

# Prediction of Remediation Rates of Microbes in Polluted Crude Oil Soil Samples

B. C. Okoro<sup>1</sup>, O. A. Nwadike<sup>1</sup> & J. C. Agunwamba<sup>2</sup>

<sup>1</sup> Department of Civil Engineering, Federal University of Technology Owerri, PMB 1526, Owerri, Nigeria

<sup>2</sup> Department of Civil Engineering, University of Nigeria, Nsukka, Nigeria

Correspondence: B. C. Okoro, Department of Civil Engineering, SEET, Federal University of Technology Owerri, PMB 1526, Imo State, Nigeria. Tel: 234-083-508-6235. E-mail: bc1okoro@yahoo.com

Received: June 13, 2013 Accepted: July 18, 2013 Online Published: July 20, 2013

doi:10.5539/enrr.v3n3p89

URL: <http://dx.doi.org/10.5539/enrr.v3n3p89>

## Abstract

The rate of removal of Total Petroleum Hydrocarbon Content (TPHC) of a crude oil polluted land was investigated using field experimental data generated from the Research Farm soil at the Federal University of Technology Owerri, Imo State, Nigeria. The soil was artificially polluted in the laboratory with crude oil - bonny light - with specific gravity of 0.8323. Petroleum contaminant present in the soil was 230 mg per kilogram of soil. The treatment variables used include: inorganic fertilizer (NPK 20:10:10), poultry manure, cow dung and a mixture of the three in equal proportion. A natural treatment was allowed to occur as the control experiment. fungi as well as bacteria played an important role in the degradation of petroleum hydrocarbon. The identified crude oil degrading Fungi are *Penicillium notatum*, *Mucor spp*, *Rhizopus stolonifer* and *Penicillium caseicolum* with *P. notatum* and *P. caseicolum* (*penicillium spp*) as the strongest fungi degraders. The identified degrading Bacteria are *Pseudomonas putida* and *Bacillus subtilis*. These can therefore be isolated and cultured and then employed on remediation sites either as indigenous or foreign degrading microbes in the engineering of bioremediation of crude oil polluted soil using the best engineering techniques. The treatment with mixture of treatment variables proved to be a better option from the results obtained with 82.38 mg/kg after 9 weeks of remediation followed by fertilizer, 83.13 mg/kg and 86.75 mg/kg for poultry manure. Cow dung had 105.5 mg/kg and the control had least with 204.50 mg/kg.

**Keywords:** total petroleum hydrocarbon content, bioremediation, microbial analysis, organic species, polluted soil

## 1. Introduction

Globally, there is a growing concern over environmental pollution and its management. The three major areas of environmental pollution include: water, air and land. One of the major causes of this environmental pollution in Nigeria is as a result of hydrocarbon exploitation and exploration (Okwuosha, 2000). This has led to the degradation of farmlands, pollution of surface and ground waters as well as air due to gas flaring. The natural recovery of crude oil polluted land is slow. Communities affected are denied meaningful and economic use of their lands a long time. Hence remediation was brought about. Remediation has been defined as “the management of a contaminant at a site so as to prevent, reduce or mitigate damage to human health or the environment which can also lead to quick recovery of the affected land” (Dodman, 1994; Ebuehi et al., 2005).

Bioremediation is a process by which chemical substances are degraded by bacteria and other microorganisms. A more expansive definition from the Joint Research Council Review of Bioremediation Research in the UK published in February 1999, defined bioremediation as being: “The elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes, to minimize the risk to human health and the environment”. The replacement of ‘microorganisms’ with ‘biological processes’ reflects the inclusion of the use of plants to include phytoremediation processes. Bioremediation processes enhance the activities of indigenous microbes, such as bacteria, via the addition of oxygen and nutrients to degrade hydrocarbon to water and harmless gases like carbon dioxide. To remediate petroleum contaminated sites, we need a low-cost, low input treatment alternative to use in conjunction with existing methods. In general, indigenous soil microbiota can degrade petroleum compounds. When soils fail to bioremediate at optimum rates, it is often a function of the water solubility of the compound and environmental limitations imposed on the microbes. Major limitations to

