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RESEARCH ARTICLE

COMPARATIVE ASSSESSMENT OF DEGRADATION POTENTIALS OF BACTERIA AND ACTINOMYCETES IN SOIL CONTAMINATED WITH MOTORCYCLE SPENT OIL

***Idemudia, M. I., Osula Amalaowa Nosagie and Odigie Omorede**

Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, PMB 1100, Benin City, Nigeria

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ABSTRACT

The degradation potentials of bacteria and actinomycetes of spent motorcycle lubricating oil were investigated using standard microbiological procedures. Ten composite soil samples were collected from ten different motorcycle mechanic workshops in Benin City. The mean heterotrophic count for bacteria and actinomycetes ranged from 68 x 10^4 cfu/g to 155 x 10^4 cfu/g and 43 x 10^4 cfu/g to 85 x 10^4 cfu/g respectively. The mean hydrocarbon degrading bacteria counted ranged from 47 x 10^4 cfu/g to 89 x 10^4 cfu/g, while that of actinomycetes ranged from 18 x 10^4 cfu/g to 46 x 10^4 cfu/g, indicating that the bacteria was greater numerically than the actinomycetes. The preliminary screening test carried out showed that the predominant hydrocarbon degrading bacteria belonged to *Micrococcus* and *Sporosarcina species* while that of actinomycetes were *Nocardia sp , Gordonia sp , Micromonospora sp* and *Rhodococcus sp.* Biodegradation test conducted revealed a range of 2.5% to 6.6% degradation and 1.035% to 7.53% degradation of the spent lubricating oil in bacteria and actinomycetes respectively, indicating a higher degradation of the spent oil by the actinomycetes.

INTRODUCTION

Petroleum products contamination of soil is a major environmental concern globally. The persistence of petroleum products in soil depend on the chemical and concentration composition of the contaminants, the chemical, geological, physical and biological characteristics of the contaminated site. The interaction of these factors provide suitable condition for microorganism to utilizing the petroleum hydrocarbon in the soil (Rahman *et al*., 2002). Consequently, fate of petroleum hydrocarbons in the soil depends on the biodiversity of hydrocarbon-utilizing microorganisms in the contaminated soil. Numerous soil inhabiting species of bacteria, fungi and actinomycetes are capable of utilizing hydrocarbon. There have been numerous report of microbial degradation of petroleum hydrocarbon especially between bacteria and fungi in a contaminated soil. Okerenandtugba and Ezeronye (2003) reported the ability of bacteria species (*Bacillus, Micrococcus and Proteus*) and Fungal species (*Aspergillus and Rhizopus*) to degrade petroleum hydrocarbon. Okoh (2003) also reported the degradation of petroleum hydrocarbon by indigenous soil bacteria and fungi. Uba and Ifeanyi (2013) reported that a bacterium (*Bacillus sp*) comparatively as better utilizers and degraders of petroleum hydrocarbon than a fungus (*Trichosporon* sp).

Corresponding author: Idemudia, M. I.,
Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, PMB 1100, Benin City, Nigeria

Kastner *et al.*, (2001) reported the degradation of petroleum hydrocarbon by actinomycetes species of *Rhodococcus and Gordonia*. However, there is less report of the comparative degradation of hydrocarbon by bacteria and actinomycetes. In developing countries like Nigeria where there is transportation problem, motor cycle is used as an alternative means of commercial transportation in some major cities. Repairs of these motor cycles lead to careless discharge of spent oil into the soil (Faboya, 1997; Adegoroye, 1997), this renders the environment unsightly and constitutes potential threats to humans, animals, and vegetations (Adelowo *et al.,* 2006). Large amounts of motorcycle engine oil, composing long-chain saturated hydrocarbons (base oil) and additives, are used in motorcycle engines (Bagherzadeh-Namazi *et al.,* 2008). As the usage of petroleum hydrocarbon products increase, soil contamination with spent engine oil is becoming one of the major environmental problems in Benin City. As a result, environmental pollution with spent engine oil continues to generate interest. This investigation is aimed at isolating, and identifying hydrocarbon degrading bacteria and actinomycetes in soils contaminated with spent motorcycle oil from selected motorcycle workshops in Benin City and also comparing their degradability potential.

