GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 6 NO. 3, JUNE 2000:489-495
COPYRIGHT (C) BACHUDO SCIENCE CO. LTD. PRINTED IN NIGERIA ISSN 1118-0579

MICROBIOLOGICAL POST IMPACT ASSESSMENT OF MOBIL QIL (IDOHO) OIL SPILL IN THE COASTAL AREAS OF SOUTH EASTERN NIGERIA

MATTHEW E. EJA

(Received 22 February 1999; Revision accepted 10 October 1999)

ABSTRACT

Following the Mobil Nigeria UNLIMITED oil spill from the 24" Idoho Qua Iboe Terminal (QIT) pipeline rupture of 12th January, 1998, a post impact study of the spill on the coastal and estuarine areas of Cross River State, South Eastern Nigeria, specifically Calabar South coastal areas, was carried out in May, 1998, using hydrocarbonoclastic microorganisms as indicators. Microorganisms which could grow in Mineral Salt Medium using Qua Iboe light crude oil as source of carbon and energy, were recognised as crude oil degraders, and the ratio of their counts on Mineral Salt agar to heterotrophic counts made on Tryptone soya agar was used to establish pollution. 50% of water samples and 58.3% of sediment samples showed ratios of crude oil degraders/heterotrophic counts far greater than 1. There was high significant correlation (r = 0.79) between respective populations of crude oil degraders in water and sediment at 0.001 probability. These results confirm gross pollution of the coastal areas. Most organisms which showed ability to utilize QIL crude oil were obligate aerobes. For example, *Bacillus* species occurred most frequently (22.3% in water, and 20.1% in sediment), although a good proportion of obligate aerobes of other bacterial genera, molds and yeasts, and a few genera of obligate anaerobes, were identified.

KEY WORDS: oil spill, hydrocarbonoclastic microorganisms, indicators.

INTRODUCTION

Some man-made activities that destabilize marine, coastal or estuarine ecosystems are industrial effluent discharge and oil pollution. In particular, these pollutants significantly alter the physico-chemical components of the ecological system, resulting in various impacts observable on the flora and fauna of the affected ecosystem.

Man, the supreme coordinator of the ecological system, immediately feels the impact of the pollution, expressed in declining economic activities and health status, loss of valuable property and resources. That is why the impact of oil pollution on the environment is of great concern to man, and creates the circumstances for compensation to be paid to affected persons, following a post impact assessment of oil spillage from a company source.

Most of the effects of oil pollution are biological, and therefore biological impact assessment of oil pollution has been applicable and useful. However, most oil pollution assessments or monitoring programmes have been applied to intertidal zones, and in some cases, off-shore areas (Baker, 1976). It follows that oil pollution impact assessments are most reliable using biological indicators. For instance, canaries in coal mines serve as early warning devices for detecting pollutants (Baker, 1976). The effects of pollutants on sensitive organisms like limpets, can also be measured. There are some organisms whose presence indicates the probability of pollution, e.g. *Enteromorpha*, which is often abundant in oil or sewage - polluted areas due to lack of competition (Baker, 1976). Also, there are bioassay organisms which are selected organisms used as laboratory 'reagents' to detect the presence and/or concentration of toxic pollutants, or to rank pollutants in order of toxicity. For example, the brown shrimp is used for testing the toxicity of oil spill dispersants (Baker, 1976).

Hydrocarbonoclastic microbes, otherwise referred to as crude oil degraders, are used as indicators. Recently, the concept of enrichment for hdyrocarbonoclastic microbes in petroleum - containing soils has been applied to the prospecting for oil and gas fields (Brisbane and Ladd, 1965). All soil samples are known to contain hydrocarbon-oxidizing microorganisms in the range of 0 to 20%, thus providing justification for the practice of using soil as the inoculum in enrichment cultures for isolating hydrocarbon-utilizing organisms (Jones and Edington, 1968).