the microbiota are temperatures that are too high or too low, excess or deficient water, insufficient or excessive nutrients, insufficient carbon in a form that microorganisms can use, poor mixing or distribution of the petroleum in the soil, and, for aerobic microorganisms, lack of oxygen (O<sub>2</sub>). The relative effectiveness of different treatment systems will vary over time. For example, lack of oxygen has been believed to be the primary limitation at depth; thus, air-injection technologies are commonly employed to overcome this. However, it is now well established that subsurface (relatively deep) microbial activity is common, and anaerobic biodegradation of water-soluble petroleum takes place without the need to inject air if alternate electron acceptors, such as oxidized species of iron or nitrogen (such as nitrate), are available.

Bioremediation treatments are successful when limitations are overcome. The key problem, however, is identifying and implementing the most cost-effective means of doing this at sites. Two important aspects in comparing low cost to more costly alternatives are time constraints and monitoring difficulties. In comparing treatments, it is important to measure effectiveness over time.

The pollution of soil, upland pollution, directly affects the quality of water due to runoff washing pollutants from soil surface into natural channels. Runoff carrying hydrocarbons and other contaminants from polluted land enters into natural channels thereby polluting the water. Treatment of such water for municipal and any local use becomes more expensive and challenging. Even the use of the water for ordinary domestic, agricultural, recreational and industrial uses may be seriously hindered. The prevention of such occurrence, therefore, is most advocated than the cleansing or expensive treatment of polluted water. Quick remediation of oil polluted land is therefore very relevant in maintaining pollution free surface water in the oil producing and oil polluted environments. Allowing crude oil polluted soil to recover naturally is dangerous to shallow water tables and open wells. Seepage down the soil though may be slow, can contaminate a whole source of water for a household or a whole community.

## 2. Material Studied

This study was done by carrying out a field study on bioremediation to ascertain the degrading elements in the bioremediation technology and comparing the field data generated with other similar works for the purpose of comparative analysis of the probable time of petroleum contaminant removal from a polluted site using different treatment variables.

### 2.1 Experimental Design

The study was done for a period of eighteen (18) weeks. Polluted crude oil soil samples were placed into five (5) different containers, of similar size and geometry, dimensioning 17 cm (height) and 18.5 cm (diameter). The 5<sup>th</sup> container, was used for the control experiment (CT). 5 kg of polluted soil was placed in each of the containers and were all exposed to the same atmospheric and environmental conditions.

(CD)	(PM)	(FZ)	(MX)	(CT)
Cow Dung	Poultry Waste	Inorganic fertilizer	Synergy /Mixture	Control Experiment

Figure 1. Layout of the experimental design

### 2.2 Soil Collection and Pollution

The soil used in the study was collected from the FUTU Research Farm from 15 cm to 20 cm depth with shovel. The soil was collected into containers and was taken to the site for treatment (greenhouse treatment). The soil was air dried for four days and 25 kg of soil was polluted with 1litre of crude oil (Bonny light) with specific gravity of 0.8343 leaving about 230 mg/kg of soil. The crude oil was allowed to cover the surface of the soil completely. This was to simulate a natural field condition of major spill. The pollution is equivalent of 73,800 litres per hectare and 200 cm<sup>-3</sup> per 5 kg of soil.

## 3. Area Descriptions

The experiment was carried out as an ex-situ treatment of polluted soil obtained from the Research Farm of Federal University of Technology Owerri (FUTO), Imo State, Nigeria. The study area is located in Owerri, Imo state and lies between latitude 5° 22' 51.5" N and longitude 6° 59' 39.3" E, with an elevation of 61 m. It is a

humid tropical environment with average annual rainfall of 2400 mm and 3 distinct months of dryness (December to February). The mean daily temperature is about 27 °C. The soils are derived from coastal plain sands called acid sands - Benin formation (Orajaka, 1975).

