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# PREVALENCE OF OIL-DEGRADING MICROBES IN SOME FILLING STATIONS IN THE LAGOS METROPOLIS

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## ABSTRACT

Soil samples were collected aseptically from twelve different oil contaminated sites (filling stations) in Lagos, Nigeria. Serial dilution of the samples was carried out and the aliquots inoculated into mineral salt medium with 1% agar impregnated with crude oil as carbon source for oil degrading bacteria, nutrient agar for heterotrophs and Knight's mineral salt medium enriched with engine oil for the isolation of Actinomycetes. The bacteria and Actinomycetes were identified by standard microbiological techniques and six genera of bacteria were recovered which included Pseudomonas, Alcaligenes, Micrococcus Flavobacterium, Acinetobacter and Proteus. Three Actinomycetes species which were Nocardia spp., Micromonospora spp. and Streptomyces spp. were also recovered. There was a high bacterial load with heterotrophs having 2.87 x 107 cfu/g and relatively low load of oil degrading bacteria of 7.22 x 106 cfu/g (approximately 4:1). The mean total bacteria count was 4.87x107 cfu/g while the total fungal count was 2.99x107 cfu/g. A consortium of bacteria were found to degrade each petroleum product with some individual bacteria degrading more than one product. The bacterial isolates exhibited high motility which was observed to play a vital role in the biodegradability and metabolic versatility of the bacteria. Pseudomonas was isolated from 60% of the samples examined followed by Flavobaterium spp. (42%) and Proteus spp from 31%. These bacteria were the most prevalent and utilized all petroleum products. Nocardia spp demonstrated similar trait while Micromonospora spp and Streptomyces spp. were slow oil-degraders. The bacteria and Actinomycetes utilized petrol, diesel and kerosene thus are likely to be useful in bioremediation and other environmental control if improved upon through genetic engineering so as to optimise their biodegradability potentials.

## INTRODUCTION

The discovery of crude oil in commercial quantity in 1956 and its exploitation with first export in 1958 is probably the best thing that heralded the Nigerian State as the "giant" of Africa at Independent in 1960. Crude oil therefore became the pivot on which the economy of the nation rotates (SPDC, 2000). Exploration and exploitation of oil have enormous ecological impact on the environment and the people, yet little or no provision has been made to cater for such by the multi-national oil companies and government in its three tiers.

Pollution of the environment due to accidental seepage, rupture of pipelines, blowout of terrestrial oil wells and sabotage are commonly reported especially in Niger Delta (Awobajo, 1983). The resulting spillage has brought huge economic loses as well as contamination of both aquatic and terrestrial ecosystems. No matter the source of contamination, some oil or oil products may reach ground water reserves or lakes providing water for domestic uses. Apart from the possible hazard to health, such contamination is objectionable because of the concentrations at which hydrocarbon arise (Hill and Greener, 1980).

Oil degrading microorganisms are ubiquitous and they naturally biodegrade numerous contaminating petroleum hydrocarbons, thereby cleansing such environment of pollutants (Atlas, 1995). According to Amund et al (1989), oil degrading microbes are microorganisms capable of breaking down hydrocarbon and use the hydrocarbon as energy source. The constituents of petroleum products are naturally occurring chemicals hence the indigenous microbial population often contains microbes capable of degrading such substances (Alexander , 1977, Atlas, 1995).

All groups of microorganisms are common in the soil but bacteria, actinomycetes and fungi make the major contribution to mineralization which may significantly according to the substrate available and the physico-chemical property of the soil.

The availability of molecular oxygen can have a profound effect but the balance of activity is also strongly influenced by temperature, pH and the presence of other nutrients, of which the most important inorganic nutrients are nitrogen

and phosphorus (Dibble and Barha, 1979) Jensen (1975) reported that the soil ecosystem is endowed with an immense versatility as regards degradation and humification of organic substances. Many microorganisms processes the enzymatic capability to degrade petroleum hydrocarbon which varies between straight chain Alkane, branched chain and the aromatics (Atlas, 1995) . The purpose of the present study was to isolate and characterise bacteria and Actromycetes involved in the degradation of some petroleum products at oil polluted soil sites, and use the microbial isolate to degrade some hydrocarbons under laboratory conditions.