Several genera of bacteria and yeasts are known to contain Aliphatic Hydrocarbon-Oxidizing species. Examples of such bacteria are *Achromobacter*, *Arthrobacter*, *Mycobacterium*, *Flavobacterium*, *Pseudomonas* etc., and yeasts such as *Candida*, *Cryptococcus*, *Selenotila*, *Torulopsis*, etc

MATTHEW E. EJA, Department of Water Resources, Ministry of Agriculture, Water Resources and Rural Development, Calabar, Nigeria.

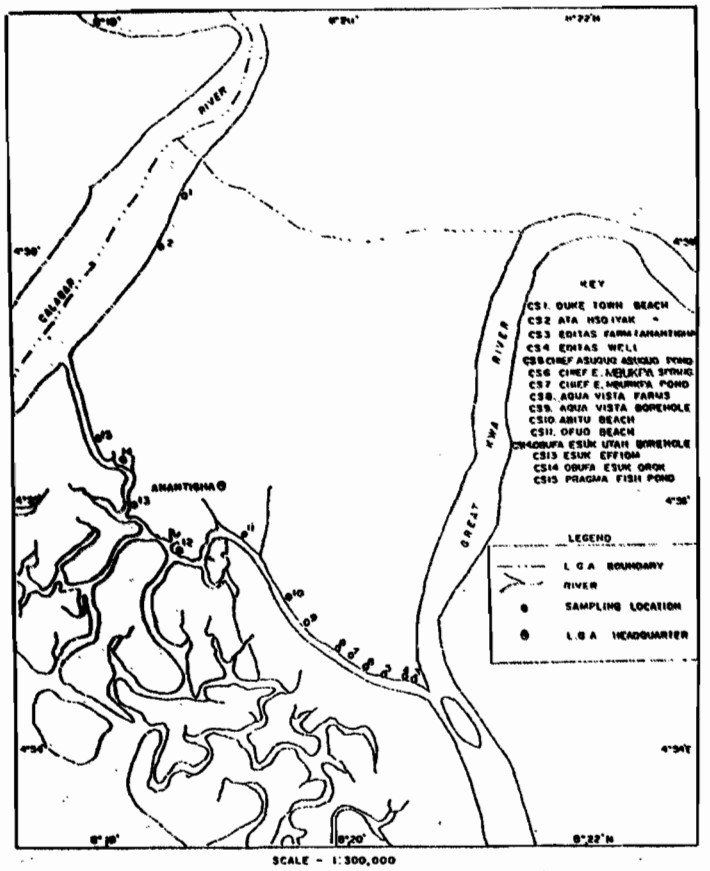

Fig. 1 MAP OF CALABAR SOUTH L.G.A. SHOWING SAMPLING LOCATIONS

(Beerstecher, 1954). Filamentous fungi are also known to be capable of degrading hydrocarbon, and as a group, appear to be more versatile than yeasts in the utilization of short-chain hydrocarbons for growth (Klug and Markovetz, 1971).

The degradation of hydrocarbon is carried out mainly by aerobic organisms. However, involvement of anaerobes in the petroleum degradation has been reported (Floodgate, 1972). The petroleum hydrocarbon-degrading microorganisms therefore have the potential for the weathering of oil spills, since hydrocarbon is their sole source of carbon and energy (Odu, 1972 and Atlas, 1981), leading to the disappearance of oil from the environment. This, however, depends on some factors such as the nature of the soil, the microbial community, temperature, salinity and nutrient availability (Atlas, 1981). There are other factors such as substrate specificity in which there are differences in hydrocarbon assimilation by different microorganisms (Foster, 1962), and growth in hydrocarbon substrate with limited solubility (Johnson, 1964).

This study is a post impact assessment of the Mobil Nigeria UNLIMITED oil spill from the 24" Idoho Qua Iboe Terminal (QIT) pipeline rupture of 12th January, 1998, on the coastal and estuarine areas of Cross River State, S.E. Nigeria; and specifically Calabar South coastal areas, using hydrocarbonoclastic microorganisms as indicators. This study was conducted five months after the spill, i.e., from May, 1998 to June, 1998. The affected areas and sampling points are shown in Figure 1.