#### 4. Methods

##### 4.1 Soil Treatment Procedure

The polluted sample was allowed to stay 14 days before the start of treatment. The amendments (treatment variables) used included: cow-dung CD, poultry waste (manure) PM, and inorganic fertilizer (NPK 20 10 10) FZ, and Synergy (mixture) MX, of the above three (ie CD, PM and FZ) in the appropriate proportion. The polluted sample was thoroughly mixed to ensure even distribution of pollutant. The various amendments were then added to 5kg of soil each and thoroughly mixed except for the control sample. The samples were thoroughly mixed twice a week with the addition of moisture to provide a conducive environment for the degrading microbes.

##### Quantities of amendments used:

- Poultry Waste (manure) - PM: 55 g of poultry manure per 5 kg of soil. This is equivalent to 20 tons/ha as recommended by Amadi and Bari (1992).
- Cow dung - CD: 55 g of cow-dung per 5 kg of soil as recommended by Amadi and Bari (1992).
- Inorganic fertilizer - FZ: 25 g of NPK per 5 kg of soil was used which is equivalent to 8.2 ton/ha. This was based on the recommendation of 4.7 - 12.5tons/ha by Ogaji, Ayotamuno, Kogbara, and Probert (2005).
- Synergy - MX: mixture of 25 g of inorganic fertilizer, 25 g of cow dung and 25 g of poultry manure.

*Experimental Soil Sampling:* The experimental soil was analyzed in the laboratory at intervals. The soil was taken to the laboratory before pollution and two weeks after pollution before the start of treatment. The rest of samplings were during treatment. The samples were thoroughly mixed and homogenized before collection into neat and well labeled polythene bags free from contamination. The soil samples were immediately taken to the laboratory for analysis.

##### 4.2 Laboratory Investigation

Both microbial and Total Petroleum Hydrocarbon (TPH) content analysis was done in the laboratory.

###### 4.2.1 Microbial Analysis

*Preparation of diluents:* diluents used for the dilution of the samples were prepared by dispensing 9 ml of distilled water into bijou bottles. This was sterilized by autoclaving at 121 °C for 15minutes and allowed to cool before use (Cheesbrough, 2000).

*Preparation of media:* nutrient agar (NA) and potato dextrose agar (PDA) were prepared according to manufacturer's specification described by Cheesbrough (2000). Mineral based petroleum agar (PA) was prepared according to the method adopted by the Institute of Petroleum Studies (IPS), Rivers State University of Science and Technology, Port Harcourt. The recipe used includes:

NH<sub>4</sub>Cl 0.5 g; K<sub>2</sub>HCO<sub>4</sub> 0.5 g; NaHPO<sub>4</sub> 2.5 g; diesel/oil 0.5%; Agar 15.0 g in 1litre of distilled water.

*Inoculation of Samples:* One gram (1 g) quantity of the sample was dispersed into 9 ml of sterile distilled water to obtain 10<sup>-1</sup> dilution. Further dilutions were made by transferring 1ml of the previous solution until 10<sup>-6</sup> was obtained. One-tenth nullilitre (0.1 nil) was collected from 10<sup>-6</sup> and inoculated into freshly prepared surface dried nutrient agar in duplicates. The same quantity was collected from 10<sup>-4</sup> dilution into potato dextrose agar and mineral based petroleum agar (International Commission on Microbiological Specification in Foods [ICMSF], 1978; Beishir, 1987; Cheesbrough, 2000). The inoculum was spread evenly with a sterile hockey stick like glass rod.

