity of the isolates to utilize hydrocarbon could be due to differences in the competence of crude oil degrading enzyme systems.

CONCLUSION

The study revealed that hydrocarbon utilizing fungi are abundant and widely distributed in Kukawa where crude oil exploration is underway, the diverse species of *Aspergillus niger, Aspergillus fumigatus, Penicillium notatum. Aspergillus oryzae,* and *Rhizopus oryzae.*

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