MATERIALS AND METHODS

(a) SAMPLING METHODS

Water and sediment samples were collected in triplicates from 15 locations in the creeks of Calabar South Local Government Area estuary (Figure 1). Water samples from low and high tides, and superficial sediment samples were collected in accordance with the recommended procedures and precautions (American Public Health Association, 1985), and the use of hand-driven auger, respectively. Samples were conveyed in icebox to the laboratory for analysis within 1 to 4 hours, or refrigerated until required for analysis.

(b) TOTAL HETEROTROPHIC BACTERIAL COUNT

Ten-fold serial dilutions in the ranges of 10^{-1} to 10^{-5} were prepared (Atlas and Bartha, 1981). 1ml aliquots of sample dilutions of from 10^{-3} to 10^{-6} were seeded in sterile petridishes and total heterotrophic bacterial count was determined by pour plate technique using tryptone soya agar which can support the growth of aerobes and anaerobes (American Public Health Association, 1985, Oxoid Manual, 1976). For the recovery of aerobes, tryptone soya agar was used, while tryptone soya agar was supplemented with 1% ($^{w}/_{v}$) cysteine hydrochloride (BDH Chemicals, U.K.) for anaerobes. Aerobic cultures were incubated at 35°C for 48 hours, while anaerobic cultures were incubated in Baird and Tat-lock anaerobic jar at 30°C for 48 to 72 hrs. Visible number of colonies (between 30 and 300) was multiplied by the reciprocal of the dilution factor, and recorded as colony-forming units (CFU) per milliliter of water, or CFU/gram of sediment (American Public Health Association, 1985).

(c) TOTAL COUNT OF PETROLEUM HYDROCARBON DEGRADERS

Aliquots of appropriate sample dilutions (in triplicates) were incubated in Mineral Salt agar (MSA) of Zajic and Supplison (1972), reconstituted with the following composition: 1.8gK_2HPO_4, 1.2gKH_2PO_4, 4.0gNH_4Cl, 0.2g$MgSO_4.7H_2O$, 0.1g$FeSO_4.7H_2O$, 1000ml distilled water. This was supplemented with filtered sterilized cycloheximide antibiotic (Sigma Company, USA) at a concentration of 40mg/ml to inhibit growth of fungi (Holm and Jenson, 1972, Ijah and Ukpe, 1992). Sterile filter paper (Whatman No.1) was saturated with Qua Iboe Light (QIL) crude oil previously filter-sterilized (Millex disposable filter unit US pat. 34709, 3386585, pressure 75 p.s.i., 0.22ml pore size). The individual saturated filter papers were aseptically placed onto the cover of the inverted petridishes (Atlas and

Bartha, 1972, Ijah and Ukpe, 1992). The plates were incubated at ambient temperature of 30°C in an inverted position, after taping round the petridishes with masking tape, in order to increase the vapour pressure within the dishes. The crude oil was the sole source of carbon and energy for the growth of the organisms through vapour-phase transfer. Growth colonies were then counted after 7 to 10 days of incubation. This treatment was for both water and sediment samples.

(d) CRUDE OIL UTILIZING ABILITY OF MICROORGANISMS

Microorganisms in the samples were screened for their ability to utilize petroleum hydrocarbon as their sole source of carbon and energy by the determination of growth turbidity. This was carried out by dispensing 9 milliliter amount of the mineral salt medium (MSM) of Zajic and Supplison, (1972) into test tubes. Following sterilization and cooling, 0.2ml amount of aliquots from 10^{-4} to 10^{-6} dilutions were seeded into the medium, followed by 0.1ml filter-sterilized QIL crude oil. The cultures were then incubated as before. Uninoculated tubes containing the filter-sterilized QIL crude oil were used as controls.