###### 4.2.2 Enumeration of Microbial Population

This was done manually by dividing the Petri-dish into four quadrants at the reverse side of the culture plates. Total colony count was expressed in colony forming units per gram (CFU/g). The mathematical expression was adopted from Harrigan and McCauce (1990).

$$\frac{CFU}{g} = \frac{N}{V} \times \frac{D}{1} \quad (1)$$

Where

N is number of colonies counted

V is volume of inoculums transferred to the plates

D is the dilution factor

#### 4.2.3 Characterization of Microbial Isolates

Colonial, microscopic and biochemical characteristics of the microbial isolates was done according to Cheesbrough (2000), Harrigan and McCauce (1990) and Beishir (1987).

#### 4.2.4 Identification of Microbial Isolates

This was done with reference to standard bacteriological and mycological manual cited in Buchanan and Gibbons (1974) and Barnet and Hunter (1987) respectively.

#### 4.2.5 Total Petroleum Hydrocarbon (TPH)

TPH is a term used for any mixture of hydrocarbons found in crude oil. There are several hundred of these compounds, but not all occur in any one sample. Because there are so many different chemicals in crude oil and in other petroleum products, it is not practical to measure each one separately. However, it is useful to measure the total amount of TPH at a site.

*Procedure:* 2 g of soil sample was weighed into a 100 ml flask and 50 ml of chloroform was added into it. After shaking vigorously for 3 minutes, the liquid phase was extracted and measured using a UV-Visible Spectrophotometer. Standard curve of the absorbance of different known concentrations of petroleum hydrocarbons in the extract was derived using fresh crude oil appropriately diluted with the solvent and was used to read off petroleum hydrocarbon content. Mathematically, Petroleum hydrocarbon concentration in soil was then calculated after reading the absorbance of the petroleum hydrocarbons in the extract from the spectrophotometer. The Total hydrocarbon content (THC) was obtained as described below:

$$\text{THC} \left( \frac{\text{mg}}{\text{kg}} \text{soil} \right) = \frac{\text{Absorbance} \times \text{DF} \times 50}{\text{Weight of soil used}} \quad (2)$$

Where DF is dilution factor

50 is the initial extraction volume

### 5. Results

Analysis of the laboratory results' changes in microbial count is almost directly proportional to changes in TPHC and hence the % reduction. The sharp increase in the microbial load of hydrocarbon degrading fungi and bacteria within the 6<sup>th</sup> and 9<sup>th</sup> weeks resulted in sharp reduction in TPHC which is seen in the increase in the % reduction. This increase continued as can be seen in the 12<sup>th</sup> week with Synergy having  $1.65 \times 10^7$  degrading bacteria and  $3.4 \times 10^6$  degrading fungi, followed by Poultry manure,  $1.21 \times 10^7$  and  $3.0 \times 10^5$  respectively. Fertilizer followed with  $6.2 \times 10^6$  and  $1.0 \times 10^6$ , then Cow dung  $6.1 \times 10^6$  and  $1.6 \times 10^5$  respectively. Table 1 provides microbial count before and after pollution.

Table 1a. Total microbial count before pollution (cfu/g)

THBC	THCBC	%Degradars	THFC	THCFC	%Degradars
$1.2 \times 10^{10}$	$6.9 \times 10^6$	0.03	$1.2 \times 10^7$	$1.4 \times 10^6$	11.67

Table 1b. Total microbial count 2 weeks after pollution (cfu/g)

THBC	THCBC	%Degradars	THFC	THCFC	%Degradars
$1.28 \times 10^{10}$	$9.1 \times 10^6$	0.07	$3.6 \times 10^7$	$2.8 \times 10^6$	7.78

Table 1c. Total microbial count during remediation (cfu/g)

