Online Publication Date: 19 February 2012
Publisher: Asian Economic and Social Society

Utilization of Petroleum Hydrocarbon Invitro by Rhizosphere Bacterial Isolates of Groundnut (*Arachis hypogeae*)

Aliyu M. B. (Department of Science Laboratory Technology, The Federal Polytechnic, Bida, P.M.B. 55, Bida, Niger State, Nigeria)

Oyeyiola G.P. (Department of Microbiology, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State Nigeria)

Citation: Aliyu M. B , Oyeyiola G.P (2011): "Utilization of Petroleum Hydrocarbon Invitro by Rhizosphere Bacterial Isolates of Groundnut (*Arachis hypogeae*)" Journal of Asian Scientific Research , Vol.2, No.2,pp.53-61.

Utilization of Petroleum Hydrocarbon Invitro by Rhizosphere Bacterial Isolates of Groundnut (*Arachis hypogeae*)

Author (s)

Aliyu M. B
Department of Science Laboratory Technology, The Federal Polytechnic, Bida, P.M.B. 55, Bida, Niger State, Nigeria.
Tel: +2348036864945;
E-mail: alymb2004@Gmail.com

Oyeyiola G.P
Department of Microbiology, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State Nigeria.
Tel: +234-803-359-7100
Email: ganiyu@unilorin.edu.ng

Abstract

The continuous increase in the use of petroleum hydrocarbon (PHC) worldwide due to industrial revolution has led to severe contamination of our terrestrial and aquatic environment. This has affected our ecosystem and human health negatively. Nigeria, one of the major PHC producing nations in the world is also facing the problem of large numbers and size of areas contaminated with PHC arising from accidental or deliberate spills and leakages such as intentional or accidental bursting of pipelines. Estimated cost for cleanup of contaminated sites using conventional techniques is enormous, insufficient and may not even be contemplated by a developing nation like Nigeria which is facing other serious economic challenges of long time infrastructural neglect that is requiring urgent attention to avoid collapse. Consequently alternative biological techniques have continued to emerge around the world like the process of using rhizosphere bacterial isolates of groundnut being described in this research. Rhizosphere soil bacterial isolates of groundnut variety RMP_{91} was sampled for their ability to utilize PHC. They were also analyzed for their ability to utilize PHC as carbon and nitrogen source. A total of 6 bacterial isolates were identified from the rhizosphere soil of groundnut to be capable of utilizing PHC namely: *Pseudomonas aeruginosa, Pseudomonas fluorescens, Bacillus cereus, Bacillus subtilis, Proteus vulgaris and Klebsiella pneumoniae.* The analysis was carried out using mineral assay medium supplemented with the PHC and measurement of optical density of the cultures at 540nm. Higher growth profile rate was observed for mixed culture which was significantly higher than when the isolates are in monoculture at ($p < 0.05$). The growth profile pattern of mixed culture exhibited is an inverse proportionality with the pH profile. *Ps. aeruginosa* showed higher growth profile rate among the monoculture with lower profile observed for *Ps. fluorescens, B. subtilis, B. cereus, and P. vulgaris* respectively. *K. pneumoniae* as monoculture was not able to utilize the various concentrations of the PHC but do in mixed culture. Only *Ps. aeruginosa* and *Ps. fluorescens* were able to utilize PHC as carbon and nitrogen source, while *B. subtilis* and *B. cereus* were able to do so as carbon source only. *P. vulgaris and K. pneumoniae* were not able to utilize PHC as either carbon or nitrogen source. The result therefore suggests that some of the isolates could serve as seed for bio-augmentation during rehabilitation of PHC polluted soil environment.