(e) TOTAL MYCOLOGICAL COUNT

Counts were carried out in triplicates, using appropriate dilutions as already described. However, fungal density was enumerated on Malt Extract Agar made more acid (pH4.8), to suppress the growth of bacteria (Oxoid Manual, 1976).

(f) CHARACTERIZATION AND IDENTIFICATION OF MICROORGANISMS

Characterization and identification of isolates were based on morphological examination and biochemical tests (Beneke and Rogers, 1970, Buchanan and Gibbons, 1974, Lodder, 1974, Talbot, 1978, DOMSCH, *et. al.*, 1980, Cowan, 1985, Samson *et al.*, 1994).

RESULT

Tables 1 and 2 show the results of the analysis of water and sediment samples respectively. In addition to microbial counts, the Tables show calculated crude oil degraders/heterotrophic ratios, and mycological/heterotrophic ratios. The ratios help to establish pollution when they are above consent limits. Many locations show ratios of more than 1, indicating that the coastal areas were heavily impacted.

A correlation analysis for total crude oil degraders in water and sediment showed a correlation coefficient, r = 0.79. This showed that there was highly significant correlation between crude oil degraders in water and sediment at 0.001 probability.

Table 3 shows the frequency of occurrence of microbial genera in the samples, and showed *Bacillus* occurring most frequently.

DISCUSSION

Either immediately or after a period of adaptation, microorganisms have the potential to mineralize any organic material of biological origin made available through excretion, death of organisms, or disposal by man. A greater part of these 'scavengers' are heterotrophic bacteria. Thus, from ecological point of view, it was necessary to examine the population of heterotrophs as physiological types, rather than taxonomic types. A few organisms within the microbial population selectively utilize some organic compounds, and may thus multiply far more than

TABLE 1: ANALYSIS OF WATER SAMPLES FROM CALABAR SOUTH COASTAL AREAS

S/N	Description of Sampling Location	Total Heterotrophic Count on TSA (Aerobes) x 10^4 CFU/ml	Total Heterotrophic Count on TSA (Anaerobes) x 10^4 CFU/ml	Total Crude Oil Degraders on MSA + QIL Crude Oil x 10^4 CFU/ml	Microbial Growth in MSM + QIL Crude Oil	Mycological Count on MEA x 10^4CFU/ml	Degraders/ Heterotrophic Ratio	Mycological/ Heterotrophic Ratio
CS1	Duke Town Beach	SPR	6.9	3.1	-	15	ND	ND
CS2	Ata Nso Iyak Beach	3.0	2.5	3.0	+	4.4	1.00	1.46
CS3	Edito's Farm	4.8	7.1	7.2	+	1.7	1.50	0.35
CS4	Edito's Well	3.0	1.6	3.1	+	3.0	1.03	1.00
CS5	Chief Asuquo Asuquo Fish Pond	2.4	3.0	7.0	-	6.1	2.91	2.54
CS6	Chief E. Mbukpa Spring	2.0	4.2	1.0	-	1.0	0.50	0.50
CS7	Chief E. Mbukpa Pond	5.6	1.6	1.0	+	4.0	0.17	0.71
CS8	Aqua Vista Farms	3.0	5.0	6.0	-	7.0	2.00	2.33
CS9	Aqua Vista Borehole	*0.0	*0.0	*0.0	-	*0.0	*0.0	*0.0
CS10	Abitu Beach	3.0	1.4	8.0	+	8.3	2.66	2.76
CS11	Ofuo Beach	25.0	17.0	23.0	-	3.5	0.92	0.14
CS12	Akani Esuk Utan Borehole	*0.0	*0.0	*0.0	-	*0.0	*0.0	*0.0
CS13	Esuk Effiom	*0.0	*0.0	*0.0	-	*0.0	*0.0	*0.0
CS14	Ebufa Esuk Orok	2.5	1.2	1.4	+ +	1.5	0.56	0.60
CS15	Pragma Fish Pond	5.6	1.1	3.5	+	5.0	0.62	0.89