MATERIALS AND METHODS

Study Area / Sample Collection

Ten motorcycle mechanic workshops in Benin City were randomly selected as sampling sites. Soil samples were

collected from the selected motorcycle mechanic workshops into sterile polythene bags and transported to the laboratory for analysis. The soil samples were air dried at room temperature for 2 days and sieved. For the isolation and enumeration of actinomycetes the soil was heated at 55^{o}c for half an hour in dry air oven.

Isolation and enumeration of heterotrophic bacteria and actinomycete

Pour plate method was used as a routine procedure. Serial dilutions of the soil samples were achieved by shaking 1.0g of the soil samples in 9ml of distilled water in test tubes and dilution was done up to 10^{-4}. 1ml of this serially diluted portion of the soil sample was transferred into sterile Petri dishes and sterilized nutrient agar and starch casein agar (soluble starch 10g, casein 0.03, NaCl 2g , K_2HPO_4 2g , KNO_3 2g, $MgSO_4$ 0.05g , $CaCO_3$ 0.02g , Agar 18g/l) were poured into the plates to solidify. 1g/l of Nystatin and streptomycin (20ug/ml) was added to the starch casein agar to prevent fungi and bacteria growth. The plates were incubated at room temperature. Distinct bacteria and actinomycetes colonies were counted after 24hours and 14 days of incubation respectively . The pure culture of the colonies where stored at 4^{o}c for further laboratory analysis.

Isolation and enumeration of Hydrocarbon Degrading Actinomycetes and Bacteria

The hydrocarbon degrading bacteria and actinomycetes were isolated using enrichment and pour plate methods. 5g of pre-heated soil samples for Actinomycetes isolation and 5g of the air dried soil samples for bacteria isolation were separately transferred into 100 ml conical flasks containing 50ml of Bushnell-Hass (BH) medium ($MgSO_4$ 0.2g, $CaCl_2$ 0.02g, KH_2PO_4 1.0g, K_2HPO_4 1.0g, NH_4NO_3 1.0g and $FeCl_3$ 0.05g, 1l of distilled water) and incubated in a shaker at 150rpm with 1% (v/v) filter sterilized spent motorcycle engine oil as carbon source. After 7days of incubation, 1ml of each enriched media were serially diluted up to 10^{-4} and inoculated into sterile starch casein agar for Actinomycetes isolation with 1g/l of Nystatin and streptomycin(20ug/ml) to prevent fungi and bacteria growth and sterilized nutrient agar for bacteria isolation using pour plate method. The plates were incubated at room temperature for 24hours for Bacteria and 14 days for actinomycetes. Distinct colonies were counted after incubation and sub-cultured on nutrient agar for the Bacteria and starch casein agar for Actinomycetes to obtain pure colonies which were then stored on slant at 4^{0}c in the refrigerator for further studies.

Determination of Hydrocarbon Utilization

The growth potentials of the Actinomycetes and Bacteria isolates in used motorcycle engine oil were performed using a modified method of Mandri and Lin (2007). A single colony of the each isolate was inoculated into 10ml of Bushnell-Hass and incubated at 130rpm at room temperature for 7days with 1% (v/v) filter sterilized spent engine oil as sole carbon in shaker. After 7days, turbidity was visually assessed and recorded as high, moderate or low depending on the level of turbidity. The bacteria and actinomycetes isolates with the highest visualized turbidity were selected for the degradation tests.