Key words: Rhizosphere, Utilization, Bio-augmentation, Optical Density, Petroleum Hydrocarbon (PHC)

Introduction

Microorganisms play an important role on nutritional chains, which form important part of life in our planet thereby sustaining biological balance. The adaptation of several abilities by microbes have made them to impact important influence on the ecological systems, making them necessary for superior organism's life in this planet (Hans-Holger and Dieter, 2006). The ability of microorganisms to utilize, transform and even degrade many types of pollutants including PHCs in different matrixes (soil, water, sediments and air) has been widely recognized during the last decades. Soil contamination with hydrocarbons causes extensive damage to local ecosystems since accumulation of pollutants in animals and plants tissues may cause progeny's death or mutation (Amadi *et al*., 1996; Ali and Maram, 2010; Zhibing *et al*., 2010). All of these concerns have made the quest for better methods of remediation and removal of pollutants from the environment even greater. In Nigerian Niger delta area and else ware around the country a number of PHC contaminated sites exist as a result of spillages and pipeline vandalization due to more than 50 years of crude petroleum oil activity (Ifeadi and Nwankwo, 1989; Olagbinde *et al*., 1999; NNPC, 2004). In recent years this problem has motivated researches using biological processes to look at possibilities of recovering these contaminated sites which is always far cheaper than the conventional methods. Microorganisms survive in contaminated habitat because they are metabolically capable of utilizing its resources and can occupy a suitable niche. Sensitivity of soil microflora to PHC is a factor of quantity and quality of oil spilled and previous exposure of autochthonous soil microbiota to oil (Bossert and Bartha, 1984; Ijah and Antai, 2003). Contaminants are often potential energy sources for microorganisms. Biodegradation, a process that exploits the catalytic abilities of living organisms to enhance the rate or extent of pollutant destruction, is an important tool in attempts to mitigate environmental contamination (Nnmachi *et al*., 2006). Biodegradation achieves contaminant decomposition or immobilization by exploiting the existing metabolic potential in microbes with catabolic functions derived through selection, or by the introduction of genes encoding such functions (Young and Cerniglia, 1995; Johnsen *et al*., 2005).

The effectiveness of biodegradation or utilization of pollutants is often a function of the extent to which a microbial population or consortium can be enriched and maintained in environment. When few or no indigenous degradative or utilizing microorganisms exist in a contaminated area and practically does not allow time for the natural enrichment of suitable population, inoculation may be a convenient option (Crawford, 2002; Aliyu and Oyeyiola, 2009[b]; Ogbulie *et al*., 2010). Studies carried out by many investigators has reveal high density of microorganism in soil associated with the plant due to nutrient in the form of exudates release by plant compared to unplanted soil. This according to Aliyu and Oyeyiola (2009[ab]) leads to a dynamic relationship in the rhizosphere between the soil microbes and plant causing accelerated utilization of pollutants. The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. The rhizosphere is also the boundary across which flow all of the water, nutrients, exudates and information exchanged between the plant and the soil community influenced by the presence of the roots. Like all boundaries the rhizosphere is complex in character, probably the most complex environment on earth (Raaijmakers *et al*., 2009; Aliyu and Oyeyiola, 2009[a]; Oyeyiola, 2010). The goal of the present work is to screen rhizosphere soil bacterial cultures of groundnut for their potentials to utilize PHC in-vitro.

Materials and Methods

Description of Study Site

The study site is situated in the biological garden of Federal Polytechnic Bida, Bida, Niger State, Nigeria. It is located at 9^0 2′ 17″ N, 6^0 0′ 27″ E with two distinct seasons viz: wet season from May to October and dry season from November to April. Bida is located in the northern Guinea savannah zone of Nigeria. The rainfall average is 1009 mm in 2009. The highest temperature occurs between the months of March and April. The soil was determined to be Sandy- loam in texture with no history of pollution for over twenty years. The trial was carried out in the wet seasons of 2009.

Land Preparation and other Management Practices

The field was disc-ploughed, harrowed and ridged at 75cm apart. The seeds were planted on 20th June 2009 at 2.5cm depth and 23cm apart.

