



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

International Journal of Current Research
Vol.8, pp.091-095, September, 2010

RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF MICROORGANISMS INVOLVED IN THE DEGRADATION OF REFINED PETROLEUM PRODUCTS POLLUTED SITES IN ELELE, RIVERS STATE, NIGERIA

¹Eze, V.C.*and ²Eze, B.N.

¹Department of Microbiology, Michael Okpara University of Agriculture, Umudike, P.M.B.7267, Umuahia, Abia State, Nigeria.

²Department of Microbiology, Madonna University Elele Campus, Rivers State, Nigeria

ARTICLE INFO

Article History:

Received 18th July, 2010

Received in revised form

19th July, 2010

Accepted 23th August, 2010

Published online 4th September, 2010

Key words:

Isolation, Characterization,
Microorganisms, Degradation,
Petroleum products,
Polluted sites.

ABSTRACT

The isolation and characterization of microorganisms involved in the degradation of refined petroleum products polluted sites in Elele was carried out. The total aerobic mean plate count for petrol, diesel and kerosene ranged from 6.16 ± 0.10 to $0.50 \pm 0.7 \text{Log}_{10}\text{cfu/g}$, 5.03 ± 0.17 - $8.01 \pm 0.18 \text{Log}_{10}\text{cfu/g}$, 4.11 ± 0.17 to $5.65 \pm 0.33 \text{Log}_{10}\text{cfu/g}$ respectively. The fungal mean count for petrol, diesel, and kerosene ranged from 5.89 ± 0.28 - $6.40 \pm 0.23 \text{Log}_{10}\text{cfu/g}$, 5.29 ± 0.07 - $6.59 \pm 0.06 \text{Log}_{10}\text{cfu/g}$, 4.01 ± 0.10 - $5.78 \pm 0.19 \text{Log}_{10}\text{cfu/g}$ respectively. The hydrocarbon-utilizing bacterial mean count for the petrol, diesel and kerosene ranged from 5.03 ± 0.03 - $6.25 \pm 0.02 \text{Log}_{10}\text{cfu/g}$, 4.01 ± 0.11 - $5.33 \pm 0.01 \text{Log}_{10}\text{cfu/g}$ and 4.01 ± 0.44 - $5.05 \pm 0.02 \text{Log}_{10}\text{cfu/g}$ respectively. The hydrocarbon-utilizing fungal mean count for the petrol, diesel and kerosene ranged from 3.97 ± 0.06 - $5.77 \pm 0.33 \text{Log}_{10}\text{cfu/g}$, 5.01 ± 0.09 - $6.01 \pm 0.11 \text{Log}_{10}\text{cfu/g}$ and 4.95 ± 0.01 - $5.25 \pm 0.03 \text{Log}_{10}\text{cfu/g}$ respectively. The bacteria isolated from the petrol polluted sites their percentage occurrence were *Bacillus* (34.2%), *Pseudomonas species* (28.9%) *Klebsiella species* (21.1%) and *Acinetobacter* (15.8%) while the fungi were *Aspergillus species* (33.3%), *Penicillin species* (44.4%) and *Saccharomyces species* (22.2%). Diesel polluted site were *Bacillus* (38.5%), *Citrobacter species* (34.6%), *Acinetobacter species* (26.9%), *Candida species* (37.8%), *Aspergillus species* (31.1%) and *Penicillin* (31.1%). Kerosene polluted sites were *Pseudomonas species* (36.8%); *Klebsiella species* (34.2%), *Citrobacter species* (28.9%), *Candida albicans* (48.1%) and *Saccharomyces species* (51.9%). The work showed that these microorganisms can be used in the degradation of these petroleum products if they contaminate the environment.

© Copy Right, IJCR, 2010 Academic Journals. All rights reserved.

INTRODUCTION

Pollution is the release of substances or energy to the environment in quantities that damage human health and resources. Pollutants are substances released into the environment in sufficient concentration as to produce measurable effects on the soil, water, plants, animals, microbes, materials or human. However the problem of pollution can be abated through proper scientific studies that generate a better management of the environment. Crude oil and its products may be regarded as pollutants when they are indiscriminately discharged into the biosphere. (Anweiller *et al.*, 2000).

