Biodegradation of Petroleum-Polluted Soils Using CNB-Tech – The Nigerian Experience

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Additional information is available at the end of the chapter

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Abstract

Remediation of petroleum-hydrocarbon-polluted soil via biodegradation process is viewed globally as an environmentally friendly process. In this study, an overview of past and present field-scale petroleum hydrocarbon biodegradation techniques utilized in Nigeria was conducted using the tools of literature review and field survey. Pilot-scale biodegradation of hydrocarbons in petroleum-impacted clay soil of up to 42-year-long contamination using novel and eco-safe CNB-Tech was carried out. This was followed by a comparative evaluation of crop growth performance on crude-oil-polluted soil remediated using a biodegradation technique adopted by a reputable oil company in Nigeria and the innovative CNB-Tech. The study revealed that CNB-Tech is an innovative, time-effective, cost-effective and eco-friendly bioremediation technique and has the potential to excel over some existing biodegradation procedures employed by many oil industries especially in the developing countries.

Keywords: Petroleum pollution, Biodegradation, Environment, CNB-Tech, Nigeria

1. Introduction

Nigeria is a constitutional federal republic, the most populous country in Africa with over 170 million people of divergent cultural values, inhabited by over 300 ethnic groups. The country comprises thirty-six states and the capital territory (Abuja) out of which nine (Abia, Akwa Ibom, Bayelsa, Cross River, Delta, Edo, Ondo, Imo and Rivers States) fall within the Niger Delta Region. The Niger Delta region is reputed for oil industry operations that commenced in 1956. The first oil well (Fig. 1) was discovered in Oloibiri, Bayelsa State, after which many oil wells were found in the other states of the Niger Delta Region. The advent of oil mining brought financial boom but afterward came trails of petroleum-based pollution. Environmental degradation due to crude oil spill on land, into the swamps and water bodies with attendant consequences on the ecosystem and public health became topical issue both at the national and international levels. Factors influencing petroleum-based environmental pollutions in the country were identified as: (i) operational failures (corrosion of pipeline, human error and equipment failure); (ii) accidental discharge; (iii) acts of sabotage (oil theft, pipeline bunkering and artisanal refining) and (iv) inappropriate handling and disposal of petroleum wastes.

Figure 1. Status of the first oil well in Nigeria 57 years after discovery, from 1956 to 2013

Most of the oil companies claim that acts of sabotage contribute the most to the release of petroleum products into the environment relative to operational failures. This is corroborated by some spill data (Fig. 2) put in the public domain by the Shell Petroleum Development Company, Port Harcourt, Nigeria [24]. These data show that oil spill incidents traceable to operational failure range from 7 to 35%; inferring that acts of sabotage are responsible for 65–93% of oil spill in the Nigerian environment. Secondary data as shown in Fig. 2a bring out the following facts: (i) the number of oil spill incidents and spill volumes are recorded on a monthly basis, (ii) a high spill incident number does not necessarily imply a high spill volume. For instance, the highest number of spill incident (28) was recorded in July 2014, but the largest volume of spill was obtained in April, 2014 with a total number of 14 spill incidents, (iii) acts of sabotage seem to be at the peak three times in a year (at the beginning of the year [92%], midyear [93%], and end of year [93%]) and (iv) the season of the year (wet or dry) does not really play a significant role in the acts of sabotage. These facts, however, require further verification by conducting more detailed analyses using statistical data of previous years.