Collection of Sample

The seed variety RMP_{91} planted for this research was bought from Institute of Agriculture, Ahmadu Bello University, Zaria. Kaduna State, Nigeria. The seeds were bought in already sealed sachet and taken to the laboratory unopened. The sachet was open in sterile atmosphere when the seeds were needed and kept sealed using sellotape. The seeds were kept at room temperature of $25^0C \pm 3^0C$ when they were not needed. The sachet was open each time, in a sterile atmosphere to prevent the introduction of foreign microorganisms onto the seeds. Rhizosphere soil samples were collected by carefully uprooting each plant and shaking the soil adhering to the roots into sterile polythene bag. Each non- rhizosphere soil sample was collected with a sterile hand trowel into a sterile polythene bag. These soil samples were collected using the methods described by Van-Elsas and Smalla (1997); Dongmo and Oyeyiola (2006). They were labeled and transported to the laboratory immediately for analysis. The petroleum hydrocarbon used was obtained from Nigerian National Petroleum Company (NNPC) Refinery and Petrochemicals Kaduna, Kaduna State, Nigeria.

Preparation of Mineral assay medium-Liquid culture medium (LCM) for screening

The LCM used in-vitro to screen for bacteria capable of utilizing petroleum hydrocarbon was prepared by mixing 10ml each of 1 molar Na_2HPO_4, KH_2PO_4, and $(NH_4)_2SO_4$. Also 1ml each of 0.01% $FeSO_4$, Ca $(NO_3)_2$ and 10g of glucose were added. The mixture was made up to 1 litre with distilled water. The pH was adjusted to 7.4 using sodium hydroxide and sterilized by autoclaving at 121^oC for 15 minutes. The details are reported in Chaudhry (1995); Ijah (1998); Ijah and Antai, 2003; Ilori *et al* (2006). The medium was also supplemented with 1% of petroleum hydrocarbon and amended with 0.002% actidione to prevent fungal contaminants from growing to form petroleum hydrocarbon supplemented liquid culture medium (PHCSLCM). The addition of 20g agar was carried out to obtain petroleum hydrocarbon supplemented agar (PHCSA).

Isolation of petroleum hydrocarbon utilizing rhizosphere bacteria

Aliquots of 1 ml groundnut rhizosphere soil suspension from dilution 10^{-3}, 10^{-4} and 10^{-5} were transferred each onto PHCSA. Sterile bent glass rod was used to aseptically spread the suspension uniformly on the agar and incubated at room temperature 28 $\pm 2^oC$ for 48-72 hours. Representative colonies were recorded and taken for purification to obtain pure cultures by repeated sub-culturing on fresh media used for primary isolation. The pure stock cultures obtained were prepared on nutrient agar (Oxoid) slants containing petroleum hydrocarbon respectively in McCartney bottles and preserved in the refrigerator at 4^oC until needed for further characterization and identification (Ibrahim *et al*., 2008; Moneke *et al*., 2010; Zhang *et al*., 2010).

Characterization and identification of isolates

These were done by carrying out a number of analyses namely: Morphological characteristics, Microscopic examination, Cultural characteristics, and Gram staining reaction. Relevant biochemical tests were also carried out including production of catalase, coagulase, oxidase, indole, utilization of citrate, fermentation of sugars, presence of spore, H_2S test, production of gas, oxidation fermentation test (OF), gelatin liquefaction, urease test, starch hydrolysis, triple sugar iron agar test, motility and methyl red-voges proskauer test. Confirmatory identities of the bacteria were made using the Bergey's Manual of determinative bacteriology (Holt *et al.,* 1994) and also as described by Collins *et al* (1995); Cheesebrough (2004).

Inoculums preparation and standardization

The cells from the above cultures were re-suspended using sterile normal saline to a 0.5 McFarland nephelometer standard (Optical density of 0.17 at 540nm).