KEY:
SPR = Spreader overgrowth, growth uncountable
* = Sample were lost
ND = Not Determined
QIL = Qua Iboe Light
\- = Absence of growth turbidity
\+ = Light growth turbidity
\+ + = Deep growth turbidity

TABLE 2: ANALYSIS OF SEDIMENT SAMPLES FROM CALABAR SOUTH COASTAL AREAS

S/No	Description of Sampling Location	Total Heterotrophic Count on TSA (Aerobes) x 10^4 CFU/g	Total Heterotrophic Count on TSA (Anaerobes) x 10^4 CFU/g	Total Crude Oil Degraders on MSA + OIL Crude Oil x 10^4 CFU/g	Microbial Growth in MSM + OIL Crude Oil	Mycological Count on MEA x 10^4CFU/g	Degraders/ Heterotrophic Ratio	Mycological/ Heterotrophic Ratio
CS1	Duke Town Beach	9.8	8.2	4.0	-	1.2	0.4	0.12
CS2	Ata Nso Iyak Beach	20.0	6.2	10.5	+	3.0	0.5	0.05
CS3	Edita's Farm	2.6	8.6	10.2	+	1.1	3.9	0.96
CS4	Edita's Well	2.5	4.8	8.9	+	2.5	3.6	0.04
CS5	Chief Asuquo Asuquo Fish Pond	1.4	1.6	1.2	+	1.0	0.8	0.71
CS6	Chief E. Mbukpa Spring	2.5	7.2	7.2	+	6.2	2.9	2.48
CS7	Chief E. Mbukpa Pond	2.8	-	3.0	+	1.0	1.1	0.36
CS8	Aqua Vista Farms	1.1	1.2	12.0	+	6.2	10.9	5.63
CS9	Aqua Vista Borehole	⊕0.0	⊕0.0	⊕0.0	-	⊕0.0	⊕0.0	⊕0.0
CS10	Abitu Beach	3.0	1.2	15.0	+	6.0	5.0	2.0
CS11	Ofuo Beach	15.0	200	106.0	+ +	4.0	7.0	0.26
CS12	Akani Esuk Utan Borehole	+0.0	+0.0	+0.0	+	+0.0	+0.0	+0.0
CS13	Esuk Effiom	*0.0	*0.0	*0.0	+	0.0	*0.0	*0.0
CS14	Ebufa Esuk Orok	2.5	1.5	5.8	+ +	1.0	0.9	0.16
CS15	Pregma Fish Pond	2.5	2.0	-	+	5.2	0.0	2.08

KEY:
- * = Sample were lost
- OIL = Qua Iboe Light
- - = Absence of growth turbidity
- + = Light growth turbidity
- + + = Deep growth turbidity
- ⊕ = Sample not taken

TABLE 3: FREQUENCY OF OCCURRENCE OF MICROBIAL GENERA IN THE SAMPLES STUDIED

S/No	Microorganisms Isolated	Water	Sediment
	BACTERIA		
1	*Bacillus*	31	24
2	*Sarcina*	2	1
3	*Serratia*	2	0
4	*Achromabacter*	1	4
5	*Clostridum*	3	16
6	*Peptostreptococcus*	2	1
7	*Micrococcus*	7	3
8	*Vibrio*	7	0
9	*Proteus*	6	0
10	*Pseudomonas*	2	4
11	*Bacteroides*	2	4
12	*Thiobaccillus*	1	8
13	*Klebsiella*	3	1
14	*Enterococcus*	6	1
15	*Enterobacter*	3	1
16	*Desulfovibrio*	0	7
17	*Aeroimonas*	4	0
18	*Brevibacterium*	0	1
19	*Corynebacterium*	1	0
	MOLDS		
1	*Penicillium*	10	10
2	*Cephalosporium*	1	0
3	*Aspergillus*	9	13
4	*Cladosporium*	5	1
5	*Nigrospora*	2	0
6	*Paecilomyces*	2	1
7	*Rhizopus*	1	0
8	*Aureobasidium*	0	1
9	*Alternaria*	2	0
10	*Phoma*	7	0
11	*Fusarium*	7	0
12	*Mucor*	1	1
13	*Absidia*	0	2
	YEASTS		
1	*Candida*	3	3
2	*Saccharomyces*	6	4
3	*Rhodotorula*	0	3

