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Research Article

Isolation and Characterization of Microorganisms from Oil Polluted Soil in *Kwata*, Awka South, Nigeria

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Abstract

Screening of hydrocarbon degrading microorganisms from oil contaminated soil was studied and the samples were obtained from three Mechanical Workshops in Awka, Anambra State. The microorganisms were isolated using the pour plate method by selective enrichment technique. All samples were cultured in solid media with crude oil as a sole carbon and energy source to isolate hydrocarbon utilizers from the sample collected from different mechanic workshop. The isolate were identified on the basis of their colonial and morphological characteristics. Bacterial strains capable of degrading hydrocarbons belonging to the genera *Bacillus spp*, *Micrococcus spp* ,*Corynebacterium spp* and *Pseudomonas spp;* as well as five genera of fungi, *Penicillin spp, Aspergillus spp, Rhizopus spp, Saccharomyces spp* and *Fusarium spp* were isolated and identified. The colony forming unit per ml ranges from 0.22x 10^7 cfu/ml and 2.83 x 10^7 cfu/ml for the total heterogenous bacteria and 0.33x10^7 cfu/ml and 3 x 10^7 cfu/ml. The ability of the organism to survive is associated with the ability of the organism to use the crude oil as the sole carbon source. Some organism isolated from the total heterogenous bacteria and fungi were not able to grow in the media with the crude oil. These include bacteria genera *Aeromonas and Staphylococcus* and the fungi genera *Cladosporium, Mucor, Geotrichium and Trichophyton.* Some factors were seen that can affect the growth of these microorganisms, which include, temperature, nutrients and oxygen. The importance of these isolates for the bioremediation of oil polluted soil including the aerobic degradation of hydrocarbon and anaerobic degradation of hydrocarbon which is of great importance for control of hydrocarbon pollution in Nigeria was ascertained.

Keywords: soil; microorganism; oil pollution; biodegradation

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Introduction

Petroleum based products are the major source of energy for industries and daily living. It is at present Nigerians and indeed, the world's most important derived energy source [1]. However, the growth and activities of petroleum and petroleum associated industries in Nigeria and other parts of the world had led to increase in oil pollution in our environment. Leaks and accidental spills occur regularly during the exploration, production, refining, transport and storage of petroleum and petroleum product. The amount of natural crude oil seepage was estimated to be 600000 metric tons per year with a range of uncertainty of 200000 metric tons per year [2]. Petroleum in its natural state is called crude oil. Crude oil, because of its characteristics, is one of the most significant pollutants in the environment as it is capable of causing serious damages to human and the ecosystem [3]. In this context, pollution is defined as the addition to any segment of the environment, any material which has detrimental effect on the ecosystem [4].The greatest single environmental problem connected with crude oil exploration in Nigeria is oil spillage both onshore and offshore. The rate of oil spillage reported in the country has been rising with a corresponding increase in petroleum production. Only a single spill was reported in 1970, when as in 2001 the number short up to 14. In 2003, 105 spills were reported, 154 in 2004 and 216 in 2005 (Nigeria Environmental study Action Team 2006). It is known that greater degradation of oil pollutant is carried out in situ by a consortium of microorganism and more than 200 species of bacteria and fungi and even biodegrade hydrocarbons [5]. The various genera that have been reported to contain hydrocarbon degrading species include *Pseudomonas, Vibrio, Corynebacterium, Arthrobacter, Brevibacterium, Flavobacterium, Sporobolomyces, Achromobacter, Bacillus, Aeromonas, Thiobacillus, Lactobacter, Staphylococcus, Penicillium And Articulosporium.* These organisms has been isolated in large numbers from many oil polluted water and soil but are found in less number in uncontaminated environment [6]. The process of bioremediation is defined as the use of microorganisms to detoxify or remove pollutants [7]. In addition, bioremediation technology is believed to be non-invasive and relatively cost effective [8]. By nature, populations of micro-organisms represent one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment and is cheaper than other technologies [9,10].

