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Full Length Research Paper

Microorganisms in the crude oil-producing areas of Ondo State, Nigeria

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The microbial population of the sampling stations was determined in the study during the rainy and dry season. It was observed that crude oil-producing areas are thickly populated with microorganism compared with the control site. This could be as a result of post-pollution microbial activity. Example is in Ayetoro recording the highest of 240 x 10^5 cfu/ml compared with Sabomi having a record of 7x10^3 cfu/ml as the highest in these periods. Various microbial species were encountered during the study including common oil degraders like *Pseudomonas* spp., *Bacillus* spp., *Proteus* spp., *Enterobacter* spp. and *Klebsiella* spp. A total of one hundred and seventy five (175) microbes were isolated and identified during the study. This includes *Bacillus* species (18), *Bacillus pasteurii (*2), *Bacillus cereus* (5), *Bacillus macerans* (9), *Bacillus circulans* (16), *Bacillus coagulans* (2), *Bacillus subtilis* (1), *Bacillus licheniformis* (3), *Bacillus alvei* (1), *Bacillus panthothemicus* (1), *Klebsiella species* (12), *Veillonella* spp. (15), *Pseudomonas* spp. (17), *Pseudomonas diminuta* (2) *Pseudomonas mallei* (2), *Pseudomonas pseudomallei* (1), *Pseudomonas aeruginosa* (1), *Micrococcus* spp. (17), *Micrococcus varians* (2), *Neisseria* spp. (4), *Streptococcus* spp. (6), *Streptococcus homonis* (1), *Proteus* spp. (9), *Staphylococcus* spp. (8), *Staphylococcus aureus* (1) *Sarcina maxima* (1), *Enterobacter* spp. (9), *Serratia* spp. (4), *Serratia marcescen* (2) and *Arthrobacter* spp. (4).

Key words: Environment, microorganisms, crude oil-producing areas, Ondo State, population.

INTRODUCTION

The study of Ajayi and Akonai (2003) emphasize the need for routine monitoring of our coastal water bodies. In order to appreciate the level of pollution and environmental degradation in the study area, an environmental impact assessment (E.I.A.) study of the area should be carried out. Munn (1979) identified E.I.A. as an activity designed to identify and predict the impact of project on the bio-geophysical programmes, operational procedures and to interpret and finally communicate the impact. The conservation of our ecosystem and natural environment is desirable for man's co-existence, hence the need to monitor its dynamics, for the sustenance of human and economic development (Ajayi and Akonai, 2003).

Industrial developments and growth in petroleum hydrocarbon exploration activities in Nigeria and all over the world has led to increased oil pollution in our environment. This can result from accidental spillage during drilling, leakages of pipelines or through vanderlization. Similarly, some discharges or outburst of petrol from some oil tankers during accidents occasionally result in flow over land, vegetation and water surfaces, polluting the soil and water, and posing serious ecological problems (Adieze et al*.,* 2003). Previous investigations have shown that crude oil and other related organic pollutants can be degraded (Philips and Stewart, 1994; Okpokwasili and Okorie, 1998; Ijah and Ukpe, 1992; Ekpo, 2002; Ekpo and Ekpo, 2006). The microbial biodegradation of crude oil and other aliphatic and aromatic hydrocarbons are carried out by both autochthonous and allochthonous species that bring about the biotransformation, reducing the complex mixture of noxious materials by breaking intermolecular bonds to simple nutrients in the soil or aquatic ecosystem (Churchill et al., 1999; Becker et al., 1999; Burland and Edwards, 1999). This process has been employed in reclaiming crude oil-polluted soil and in clearing-up polluted aquatic ecosystem, a process termed bioremediation (Ekpo and Ekpo, 2006).

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To this end that with regards to the devastating effects of oil pollution on ecology and adverse health, management schemes are being organized by government sectors to bring such effects under control. According to Fasasi (2006), oil spill also destroys the biodiversity of the delicate ecosystem of the Niger Delta. Oil spill blocks oxygen supply and destroys some essential nutrients in the soil. At chronic levels exposure to hydrocarbons, even from skin contact causes injury to internal organs (Fasasi, 2006).

Large scale production of fossil fuel requires a large initial accumulation of organic matter, which is rich in carbon and hydrogen. Another requirement is that the organic debris is build-up quickly to protect it from air so that decay by biological means or reaction with oxygen will not destroy it. Microscopic life is abundant over much of the oceans. When these organisms die, their remains can settle to the sea floor. Oil and gas are believed to form from such accumulated microorganisms (Montgomery, 2000).