other 'scavengers'. The ratio of such organisms to the entire heterotrophic population can indicate whether such organisms which selectively utilize some organic compounds are present in quantities above the ambient level. Of considerable practical interest is the biological degradation of accidentally spilled oils and petroleum, as many hydrocarbon degrading bacteria are common in coastal areas where oil spills are more or less chronic (Gundersen, 1976).

The ratio of crude oil degraders to heterotrophic organisms (Tables 1 and 2) was adopted as an index of oil pollution (Wakama *et al.*, 1989). The rationale is from the understanding that the proportion of bacteria and fungi in sediment is generally greater than 1% for hydrocarbon-polluted ecosystem.

To validate the use of the ratio, it has been shown that significant numbers of soil microbes can use selected hydrocarbons as sole source of carbon and energy, and depending on the soil sample used as inoculum in enrichment cultures, and the hydrocarbon employed, the percentage of hydrocarbon oxidizers ranges from 0 to 20% of the total population (Britton, 1984). It has been further demonstrated that soils taken from oil fields contain a higher percentage of hydrocarbon oxidizers (Britton, 1984). Hydrocarbon oxidizers are also known to be prevalent in marine environments (Colwell and Walker, 1977), and as in soil, the microbial flora of oil-polluted and unpolluted marine sites will differ (Britton, 1984).

It follows from the ratios (Tables 1 and 2) that, on average, the coastal environment of Calabar South Local Government Area, was significantly impacted following the oil spill, with the pollution persisting even five months after the incident. Ignoring sampling locations whose samples were lost, about 50% of the coastal areas were grossly polluted (Table 1), while sediment samples (Table 2) showed that 58.3% of the coastal areas were grossly polluted. There was significant correlation ($r = 0.79$) between the populations of crude oil degraders in water and sediment at 0.001 probability. This confirms gross pollution of the coastal areas, Aqua Vista Farm (CS_8), Ofuo Beach (CS_{11}), Abitu Beach (CS_{10}), Edita's farm (CS_3), Edita's well (CS_4), and Ata Nso Iyak Beach (CS_2), being most highly polluted in order of magnitude of pollution. On the other hand, it is not clear why there were low ratios of mycological to heterotrophic counts in some locations. Although physico-chemical investigation was not a part of the primary design and objective of this study, it is reasonable, however, to attribute the low ratios in some locations to unfavourable intertidal fluctuations of some environmental factors such as pH, temperature and salinity, especially as this impact assessment was restricted to the marine environment. Moreover, physical parameters do not measure the concentrations of specific pollutants in water; rather, they assess the changes that may take place in well-defined characteristics as a result of the addition of potential pollutants (Mckee, 1967). Thus, it is probable that intertidal fluctuations, especially of the factor of salinity, in view of its overwhelming level in natural marine water (Mckee, 1967), could have adversely affected the fungal population, resulting in the low ratios in some locations. Furthermore, there is substrate specificity in the utilization of individual hydrocarbons (Foster, 1962), e.g. filamentous fungi preferentially utilize long-chain *n*-alkanes more readily than short-chains for growth (Foster, 1962, Klug and Markovetz, 1971, Kachholz and Rehm, 1978). Therefore, the fungal population could partially depend on the nature of substrate which probably could have been responsible for the low ratios in a few sampling locations in this study.