The principle by which a consortium of microorganisms act to bring about the oxidation of complex compound is known as co-metabolism. This principle is employed by oil companies in Nigeria to remediate oil polluted sites in a process known as remediation by enhanced natural attenuation (RENA) this is because the microbial degradative mechanism appear to be a natural processes which eliminate the bulk of oil pollutant after initial physical and chemical breakdown has occurred. The success of oil spill bioremediation depends on one's ability to establish and maintain condition that favors enhanced oil biodegradation rate in the contaminated environment. Numerous scientific articles have covered various factors that influence the rate of biodegradation. One important requirement is the presence of microorganisms with the appropriate metabolic capabilities, if these microorganisms are present, then optimal rate of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentration of nutrient and oxygen is present and that the pH is between 6 and 9. The ability of microbes to degrade organic contaminants into harmless constituents has been explored as a means to biologically treat contaminated environments. It is the subject of many research investigations and real-world applications and is the basis for the emergent field of bioremediation. The physical and chemical characteristics of the oil and the oil surface area are also important determinants of bioremediation success. There are two main approaches to oil spill bioremediation (a) bio-augumentation in

which known oil degrading bacteria are added to supplement the existing microbial population and (b) bio-stimulation in which the growth of indigenous oil degraders is stimulated by the addition of nutrient and other growth limiting co-substrate.

The objective of this study is to identify and characterize micro-organisms in oil polluted soil which are capable of degrading hydrocarbon in Awka Metropolis.

Materials and methods

Sample Collection

Soil samples were obtained from an Oil Polluted, Mechanic Workshop in *Kwata*, Awka, Anambra state, Nigeria. The samples were aseptically collected using soil auger to a depth of 2-5cm, stored in sterile aluminum foils and transported to the laboratory within 24 hours of collection. The polluted site was cleared and divided into 3 portions which were labeled A, B and C, respectively. The spill site was cleared so as to allow for easy access to the site.

Enumeration of Total Heterotrophic Bacteria and Fungi in the Soil Samples.

10 g of each sample was measured into a conical flask and 90ml of distilled water was mixed with the sample. This was placed in a laboratory shaker (S150) for 3 hours to homogenize the solution and this served as the stock solution. Ten fold serial dilution of all the homogenized mixture was carried out using sterile distilled water as diluents. Seven test tubes containing 9ml of distilled water was used for the serial dilution.

Inoculation of culture medium

One milliliter aliquot from dilutions of 10^{-2} and 10^{-3} were plated in duplicates on sterile Sabouraud Dextrose Agar plates while dilutions of 10^{-4} and 10^{-5} were plated in duplicates on sterile Nutrient Agar plates, using the pour plate technique. Incubation was carried out at 37 ℃ for 7 days for the Sabouraud Dextrose Agar plates and 37 ℃ for 24 h for the Nutrient Agar plates. Colonies on the plates were enumerated according to the [11].

Enumeration of Hydrocarbon-Utilizing Bacteria and Fungi

1ml each of homogenized samples were diluted serially (10^{-1} to 10^{-7}). 0.1 milliliter from dilutions of 10^{-2} to 10^{-5} were plated in duplicates on Mineral Salt Agar using the spread plate technique. The composition of the medium was (Nacl, 2.0g, $MgSO_4.7h_20$, 0.1g, K_2HPO_4, 0.3g, KH_2PO_4 0.2g, $NaNO_3$, 0.1g, Agar-Agar, 4.0g, distilled water, 200ml). For the 10^{-2} and 10^{-3} dilutions, 0.5 ml of Chloramphenicol was added to the mineral salt agar to suppress bacterial growth and also Nystatin was added to the Mineral Salt Agar for the 10^{-4} and 10^{-5} dilutions.

A filter paper saturated with sterile crude oil was aseptically placed on the inside of the inverted Petri dishes and the culture plates were incubated for 14 days at 37 ℃ for fungi and 14 days at 37 ℃ for bacteria. Plates yielding 30 to 300 colonies were afterwards enumerated for bacterial isolates. Plates with fungal colonies were also enumerated.