Determination of Spent Motorcycle Engine oil Degradation

The level of degradation of the spent motorcycle engine oil was determined, using spectrophotometric (Rahman *et al.,* 2002), gravimetric (Marquez-Rocha *et al.,* 2001) and cell count (Emtiazi and Shakarami, 2004) methods. 1ml each from the selected bacteria and actinomycetes isolates from the utilization test were transferred to conical flasks containing 100ml of Bushnell-Hass medium with 1% spent engine oil and incubated at room temperature at 130rpm in a shaker for a short period of 5 days. The level of engine oil degradation by the isolate was determined gravimetrically by weighing the incubated conical flasks at interval of 24hours for 5days. The percentage of engine oil degradation was calculated using the formula below:

$$\frac{D_b - D_a}{D_b} \times 100\%$$

Where D_b is weight before and D_a is weight after each day (hour).

Assessment of growth rate of Degradation

Growth of the isolates during degradation was monitored by spectrophotometric measurement of absorbance at 560nm and counting of the viable cells by plating 1ml of 10^{-4} serial dilution of BH medium into nutrient agar and starch casein agar for bacteria and actinomycetes respectively at an interval of 24hours for 5days.

Identification of Isolates

Purified isolates of actinomycetes were identified to their generic names by microscopic examination using the cover slip method. Sterilized cover slips were carefully inserted at an angle of about 45^0 into solidified medium in Petri dish until about half of the cover slip was buried in the medium (William and Cross, 1971). The isolates were incubated along the line where the medium meets the upper surface of the cover slip. After incubation for 7-10days, the cover slip was carefully moved and placed downwards on the slide and directly examined under the microscope. Biochemical test(Urease test, Oxidase test, Catalase test, indole test and citrate test) was done. Microscopic and morphologic and biochemical identification of the bacterial isolates was done according to Chessbrough (2002).

RESULTS

Microbial Count

The total heterotrophic counts for the Bacteria and Actinomycetes is presented in table 1 below:

Table 1. Total Heterotrophic Bacteria and Actinomycetes count

Sample sites	Total Heterotrophic bacteria Count (x 104cfu /g)	Total Heterotrophic Actinomycetes count (x 104cfu /g)
Site 1	132	78
Site 2	89	85
Site 3	120	48
Site 4	68	56
Site 5	95	50
Site 6	85	43
Site 7	145	69
Site 8	152	72
Site 9	136	57
Site 10	155	69
Mean	214	114

The total heterotrophic count for bacteria and actinomycetes ranged from 68×10^4 cfu/g to 155×10^4 cfu/g and 43×10^4cfu/g to 85×10^4 cfu/g respectively indicating a comparatively higher bacteria population density. The total count for the hydrocarbon degrading Bacteria and Actinomycetes is presented in table 2 below:

Table 2. Total Hydrocarbon Degrading Bacteria and Actinomycetes counts

Sample sites	Total Hydrocarbon Degrading Bacteria (x 10^4cfu /g)	Total Hydrocarbon Degrading Actinomycete (x 10^4cfu /g)
Site 1	83	55
Site 2	57	56
Site 3	87	33
Site 4	47	38
Site 5	55	42
Site 6	47	18
Site 7	82	43
Site 8	68	49
Site 9	89	37
Site 10	87	47
Mean	127.63	76
% degraders	59.35	66.67

The total hydrocarbon degrading bacteria ranged from 47×10^4 cfu/g to 89×10^4 cfu/,g while the total count for hydrocarbon degrading actinomycetes ranged from 18×10^4cfu/g to 56×10^4 cfu/g. the percentage of spent oil degraders in the soil were 59.35% and 66.67% for the bacteria and actinomycetes isolates respectively. Comparatively, the population density of hydrocarbon degrading bacteria in the soil was more than that of the hydrocarbon degrading actinomycetes.