Utilization of different concentrations of petroleum hydrocarbon by mixed bacterial cultures

Aliquots (1.0 ml) of 24 hours old bacterial cultures each from the above (0.5 MacFarland standards) were inoculated into 250 ml Erlenmeyer flasks containing 150 ml of LCM supplemented with various concentrations of petroleum hydrocarbon separately (2, 4, 6, 8, and10 %). LCM supplemented with 1% of petroleum hydrocarbon only, without the inoculum's serve as control. Bacterial growth was monitored by withdrawal of 5.0 ml of the culture sample immediately after inoculation for the 0 hour to measure the optical density (OD) and every 24 hours up to 120 hours of incubation in a Gallenkamp orbital shaker (Cat no: IH-460, made in England) at 28^{o} $\pm 2^{o}$C 100rpm (Liu *et al*., 1991; Zboinska *et al*., 1992; Atlas *et al*., 1995; Moneke *et al*., 2010). Growth and utilization were monitored by measuring the turbidity using spectrophotometer (Spectronic 21D Milton and Roy made in USA) set at 540nm. The changes in pH were monitored using Pye Unicam pH meter (model 90, MKZ, Cambridge UK) (Okpokwasili and Okorie, 1988; Ibrahim *et al*., 2008). The total viable count was estimated by plating out onto nutrient agar (Oxiod). The plates were incubated at 37^{o}C for 48 to 72 hours. Representative colonies were enumerated and also taken for purification and identification.

Utilization of different concentrations of petroleum hydrocarbon by monoculture of bacteria

The same procedure followed for mixed bacterial cultures were adopted using monoculture of the individual isolates that make up the mixed culture. Growth and utilization was monitored by measurement of optical densities and estimation of viable counts of the growth media.

Utilization of petroleum hydrocarbon as carbon and nitrogen Sources by mixed culture and monoculture of the bacterial isolates

PHCSLCM was prepared without addition of glucose as one of its components to test for the ability of bacterial cultures to utilize petroleum hydrocarbon as their carbon source. Similarly, another PHCSLCM was prepared without addition of $(NH_4)_2S0_4$ as one of its components to test for the ability of bacterial cultures to utilize petroleum hydrocarbon as nitrogen source. From each of the preparations above, aliquots (1.0 ml) of 24 hours old mixed bacterial cultures isolated from above (0.5 McFarland standards) were inoculated into 250 ml Erlenmeyer flasks containing 150 ml of PHCSLCM. The control was uninoculated. Incubation was at 28 ± 2^{o}C for 120 hours. Utilization was monitored by measuring the optical density (OD) at 540nm for 120 hours at 24 hours interval using spectrophotometer (Chaudhry, 1995). The above procedure was also followed for monoculture.

Statistical Analysis

The statistical analysis of the results was carried out using Package for Social Sciences (SPSS) for Windows version 12.0 software by analysis of variance (ANOVA) and Duncan multiple range tests.

Result

The percentage frequency of occurrence of the various bacterial isolates is presented in Table 1 with *Ps. Aeruginosa* indicating the highest utilization rate. The growth profile of mixed bacterial cultures in different concentrations of petroleum hydrocarbon measured with spectrophotometer is presented in figure 1, while that of the individual monocultures are presented in figures 3-6 respectively. The pH changes in the mineral assay petroleum hydrocarbon supplemented medium inoculated with mixed bacterial isolates is presented in figure 2.

Discussion

Only six isolated bacterial cultures showed the capacity to grow in the presence of the petroleum hydrocarbon. The six isolates were identified as *Pseudomonas aeruginosa, Ps. fluorescens, Bacilus subtilis, B. cereus, P. vulgaris* and *K. pneumoniae.* The percentage frequency of occurrence of the various bacterial isolates is shown in Table 1. The growth profile of mixed cultures in different concentrations of petroleum hydrocarbon through observation of the growth medium turbidimetrically, as

compared with the uninoculated control by optical density measurement at 540 nm is shown in Figure 1. The result in figure 1 indicated a significantly ($p < 0.05$) higher utilization by mixed culture isolates compared to the individual monoculture isolates as presented in figures 3, 4 and 5 respectively. This observation indicates that the mixed bacterial cultures could in concert utilize the petroleum hydrocarbon more than the monoculture. Similar observations were made in the findings of Hans-Holger and Dieter (2006); Ogbulie *et al* (2010).