Isolation and Characterization of Heterogenous Bacteria and Fungi

Colonies of different heterogenous bacteria and fungi were picked randomly using a sterile inoculating wire loop and purified by repeated subculture technique. The plates were incubated at 37 ℃ for 24 h and at room temperature for 3 days, respectively to obtain pure colonies. All the isolates were characterized using the techniques of [12-14].

Isolation and Characterization of Hydrocarbon-Utilizing Bacteria and Fungi

Colonies of different hydrocarbon utilizing bacteria and fungi were picked randomly using a sterile inoculating wire loop and purified by repeated subculture technique. The plates were incubated at 30 ℃ for 24 h and at room temperature for 3 days, respectively to obtain pure colonies. All the isolates were characterized on the basis of their colony morphology and microscopic examination

Moisture Content Determination (AOAC)

A known amount of soil sample was placed in a weighed crucible and dried at 105 ℃ in the oven until a constant weight was reached. From the difference in weight, the percentage moisture content was calculated [15].

$$\text{Formula} = \frac{W1-W2}{W1} \times 100$$

Where:

W1= Initial weight of soil sample before drying

W2= Final weight of soil sample after drying

Determination of percentage occurrence of the fungal isolates

This was done to determine the incidence of occurrence of the different fungal isolates. The frequency of occurrence of the isolates from the soil was determined.

The total number of each isolate in the soil sample was obtained against the total number of all the isolates in the sample screened. The mean value of this yielded the percentage of occurrence as the following equation shows:

% of occurrence = X/N × 100; where X = total number of each isolate in the sample and N = total number of all the isolates in the sample.

Identification of isolated fungi The identification of the isolated fungi was done both macroscopically and microscopically. The gross morphology of the fungal growth on plates was studied including their colors. Small portions of the fungal pure culture were teased and mounted in lactophenol cotton blue stain on a clean grease-free glass slide and covered with a clean cover slip and observed under the microscope. Referencing to the Manual of Fungi Atlas (watanabe 2002) [21].

The identities of the fungi were certified using cultural method as well as comparing them with confirmed representative identified by means of keys [16, 17]. The incidence of occurrence of each fungus was calculated using a formula [17, 18].

Identification of isolated bacteria

The bacterial isolates after obtaining pure culture was further subjected to biochemical test (Gram staining reactions, Coagulase test, Oxidase test, Spore test, Motility test, IMVC (Indole test, Methyl red test, Vogues

proskauer test, Citrate utilization test), Sugar fermentation test (Sucrose, Lactose And Glucose. The bacterial isolates were also identified by comparing their characteristics with those of known taxa, as described by [19,20].

Results

Microbial Counts and Identification

The hydrocarbon utilizing microbial isolates encountered were five fungi, *Penicillium spp, Aspergillus spp, Rhizopus spp, Saccharomyces spp* and *Fusarium spp*, as well as four genera of bacteria, *Bacillus spp, Micrococcus spp ,Corynebacterium spp* and *Pseudomonas spp* were isolated and identified. *Bacillus* spp and *Aspergillus spp* were more frequently isolated among the bacteria and fungi respectively. The counts of the total heterotrophic bacteria (THB) in crude oil polluted soil ranged from $0.22x10^7$ cfu/ml to $2.83x10^7$ cfu/ml, the counts ranged from $0.33x10^7$ cfu/ml to $3x10^7$ cfu/ml for the Hydrocarbon Utilizing Bacteria.