## MATERIALS AND METHODS

### COLLECTION OF SAMPLES

Oil contaminated soil samples were collected from twelve different filling stations (location) in Lagos into sterile screw capped bottles at the discharge units using sterile spatula. Samples were collected from four locations between Alakija and Volkswagen bus stops along Badagry Expressway, four locations between L.A.S.U. and Agbara, four locations at Egbeda and the last four locations between Festac First Gate and Oluti bus stops. The samples were collected from three different sources in each filling station i.e (i) Petrol (ii) Diesel and Kerosene. They were all transported to the laboratory promptly for microbiological analysis.

### DETERMINATION OF MICROBIAL POPULATION IN THE SOIL

About 1g of each soil sample from each source was weighed and added to 9 ml of sterile distilled water in test tubes. It was shaken vigorously and allowed to stand. Serial dilutions were made from each stock up to 10-6. An aliquot of 0.1ml of each of the 10-4 and 10-5 dilutions (suspensions) were taken and analysed by spread plating on nutrient agar for heterotrophic count nutrient agar with 1mcg/100ml of fungizone for bacterial counts, potato dextrose agar for fungi, minimal salt agar with motor oil as sole carbon source for oil degrading bacteria and Knight's minimal salt agar of pH 7.2 for Actinomyetes incubated at room temperature for four days. A loopful of the 10-4 and 10-5 suspensions were also streaked on the media for discrete colonies. Pure cultures were obtained from the mixed culture for further identification (Amund and Adebiyi, 1991).

### IDENTIFICATION OF MICROORGANISMS

All bacterial isolates were identified based on morphological, cultural and biochemical characteristics according to Buchanan and Gibbons (1974) while pure cultures to Actinomycetes isolated were identified on their morphological characteristics and microscopic examination according to the schemes by Hirsch and Christensen, 1983.

### HYDROCARBON UTILIZATION STUDIES

The ability of bacteria and Actinomycetes isolated to degrade and utilize hydrocarbons as sole carbon source in the laboratory was tested using sterile minimal salt medium and Knights minimal salt both at 0.5(v/v) using engine oil, diesel oil and kerosene as carbon source. Bacterial growth was monitored by viable counts on nutrient agar. The plates were incubated at room temperature (28+- 20c) for seven days.

## RESULTS

A total of 12 filling stations were used for this study and three samples were collected from each location making a total of 36 samples. Four filling stations were studied within every given area.

The distribution of heterotrophs in the sample sites are shown in figure 1. Petrol source had the highest heterotroph count of $3.56 \times 10^7$ cfu/g followed by Kerosene source with $2.75 \times 10^7$ cfu/g with diesel having the least.

Diesel oil source however had the highest bacterial count of 5.5 x 107 cfu/g and kerosene source with 5.03 x 107 cfu/g while petrol source had the least of 4.0 x 107cfu/g. (Fig. 2)

The total fungal count was highest from kerosene source 3.43 x 107cfu/g, petrol with 2.98x107 cfu/g while diesel had the least of 2.56 x 107 cfu/g (Fig. 3) Figure 4 shows the distribution of oil degrading bacterial count from the various Petrol had the highest count of 9.56 x 106 cfu/g diesel oil 6.65 x 106 cfu/g with kerosene having the least of 5.45 x 106 cfu/g. There was a high microbial load with heterotrophs having 2.87 x 107 cfu/g and relatively low load of oil degrading bacteria which was 7.22 x 106 cfu/g which was approximately ratio four to one (Fig 1 and Fig. 4).

Similarly, the mean total bacterial count was 4.87 x 107cfu/g while the mean total fungal count was 2.99 x107 cfu/g (fig. 2 and 3). Six bacteria general were recovered from the oil contaminated soils which included: Pseudomonas spp from 18(60%) of the 30 samples analysed, Flavobacterium spp from 13(43.3%) Proteus spp from 9(30%), Acinetobacter spp 6(20%) and Alcaligenes spp 8(26.7%) (Table 1). This shows that Pseudomonas spp was the most common hydrocarbon degrading isolates while Alcaligenes spp was the least.