TRT	THBC	THCBC	%Degradars	THFC	THCFC	%Degradars
1 week of Remediation						
CD	$7.2 \times 10^9$	$4.1 \times 10^5$	0.006	$1.2 \times 10^7$	$1.0 \times 10^5$	0.83
PM	$4.9 \times 10^{10}$	$3.9 \times 10^6$	0.008	$3.6 \times 10^7$	$1.2 \times 10^6$	3.3
FZ	$2.8 \times 10^9$	$1.2 \times 10^5$	0.004	$1.1 \times 10^7$	$5.0 \times 10^6$	45.45
MX	$9.8 \times 10^{10}$	$5.1 \times 10^6$	0.005	$4.1 \times 10^7$	$2.1 \times 10^6$	5.12
CT	$4.9 \times 10^9$	$1.6 \times 10^5$	0.003	$2.8 \times 10^7$	$1.0 \times 10^5$	0.36
3 weeks of Remediation						
CD	$1.3 \times 10^{10}$	$3.0 \times 10^6$	0.02	$4.0 \times 10^7$	$1.0 \times 10^5$	0.25
PM	$6.9 \times 10^9$	$9.0 \times 10^6$	0.15	$5.0 \times 10^7$	NG	-
FZ	$3.3 \times 10^9$	$3.0 \times 10^5$	0.01	$2.0 \times 10^7$	NG	-
MX	$1.7 \times 10^{10}$	$7.9 \times 10^6$	0.05	$6.0 \times 10^7$	NG	-
CT	$2.3 \times 10^{10}$	$3.0 \times 10^5$	0.001	$1.0 \times 10^7$	$1.0 \times 10^6$	10
6 weeks of Remediation						
CD	$2.48 \times 10^{10}$	$5.0 \times 10^6$	0.02	$1.8 \times 10^6$	$1.0 \times 10^5$	5.56
PM	$3.4 \times 10^9$	$9.6 \times 10^6$	0.28	$3.4 \times 10^6$	$1.0 \times 10^5$	2.9
FZ	$2.7 \times 10^9$	$4.0 \times 10^5$	0.01	NG	$1.0 \times 10^5$	-
MX	$2.61 \times 10^{10}$	$1.1 \times 10^7$	0.04	$1.69 \times 10^9$	$2.0 \times 10^6$	0.11
CT	$1.21 \times 10^{10}$	$3.0 \times 10^5$	0.002	NG	$1.0 \times 10^5$	-
9 weeks of Remediation						
CD	$1.96 \times 10^{10}$	$5.2 \times 10^6$	0.03	$2.8 \times 10^6$	$1.0 \times 10^5$	3.57
PM	$4.2 \times 10^9$	$9.0 \times 10^6$	0.21	$4.7 \times 10^6$	$1.0 \times 10^5$	2.13
FZ	$2.1 \times 10^9$	$4.5 \times 10^5$	0.02	NG	$1.0 \times 10^5$	-
MX	$2.72 \times 10^{10}$	$9.5 \times 10^6$	0.03	$1.81 \times 10^9$	$1.0 \times 10^6$	0.06
CT	$9.6 \times 10^9$	$4.2 \times 10^5$	0.004	NG	$1.0 \times 10^5$	-
12 weeks of Remediation						
CD	$2.95 \times 10^{10}$	$6.1 \times 10^6$	0.02	$1.11 \times 10^7$	$1.6 \times 10^6$	14.41
PM	$7.2 \times 10^9$	$1.21 \times 10^7$	0.17	$6.5 \times 10^6$	$3.0 \times 10^5$	4.62
FZ	$6.4 \times 10^9$	$6.2 \times 10^5$	0.1	-	$1.0 \times 10^6$	-
MX	$2.61 \times 10^{11}$	$1.65 \times 10^7$	0.002	$1.69 \times 10^7$	$3.4 \times 10^6$	20.12
CT	$1.65 \times 10^{10}$	$4.2 \times 10^6$	0.02	-	$1.0 \times 10^5$	-

THBC, Total Heterotrophic Bacteria Count; THCBC, Total Hydrocarbon Bacteria Count; THFC, Total Heterotrophic Fungi Count; THCFC, Total Hydrocarbon Fungi Count; TRT, Treatment; NG, No Growth; CD, cow-dung; PM, poultry waste( manure); FZ, fertilizer; MX, Synergy(mixture); CT, control.