The result of this study showed that increase in the concentrations of the petroleum hydrocarbon led to a concomitant decrease in the growth profile of both the mixed bacterial isolates and the monoculture counterpart. Ilyina *et al* (2006) observed that petroleum hydrocarbon can inhibit rhizosphere soil microorganisms depending on the soil type, concentration and other environmental variables. The short lag phase in the growth pattern was observed especially at a high concentration of 10% in the first 24 hours of incubation (figure 1 and figures 3-6). This is in agreement with the finding of Dileep (2008) that microbes require a period of adjustment when introduced into a new environment. The growth pattern also showed an inverse proportionality with the pH profile as observed in figure 2.

This study revealed that *K. pneumoniae,* one of the members of the mixed cultures was not able to utilize the PHC when in monoculture but do so in mixed culture. This bacterium therefore does not possess the requisite enzyme or coenzymes necessary to carry out the utilization of PHC. Consequently it could be utilizing the PHC when in mixed culture with other bacteria at the expense of metabolites secreted by these mixed cultures via the process of comatabolism. Similar phenomenon was reported by Ogbulie *et al* (2010).

P. aeruginosa exhibited higher utilization rate than all the other monoculture isolates with *P. vulgaris* exhibiting the lowest utilization rate. This is in line with the findings of Hans-Holger and Dieter (2006); Moneke *et al* (2010) in their various researches that *Pseudomonas* genus generally can grow and multiply more than most microorganisms because of their fast colonizing nature even in the presence of pollutant or xenobiotics.

The mixed culture was able to utilize petroleum hydrocarbon as carbon and nitrogen source. However it was able to utilize it as carbon source at a higher rate compared to as nitrogen source. The reason for this difference is not clear from the various literatures available. *P. aeruginosa* and *P. fluorescens* were able to utilize petroleum hydrocarbon as carbon and nitrogen source among all the monoculture, while *B. subtilis* and *B. cereus* were able to do so as carbon source only. This mean that the other isolates lack enzymatic properties responsible for utilization of petroleum hydrocarbon as nitrogen source but could only be doing so in mixed cultures at the expense of metabolites release by Pseudomonas genus as reported in similar study by Kanaly and Harayama (2000); Ogbulie *et al* (2010). Higher growth profile exhibited by *P. aeruginosa* and *P. fluorescens* as well as their abilities to utilize the pollutant as carbon and nitrogen is consistent with the findings of Young and Cerniglia (1995) that *Pseudomonas* sp utilizes wide range of xenobiotics. *P. vulgaris* and *K. pneumoniae* were not able to utilize PHC as both carbon and nitrogen source. This study indicates conclusively that rhizosphere bacterial isolates of groundnut was able to utilize all concentrations of petroleum hydrocarbon analyzed. The Plant therefore has a potential of being use for phytoremediation of contaminated soil with petroleum hydrocarbon and the isolates could also serve as seed for bio-augmentation during remediation. However, further researches are clearly needed to translate in-vitro findings to the field situation due to the complexity of field environmental conditions.

Online Publication Date: 19 February 2012
Publisher: Asian Economic and Social Society

Table -1 Hydrocarbon utilizing rhizosphere bacteria (percentage frequency of occurrence)

Isolates	**Hydrocarbon utilizing rhizosphere bacteria (%)**
Bacillus subtilis	22
Bacillus cereus	20
Pseudomonas aeruginosa	31
Pseudomonas fluorescens	9
Klebsiella pneumoniae	11
Proteus vulgaris	7