MATERIALS AND METHODS

Study site

The study research was carried out in a sizeable riverine area of Ilaje community located on the Southern part of Ondo State, a Delta region with most rivers flowing into the Atlantic Ocean. Sample was obtained from different sites in the four major kingdoms which constitute Ilaje community namely Mahin, Ugbo, Etikan and Aheri kingdoms. The Local Government headquarters of Ilaje is at Igbokoda; Ilaje comprises many towns and villages. Among the towns are: Araromi seaside, Ode-Etikan, Abereke, Ayetoro, Bijimi, Ilepete, Obe-iji, Awoye, Ubales and Oghoye among many other communities.

The control site for this study covers the neighboring areas of Ese-Odo riverine communities, Ondo State, which is relatively free from oil exploration activities.

Methods of sample collection

Water samples were collected using sterile sample bottles of about 200 ml in triplicates from each sample source. Similarly, sediment (soil) samples were obtained using sterile plastic nylon seals from sampling points where water was collected. About 100 g of soil samples were collected from each source and stored into a cooler containing ice on board in the boat (transport ferry) to pressure the samples. Similarly, some fish samples were obtained from the sampling sites for analysis in this study. Temperature of the water was measured with the aid of a thermometer calibrated in degree centigrade (^{0}C) during the period of collection

Laboratory procedure

The water, fish and economic plant sources obtained from the sampling sites were analyzed in the laboratory for some biological attributes. The microbial isolates recovered from these sources were characterized for identification purposes following some morphological and biochemical tests and occurrence at different sites in the oil producing riverine Ilaje community.

Culture preservation

Microbial strains isolated were maintained on nutrient agar slants at 4^{o}C. The stock culture was prepared in duplicates, one set serving as working cultures. They were sub-cultured every three months or when necessary. Nutrient agar, plate count agar, MacConkey agar and Eosin Methylene blue (E.M.B) agar were generally used during the study.

RESULT AND DISCUSSION

Various riverine communities of Ilaje Local Government area which are oil producing areas of Ondo State and Eseodo Local Government Area, which is the control area, were visited during the study. The microbial population of the sampling stations was determined during the study. This was coupled with subsequent isolation and identification of the organisms encountered in different sampling sites. Table 1 shows the total bacterial and coliform counts during the rainy season in the month of June. The total bacterial count ranged from 3 x 10^3 cfu/ml in Ilepete to 200 x 10^3 cfu/ml in Awoye. Similarly for the control sites the lowest recorded was 2 x 10^3 cfu/ml and highest was 7 x 10^3 cfu/ml both at different sites of Sabomi via Igbobini.

The coliform counts of the study sites for rainy season in the month of June also range from low counts of 0 and 1 x 10^2 cfu/ml in areas like Odofado, Ayetoro and Bijimi to a high counts up to 44 and 45 x 10^2 cfu/ml in Odeetikan and Abereke, respectively. The control site, however, showed coliform value that varied from low counts of 0 to 4 x 10^2 cfu/ml (Table 1a). Table 1b shows the viable bacterial counts of the water samples from Ayetoro. It was determined using different media like Plate count agar (P.C.A.) to enumerate the total bacterial count, McConkey Agar for enumeration of the Gram negative organisms which could be lactose or nonlactose fermenting organisms and Eosin Methylene Blue (E.M.B.) Agar which is a differential media for culturing coliform group of organism.

In the five site studied for Ayetoro, high total bacterial counts of 240 x 10^5 cfu/ml was recorded compared with 160 and 34 x 10^3 cfu/ml for coliform counts, thus showing the range of organism present in the water sample for this station which can also be representative of others (Table 1b). The laboratory media used, P.C.A., McConkey and E.M.B agar were equally tested for viability of some organism species from soil sample. This also recorded the highest value of 200, 37 and 21 x 10^5 cfu/ml for total counts and coliform counts respectively for this group of organisms from soil sources (Table 1b).

Table 2 shows the microbial load of the microorganisms during the dry season. The bacterial count ranged from low of 5 x 10^5 cfu/ml in Abereke 1 to a high of 81 x 10^5 cfu/ml in Ayetoro. The coliform counts also varied from low counts of 6 x 10^3 cfu/ml in Awoye 1 to 48 x 10^3 cfu/ml in Ode-Etikan.

The total bacterial count was determined for the sedi-

Table 1a. Total bacterial and coliform counts in some sample stations during rainy season (month of June).