Spent oil Utilization Test

Result of the visual assessment of the ability of the bacteria and actinomycetes isolates to utilize the motorcycle spent oil are presented below in table 5 and table 6

Table 5. Utilization Test of Spent oil by selected Bacteria isolate

Isolates code	Turbidity
B1	+
B2	+++
B3	+
B4	+
B5	+
B6	+
B7	+

Keys: B-Bacteria + Low ++ Moderate +++Heavy

Table 6. Utilization of Spent oil by the Actinomycetes isolate

Isolates code	Turbidity
A1	+
A2	+++
A3	+
A4	+
A5	++
A6	+
A7	++

Keys: A- Actinomycetes + Low ++ Moderate +++Heavy

The results in table 5 and 6 indicate utilization of the spent oil by all the bacteria and actinomycetes isolates tested. However, the bacteria isolate B2 and the actinomycetes isolate A2 had the highest turbidity based on visual assessment

Identification of Hydrocarbon degraders

Morphological and bacteriological characteristics of selected hydrocarbon degrading Bacteria and Actinomycete isolates are presented below in tables 7 and table 8 respectively:

Determination of Degradation rate

Based on gravimetric analysis, the percentages of degradation for the selected bacteria and actinomycetes isolate is presented below in figure 1.

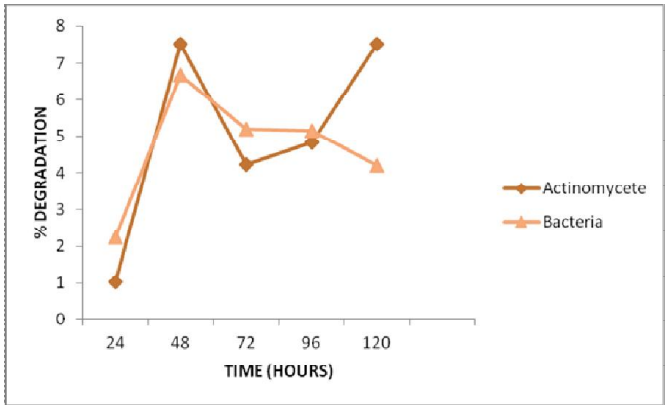

Figure 1. Percentage of degradation of spent oil by the Bacteria and Actinomycete isolates

As shown in figure 1 above, the percentage of degradation of the spent oil by the bacteria isolate range from 2.55% to 6.67% while that of the actinomycetes isolate range from 1.03% to 7.53%. Comparatively, the actinomycete isolate showed a higher percentage of degradation than the bacteria isolate indicating that the actinomycete has greater ability to utilize the spent motorcycle oil. The highest rate of degradation was observed for the bacteria (6.67 %) and the Actinomycetes after 48 hours between 72hours and the 96 hours. There was observed gradual decline in degradation of the oil bt both isolates.

Assessment of growth rate

Based on colony count, the degradation level of the selected bacteria and actinomycetes isolates is presented below in figure 2.

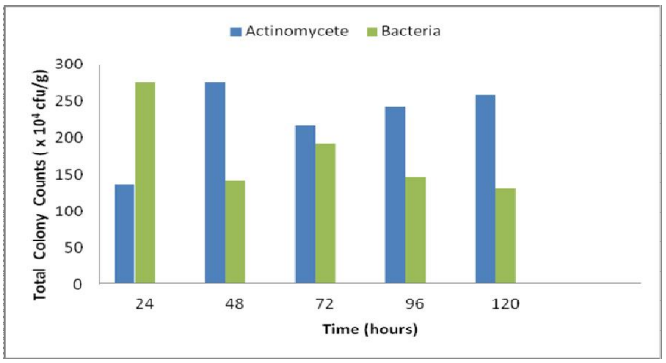

Figure 2. Cell counts of selected Bacteria and Actinomycete isolates during growth