Table 1 Colonial and Morphological Characteristics of the Heterogenous Fungi

Isolates	Colonial Morphology	Morphological Characteristics	Organism
1	Yellow at first but quickly becoming brown to yellow green with radial grooves cottony and powdery colony	Conidia heads are large, globose and dark brown hyaline hyphae and septate	*Aspergillus spp*
2	Yellow at fist with white edges and flat periphery rapidly becoming dark green	Conidiophores with dark hyphae, non septate conidiophores with sparsely branching long chains of conidia	*Cladosprium spp*
3	Black colony with white edges, creamy underside, powdery and flat	Conidia heads are large, globose and dark brown conidiophores	*Aspergillus spp*
4	Creamy colonies that are smooth ,convex and opaque with a yeasty odour	Budding, spherical to elongated cells forming pseudomycelium	*Candida spp*
5	White cottony colony with white underside, mucoid and rapidly filling the plate	Single and branching spora ngiophores that at the tip have a round sporangium filled with sporangiospores	*Mucor spp*
6	White colony at first, rapidly becoming blackish-grey, rapidly filling the plate	Erect sporangiophores term inated by dark sporangia and sporangiospores	*Rhizopus oryzae*
7	Wooly white colony with orange spots, rapidly filling the plate and produces spores, rhizoid and umbonate	Non septate hyphae, sporran- giospores are ovoid in shape and are directly opposite the branched rhizoid	*Rhizopus spp*
8	White spongy colony with creamy underside, filament ous and slowly growing not filling the plate	Septate hyphae that produce numerous arthroconidia that are rectangle in shape	*Geotrichium spp*
9	White powdery colony, flat, filamentous and yellow underside	Non septate hyphae with microconidia.	*Trichophyton spp*

Isolation and identification of fungi

The fungi isolated from the soil of both heterogenous and hydrocarbon utilizing samples showed that lesser species of fungi were isolated from the hydrocarbon utilizing sample than the heterogenous sample. These included: *Aspergillus niger, Penicillium spp., Aspergillus spp., Rhizopus spp., Saccharomyces spp.* and *Fusarium spp.*, while the heterogenous isolates include *Aspergillus spp., Cladosporium spp., Candida spp., Mucor spp., Rhizopus oryzae, Rhizopus spp., Geotrichium spp.* and *Trichophyton spp.* The morphological characterization of the isolated fungi from the soil is shown in Tables 1, 2.

Table 2 Colonial and Morphological Characteristics of the Hydrocarbon- utilizing Fungi

Isolates	Colonial Morphology	Morphological Characteristics	Organism
1	White colony with a raised center, having a black dot at the center. Slow growing with flat periphery	Branched septate hyphae with hyaline conidiophores, conidia are globose	*Penicillium* spp
2	White cottony colony rapidly filling the plate, producing spores.	Non septate hyphae with branched sporangiophore, dark sporangia and sporangiospores	*Rhizopus* spp
3	Dirty-yellow to brown colony having a yellow periphery followed by a white periphe ry and creamy underside.	Large globose sporangia head with dark sporangiospores	*Aspergillus* spp
4	White colony with a raised center, which is white to pinkish and white underside	Intertwined, septate hyphae, branching sporangiophore and dark sporangiospores	Un-identified
5	Dense white budding colony seperated by a brown line.	Non septate hyphae with large sporangia heads having numerous sporangiospores	*Saccharomyces* spp
6	Brown-yellow colony with raised center and a flat white pheriphery followed by a yellow edge then a white edge	Micro-conidia are ovoid in shape borne on phialides on branched conidiophores with septate hyphae	*Fusarium* spp
7	Filamentous brown colony with yellow periphery and white edges	Dark sporangia with numerous sporagiospores, non septate hyphae	*Aspergillus* spp

Isolation and identification of bacteria

The bacteria isolated from the soil of both heterogenous and hydrocarbon utilizing samples also showed that lesser species of bacteria were isolated from the hydrocarbon utilizing sample than the heterogenous sample. These included: *Corynebacterium spp, Pseudomonas spp, Micrococcus spp,* and *Bacillus spp.* While the isolated bacteria from heterogenous sample included *Corynebacterium spp, Pseudomonas spp, Aeromonas spp , Staphylococcus spp, Micrococcus spp, and Bacillus spp*. The incidence of *Corynebacterium spp,* was highest in both samples, with 25% in the heterogenous sample and 33.3% in the hydrocarbon utilizing sample, and 25% in the THB.

Frequency of occurrence of microorganism

For the bacteria isolates, *Bacillus and Corynebacterium* occurred more frequently in both the heterogenous bacteria and hydrocarbon utilizing bacteria (Tables 3,4) while for the fungi isolates, *Aspergillus and Rhizopus* (Tables 1, 2) were predominant in both the heterogenous and hydrocarbon utilizing bacteria. The *Aspergillu spp* occurred mostly in all the samples likewise the corynebacterium as shown in Tables 1-4.