The demand for crude oil as a source of energy and raw materials for industries took a dramatic term in the 20th century with an increase in the world output from 29 to over 3,000 million tons per annum. This increase in crude oil production has brought an increasing problem of

environmental pollution. A greater part of the problem stems from the fact that the major oil producing countries are not the major consumers as a result massive movement of petroleum has to be made from areas of high production to the areas of high consumption. In 1997, it was estimated that approximately 0.5%, (12 million tons per annum) of the transported crude oil found its way into the sea through accidental spills and deliberate change of ballasts (heavy materials used for stabilizing the ship) (Van *et al.*, 2003).

Oil pollution has steadily increased with the increased use of oil. Petroleum hydrocarbons are grossly being introduced into the biosphere as a consequence of oil exploration, exploitation, refining and transportation. Other sources of hydrocarbons contamination include formalin waters, industrial effluents, urban runoffs, cleaning operations, seep around oil bearing rocks, automobiles etc. crude oil is also added into the environment through oil well blow out breakage of oil pipeline and as deliberate policy of waste disposal on

*Corresponding author: vin13eze@yahoo.com

land. Leakage of petroleum products from underground storage tanks is pervasive source of ground water contamination in the USA. Sabotage or vandalization as a means of oil pollution mainly cannot stop activities on flow lines. Nigeria has experienced several oil pollution from sabotage. The activities of automobile technicians in various mechanic workshops also contribute to the discharge of oil into the environment (Sarkar *et al.*, 2005).

Oil pollution causes environmental devastation, loss of lives and other untold hardship. Cases of burst pipes and serious blowouts in Nigeria oil fields and pipelines had been reported. The Funiwa off shore oil well blow out of January, 1980 had been described as the worst in Nigeria. Nigerians cannot easily forget the Jesse oil spillage also in 1980 and its consequences. The Nigerian national petroleum corporation recorded 121 incidents of oil pipeline vandalization, 82 of which resulted in explosion across the country between January to august, 2000. The Exxon Valdez tanker that ran aground and oiled the rocks of prince Williams sound Alaska in 1987 is an their serious oil spillage that had occurred (Atlas,1994; Atlas and Philip, 2005). There is no doubt that the petroleum industry accounts for a sizeable pollution of terrestrial and aquatic environment. Major improvements are required in the technologies employed to fight oil spills. However, useful biotechnological approaches have been developed to crude have been known to be most important process involved in weathering and eventually disappearance of oil pollutants from the ecosystem and the process occur naturally (Ghazali,2004).

This has stimulated interest in the study of biodegradation of crude oil, specifically focusing on optimizing their degradation potential for biodegradation purposes. Microorganisms are capable of utilizing crude oil and its products as sources of carbon and energy thereby increasing in biomass. The rate of biodegradation of oil is influenced by the physical state of the oil pollutants, chemical composition, moisture, temperature, nutrient availability, aeration, nature and abundance of oil degraders (Delille *et al.*, 2000). The purpose of bioremediation is to optimize these factors so that mineralization will occur. Although biodegradation pathways for crude oil have evolved naturally, there is need for genetic manipulation of microorganisms to produce strains that are capable of dealing with broader range of higher concentration of crude oil and its products. The microbial activities at the polluted site are governed by microbe's ability to synthesize enzymes that will catalyze the metabolic reactions. This is genetically determined. Many different enzymes and metabolic pathways are required to degrade components of crude oil (Nester *et al.*, 2005). The aim of this work is to isolate and characterize microorganisms involved in the degradation of refined petroleum products polluted sites.

MATERIALS AND METHODS

Collection of samples

The soil samples polluted with diesel, kerosene and petrol were collected from five different sites in Elele, Ikwere L.G.A of Rivers State, Nigeria. The samples were collected in sterile containers. Control sample not polluted with the petroleum was collected.

Chemical Reagents

The chemical reagents used in the study were of analytical grade and were products of BDH Chemicals, Poole's, England and Sigma Chemical Company, St. Louis Missouri, USA. The microbiological media used were products of Oxoid and Difco Laboratories, England. They include nutrient agar used for the estimation of total heterotrophic aerobic, bacteria, purification of hydrocarbon utilizes and for pure culture; Saboured dextrose agar (SDA) used for the isolation of fungi. The modified mineral salt agar was used for the isolation of hydrocarbon-utilizing bacteria and fungi.

Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the polluted soils were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined by plating in duplicate using pour plate technique. Then molten nutrient agar at 45°C was poured into the Petri-dishes containing 1ml of the approximate dilution for the isolation of the total heterotrophic bacteria and fungi respectively. They were swirled to mix and colony counts were taken after incubating the plates at room temperatures for 48h.

Enumeration of Hydrocarbon-Utilizing Bacteria and fungi

The hydrocarbon-utilizing bacteria and fungi were determined. The mineral salt agar of Mills *et al.* (1978) as modified by Okpokwasili and Amanchukwu (1988) comprising per litre of distilled water NaCl, 10g; MgSO₄.7H₂O, 0.42g; KCL, 0.29g; K₂HPO₄, 1.2g; KH₂PO₄, 0.83; NaNO₃, 0.42g; agar,15g; pH 7.2 was used. To 990ml of the mineral salt medium in conical flasks was added to 10ml each of the petrol, diesel and kerosene respectively which served as sources of carbon. However, for the hydrocarbon-utilizing fungi, the medium was supplemented with an antibiotic chloramphenicol. The hydrocarbon-utilizers were then enumerated after plating in duplicate using pour plate technique, 1ml of the appropriate dilutions of the samples on Petri dishes. The molten mineral salt agar medium and the ones containing antibiotic at 45°C were poured into the Petri dishes for the isolation of hydrocarbon-utilizing bacteria and fungi respectively. These were swirled to mix, allowed to solidify and incubated. Enumeration of the hydrocarbon-utilizes was performed after incubation at room temperature for 7 days. Colonies of the hydrocarbon-utilizing bacteria growing on the agar plates were counted, isolated, purified by streaking on nutrient agar plates and kept on nutrient agar starts as stock cultures for characterization and identifications. In the case of hydrocarbon-utilizing fungi, the isolates were streaked to purify onto Sabouraud dextrose agar plates and kept on sabouraud and agar slants as shock cultures for characterization and identification.

Characterization and Identification of Hydrocarbon Utilizing Isolates

Bacterial isolates were characterized and identified after studying their Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, and catalase production. Citrate utilization,

Table 1. Total Mean Counts of Microorganisms Isolated from the Sites Polluted with Petrol

Locations	THB	Log ₁₀ cfu/g FC	HUB	HUF
RES	6.50 ± 0.07	6.25 ± 0.07	6.25 ± 0.02	5.01 ± 0.28
MW	6.28 ± 0.10	6.40 ± 0.23	5.03 ± 0.15	4.39 ± 0.55
SFS	6.22 ± 0.06	6.37 ± 0.11	6.00 ± 0.15	5.77 ± 0.33
UGR	6.16 ± 0.03	6.08 ± 0.10	5.36 ± 0.01	4.74 ± 0.80
IMW	6.32 ± 0.10	5.89 ± 0.28	5.46 ± 0.44	3.97 ± 0.06

Legend: THB= Total Heterotrophic Bacteria; FC= Fungal count; HUB= Hydrocarbon-utilizing Bacteria; HUF=Hydrocarbon-utilizing Fungi; RES= Real oil filling Station; MW=Motorcycle workshop; UGR= Uche generator repairs; IMW=Ikye Motor workshop.

Table 2. Total Mean Counts of Microorganisms isolated from the Sites Polluted with Diesel

Location	THB	Log ₁₀ cfu/g FC	HUB	HUF
RES	7.53 ± 0.01	6.44 ± 0.25	5.33 ± 0.01	5.74 ± 0.01
MW	5.78 ± 0.08	6.31 ± 0.01	4.01 ± 0.11	6.01 ± 0.11
SFS	8.01 ± 0.18	5.74 ± 0.35	5.28 ± 0.18	5.53 ± 0.12
UGR	5.95 ± 0.12	5.29 ± 0.07	5.23 ± 0.15	5.01 ± 0.09
IMW	5.03 ± 0.17	6.59 ± 0.06	5.00 ± 0.11	5.97 ± 0.13

Legend: THB= Total Heterotrophic Bacteria; FC= Fungal count; HUB= Hydrocarbon-utilizing Bacteria; HUF=Hydrocarbon-utilizing Fungi; RES= Real oil filling Station; MW=Motorcycle workshop; Shalom filling station UGR= Uche generator repairs; IMW=Ikye Motor workshop.