*TPHC Values: In Table 2, the TPHC values during treatment for 9 weeks are stated. The TPHC (mg/kg) before pollution is 1.6 and TPHC (mg/kg) 2 weeks after pollution is 230*

Table 2. TPHC Values during treatment

Week 1 of Remediation		
Variables	TPHC mg/kg	% Reduction
Cow dung	209.5	8.91
Poultry manure	208	9.56
Fertilizer	209.25	9.02
Synergy	217.63	5.38
Control	223	3.04
Week 3 of Remediation		
Cow dung	156.25	32.06
Poultry manure	163.5	28.91
Fertilizer	146.63	36.25
Synergy	158.5	31.08
Control	221.13	3.85
Week 6 of Remediation		
Cow dung	117.63	48.85
Poultry manure	111.13	51.68
Fertilizer	101.75	55.76
Synergy	142.88	37.88
Control	204.4	11.13
Week 9 of Remediation		
Cow dung	105.5	54.13
Poultry manure	86.75	62.28
Fertilizer	83.13	63.85
Synergy	82.38	64.18
Control	204.4	11.13

Figures 2a to 2e show the graphs of the TPHC with time for the different treatment variables. Figures 2a and c showed a pretty gradual reduction in TPHC with time with  $R^2$  as 0.889 and 0.906 respectively. The regression equations are also shown,  $y = 207.1 - 12.61x$  and  $y = 207.6 - 15.26x$  respectively. Figure 2b also showed a steady decrease in TPHC with time with  $R^2$  as 0.962 and the regression equation  $y = 214.5 - 15.19x$ . However, Figure 2d shows that the removal of TPHC from the soil medium was initially slow but suddenly increased sharply around the 7<sup>th</sup> week of treatment. This is as a result of the sharp increase in the activities of the petroleum contaminant degrading micro-organisms which probably were acclimatizing before now. The  $R^2$  is 0.936 and the regression equation given by  $y = 223.3 - 15.36x$ . Then, the Figure 2e which shows a curve quite different from the rest is the control. The reduction of TPHC was slow.  $R^2 = 0.850$  and  $y = 226 - 2.694x$ . 'y' =TPHC and "x" = time (duration) covered during treatment. The higher the value of x, the lower the value of y. From the prediction equations, the rates of remediation for the different treatments could be ascertained at any time x, till maximum treatment is achieved.