Sampled stations	Total bacterial count(cfu/ml x 10^3)	Coliform count (cfu/ml x 10^2)
Study site		
Awoye 1	Swarmy organisms[a]	3[c]
Awoye 2	200[a]	8[c]
Awoye 3	30[ab]	-
Awoye 4	10[c]	2[c]
Awoye 5	Swarmy[a]	-
Ilepete 1	30[ab]	17[ab]
Ilepete 2	3[c]	1[c]
Ayetoro 5	13[c]	1[c]
Odofado	3[c]	-
Bijimi 1	8[c]	-
Bijimi 2	Multiple[a]	2[c]
Bijimi 3	8[c]	1[c]
Abereke 1a	91[ab]	41[ab]
Abereke 1b	96[ab]	44[ab]
Abereke 2a	72[ab]	36[ab]
Abereke 2b	89[ab]	37[ab]
Abereke 3	174[a]	100[a]
Odeetikan	24[ab]	-
Odeetikan 1b	20[ab]	4[c]
Odeetikan 2a	46[ab]	34[ab]
Odeetikan 2b	55[ab]	45[ab]
Odeetikan 3a	13[c]	8[c]
Odeetikan 3b	36[ab]	31[ab]
Control sites		
Sabomi 1	2[c]	-
Sabomi 2	5[c]	4[c]
Sabomi 3	7[c]	-
Iluagbo	6[c]	4[c]

Variables with 'a' superscript shows sampling sites with high microbial load (statistically significant in the same group); 'ab' superscript shows sampling sites with moderate microbial load (statistically significance); and 'c' superscript shows low statistical significance by Duncan statistical analysis.

Table 1 b. Viable bacterial count of soil and water samples from Ayetoro community in the oil producing area of Ondo State.

Sample site	Water sample (cfu/ml x 10^5)			Soil sample (cfu/ml x 10^6)		
	P.C.A.	McConkey	E.M.B.	P.C.A.	McConkey	E.M.B.
1	240	160	34	60	37	21
2	8	-	-	6	-	-
3	50	42	31	23	17	15
4	-	12	-	200	24	-
5	14	-	-	-	-	-

Table 2. Total bacterial and coliform counts of water samples during dry season (month of February).

Sample	Total bacterialcount (cfu/ml x 10^5)	Coliform count (cfu/ml x 10^3)
Ayetoro 1	62	16
Ayetoro 2	20	40
Ayetoro 3	81	17
Awoye 1	48	6
Awoye 2	16	20
Awoye 3	60	18
Ilepete 1	50	20
Ilepete 2	70	31
Ilepete 3	30	24
Abereke 1	5	44
Abereke 2	28	36
Abereke 3	40	46
Odeetikan 1	24	20
Odeetikan 2	62	48
Odeetikan 3	52	8

Table 3a. Comparative total bacterial counts of study site and control areas sediment samples.

Sample code	Total bacterial count (cfu/ml x 10^5)
Oil producing study sites	
Ilepete 2	11
Ilepete 2c	29
Ilepete 3	28
Bijimi 1	26
Bijimi 2	43
Awoye 1	11
Awoye 2	22
Awoye 3	12
Awoye 4	Swarm
Ayetoro 5	Numerous
Odeetikan	12
Odofado	4
Abereke	5
Control riverine sites	
Sabomi 1	30
Sabomi 2	35
Sabomi 3	38
Iluagbo 1	35
Iluagbo 2	37

ment samples of oil producing areas in Ilaje riverine communities and Eseodo riverine communities, which serve as the control site. Table 3 shows the lowest total

Table 3b. Bacteria count of fish samples obtained from study site.

Fish	Part of fish	Total heterotrophic count (cfu/g/ml)	Coliform count	*E. coli* count (cfu/g/ml)
Tilapia zilli	Gut	152 ×10^5	92 ×10^5	
	Gill	164 × 10^5	122 × 10^5	
	Skin	276 × 10^5	136 × 10^5	
Croaker	Gut	18 ×10^6		18 ×10^6
	Gill	16 × 10^6		16 × 10^6
	Skin	10 × 10^6		10 × 10^6
Ethmaloza fimbrata	Gut	14 ×10^6		18 ×10^6
	Gill	13 × 10^6		16 × 10^6
	Skin	18 × 10^6		10 × 10^6
Marine fish	Gut	6 ×10^6		18 ×10^6
	Gill	11 × 10^6		16 × 10^6
	Skin	12 × 10^6		10 × 10^6

bacterial count of 5 x 10^5 cfu/ml in Abereke to a high of 43 x 10^5 cfu/ml and other numerous counts in Bijimi and Ayetoro. In Iluagbo and Ayetoro, which are riverine areas in Okitipupa and Eseodo riverine communities, the total bacterial counts range from 30 to 38 x 10^5 cfu/ml (Table 3). However, the ecological distribution pattern of microorganisms in the oil producing areas compared with the control sites is significant, because a lot of oil utilizing bacteria were encountered in the study area which suggested the possibility of reactivating or amplifying the genes of such organisms for clean up purposes in case of oil or petroleum hydrocarbon pollution.