Table 3 Biochemical Results of the Heterogenous Bacteria

Samples	Gram= reaction	Spore stain	Catalase	Coagulase	Oxidase	Indole	Motility	Methyl red	Vogues	Citrate	Urease	Glucose	Lactose	Sucrose	Suspected organism
$3A^5$	Gram negative rod	-	+	-	+	+	+	-	-	+	+	AG	A	A	*Aeromonas spp.*
$3A^5$	Gram positive cocci in cluster	-	+	+	-	+	-	-	-	+	-	A	A	AG	*Staphylococcus spp.*
$1B^4$	Gram positive Rod in luster	-	+	+	-	+	-	-	-	+	+	AG	AG	AG	*Corynebacterium spp.*
$1A^5$	Gram positive Rod in V shape	-	+	+	+	+	-	-	-	+	+	AG	AG	A	*Corynebacterium spp.*
$2B^5$	Gram negative rod and Positive cocci	+	+	-	+	+	+	-	-	+	+	A	A	A	*Aeromonas spp.*
$3B^5$	Gram positive cocci cluster	-	+	-	+	+	-	-	-	+	-	A	G	-	*Micrococcus spp.*
$2B^4$	Gram positive rod single	+	+	+	+	+	-	-	-	+	+	-	-	-	*Bacillus spp.*
$3B^4$	Gram negative short rod	_	+	-	+	+	+	-	-	-	+	-	-	-	*Pseudomonas spp.*

(+) = positive; (-) =negative; A= Acid Production; G= Gas Production; AG= Acid and Gas Production

Hydrocarbon utilizing microorganisms

All the soil samples studied contains hydrocarbon utilizing microorganisms which includes both fungi and bacteria isolates and no of occurrence as shown in Table 4 and Table 2 below. The microorganisms isolated during this study are the same with those reported previously [6]. The action of the microorganisms is in accordance with [9].

Effect on hydrocarbon on the moisture content of soil

The moisture content of crude oil polluted soil was low. This may be due to the fact that crude oil can coat the

soil and consequently prevent the penetration of water compared to kerosene that was used in their study as reported by [22]. The weight of the soil reduced from 20gram to 18.9gram after drying in an hot air oven hence the moisture content is 5.5%.

Table 4 Biochemical Results of the Hydrocarbon Utilizing Bacteria

Samoles	Gram reaction	Spore stain	Catalase	Coagulase	Oxidase	Indole	Motility	Methyl red	Vogues	Citrate		Glucose	Lactose	Sucrose	Suspected organism
$1C^5$	Gram negative Rod in single & cluster	-	+	-	+	+	+	+	-	-	+	-	-	AG	*Pseudomonas spp*
$1D^4$	Gram positive Rod	+	+	+	+	-	+	+	-	+	+	AG	AG	AG	*Bacillus Spp*
$3D^4$	Gram positive cocci in cluster	-	+	-	+	-	-	+	-	-	+	AG	AG	AG	*Micrococcus spp*
$3C^4$	Gram positive Rod in charin	+	+	-	+	-	-	+	-	+	+	AG	AG	AG	*Corynebacterium spp*
$2D^4$	Gram Positive Rod in cluster	+	+	-	+	-	-	+	-	+	+	A	AG	AG	*Bacillus spp*
$2C^4$	Gram positive cocci in single & cluster	+	+	-	+	-	+	+	-	+	+	AG	AG	AG	*Corynebacterium spp*

(+) = positive; (-) =negative; A= Acid Production; G= Gas Production; AG= Acid and Gas Production