Table 7. Characterization of Hydrocarbon Degrading Actinomycetes isolate

MORPHOLOGICAL CHARACTERISTICS	A1	A4	A3	A2
Colour of aerial mycelium	Cream	Brown	Black	White
Shape	Round but not entire	Round	Round	Round
Margin	Smooth	Rough	Smooth	Smooth
BIOCHEMICAL CHARACTERIATICS				
Indole production	–	–	–	–
Urease	+	+	–	+
Catalase	+	+	+	+
Oxidase	+	–	+	+
Citrate	+	+	+	–
CARBON SOURCE UTILIZATION				
Lactose	–	–	–	–
Glucose	–	–	–	A/NG
GRAM STAIN	+	+	+	+
Cell Shape	Short Rods	Cocci	Cocci	Cocci
PROPABLE ISOLATE	*Nocardia sp.*	*Gordonia sp.*	*Micromonospora sp.*	*Rhodococcus sp.*

As shown in table 7 above, the predominant hydrocarbon degrading bacteria isolates are; *Nocardia sp., Gordona sp., Micromonospora sp.* and *Rhodococcus sp.*

Table 8. Characterization of Hydrocarbon Degrading Bacteria

MORPHOLOGICAL CHARACTERISTICS	B1	B2	B5	B6
Colour	Cream	Cream	Cream	Yellow
Shape	regular	Irregular	Round	Irregular
Margin	Smooth	Rough	Smooth	Rough
BIOCHEMICAL CHARACTERISTICS				
Indole production	+	+	+	+
Urease	+	+	+	+
Catalase	+	+	+	+
Oxidase	+	+	–	–
Citrate	+	+	+	+
CARBON SOURCE UTILIZATION				
Lactose	A/G	–	A/G	A/NG
Glucose	A/G	–	A/G	A/G
GRAM STAIN	+	+	+	+
Cell shape	Cocci	Cocci	Cocci	Cocci
PROBABLE ISOLATES	*Micrococcus sp.*	*Micrococcus sp.*	*Sporosarcina sp.*	*Micrococcus sp.*

As shown in table 8 above, the predominant hydrocarbon degrading bacteria isolates are; *Micrococcus spp.* and *Sporosarcina sp.*

As shown in figure 2 above, the cell counts for the bacteria isolate range from 276×10^4cfu/g to 129×10^4cfu/g while that of the actinomycete ranged from 136×10^4cfu/g to 258×10^4cfu/g. There was observed increased after 48 hours and declined growth at 72hours of degradation in the population density of the bacteria and actinomycete isolates Comparatively, the actinomycetes isolate increased in population density after 72 hours of incubation more than that of the bacteria isolate indicating that the actinomycete isolate has greater ability to utilize the motorcycle spent oil.

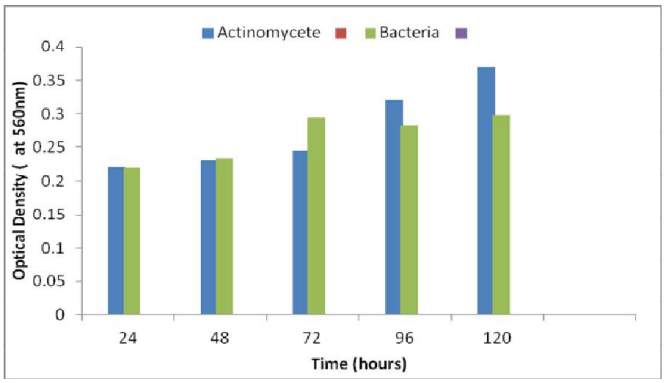

Figure 3. Optical density assessment of the isolates growth during degradation

Based on optical density (OD_{560nm}), the the growth of the by the selected bacteria and actinomycetes isolates degradation is presented below in figure 3. The growth rate of the isolates during degradation ranged from 0.212nm to 0.298nm and 0.215nm to 0.370nm for the bacteria isolate and Actinomycete isolate respectively. Comparatively, the actinomycete isolate had a higher growth. This indicates that the actinomycete isolate has higher ability to utilize the spent motorcycle oil than the bacteria isolate.