In any case, it has been observed in this study that, mycological count is only a fraction of total crude oil degraders counts (Tables 1 and 2), and cannot alone wholy be relied on, in validating the claim of oil pollution from its ratio to heterotrophic count. Where its ratio to heterotrophic count, in conjunction with crude oil degraders/heterotrophic ratio, is equal to, or greater than 1, then oil pollution is confirmed. Therefore the apparently low ratios of mycological to heterotrophic counts in some locations may not be unusual, since the fungal population forms a fraction of the total crude oil degraders in any polluted location, as in CS3 (Tables 1 and 2), CS4, CS7 and CSII (Table 2).

It is not clear why there was apparent lack of correlation of growth in MSM and MSA (Tables 1 and 2). Based on the fact that aliphatic hydrocarbons are the major components of crude oils and petroleum products, and whereas not all species and strains of the genera of hydrocarbonoclastic organisms are capable of utilizing aliphatic hydrocarbons as growth substrates (Britton, 1984), it has been established that the isolation of these organisms is usually based on competitive growth in selective conditions of hydrocarbon enrichment cultures (Foster, 1962), while the range of hydrocarbon utilization for growth by individual organisms is usually limited (Britton, 1984). This calls for caution in interpreting substrate specificity studies based on growth or absence of growth, because several criteria must be fulfilled for growth of microorganisms which could show colonial appearance on mineral salt agar (MSA) and growth turbidity in Mineral salt medium (MSM) since some could not readily utilize QIL crude oil as source of carbon and energy. On the other hand, a few others could grow both on MSA and MSM with growth turbidity because they could utilize the crude oil as source of carbon and energy.

Most organisms which showed ability to utilize the

QIL crude oil were obligate aerobes (Table 3). An example is *Bacillus* species which occurred most frequently (22.3% in water, and 20.15 in sediment). A good proportion of other bacterial genera, molds and yeasts are obligate aerobes. This points to the fact that hydrocarbons can be oxidized by many microbes, including bacteria, yeasts and molds. Since the initial reaction step requires the participation of oxygen (Britton, 1984) hydrocarbon degradation is only possible under aerobic conditions.

In this study, a few anaerobes, e.g. *Clostridium* were also encountered (Table 3). Involvement of anaerobes in petroleum degradation of aromatic compounds is fairly well established, and can occur by photometabolism involving *Athiorhodaceae*, by nitrate respiration as in *Pseudomonas* and *Moraxella* species, and by methanogenetic fermentation (Van der Linden and Thijssee, 1965). Also, *Desulfovibrio* species, sulphate - reducing obligate anaerobes have been implicated in the anaerobic degradation of aliphatic petroleum products. For instance, mud samples from a marine bay have shown sulphate reduction when incubated anaerobically with paraffin oil (Zobell and Prokop, 1966). It was concluded that the decomposition of mineral oils under anaerobic conditions was accompanied by the growth of sulphate reducers. Therefore the isolation of *Desulfovibrio* and other anaerobes in this study was in agreement, and confirms oil pollution as well.

ACKNOWLEDGEMENT

I thank the Cross River State Government and Mobil Producing Nigeria UNLIMITED for the provision of resources and the wonderful opportunity given to me to undertake this study.

REFERENCES

American Public Health Association, 1985. *Standard Methods for the Examination of Water and Wastewater*. 16th ed., APHA, Washington.

Atlas, R.M., 1981. Microbial Degradation of Petroleum Hydrocarbon: An environmental perspective. *Microbiology Review*.

Atlas R.M. and Bartha, R., 1972. Degradation and Mineralization of Petroleum by two bacteria isolated from coastal waters. *Biotechnology and Bioengineering*. 14:297-308.

Atlas, R.M., 1981. *Microbial Ecology Fundamentals and Application*. Addison-Wesley Publishers Co. california.

Baker, J.M., 1976. Biological Monitoring Principles: Methods and Difficulties. In : *Marine Ecology and oil Pollution*. Ed. Jenifer M. Baker, 41 - 52. Institute of petroleum, London.