Table 5 Colonial and Morphological Characteristics of the Heterogonous Bacteria

Isolates	Colonial Characteristics	Identity	No. of Colonies	Size	Cfu/ml(10^7)
$3A^5$	Creamy, circular, smooth raised convex, entire and opaque and mucord	*Staphylococcus* spp.	233	2mm	1.79
$3A^5$	White, circular, coarse raised, convex, entire and opaque	*Staphylococcus* spp.	233	2mm	1.79
$1B^4$	Creamy yellow, irregular, rough, flat, undulate, mucoid, slimy & translucent	*Corynebacterium* spp.	222	1mm	0.22
$1A^5$	White, circular,, slimy, dry, convex, entire, raised and opaque	*Corynebacterium* spp.	283	2mm	2.83
$2B^5$	Brown, Irregular, Coarse, flat, convex, entire & Opaque	*Aeromonas* spp.	231	1mm	2.31
$3B^5$	Pink, Irregular, Shiny, mucoid, raised, convex, entire and translucent	*Micrococcus* spp	73	2mm	1.79
$2B^4$	Milkish, circular, coarse, flat, convex, entire and opaque	*Bacillus* spp.	220	Punctiform	0.22
$3B^4$	Brown, circular, mucoid, flat, convex, entire and translucent	*Pseudomonas* spp.	45	Punctiform	0.45

Table 6 Colony and Morphological Characteristics of the Hydrocarbon Utilizing Bacteria

Samples	Colonial Characteristics	Identity	No. of Colony	Size	Cfu/ml (10^7)
$1C^5$	Creamy, irregular, smooth shiny, flat, convex, entire and opaque	*Pseudomonas* spp.	30	2mm	3
$1D^4$	White, circular, smooth, shiny, glistering, mucoid, raised, convex, entire and opaque	*Bacillus* spp.	60	2mm	0.6
$3D^4$	Yellow-brown, circular, dry, rough, dull, flat, convex, entire and opaque	*Micrococcus* spp.	32	Punctiform	0.33
$3C^4$	Yellow, circular, smooth, mucoid, raised, convex, entire and opaque	*Corynebacterium* spp.	35	Punctiform	0.33
$2D^4$	Pink, circular, smooth, raised, convex, entire and opaque	*Bacillus* spp.	36	1mm	0.38
$2C^4$	White, circular, smooth, mucoid, raised, convex, entire and opaque	*Corynebacterium* spp.	40	1mm	0.38

Discussion

The present study has revealed however, that the number of Hydrocarbon Utilizing Bacteria (HUB) in crude oil polluted soil were higher than those of the non utilizing bacteria. The reason for higher counts in crude oil polluted soil may be due to the presence of residual crude oil in the polluted soil which boosts its carbon supply, hence favor the growth of the hydrocarbon utilizing bacteria as compared to crude oil free soil [22].

The bacterial counts in the polluted soil were higher than the fungal counts in the soil and the hydrocarbon utilizing fungi (HUF) in the polluted soil was higher than that of the non utilizing fungi soil. The higher counts of bacteria compared to fungi may be as a result of the nutrient status of the soil [23] and the presence of some toxic components which do not favor fungal growth [24].

Table 5, shows the colonial and morphological characteristics of the heterogonous bacteria. The heterogenous bacteria isolated include *Aeromonas* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Micrococcus* spp., *Bacillus* spp. and *Pseudomonas* spp. The samples used were three in number and a total of 6 bacteria species were obtained from the heterogeneous bacteria which includes *Corynebacterium spp, Pseudomonas spp, Aeromonas spp, Staphylococcus spp, Micrococcus spp, and Bacillus spp*. The total count ranged from $0.22x10^7$ to $2.83x10^7$. Also a total of 4 bacteria species were isolated from the hydrocarbon utilizing bacteria which includes *Corynebacterium spp, Pseudomonas spp, Micrococcus spp,* and *Bacillus spp.* Having a count ranging from $0.33x10^7$ to $3 x10^7$ (Table 6). The heterogenous fungi isolated is given in Table 1 which include *Aspergillus spp, Cladosporium spp, Candida spp, Mucor spp, Rhizopus oryzae, Rhizopus spp, Geotrichium spp* and *Trichophyton spp* The hydrocarbon utilizing fungi is shown in Table 2 which include *Penicillin spp, Aspergillus spp, Rhizopus spp, Saccharomyces spp* and *Fusarium spp.* Also the percentage frequency of occurrence is shown in Table 7 and 8

However, the moisture content of crude oil polluted soil was low. This may be due to the fact that crude oil

can coat the soil and consequently prevent the penetration of water compared to kerosene that was used in their study. The weight of the soil reduced from 20gram to 18.9gram after drying in an hot air oven hence the moisture content is 5.5%.