Table 3. Total Mean Counts of Microorganisms Isolated from Sites polluted with Kerosene

Location	THB	Log ₁₀ cfu/g FC	HUB	HUF
RES	5.11 ± 0.07	5.25 ± 0.13	5.05 ± 0.02	5.25 ± 0.03
MW	5.65 ± 0.33	5.45 ± 0.12	4.07 ± 0.03	5.15 ± 0.01
SFS	4.11 ± 0.17	5.78 ± 0.19	4.01 ± 0.44	5.00 ± 0.16
UGR	5.78 ± 0.01	4.95 ± 0.01	4.77 ± 0.15	4.01 ± 0.10
IMW	5.05 ± 0.15	5.03 ± 0.10	4.89 ± 0.14	5.21 ± 0.10

Legend: THB= Total Heterotrophic Bacteria; FC= Fungal count; HUB= Hydrocarbon-utilizing Bacteria; HUF=Hydrocarbon-utilizing Fungi; RES= Real oil filling Station; MW=Motorcycle workshop; Shalom filling station UGR= Uche generator repairs; IMW=Ikye Motor workshop.

Table 4. Bacteria isolated from Petroleum Products Polluted Sites and their Percentage Occurrence.

ORGANISMS ISOLATED	RFS	MW	SFS	IMW	UGR	TOTAL	% OCCURRENCE
Petrol							
<i>Bacillus</i> species	2(15.4%)	1(7.69%)	5(38.5%)	4(30.8%)	1(7.69%)	13	34.2
<i>Pseudomonas</i> species	3(27.3%)	3(27.3%)	2(18.2%)	1(9.09%)	2(18.2%)	11	28.9
<i>Klebsiella</i> species	1(12.5%)	3(37.5%)	1(12.5%)	1(12.5%)	2(25.0%)	8	21.1
<i>Acinetobacter</i> species	0(0.00%)	0(0.00%)	2(33.3%)	2(33.3%)	2(33.3%)	6	15.8
Total	6	7	10	8	7	38	100
Diesel							
<i>Acinetobacter</i> species	0(0%)	0(0%)	4(57.1%)	1(14.3%)	2(28.6%)	7	26.9
<i>Bacillus</i> species	5(50%)	1(10%)	2(20%)	1(10%)	1(10%)	10	38.5
<i>Citrobacter</i> species	3(33.3%)	1(11.1%)	2(22.2%)	1(11.1%)	2(22.2%)	9	34.6
Total	8	2	8	3	5	26	100
Kerosene							
<i>Pseudomonas</i> species	3(21.4%)	1(1%)	6(50%)	3(21.4%)	1(1%)	14	36.8
<i>Citrobacter</i> species	1(9.1%)	0(0%)	3(27.3%)	4(45.5%)	2(18.2%)	11	28.9
<i>Klebsiella</i> species	4(30.8%)	2(15.4%)	1(7.7%)	2(15.4%)	4(30.8%)	13	34.2
Total	8	3	10	10	7	38	100

KEYS: RFS= Realoil filling station, MW= Motorcycle workshop, SFS= Shalom filling stations; IMW= Ikye motor workshop, UGR= Uche generator repairs

oxidative/fermentative utilization of glucose, indole production, methyl red - Voges Proskauer reaction, urease and coagulase production, starch hydrolysis, production of H₂S from triple sugar iron (TSI) agar and sugar fermentation. The tests were performed according to the methods described by Cheesborough, (2005), Margesin and Schimme (1997), and Atlas (1994). Microbial identification was performed using the keys provided in the *Bergey's Manual of Determinative Bacteriology* (1994). Fungal isolates were examined macroscopically and microscopically using the needle mounts technique.

Their identification was performed according to the scheme of Barnett and Hunter (1972) and Larone (1986).

RESULTS

The results of the isolation and characterization of microorganisms involved in the degradation of refined petroleum products polluted sites are presented. Table 1 shows the mean counts of microorganisms isolated for the sites polluted with petrol. The mean of the total heterotrophic bacterial count ranged from $6.16 \pm 0.10 - 6.50 \pm 0.7 \text{Log}_{10}\text{cfu/g}$. The oil filling station had the

Table 5. Fungi isolated from Petroleum Products Polluted Sites and their Percentage occurrence