DISCUSSION

This study investigated the ability of actinomycetes and bacteria to degrade motor cycle spent oil under a time course experiment, with a view of having a comparative response of these microorganisms to discharge of spent oil into the soil, toxicity and bioavailability of the spent oil on the bacteria and actinomycetes biodegradation capability. According to table 1 and 2, the respective mean heterotrophic and hydrocarbon degraders population density for bacteria isolates were 214x 10^4 cfu/g and 127.6 x10^4 cfu/g while that of the actinomycetes isolates was 114 x10^4cfu/g and 76 x10^4cfu/g indicating a comparatively higher population of heterotrophic and hydrocarbon degrading bacteria in the contaminated soil. However, contrary to expectation the percentage of

hydrocarbon degrading of the actinomycetes (66.6 %) was relatively higher than that of the bacteria (59.4%). The relatively low population of heterotrophic bacteria and actinomycetes recorded in this study is in agreement with Umanu, *et al*., (2013) report of low level of heterotrophic bacteria due to the toxic and unfavaourabl effect of oil contamination in the soil. Bacteria and actinomycetes have been found to utilize hydrocarbon and its derivatives as carbon sources which is a criteria for their multiplication. They are usually present in soil samples contaminated with hydrocarbons, with low molecular weight hydrocarbons that are bioavailable for their use (Bundy *et al*., 2002).

Therefore, the relatively high percentage of hydrocarbon degraders recorded in the soil may be due to the sensitivity of the microorganism to oil contamination and the stimulating effect of additional carbon source for energy and growth (Rahman *et al.,* 2002; Umanu *et al*. 2013), The observed ability of the bacteria and actiomycetes isolates to utilize the spent oil suggest that the indigenous microorganism have the potential to be used in bioremediation of the soil (table5 and 6). In figure 1, the degradation of the spent motorcycle lubricating oil by bacteria and actinomycetes was expressed in percentage; with a range of 2.55% - 6.67% for bacteria and 1.03%-7.53% for actinomycetes indicating that actinomycetes isolate displayed a greater ability to utilize the spent motorcycle lubricating oil and this may be responsible for the higher percentage of degradation observed. In figure 3 also, the level of degradation of both actinomycetes and bacteria based on absorbance was 0.215nm – 0.307nm and 0.122nm – 0.298nm respectively, indicating that actinomycetes had higher ability to utilize the spent lubricating oil The cell counts for the bacteria isolate range from 276×10^4cfu/g to 129×10^4cfu/g while that of the actinomycete ranged from 136×10^4cfu/g to 258×10^4cfu/g.

Comparatively, the actinomycetes isolate increased in population density after 72 hours of incubation more than that of the bacteria isolate indicating that the actinomycete isolate has greater ability to utilize the motorcycle spent oil. (figure 2) Contrary to expectations that when bacteria are provided with suitable environment, they multiply. The reduction in the population density of the bacteria isolate may have been due to some factors. Ogunbayo *et al*. (2012) reported that 80% degradation of used and unused engine oil by actinomycetes specie (*Rhodococcus* sp). and 60 % degradation by a bacterial specie (*Pseudomonas sp*) and concluded that that the actinomycetes isolate degraded better than the bacterial isolate. A close look at tables 3 and 4 may give an insight to the difference in the degradation potentials noticed among the actinomycetes and bacteria. In the tables 3 and 4, the probable isolates revealed a wider diversity of species among the actinomycetes than from the bacteria isolates, and research has shown that the efficiency of the degradation or hydrocarbon removal is favoured more by a consortium than isolates that are less diverse (Bautista *et al*., 2009). This is because the individual potentials of all participating species will be factored in the degradation process. Stringfellow and Alvarez-Cohen (1999), reported that aromatic hydrocarbon degradation is determined by biosorption of the hydrocarbon constituents and this biosorption capacity is varied with the bacteria species and strains. The species diversity of actinomycetes isolated was more diverse than the bacteria as stated earlier and this could be responsible for a higher percentage of biosorption of hydrocarbon by the actinomycetes than the bacteria isolates and subsequently a higher degradation among the actinomycetes. Also, Ogunbayo *et al*. (2012) in agreement with Stringfellow and Alvararez -Cohen, (1999) attributed the better degradation of used engine oil by *Rhodococcus* sp. than *Pseudomonas* sp. to the more hydrophobic and higher affinity for hydrocarbon water interfaces than the *Pseudomonas* sp.