Beerstecher, E., 1954. *Petroleum Microbiology*. Elsevier, New York.

Beneke, E.S. and Rogers, A.L.C., 1970. *Medical Mycology Manual*. 3rd Ed., Burgers Publishing Co., Mineapolis, 229pp.

Brisbane, P.G. and Ladd, J.N., 1965. *Ann. Rev. Microbiol*. 19:351.

Britton, L.N., 1984. Microbial Degradation of Aliphatic Hydrocarbon. *Microbiology Series*. vol. 13. Marcel Dekker Inc., New York.

Buchanan, R.E. and Gibbons, M.E., 1974. *Bergey's Manual of Determinative Bacteriology*. 8th Ed., Williams and Wilkins Publishers, Baltimore MD. 1246 pp.

Colwell, R.R. and Walker, J.D., 1977. *Crit. Rev Microbiol*. 5:423.

Cowan, S.T., 1985. *Cowan and Steel's Manual for Identification of Medical Bacteria*. 2nd Ed. Cambridge University Press.

Domsch, K.H., Gam, W. and Anderson, T., 1980. *Compendium of Soil Fungi*. Academic Press, London.

Floodgate, G.D., 1972. In: *Water Pollution Microbiology*. Ed. R. Mitchell Wiley, New York, p. 153.

Foster, J.W., 1962. *Antonie van Leeuwenhoek*. New York, p.241.

Gundersen, K.I., 1976. Cultivation of Microorganism. In: *Marine Ecology*. Ed. Otto Kinne. Vol.111. John Wiley and sons, London.

Holm, E. and Jensen, V., 1972. Aerobic chemoorganotrophic Bacteria of a Danish Beach Forest. *Oikos* 23:248-260.

Ijah, U.J.J. and Ukpe, L.I., 1992. Biodegradation of crude oil by *Bacillus* strains 28A and 61B isolated from spilled soil. *Waste Management*. 12:55-60.

Johnson, M. J., 1964. *Chem.Ind*. 36:1532.

Jones, J. G. and Edington, M.A., 1968. *J.G. Microbial* 57: 381.

Kachholz, T. and Rehm, H.J., 1978. *Eur. J. Appl. Microbiol*. 6:39.

Klug, M. J. and Markovetz, A.J., 1971. *Adv. Microbial Physiol*. 5:1.

Lodder, J., 1974. *The Yeast*. 2nd edn. Northern Holland Publishing Co. Amsterdam, and

American Publishing Co. Incorporated, New York.

Mckee, J. E., 1967. Parameters of Pollution An overall Evaluation. In: *Pollution and Marine Ecology*. Ed. Theodore A. Olson. Interscience Publishers, New York. 25:259 - 266.

Odu, C. T. I., 1972. Microbiology of Soils Contaminated with Petroleum Hydrocarbon 1. Extent of contamination and some soil and microbial properties after contamination. *Journal of Institute of Petroleum*. 5:20.

Oxoid Manual, 1976. 3rd edn. (Revised). Published by Oxoid Ltd, Wade Road, Basingstoke.

Samson, R A., Hoestra, E.S. and Vanpoerschot, C.A.N 1984. *Introduction to Foodborne Fungi* ntre Bereau Voor Schimelaltrores Nether ds.

Van der Linden, A.C. and Thijssee, G.J.E., 1965. *Adv. Enzyrnol.*, 27:469.

Wakama, W.T., Odu, E.J., Adok, A., and Braide, S.P., 1989. Ecological Post Impact Study of Ebubu-Olhani Oil.Report submitted to Shell Petroleum Dev. Co. Nig. Ltd, Lagos, 236p.

Zajic, J.E. and Supplison B., 1972. Emulsification and Degradation of Bunker C. fuel oil by microorganisms. *Biotechnology and Bioengineering*. 14:331-343.

Zobell, C.E., and Prokop, J.F., 1966. *Allg. Mikrobiol.* 6:143.