ORGANISMS ISOLATED	RFS	MW	SFS	IMW	UGR	TOTAL	% OCCURRENCE
Petrol							
<i>Aspergillus</i> species	4(33.3%)	3(25%)	1(8.3%)	2(16.7%)	2(16.7%)	12	33.3
<i>Penicillin</i> species	4(25%)	2(12.5%)	4(25%)	2(12.5%)	4(25%)	16	44.4
<i>Saccharomyces</i> species	3(37.5)	1(12.5%)	0(0%)	1(12.5%)	1(12.5%)	8	22.2
Total	11	6	5	5	9	36	100
Diesel							
<i>Aspergillus</i> species	3(21.4%)	3(21.4%)	5(35.7%)	2(14.3%)	1(7.1%)	14	31.4
<i>Penicillium</i> species	4(28.6%)	2(14.3%)	2(14.3%)	3(21.4%)	3(21.4%)	14	31.1
<i>Candida albicans</i>	5(29.4%)	3(17.6%)	2(11.8%)	4(23.5%)	3(17.6%)	17	37.8
Total	12	8	9	9	7	45	100
kerosene							
<i>Candida albicans</i>	4(30.8%)	3(23.1%)	2(15.4%)	1(7.7%)	3(23.1%)	13	48.1
<i>Saccharomyces</i> species	2(14.3%)	5(35.7%)	3(21.4%)	3(21.4%)	3(21.4%)	14	51.9
Total	6	8	5	3	5	27	100

KEYS: RFS= Real oil filling station, MW= Motorcycle workshop, SFS= Shalom filling station; IMW= Ikye motor workshop, UGR= Uche generator repairs

highest count of $6.50 \pm 0.7\text{Log}_{10}\text{cfu/g}$ while the Uche generator repairs workshop had the least count of $6.16 \pm 0.10\text{Log}_{10}\text{cfu/g}$. The ANOVA, $P > 0.05$ showed that there was no significant count in the mean values among the locations. The mean fungal count ranged from 5.89 ± 0.28 – $6.40 \pm 0.23\text{Log}_{10}\text{cfu/g}$. The motorcycle workshop had the highest of $6.40 \pm 0.23\text{Log}_{10}\text{cfu/g}$ while the Uche generator repair workshop had the least count of $5.89 \pm 0.28\text{Log}_{10}\text{cfu/g}$. The ANOVA, $P > 0.05$ showed that there was no significant difference in their mean values among the locations. The mean count for hydrocarbon-utilizing bacteria and fungi ranged from 5.03 ± 0.03 – $6.25 \pm 0.02\text{Log}_{10}\text{cfu/g}$ and 3.97 ± 0.06 – $5.77 \pm 0.33\text{Log}_{10}\text{cfu/g}$. The real oil filling station recorded the highest count of $6.25 \pm 0.02\text{Log}_{10}\text{cfu/g}$ and the motorcycle mechanic workshop had the least count of $5.03 \pm 0.03\text{Log}_{10}\text{cfu/g}$ for HUB while the shalom filling station had the highest of $6.77 \pm 0.33\text{Log}_{10}\text{cfu/g}$ and the ikye motor workshop had the least of $3.97 \pm 0.06\text{Log}_{10}\text{cfu/g}$ for HUF. The ANOVA, $P < 0.05$ showed that there was significant value in the mean count for both parameters among the locations. Table 2 shows the mean counts of microorganisms from the sites polluted with diesel, the mean counts of the total heterotrophic bacteria and fungi ranged from 5.03 ± 0.17 – $8.01 \pm 0.18\text{Log}_{10}\text{cfu/g}$ and 5.29 ± 0.07 – $6.59 \pm 0.06\text{Log}_{10}\text{cfu/g}$ respectively. The shalom filling station had the highest count of $8.01 \pm 0.18\text{Log}_{10}\text{cfu/g}$ and ikye motor cycle workshop had the least count of $5.03 \pm 0.17\text{Log}_{10}\text{cfu/g}$ for total heterotrophic count. The ikye motor workshop had the highest count of $6.59 \pm 0.06\text{Log}_{10}\text{cfu/g}$ and Uche generators repairs workshop had the least count of $5.29 \pm 0.07\text{Log}_{10}\text{cfu/g}$.