Similarly, three species of Actinomycetes which included Nocardia spp, Micromonospora spp and Streptomyces spp were also recovered . The ability of bacteria and Actnomycetes to utilize hydrocarbons shows their potentials as good materials for bioremediation of oil spills if genetically engineered (Table 2)

Fig. 1: Distribution Means heterophs in the various Sources

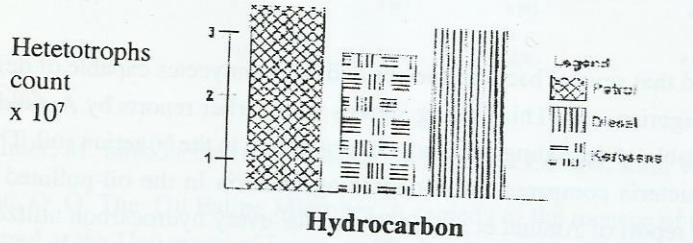


Fig 2 Mean total Bacterial count in various sources

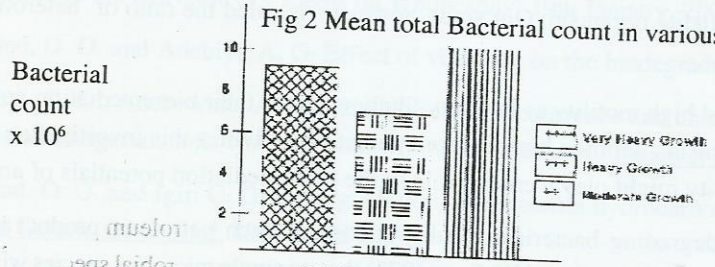


Table 1. Prevalence of bacterial isolates in soil samples n = 30

| | Organism | Number | Percentage |
|---|---|---|---|
| 1. | Pseudomonas spp | 18 | 60 |
| 2. | Flavobactterium spp | 13 | 43.3 |
| 3. | Proteus spp | 9 | 30 |
| 4. | Acinetobacter spp | 6 | 20 |
| 5. | Alcaligenes spp | 8 | 26.7 |
| 6. | Micrococus spp | 5 | 16.7 |

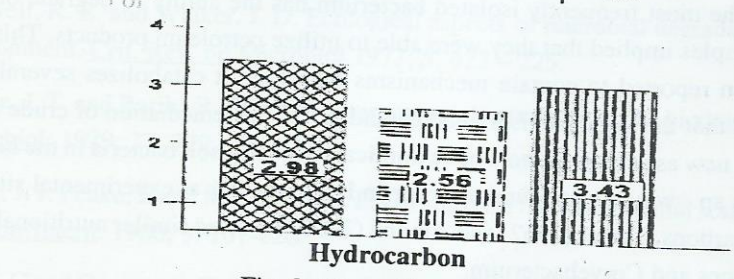


Fig. 3: Means Fungal count

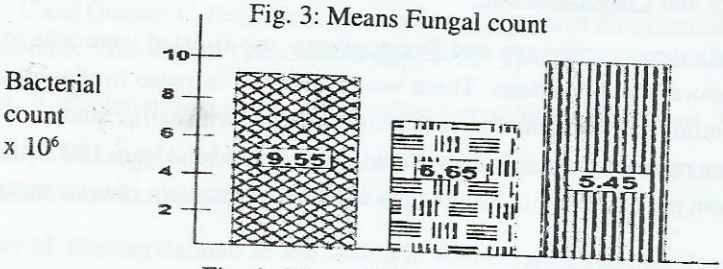


Fig. 4: Means Oil degrading bacterial Count

Table 2. Actinomycetes recovered from the soil samples and their hydrocarbon utilization.

| Actinomycetes | substrate | | |
|---|---|---|---|
| | Engine | Diesel Oil | Kerosine |
| Nocardia spp | +++ | ++++ | ++++ |
| Streptomyces spp | ++ | ++ | +++ |
| Micromonospoda spp | +++ | ++ | +++ |

## DISCUSSION

The impact of petroleum prospecting and production operations on the environment has produced ecological problems of great dimension. Oil spillages often bring about economic losses as well as serious environmental problems with living organisms within both aquatic and terrestrial habitats adversely affected.

Duffy et al (1980) reported that oil spills may be regarded as an inevitable consequence of the ever increasing demand for exploration, production, transportation and use of oil. The physico - chemical properties of soils vary greatly which thus affects the "in situ" microbiology and have profound implication for the biotechnologists intending to enhance biodegradation rate in order to clean up contaminated sites. Interestingly, most environments have indigenous population of oil-degrading microorganisms that can multiply rapidly under appropriate conditions (Rosenberg, 1997). The soil is endowed with millions of microorganisms, only few of which are however capable of degrading oil.