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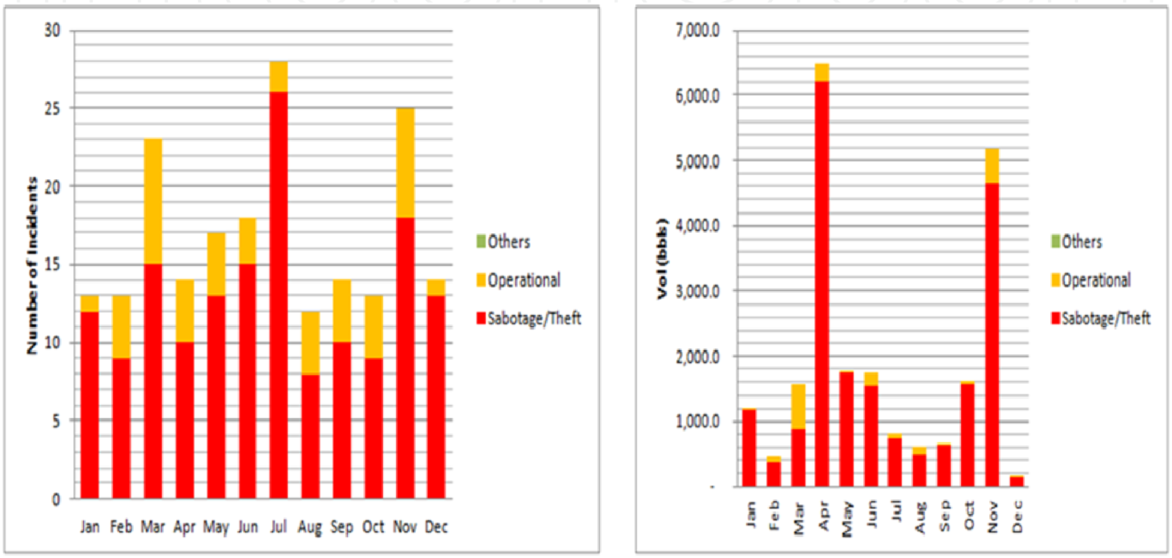
Irrespective of the oil spill causative factor, petroleum-based pollution endangers the entire environment including the human population [25]. Once petroleum product (crude or refined) is either intentionally or unintentionally released into the environment, the consequences remain the same. In any community impacted by oil spill, the degree of response to such an incident plays an important role in ensuring environmental safety, protection, and sustainability. From the environmental standpoint, the most important issue is that swift, positive and appropriate action aimed at safeguarding the ecosystem be taken once an oil spill occurs.

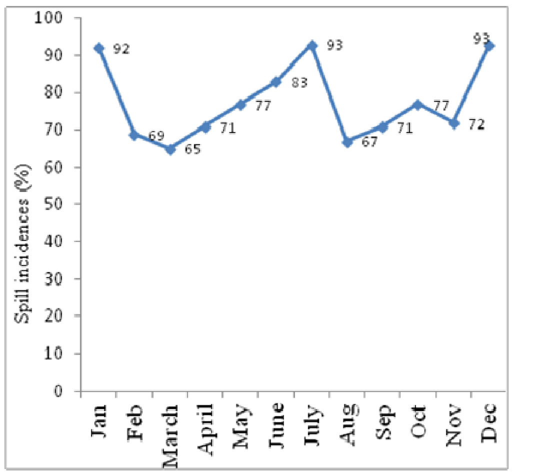


Monthly incidents of oil spill

Volume of spill per month

(a)

S N	Month	Total spill incident	Operational failure		Acts of sabotage	
			No	%	No	%
1	January	13	1	8	12	92
2	February	13	4	31	9	69
3	March	23	8	35	15	65
4	April	14	4	29	10	71
5	May	17	4	24	13	77
6	June	18	3	17	15	83
7	July	28	2	7	26	93
8	August	12	4	33	8	67
9	September	14	4	29	10	71
10	October	13	3	23	10	77
11	November	25	7	28	18	72
12	December	14	1	7	13	93



(b)

Figure 2. a: Oil spill incidents and volume of spill for 2014 in the Niger Delta region of Nigeria as presented by the Shell Petroleum Development Company, Port Harcourt, Nigeria. b: Secondary data showing numerical values of oil spill incidents, volume of spill, and trend in oil spill incident due to sabotage for 2014 in the Niger Delta region of Nigeria

Response actions include site cleanup via recovery of free phase oil, subsequent reduction of the residual petroleum hydrocarbon concentrations to an acceptable value, followed by restoration of the environment to its previous utility status. Options for the reduction of residual petroleum hydrocarbon concentrations are preferably eco-safe techniques. After a cleanup exercise, detoxification of soils polluted with residual petroleum hydrocarbon compounds is necessary. There are different methods by which the concentrations of these pollutants (total petroleum hydrocarbon – TPH, and polynuclear aromatic hydrocarbon – PAH) could be reduced to fall within the acceptable level. The major mechanism involves degradation processes. Degradation generally applies to the breakdown or transformation of complex materials into simpler ones.