Various microbial species were encountered during the study including common oil degraders like *Pseudomonas* spp., *Bacillus* spp., *Proteus* spp., *Enterobacter* spp. and *Klebsiella* spp. This suggested the possibility of some natural process of oil degradation in the oil producing areas, due to the presence of some hydrocarbon pollutants. This is in consistence with the study of Alofe and Ajisebutu (2004) and Antai and Crawford (1983) who demonstrated the ability of some microbial oil degraders in cleaning up oil polluted areas. A total of one hundred and forty (175) microbes were isolated and identified during the study using standard microbiological techniques (Table 4). These include *Bacillus* species (18), *Bacillus pasteurii (*2), *Bacillus cereus* (5), *Bacillus macerans* (9), *Bacillus circulans* (16), *Bacillus coagulans* (2), *Bacillus subtilis* (1), *Bacillus licheniformis* (3)*, Bacillus alvei* (1), *Bacillus panthothemicus* (1), *Klebsiella species* (12)*, Veillonella* spp. (15), *Pseudomonas* spp. (17), *Pseudomonas diminuta*(2) *Pseudomonas mallei* (2), *Pseudomonas pseudomallei* (1), *Pseudomonas aeruginosa* (1), *Micrococcus* spp. (17), *Micrococcus varians* (2), *Neisseria* spp. (4), *Streptococcus* spp. (6),

Table 4. Distribution pattern of microbial isolates in the oil producing areas of Ondo State.

Isolates	Occurrence	Percentage
Bacillus species	17	9.71
Bacillus circulans	16	9.14
Bacillus macerans	9	5.14
Bacillus pasteurii	2	1.14
Bacillus cereus	5	2.86
Bacillus coagulans	2	1.14
Bacillus subtilis	1	0.57
Bacillus licheniformis	3	1.71
Bacillus alvei	1	0.57
Bacillus panthothemicus	1	0.57
Klebsiella species	12	6.86
Veillonella spp.	15	8.57
Pseudomonas spp	17	9.71
Micrococcus spp.	16	9.14
Micrococcus varians	2	1.14
Neisseria spp.	4	4.57
Streptococcus spp	6	3.43
Streptococcus homonis	1	0.57
Proteus spp.	9	5.14
Staphylococcus spp.	8	4.57
Staphylococcus aureus	1	0.57
Sarcina maxima	1	0.57
Enterobacter spp.	9	5.14
Serratia spp.	4	4.57
Serratia marcescens	2	1.14
Arthrobacter spp.	4	4.57
Pseudomonas diminuta	2	1.14
Pseudomonas mallei	2	1.14
Pseudomonas pseudomallei	1	0.57
Pseudomonas aeruginosa	1	0.57
Total	175	100

Streptococcus homonis (1), *Proteus* spp. (9), *Staphylococcus* spp. (8), *Staphylococcus aureus* (1) *Sarcina maxima* (1), *Enterobacter spp.* (9) and *Serratia spp.* (4), *Serratia marcescen* (2) *Arthrobacter spp.* (4) (Tables 4). This correlates with the study of Ekpo and Ekpo (2006) which shows the versatility of various species of microorganisms in oil producing areas.

Various riverine communities of Ilaje and Ese-odo in the oil producing area of Ondo state were visited during the study. Some communities in Eseodo serve as control site. It was observed during the study that oil-producing areas are thickly populated with microorganism compared with the control site. This could be as a result of post-pollution microbial activity. Example is in Ayetoro recording the highest of 240 x 10^5 compared with Sabomi having a record of 7 x 10^3 cfu/ml as the highest these periods. This is consistent with the study of Obiaku and Abu (2003) which shows that a lot of mesophilic microorganisms have being reported to degrade pollutant from petroleum hydrocarbon.

The sites recording the highest number of coliforms are Ode-Etikan 48 x 10^3 cfu/ ml, Abereke 46 x10^3 cfu/ml and Ayetoro, 40 x 10^3 cfu/ ml. This signified contamination of the water body from feacal sources making the affected site, water undesirable for domestic use unless properly treated. The prevalence of coliforms in this area may be as a result of feacal pollutants from domestic sources; however some of these coliforms can participate actively in the degradative processes of oil pollutants. This is consistent with the study of Churchill et al. (1999) and Burland and Edwards (1999), which reveals that both autochthonous and allochthonous species bring about the biotransformation and reducing the complex mixture of noxious materials by breaking intermolecular bonds to simple nutrients in the soil and aquatic ecosystems.

Government bodies and health management authori-

ties can, however, embark on environmental monitoring in this oil producing riverine areas of Ondo State, using environmental benchmark data obtained in this kind of research for possible environmental amelioration in case of oil pollution in these areas

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