REFERENCES

Adegoroye, G. 1997. Environmental considerations in property design, urban development and renewal. In: *Dimensions of Environmental Problems in Nigeria*, Akinjide, O. (Ed). Friedrich Ebert Foundation, pp 12-25.

Adelowo, O.O., Alagbe, S.O. and Ayandele, A.A. 2006. Time-dependent stability of used engine oil degradation by cultures of *Pseudomonas fragi* and *Achromobacter aerogenes*. Afri. J. Biotechnol. 5(24): 2476-2479.

Bagherzadeh-Namazi, A., Shojaosadati, S.A., Hashemi-Najafabadi, S. 2008. Biodegradation of used engine oil using mixed and isolated cultures. Int. *J. Environ. Res.,* 2(4): 431-440.

Beacker, J.O. and Schwinn F.L. 2003. Control of soil-borne pathogens with living bacteria and fungi: status and outlook. *Pestic. Sci.* 37:355-363.

Berdy, J. 2005. Bioactive microbial metabolites. *J. Antibiot.* 152: 675-683.

Bhadbhade, B.J, Sarnaik, S.S. and Kanekar, P.P. 2002. Bioremediation of an industrial effulent containing monocrotophos. *Current Microbiology.* 45:346-356.

Blodgett, W.C. 2001. Water-soluble mutagen production during the bioremediation of oil contaminated soil. *Florida Scientist* 60(1):28-36

Boonchan, S., Britz, M.L. and Stanley, G.A. 2000. Degradation and Mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. *Appl. Environ. Microbiol.* 66(3):1007-1019.

Bundy, J.G., Paton, G.I. and Campell, C.D. 2002. Microbial communities in different soil types do not converge after diesel contamination. *J. Appl. Microbiol*. 92: 276-288

Chessbrough, M 2002. Laboratort Manual for Tropical Countries, Volune II Microbiology Tropical Health ,ELBS London

Coral, G. and Serap, K. 2005. Isolation and characterization of phenanthrene–degrading bacteria from a petroleum refiney soil. *Ann. Microbio*l., 5(4): 225-259.

Duraes Sette, L., Mendonaca Alves da Costa, L.A., Marsaioli, A.J. and Manfio, G.P. 2004. Biodegradation of alachlor by soil Streptomycetes. *Applied Microbology and Biotechnology.* 64: 712-717.349.

Emitiazi, G. and H. Shakarami, 2004. Utilization of petroleum by Pseudomonas sp. and transformed Escherichia coli. *Afr. J. Biotechnol.,* 4: 172-176.

Faboya, O.O.P. 1997. Industrial Pollution and Waste Management. In: *Dimensions of Environmental Problems in Nigeria,* Akinjide O (ed). Friedrich Ebert Foundation; pp12-2

Ghazali, M.F., Zaliha, N.R., Abdul, R.N., Salleh, A.B. and Basri, M. 2004. *Biodegradation of hydrocarbons in soil by microbial consortium. International Biodeterioration and Biodegradation.* 54:61-67.

Hamme, D.L., Singh, A. And Ward, P.O. 2003. Recent advances in Petroleum Microbiology. *Microbiology and Molecular Biology Reviews.* 67:503-548.

Holt Javid, H. 2005. Isolation and identification of halophyl microorganisms from Lake Bakhtegan and their impact on bioremediation. M.Sc. Thesis, Islamic Azad University Jahrom, Jahrom, Iran, pp. 1-17.

Johnsen, A.R., Winding, A., Karlson, U. And Roslev, P. 2002. Linking of microorganisms to phenanthrene metabolism in soil by analysis of C-labelled cell lipids. *Applied and Environmental Microbiology*. 68:6106-6113.