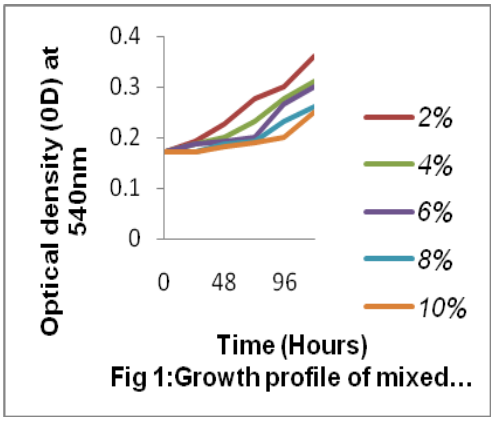

Fig 1:Growth profile of mixed…

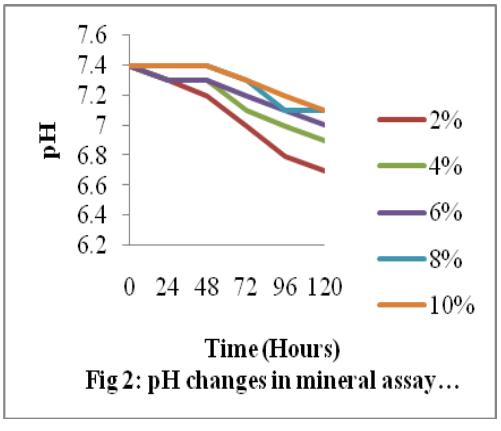

Fig 2: pH changes in mineral assay…

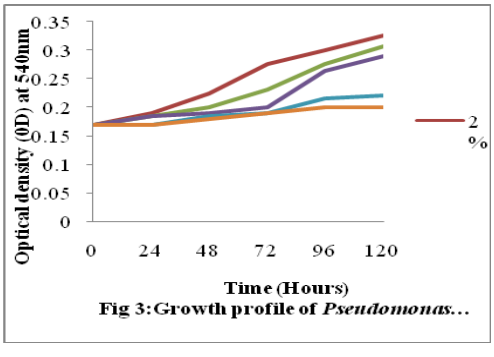

Fig 3:Growth profile of *Pseudomonas*…

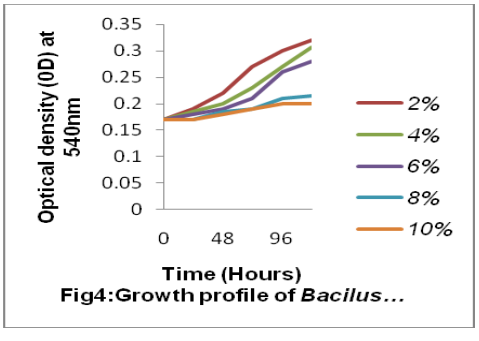

Fig4:Growth profile of *Bacilus*…

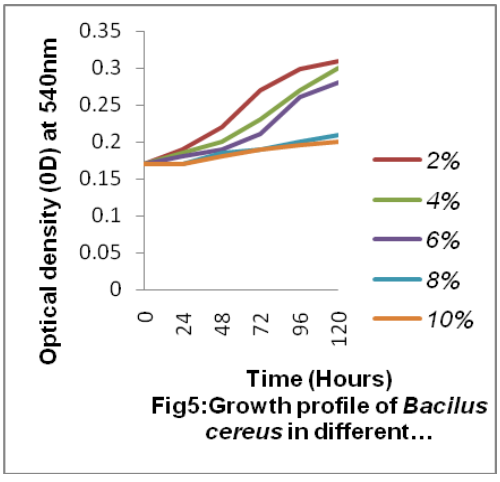

Fig5:Growth profile of *Bacilus cereus* in different…

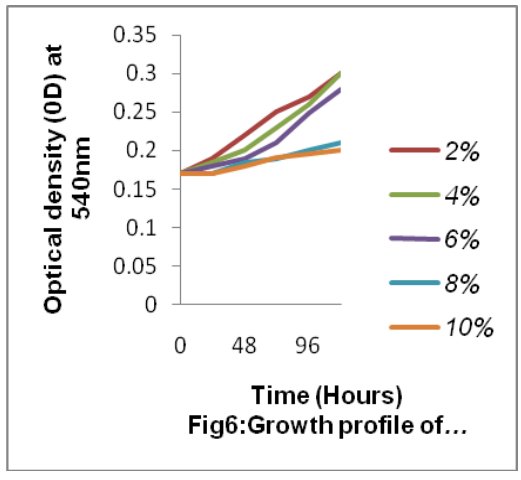

Fig6:Growth profile of…

References

Ali Diab and Maram, S. (2010) Biodegradation of Polycyclic aromatic hydrocarbons (PAHs) in the Rhizosphere soil of *Cyperus conglomeratus*, an Egyptian Wild Desert Plant. Nature and Science Vol.8, No.12, pp. 144-153.