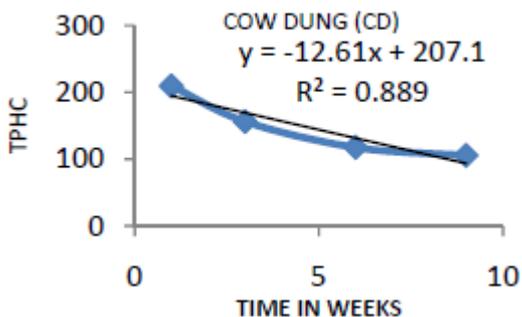


Figure 2a. TPHC for cow-dung treatment

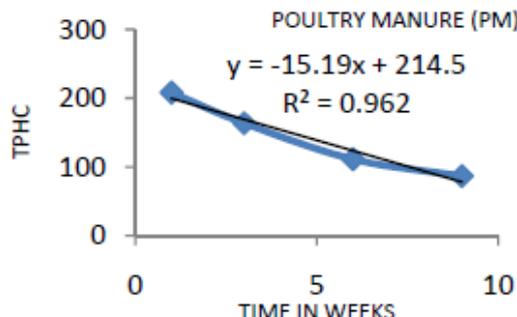


Figure 2b. TPHC for poultry manure treatment

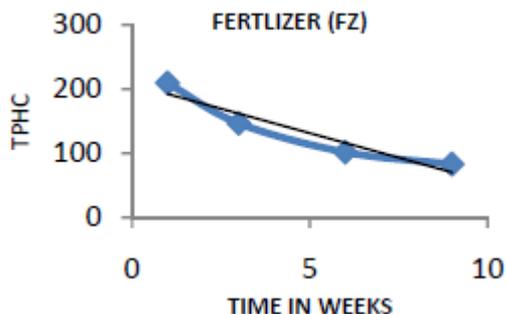


Figure 2c. TPHC for NPK fertilizer treatment

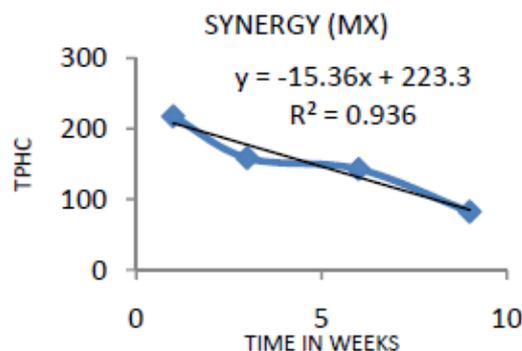


Figure 2d. TPHC for synergy treatment

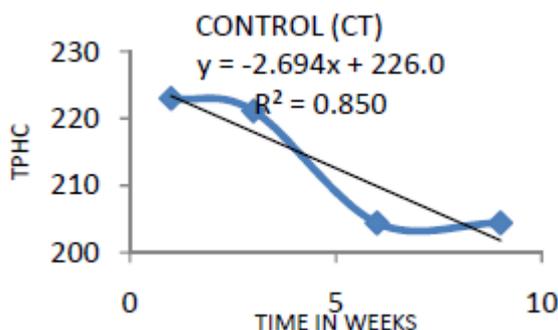


Figure 2e. TPHC for control experiment

**6. Discussion**

From the laboratory results and analysis, it can be seen that the percentage (%) degraders are higher with fungi than bacteria. THBC is higher than THFC but % of THFC is higher than %THCBC. The % degraders from Table 1 are a reflection of the degrading microbial counts. The Synergy remediated fastest with time. Initially the remediation was faster with fertilizer within the first 6 weeks of treatment. From the % reduction at the 6<sup>th</sup> week, Fertilizer had 55.76 while Synergy had 37.88 but after the 6<sup>th</sup> week, there was a sharp increase in the microbial activities of the Synergy and the percentage reduction increased to 64.18 while Fertilizer stood at 63.85 followed by Poultry manure, 62.28. Cow-dung had 54.13 while the control remained constant at 11.13. The identification of degrading microbes is a very important aspect of the results. Identified microbes can be isolated, whether as indigenous or non-indigenous and can be cultured and then introduced into a polluted site for hydrocarbon degradation. The identified microbes that survived and multiplied (cultivable) throughout the period of remediation are suitable for the remediation. This is because they could eat up and use the hydrocarbon, thereby degrading the contaminant. Many microbes both indigenous and non-indigenous (non cultivable) phased out with time and so are not suitable for bioremediation of crude oil. The following microbes have therefore been seen to be good and suitable fungi and bacteria for the bioremediation of crude oil polluted land: Fungi:

*Penicillium notatum*, *Mucor spp*, *Rhizopus stolonifer* and *Penicillium caseicolum* with *P.notatum* and *P.caseicolum* (*penicillium spp*) as the strongest fungi degraders. Bacteria: *Pseudomonas putida* and *Bacillus subtilis*. These fungi and bacteria (cultivable) can therefore be isolated and cultured in the laboratory and then introduced to a polluted site with the best engineering method as a less laborious alternative to the use of inorganic fertilizers, solid poultry or animal wastes or other input variables where the variables are not readily available or are not in large quantities. The curves also revealed the rates of the microbial degradations with time