Kastner, M. 2001. Degradation of aromatic and polyaromatic compounds. In: *Biotechnology: The Science and the Business*, Harwood Academic Press, Switzerland, pp. 274-304.

Kimberley, Q.S. 2005. Source of spilled oil. *Journal on Environmental Biology.* 2:4-5.

Madigan, M. and Martinko, J. 2005. *Brock Biology of Microorganisms* (11th ed.). Prentice Hall. New Jersey, pp. 1350.

Mandri, T. and Lin, J. 2007. Isolation and Characterization of Engine Oil Degarding Indigenious Microorganisms in KwaZulu-Natal, South Africa, *Afr. J. Biotechnol*. 6(1): 23-27.

Marquez-Rocha, F.J., V. Hernandez-Rodriquez and M.T. Lamela, 2001. Biodegradation of diesel oil in soil by the microbial consortium. *Water Air Soil Pollut,* 128: 313-320.

McMurry, J. 2000. Aromatic hydrocarbons. In: *Organic Chmistry*. 5th ed.. Brooks/cole: Thomas learning. New York, pp. 120-180.

Ogunbayo; A O Bello, R. A. and Nwagbara, U. ioremediation of Engine Oil Contaminated Site *Journals of Emerging Trends in Engineering and Applied Sciences* (JETEAS) 3 (3): 483-489

Okerentugba, P.O. and Ezeronye, O. U. 2003. Petroleum Degradation of Potentials of Single and Mixed Microbial Cultures Isolated from Rivers and Refinery Effluent in *Nigeria. Afr. J. Biotechnol.* 2(9): 288 – 292.

Okoh, I. A. 2003. Biodegradation of Bonny Light Crude Oil in Soil Pits in Nigeria *Afr. J. Biotechnol*. 2 (5): 104 – 108.

Rahman, K.S.M., Banat, I.M. and Thahira, J. 2002. Bioremediation of gasoline

contaminated with poultry litter, coir pith and rhamnolipid biosurfactant. *J. Environ. Quality,* 24: 19-28.

Roling, W.F.M., Milner, M.G., Jones, D.M., Lee, K., Daniel, F., Swannell, R.J.P. and Head, I.M. 2002. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. *Appl. Environ. Microbiol.* 68 (11): 5537-5548.

Sahin, N. and Ugur, A. 2003. Investigation of the Antimicrobial Activity of some Streptomyces isolates. *Turk. J. Biol.* 27: 7984.

Stegmann, R., Lotter, S. and Heerenklage, J. 1991. Biological treatment of oil contaminated soils in bioreactors. *J. Inter. Microbiol.,* 4(3): 231-235.

Steve, F.J. 1994. Human Chemistry. John Willey and Sons. Incorporation, New York, pp. 312-314.

Stringfellow, W.T and Alverez-Cohen, L. 1999. Evaluating the relationship between the sorption of PAHs to biomass and biodegradation. *Water Research* 33 (11) : 2535 – 2544.

Survery, S., Samia, A. and Syed, A.A. 2004. Hydrocarbon Degrading Bacteria from Pakestani soil: Isolation, Identification, screening and Genetical Studies. *Pak. J. Biol. Sci.,* 7(9): 1518–1522.

Trindade, P.V.O., Sobal, L.G., Rizzo, A.C.L., Leite, S.G.F. and Soriano, A.U. 2005. Bioremediation of a weathered and recently oil-contaminated soil from Brazil: a comparison study. *Chemosphere,* 58: 515-522.

United State Environmental Protection Agency, 2001. An Analysis of Composting as an Environmental Remediation Technology. USEPA Solid Waste and Emergency Response (5305W). EPA 530-R-98-008, pp. 2-38.

Williams, S.T. and Cross, T. 1971. Actinomycetes. In: Norris, J.R., Robbins, D.W. (eds.) *Methods in Microbiology,* Vol. 4. Academic Press, London and New York. Pp 295-334.

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