Aliyu, M.B. and Oyeyiola, G.P. (2009)[a] Studies on the application of selected pesticides on the microflora composition in the rhizosphere of groundnut. International Journal of Bioscience Vol.4, No.2, pp. 98-103.

Aliyu, M.B and Oyeyiola, G.P. (2009)[b]. Effect of selected pesticides on biodegradative potentials of microbes in the soil previously cropped to groundnut. Journal of Agricultural Research and policies Vol. 4, No.3, pp. 8-12.

Amadi, A., D.A. Samuel and N. Anthony (1996) Chronic effects of oil spill on soil properties and microflora of a rainforest ecosystem in Nigeria. Journal of Earth and Environmental science Water, Air, Soil and Pollution Vol. 86, No.4, pp. 1-11.

Atlas, R.M. (1995) Bioremediation of petroleum pollutants. International Journal of Biodegradation Vol.35, pp. 317-327.

Bossert, I and Bartha, R. (1984) The fate of petroleum in soil ecosystems. In R. M. Atlas (ed) Petroleum Microbiology. Macmillan Publishing Company, NewYork. pp 435-473.

Chaudhry, G.R. (1995) Biological degradation and bioremediation of toxic chemicals. Timber Press. Portland. pp 15-17.

Cheesebrough, M. (2004) Biochemical Test to Identify Bacteria. In District Laboratory Practices in Tropical Countries. Cambridge University Press. UK pp. 62-291.

Collins, C.H., P.M. Lyne and J.M. Grange (1995) Collins and Lyne's Microbiology Methods (7th ed.). Butterworth-Heinemann, Oxford. pp: 102-103.

Crawford, R.L. (2002) Overview: Biotransformation and Biodegradation. In C.J. Hurst, R.C Crawford, G.R. Knudsen, M.J. Mclnerney and L.D. Stetzenbach, eds, Manual of Environmental Microbiology, 2nd ed. American Society for Microbiology, Washington, USA. pp 901-902.

Dileep, K.S. (2008) Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Indian Journal of Microbiology, 48: 35-40.

Dongmo, J.C and Oyeyiola, G.P. (2006) Fusarium in the rhizosphere and rhizoplane of groundnut (*Arachis hypogaea*). Journal of Agricultural Research and Development Vol.5, pp. 153-164.

Hans-Holger, L and Dieter, F. (2006) Crop growth, culturable bacteria, and degradation of petrol hydrocarbon (PHCS) in a long term contaminated field soil. Applied Soil and Ecology Vol.31, No.1-2, pp. 43-52.

Holt, J.G., N.R. Krieg, P.H.A Sneath, J.T. Staley and S.T. Williams (1994) Bergeys Manual of Determinative Bacteriology. Willimans and Wilkins Press, Baltimore, USA 9th edn.

Ibrahim, M.L., U.J.J. Ijah, S.B. Manga and A.B. Rabah (2008) Occurrence of hydrocarbon utilizing bacteria in the rhizosphere of *Eucalypus camaldulensis, Lablab parpureus and Moringa oleifera*. International Journal Pure and Applied Science Vol.2, No.3, pp.21-26.

Ifeadi, C.N and Nwankwo, J.N. (1989) Oil spill incident in Nigerian Petroleum Industry: A critical analysis. Nepector Vol.8, pp. 11-45.

Ijah, U.J. J. (1998) Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. Waste Management Vol.18, pp. 293-299.

Ijah, U.J.J and Antai, S.P. (2003) Removal of Nigerian light crude oil in soil over a 12 month period. International Journal of Biodeterioration and Biodegradation Vol.51, No.2, pp. 93-99.

Ilori M.O., O.O. Amund, C.J. Ezeani, S. Omoijiahina and S.A. Adebusoye (2006) Occurrence and growth potentials of hydrocarbon degrading bacteria on the phylloplane of some tropical plants. African Journal of Biotechnology Vol.5, No.7, pp. 542-545.