For the bacteria isolates, *Bacillus* and *Corynebacterium* occurred more frequently in both the heterogenous bacteria and hydrocarbon utilizing bacteria (Tables 3 and 4) while for the fungal isolates, *Aspergillus* and *Rhizopus* (Tables 1 and 2) were predominant in both the heterogenous and hydrocarbon utilizing bacteria. The *Aspergillus* spp., occurred mostly in all the samples likewise the *Corynebacterium* as shown in Tables 1-4.

Table 7: Percentage Occurrence of Heterogenous Bacteria and Hydrocarbon-utilizing Bacteria

Bacteria isolated	% Occurrence in THB	% Occurrence for HUB
Aeromonas	25	not isolated
Staphylococcus	12.5	not isolated
Corynebacterium	25	33.3%
Micrococcus	12.5	16.7%
Bacillus	12.5	33.3%
Pseudomonas	12.5	16.7%

Table 8 Percentage Occurrence of Heterogenous and Hydrocarbon-utilizing Bacteria.

Fungal Isolates	% Occurrence in THF	% Occurrence of HUF
Aspergilus spp	22.2%	28.6%
Cladosporium spp	11.1%	not isolated
Candida spp	11.1%	not isolated
Mucor spp	11.1%	not isolated
Rhizopus	22.2%	14.3%
Geotrichium	11.1%	not isolated
Trichophyton	11.1%	not isolated
Penicillium spp	not isolated	14.3%
Saccharomyces spp	not isolated	14.3%
Fusarium spp	not isolated	14.3%
Un-identified	not isolated	14.3%

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[26].

Crude oil contains hydrocarbon and does not resist attack by microorganisms [25]. The hydrocarbon utilizing microorganisms isolated from the soil were species of *Corynebacterium, Bacillus, Pseudomonas* and *Micrococcus. Bacillus* species 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.

Since this organisms are able to utilize the crude oil as their sole carbon source, the can be used for bioremediation of polluted sites.

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Again, this work has shown that the frequency of occurrence heterogenous and hydrocarbon utilizing micro organisms from the crude oil polluted soil ranged from 6 to 8 for bacteria and 7 to 9 for fungi, connoting that fungi are better crude oil utilizing microbes than bacteria (Fig. 1). Meanwhile, Figs 2 and 3, showed the Mean percentage occurrence of bacteria isolated from the crude oil polluted soil and the Mean percentage occurrence of fungi isolated from the crude oil polluted soil.

Since this organisms are able to utilize the crude oil as their sole carbon source, the can be used for bioremediation of polluted sites.

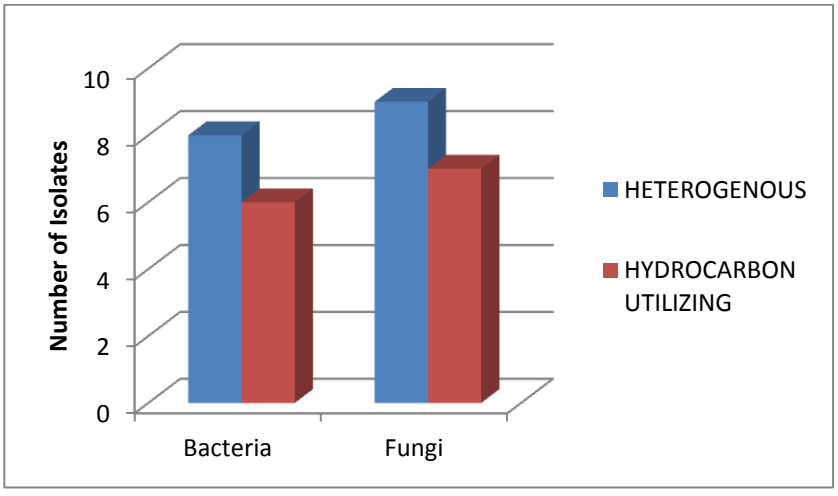

Fig. 1 Frequency of Occurrence of Heterogenous and Hydrocarbon Utilizing Micro organisms.