The results of this investigation showed that several bacteria species and Actinomycetes capable of degrading oil in oil polluted soils are available in the Nigerian soil. This finding agrees with earlier reports by Amund et al (1987) of increased occurrence of bacteria capable of degrading petroleum hydrocarbon in the Nigerian soil. The occurrence of higher number of heterotrophic bacteria compared to oil-degrading bacteria in the oil-polluted soil samples investigated in this study confirms the report of Amund et al (1987) that for every hydrocarbon utilizer there are at least eight other non-hydrocarbon utilising organism. Our study however revealed the ratio of heterotrophs to oil-degrading bacteria as 4:1.

Most of the bacterial isolates exhibited high motility as this may likely enhance their bioremediation potentials. It is interesting to know that the two organisms with the highest bioremediation activities this investigation were motile giving rise to the suspicion that motility might play a crucial role in the bioremediation potentials of an organism.

The involvement of two or more oil-degrading bacteria in the degradation of each petroleum product as seen in this experiment corroborates the observation of Colwell and Walkes (1977) that no single microbial species will completely degrade any particular oil. This also agrees with report by Amund (2000) that the biodegradation of both crude and refined oil involves a consortium of organisms due to substrate specificity exhibited by these microbial species. This finding also agrees with report by Rosenberg (1997) that the requirement for a mixture of different microorganisms arises from the fact that petroleum is composed of a wide variety of different group of hydrocarbons whereas any specific microorganism is highly specialized with regard to the type of hydrocarbon it can break down.

Another interesting observation is the higher bioremediation potentials exhibited by Pseudomonas sp. and Micrococcus sp. Pseudomonas sp. which was the most frequently isolated bacterium has the ability to degrade organic matter hence their presence in the soil samples implied that they were able to utilize petroleum products. This organism is nutrionally versatile and have been reported to contain mechanisms by which it catabolizes several organic and inorganic substrates which implies that the organism is a good candidate for bioremediation of crude or refined oil spills. This observation may not be new as several authors have implicated several soil bacteria in the biodegradation process, particularly Pseudomonas sp. which was found to occur in large number at experimental sites capable of degrading a wide variety of hydrocarbons. Taxler (1962) and Amund (2000) reported similar nutritional versatility in species of Acinetobacter, Alcaligenes and Coryebacteruim.

The Actinomycetes Nocardia spp, Micromonospora spp and Streptomyces spp isolated were able to degrade and utilize some hydrocarbons under laboratory conditions. There was a constant increase in the population of the Actinomycetes isolates on Knight's minimal salt broth. This is finding correlates with earlier studies of a significant increase in population of oil degrading microorganisms in oil polluted sites (Amund and Igiri, 1990 Atlas, 1995) The pH of the medium also decreased from pH of 7.2 to 5.2, which may be due to production of toxic metabolites in the medium.

It is noteworthy that all the bacterial and Actinomycetes species isolated in the work are normal flora of the soil which are able to degrade oil consequent on adaptation to the new environment of oil polluted soils. The pollutant oil could have also stimulated the growth of the adapted strains bacteria (Jobson et al; 1974). Similar bacteria were isolated by Amund and Adebiyi (1991) from the Lagos Lagoon. With the recent establishment of biotechnology centres in Nigeria, further studies on genetic engineering of normal soil microflora on spillages hold a ray of hope for the bioremediation of both crude and refined spills in the nearest future with minimal cost.

**Table III: Resistance pattern of bacteria isolated from undergraduates with asymptomatic bacteriuria**

| Antibiotic | Organisms and number (Percentage) | | | | | | |
|---|---|---|---|---|---|---|---|
| | E coli (100) | Ps aeruginosa (70) | Serratia (64) | Enterobacter (48) | Proteus (36) | Klebsiella (20) | Citro bacter (20) |
| Ampicilli | 20 (20) | 8 (11.4) | 6 (8.6) | 5 (7.1) | 7 (19.4) | 4 (20) | 5 (25) |
| Amoxicillin | 18 (18) | 6 (8.6) | 4 (5.7) | 5 (7.1) | 5 (14.0) | 2 (10) | 3 (15) |
| Cotromoxazole | 16 (16) | 22 (31.4) | 4 (5.7) | 8 (11.4) | 8 (22.2) | 2 (10 | 2 (10) |
| Cefactor | 5 (5) | 16 (22.9) | 3 (4.3) | 2 (2.9) | 1 (2.8) | 3 (15) | 1 (5) |
| Nitrofurantoin | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Oflexacin | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Ceprofloxacin | 0 (0) | 0 (0) | 0 (0) | 0(0) | 0 (0) | 0 (0) | 0 (0) |

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