## 7. Conclusion

It is obvious from the foregoing that it is most appropriate to induce and facilitate remediation of crude oil polluted land by use of organic or inorganic amendments than to allow the polluted land natural recovery. Natural recovery most likely will lead to economic loss of land. Organic variable can remediate in record time and is environment friendly. These species of fungi and bacteria, namely: *Penicillium notatum*, *Mucor spp*, *Rhizopus stolonifer* and *Penicillium caseicolum* and *Pseudomonas putida* and *Bacillus subtilis* can therefore be isolated and cultured (cultivable) and can be directly employed in the remediation of the polluted land using the best engineering and aeration techniques. This process has no pollution effects on land, air and water. It will be less cumbersome and laborious than the application of inorganic fertilizers, animal wastes and municipal wastes which are normally introduced to stimulate the action of the microbial population in a polluted site. Government, oil servicing and producing companies and environmental protection agencies should endeavor to work together to ensure minimum occurrence of oil spills in the environment both on land and in the water. Offenders should be sanctioned and made to face the responsibility of fast recovery of crude oil contaminated land and surface water. Above all, the sustainability of whatever strategy employed in the remediation, in terms of environmental impacts, should be seriously taken into consideration. This is because remediation of contaminated land is only an integral part of sustainable development.

## Acknowledgement

The authors are grateful to Institute of Petroleum Studies (IPS), Rivers State University of Science and Technology, Port Harcourt for providing the recipe for the petroleum agar used in this study and the Industrial Chemistry Laboratory of the Federal University of Technology Owerri (FUTO), Imo State, Nigeria for the use of her facilities for analysis of samples.

## References

- Amadi, A., & Bari, Y. U. (1992). Use of poultry manure for the amendment of oil polluted soils in relation to growth of maize. *Environment*, 18, 521-527.
- Barnet, H. I., & Hunter, B. B. (1987). *Illustrated Genera of Imperfecti Fungi* (4th ed.). Macmillan Publishing Company New York, USA, pp. 106, 130.
- Beishir, I. (1987). *Microbiology in Practice. A self instructions laboratory course* (4th ed.) (pp. 96-111, 120-130, 238-272). New York: Harper and Row Publisher.
- Buchanan, R. E., & Gibbons, N. E. (1974). *Bergey's Manual of Determinative Bacteriology* (pp. 522-568) Williams and Wilkins Co. Baltimore, USA.
- Cheesbrough, M. (2000). *District Laboratory Practice in Tropic Countries Part 2* (pp. 35-38, 62-69). Cambridge University Press.
- Dodman, P. (1994). European Perspectives of Field Research on Bioremediation Special Attention the Netherlands (p. 15). Invited paper for the International congress of soil Science, Acapulco, Mexico.
- Ebuehi, O. A. T., Abibo, I. B., Shekwolo, P. D., Sigismund, K. I., Adoki, A., & Okoro, I. C. (2005). Remediation of Crude Oil Contaminated Soil by Enhanced Natural Attenuation Technique. *Journal of Applied. Science & Environmental Management*, 9(1), 103-106. <http://hdl.handle.net/1807/6428>
- Harrigan, W. F., & McCance, M. E. (1990). *Laboratory Method in Food and Dairy microbiology* (8th ed.) (pp. 7-23, 286-303). London: Academic Press Inc.
- International Commission on Microbiological Specification in Foods [ICMSF]. (1978). Microorganisms in Foods 1: Their Significance and Methods of Enumeration (pp 100-107.), Toronto Canada: University of Toronto Press.
- Ogaji, S. O. T., Ayotamuno, M. J., Kogbara, R. B., & Probert, S. D. (2006). Bioremediation of a Crude oil Polluted Agricultural Soil at Port Harcourt, Nigeria. *Applied Energy*, 83(11), 1249-1257. <http://dx.doi.org/10.1016/j.apenergy.2006.01.003>

- Okwuosha, S. C. (2000). Physio-chemical Characterization of Soil under the Influence of Gas Flaring. A thesis submitted to the department of crop and soil technology (pp. 74-88). Federal University of Technology Owerri. Imo State, Nigeria.
- Orajaka, S. O. (1975). Geology in Ofomata, G. E. K. (Ed.), *Nigeria in Maps: Eastern states* (pp. 5-7). Benin City: Ethiope Publishing House.

### **Copyrights**

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).