The ANOVA, $P < 0.05$ showed that there was significant difference in the mean values for both total heterotrophic bacteria and fungi among the locations. The mean values for hydrocarbon-utilizing bacteria and fungi range from 4.01 ± 0.11 – $5.33 \pm 0.01\text{Log}_{10}\text{cfu/g}$ and 5.01 ± 0.09 – $6.01 \pm 0.11\text{Log}_{10}\text{cfu/g}$ respectively. The real oil filling station had the highest count of $5.33 \pm 0.01\text{Log}_{10}\text{cfu/g}$ and the motorcycle workshop recorded the highest count of $6.01 \pm 0.11\text{Log}_{10}\text{cfu/g}$ while Uche generators repairs had the least count of $5.01 \pm 0.09\text{Log}_{10}\text{cfu/g}$. The ANOVA, $P > 0.05$ showed that there was no significant difference in the mean values for both the HUB and HUF. Table 3 shows the mean counts of microorganisms isolated from the sites polluted with kerosene. The total heterotrophic bacterial and fungal mean counts ranged from 4.11 ± 0.17 – $5.65 \pm 0.33\text{Log}_{10}\text{cfu/g}$ and 4.01 ± 0.10 – $5.78 \pm 0.19\text{Log}_{10}\text{cfu/g}$ respectively. The highest count of $5.78 \pm 0.19\text{Log}_{10}\text{cfu/g}$ was observed in Uche generator repairs while shalom filling station had the least count of $4.11 \pm 0.17\text{Log}_{10}\text{cfu/g}$ for total heterotrophic bacteria. The highest

fungal count of $5.78 \pm 0.19\text{Log}_{10}\text{cfu/g}$ was observed in shalom filling station while the least count of $4.01 \pm 0.10\text{Log}_{10}\text{cfu/g}$ was seen in Uche generator repairs.

The ANOVA, $P > 0.05$ showed that there was no significant difference in the mean counts for both the total heterotrophic bacteria and fungi among the locations.

The mean counts for hydrocarbon-utilizing bacteria and fungi ranged from 4.07 ± 0.03 – $5.05 \pm 0.02\text{Log}_{10}\text{cfu/g}$ and 4.01 ± 0.10 – $5.25 \pm 0.03\text{Log}_{10}\text{cfu/g}$ respectively. The real filling station had the highest count of $5.05 \pm 0.03\text{Log}_{10}\text{cfu/g}$ while the Uche generator repairs had the least count of $4.01 \pm 0.44\text{Log}_{10}\text{cfu/g}$ for hydrocarbon-utilizing bacteria. The real oil filling station recorded the highest hydrocarbon-utilizing fungal mean count of $5.25 \pm 0.03\text{Log}_{10}\text{cfu/g}$ while the uche generator repair recorded the least mean count of $4.01 \pm 0.10\text{Log}_{10}\text{cfu/g}$. The ANOVA, $P > 0.05$ showed that there was no significant difference in the mean counts of the two parameters among the locations. The result of the bacteria isolated from the sites polluted with the different refined petroleum products are shown in table 4. Petrol polluted sites had *Bacillus* species (34.2%); *Pseudomonas* species (28.9%), *Klebsiella* species (21.1%); *Acinobacter* species (15.8%). Diesel polluted sites recorded *Acinobacter* (26.9%); *Bacillus* species (38.5%); *Citrobacter* species (34.6%) while kerosene polluted sites had *Pseudomonas* species (36.8%); *Citrobacter* species (28.9%); *Klebsiella* species (34.2%). The results of the fungi isolated for the sites polluted with the different refined petroleum products are shown in table 5. Petrol polluted sites had the following fungi *Aspegillus* species (33.3%); penicillin species (44.4%) and *Saccharomyces* species (22.2%). Diesel polluted sites had *Aspergillus* species (31.1%); penicillin species (31.1%) and *Candida* species (37.8%) while kerosene polluted sites recorded *Candida albicans* (48.1%) and *Saccharomyces* species (51.9%).

DISSCUSSION

The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility and the ability of microorganisms to degrade or convert such compounds (Diaz, 2008). It has been observed that the aquatic and terrestrial environment contains hydrocarbon degraders as well as other microbial communities (Odokuma and Okpokwasili, 1993; Nnubia and Okpokwasili, 1997).