Ilyina A., M.I. Castillo, J.A. Villarreal, G. Ramirez and J. Candelas (2006) Isolation of soil bacteria for bioremediation of hydrocarbon contamination. *Вестн. Мос*. Vol.44, No.1, pp. 88-91.

Johnsen, A.R., L.Y. Wick and H. Harms (2005) Principles of microbial PAH-degradation in soil. Environmental Pollution Vol.133, pp. 71-84.

Kanaly, R and Harayama, S. (2000) "Biodegradation of high molecular weight PAHs by bacteria." Journal of Bacteriology Vol.182, pp. 2059-2067.

Liu, C.M., P.A. Mclean, C.C. Sookdeo and F.C. Cannon (1991) Degradation of the herbicide glyphosate by members of the family Rhizobiaceae. Applied Environmental Microbiology Vol.57, pp.1799-1800.

Moneke, A.N., G.N. Okpala and C.U. Anyanwu (2010) Biodegradation of glyphosate herbicide in *vitro* using bacterial isolates from four rice fields. African Journal of Biotechnology Vol.9, No.26, pp. 4067- 4074.

Nnamchi, C.I., J.A.N. Obeta and L.I. Ezeogu (2006) Isolation and characterization of some polycyclic aromatic hydrocarbon degrading bacteria from Nsukka soils in Nigeria. International Journal of Environmental Science and Technology Vol.3, No.2, pp.181-190.

NNPC (2004) PPMC loses N34 billion to pipeline vandalization. NNPC News- official monthly newsletter of the Nigerian National Petroleum Cooperation (NNPC) Vol.25, No.8, pp.1-2.

Ogbulie, T.E., H.C. Nwigwe, M.O.E. Iwuala and G.C. Okpokwasilli (2010) Study on the use of monoculture and multispecies on bioaugmentation of crude oil contaminated agricultural soil. Nigerian Journal of Microbiology Vol.24, No.1, pp. 2160-2167.

Okpokwasili, G.C and Okorie, B.B. (1988) Biodeterioration potential of microorganism isolated from Engine Lubricating oil. Tribology International Vol.21, pp. 215-217.

Olagbinde, O.T., G.O. Ede, L.E.D. Iyang, E.S. Gundlach and D.S. Page (1999) Scientific and cleanup response to the Idoho-Qua Iboe Terminal oil spill, Nigeria. Environmental Technology Vol.20, pp. 1213-1222.

Oyeyiola, G.P. (2010) Rhizosphere bacterial flora of *Amaranthus hybridus*. Research J. of Microbiology Vol.5, No.2, pp. 137-143.

Raaijmakers, J.M. C.P. Timothy, S. Christian, A. Claude and M.L. Yvan (2009) The Rhizosphere: a playground and battlefield for soil borne pathogens and beneficial micro organisms. Plant Soil Vol. 321, pp. 341-361.

Van-Elsas J. D and Smalla, K. (1997) Methods for sampling soils microbes. In: Manual of Environmental Microbiology (Hurst, C.H., G. Knudsen, M. McInerney, L. Stetzenbach and M. Walter, Eds.). American Society of Microbiology. Washington, D.C. pp. 383-390.

Young, L.Y and C.E. Cerniglia C.E. (1995) Microbial transformation and degradation of toxic organic chemicals. Witey –liss, N.Y Press. pp 22-25

Zboinska, E., B. Lejczak and P. Kafarski (1992) Organophosphonate utilization by the Wild-Type strain of *Pseudomonas fluorescens*. Applied Environmental Microbiology Vol.58, No.9, pp.2993-2999.

Zhang, C., Z. Qixing, P. Shengwei and L. Kenan, (2010) Promoted biodegradation and microbiological effects of petroleum hydrocarbon by impatiens balsamina L. with strong endurance. Journal of Hazardous Materials Vol.183, No13, pp.713-737.

Zhibing, J., H. Yijun, X. Xiaoqun, L. Yibo and Z. Jiangning (2010) Advances in the toxic effect of petroleum water accumulated fraction on marine plankton. Acta Ecologica Vol.30, No1, pp.8-15.