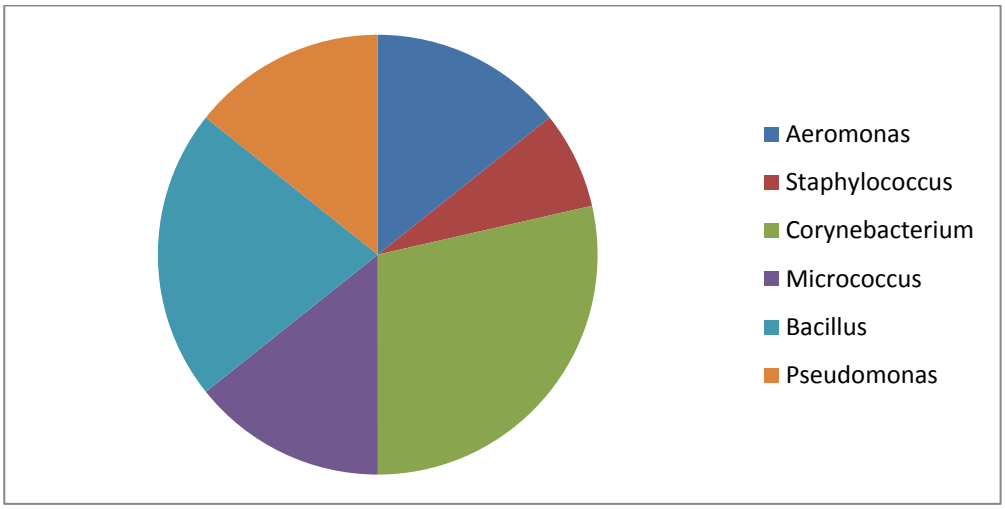

Fig. 2 Mean Percentage Occurrence of Bacteria Isolated from the Crude oil Polluted Soil

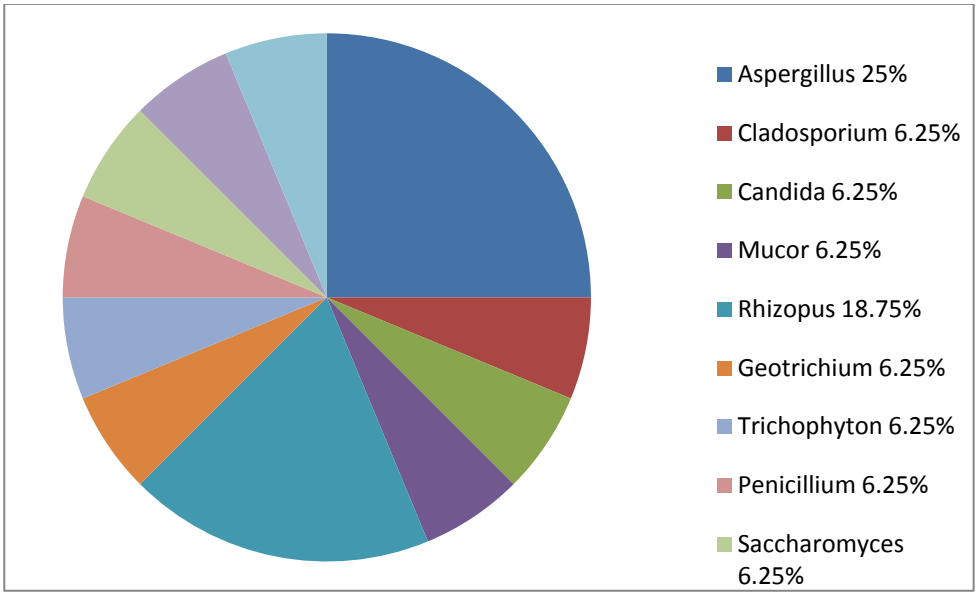

Fig. 3 Mean Percentage Occurrence of Fungi Isolated from the Crude oil Polluted Soil

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