Thus the mean counts of hydrocarbon-utilizing bacteria and fungi obtained from the refined petroleum products were very high when compared with the control. This may be attributed to the fact that contaminated soils often harbour a vast array of microbial flora that is capable of utilizing the hydrocarbon as energy and carbon source. It has been established that the discharge of hydrocarbon to any ecosystem may result in the selective increase or decrease in the microbial population (Okpokwasili and Nnubia, 1995; Okpokwasili and Odokuma, 1996). The hydrocarbon-utilizers isolated were dominated by Gram negative bacteria belonging to a wide range of taxa. The existence of wide taxa of hydrocarbon degraders might result in co-oxidation, commensalitic or complimentary degradation of the hydrocarbons in the ecosystems. Such co-oxidation may be a process through which undegraded and otherwise recalcitrant hydrocarbon can easily be removed from the oil contaminated ecosystem (Westlake *et al.*, 1976; West *et al.*, 1984; Eze *et al.*, 2006).

The hydrocarbon-utilizing bacterial genera isolated from the polluted sites included *Bacillus* species, *Pseudomonas* species, *Klebsiella* species, *Acinetobacter* species and *Citrobacter* species. Okpokwasili and Nwosu (1990) isolated similar hydrocarbonoclastics bacteria from the Niger Delta aquatic systems. Chikere and Okpokwasili (2004) also made similar findings when they worked on petroleum effluents. It has also been observed that some microorganisms are more abundant in areas of high concentration of hydrocarbons. These micro flora are actively oxidizing the hydrocarbons and this is considered as another source of carbon for use in the ecosystem. These organisms constitute an important link in the food chain and in the productivity cycle. Therefore, the presence of excess hydrocarbon could be considered to be a positive factor in this process. Another favourable effect of oil contamination on the soil is the generally increased fertility of the soil after most of it has been decomposed (Bhattacharya *et al.*, 2002).

The hydrocarbon-utilizing fungi isolated from the polluted sites were *Aspergillus* species, *Penicillium* species, *Candida* species, *Candida albicans* and *Saccharomyces* species. These genera of fungi have been implicated by other workers in the degradation of hydrocarbon (Bossert and Bartha, 1984; Okpokwasili and Amanchukwu, 1988; Eze and Okpokwasili, 2010). It has been observed that in the ecosystem, different substrates are attacked at different rates by consortia of organisms from different kingdoms. *Aspergillus* and other moulds play important role in these consortia because they are adept at recycling starches, hemicelluloses, pectin and keratin. Some aspergilli are capable of degrading more refractory compounds such as fats, oils, chitins and keratin. Maximum decomposition occurs when there is sufficient nitrogen, phosphorus and other essential inorganic materials. Fungi also provide food for many soil organisms (Machida and Igomi, 2010). The study has revealed that the indigenous microbial populations are capable of removing these pollutants from the environment. The activities from these locations should be properly monitored to avoid possible harm to the ecosystem.

REFERENCES

Anweiler, E, Richnow, H.H., Antranikian, G., Brock, S. and Grams, C. 2000. Naphthalene Degradation and Incorporation of Naphthalene Derived Carbon into Biomass by the Thermophile *Bacillus Thermoleovorans*. *Appl. Environ. Microbiol.*, 68(66):33.
 Atlas, R.M. 1994. Handbook Biological Media; Parks Edition, CRC Press, p. 175.
 Atlas. R.M. and Philip, J. 2005. Biodegradation: Applied Microbial Solutions for Real-World Environmental Cleanup, ASM Press, Washington, DC, pp. 1 - 292.
 Barnett, H.L. and Hunter, B.B. 1972. Illustrated genera of imperfect Fungi, 3rd edition, Burgess Publishing Company Minnesota, USA.
 Bergey's Manual of Determinative Bacteriology. 1994. 9th edition, Holt, J.D. (Ed.), Williams Wilkins CO. Baltimore, p. 783

Bhattacharya, D., Sarma, P.M., Krishnan, S., Mishra, S. and Lal, B. 2002. Evaluation of genetic diversity among *Pseudomonas citronellolis* strains isolated from oily sludge-contaminated sites. *Appl. Environ. Microbiol.*, 69(3): 1435 – 1441.
 Bossert, I. and Bartha, R. 1984. The fate of petroleum in soil ecosystems. In: R.M. Atlas (Ed.) Petroleum Microbiology, Macmillan, New York, pp. 435 – 473.
 Cheeshrough, M. 2005. District Laboratory Manual for Tropical Countries, Part 2, Cambridge University Press, UK, pp. 63-70.
 Chikere, B.O. and Okpokwasili, G.C. 2004. Frequency occurrence of microorganisms of a petrochemical effluent outfall site. *J. Trop. Biosci.*, 4: 12 – 18.
 Delle, D., Delle, B. and Pelleier, E. 2000. Effectiveness of Bioremediation of Crude Oil Contaminated Subantarctic Intertidal Sediment: The Microbial Response. *Microbial Ecol.*, 44:118-126.
 Diaz, E. 2008. Microbial biodegradation; Genomics and Molecular Biology, 1st edition, Caister Academic Press.
 Eze, V.C. and Okpokwasili, G.C. 2010. Microbial and other related changes in a Niger Delta River sediment receiving industrial effluents. *Continen. J. Microbiol.*, 4: 15 – 24.
 Eze, V.C., Okwulume, C.O. and Agwung, F.D. 2006. Biodegradation of Palm Oil Polluted sites. *Int. J. Biotechnol. Allied Sci.*, 1(1): 58 - 65.
 Ghazali, F.M., Rahman, R.A., Salleh, A.B. and Basri, M. 2004. Biodegradation of Hydrocarbons in Soil by Microbial Consortium, *Int. Biodeter. Biodegrad.*, 54:61-67.
 Larone, B.H. 1986. Important Fungi: A Guide to Identification, Harper and Row Publishers, Hagerstown, Maryland, pp. 7- 26.
 Machida, M. and Igomi, K. 2010. *Aspergillus*; Molecular Biology and Genomics, Caister Academic Press.
 Margesin, R. and Schinner, F. 1997. Efficiency of indigenous and inoculated cold-adapted soil microorganisms for biodegradation of diesel oil in alpine soils. *Appl. Environ. Microbiol.*, 63(7): 2660-2664.
 Mills, A., Breuil, Lu C. and Colwell, R.R. (1978). Enumeration of petroleum degrading marine and estuarine microorganisms by the most probable number method. *Can. J. Microbiol.*, 24: 552 - 557.
 Nester, E.W., Anderson, D.G., Roberts, C.C., Pearsall, N.N. and Nester, N.T. 2005. Microbiology: A Human Perspective 4th Edition, McGraw-Hills Companies, New York; pp.627 - 629.
 Nnubia, C. and Okpokwasili, G.C. 1997. Effects of oil spill dispersants on growth of marine bacteria from a Nigerian offshore oilfield. *J. Sci. Res. Dev.*, 3: 103 – 104.
 Odokuma, L.O. and Okpokwasili, G.C. 1993. Seasonal influences on inorganic anion monitoring of the New Calabar River, Nigeria. *Environ. Manage.*, 17: 491 – 496.
 Okpokwasili, G.C. and Amanchukwu, S.C. 1988. Petroleum Hydrocarbon degradation by *Candida* Species. *Environ. Int.*, 14: 243-247.
 Okpokwasili, G.C. and Nwosu, A.I. 1990. Degradation of aldrin by soil and aquatic microorganisms. *Nig. J. Techn. Res.*, 1: 2 – 6.
 Okpokwasili, G.C. and S.C. Amanchukwu (1988). Petroleum Hydrocarbon degradation by *Candida* Species. *Environ. Int.*, 14: 243-247.
 Okpokwasili, G.C. and Nnubia, C. 1995. The effects of drilling fluids on marine bacteria from a Nigerian offshore oilfield. *Environ. Manage.*, 19: 923 – 929.
 Okpokwasili, G.C. and Odokuma, L.O. 1996. Effects of oil spill dispersants and drilling fluids on substrate specificity of marine bacteria. *Waste Manage.*, 15: 515 – 520.
 Sarkar, D., Ferguson, M. and Datta, R. 2005. Bioremediation of Petroleum Hydrocarbons in Contaminated Soils, *Environ. Pollut.* 136:187-195.
 Van, J.D., Singh, A. and Ward, O.P. 2003. Recent Advances in Petroleum Microbiology. *Microbiol. Mol. Biol. Rev.*, 67(4): 503-549.
 West, P.A., Okpokwasili, G.C., Brayton, P.R., Grimes, D.J. and Colwell, R.R. 1984. Numerical taxonomy of phenathrene degrading bacteria isolated from Chesapeake Bay. *Appl. Environ. Microbiol.*, 48: 988 – 883.
 Westlake, D.W.S., Jobson, A., Phillippe, R. and Cook, F.D. 1976. Biodegradability and crude oil composition, *Can. J. Microbiol.*, 20: 915 – 926.