Various types of degradation processes include (i) thermal degradation that occurs via the application of heat, (ii) mechanical degradation, which takes place by the application of mechanical force, (iii) photo degradation, which is the transformation of complex compounds by the action of sunlight, (iv) oxidation/chemical degradation that occurs by the addition of chemicals and (v) biodegradation, which proceeds by the action of microorganisms (yeast, fungi, or bacteria). Organic substances that can be broken down by the action of microorganisms are said to be biodegradable. The technique that enables the application of biodegradation to clean up biodegradable organic pollutants from the environment is referred to as bioremediation. An example of a class of organic compounds that can be detoxified via biodegradation is petroleum-derived hydrocarbons. Petroleum-based hydrocarbons generally belong to the normal hydrocarbons known in organic chemistry. Hydrocarbons vary in their degree of susceptibility to microbial degradation. Some high molecular weight polynuclear aromatic hydrocarbons (PAHs) may not be degraded by microorganisms at all. Biodegradation of hydrocarbons proceeds through the major pathways presented in Fig. 3 [19]. A given hydrocarbon is eventually transformed to an acid, which is finally converted to innocuous end product(s).

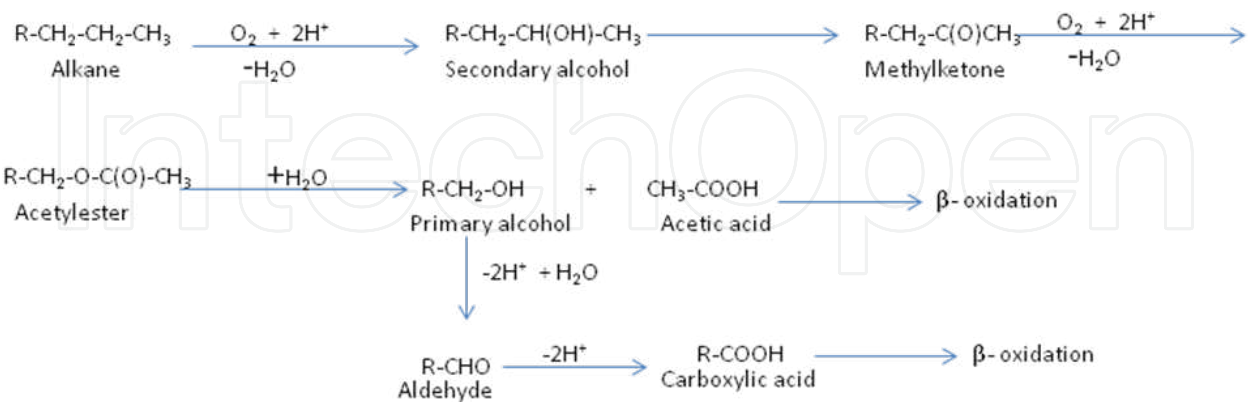


Figure 3. Major processes involved in biodegradation of a typical hydrocarbon compound

For a given biodegradation process, a hydrocarbon compound is generally transformed, through biochemical processes, to more polar organic compounds such as alcohol, ketone, aldehyde and organic acid. Essentially, biodegradation of an organic pollutant depends on the

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nature of the target compounds, environmental factors and microorganisms as highlighted in Table 1 [9, 23, 26, 27]. The success of biodegradation of petroleum hydrocarbons at the field-scale platform is highly dependent on effective maneuvering of these three factors. Doing otherwise would endanger the environment.

S/N	Target Compound (Petroleum Hydrocarbon)	Environmental Factor	Microorganism Factor
1.	**Chemical characteristics**: The nature or type of hydrocarbon compounds determines chemical properties that ultimately affect biodegradation. Hydrocarbons could be alkenes or alkanes, cyclic aliphatics or acyclic aliphatics, mononuclear aromatics or polynuclear aromatics in nature. Generally, the extent of biodegradation tends to decrease with increase in number of rings, the degree of condensation and the number of alkyl substituents on the aromatic nucleus. An acceptable sequence for the degree of susceptibility to microbial degradation is: alkanes > branched alkanes > small aromatics > cyclic alkanes	**Temperature**: This affects the viscosity, solubility and bioavailability of hydrocarbons. It also affects the physiology and diversity of microorganisms. Although hydrocarbon biodegradation can occur over a wide range of temperature, the rate of biodegradation generally decreases with decreasing temperature	**Type of microbes**: Microbes involved in biodegradation are fungi, yeast and bacteria. They have their potentials and limitations
2.	**Concentration**: The higher the concentration of a given type of hydrocarbon compound, the slower is the biodegradation	**Nutrients**: Major nutrients required to enhance or stimulate microbial activities are C, N, P. These are important for metabolism. This explains why many stakeholders apply inorganic NPK fertilizers for nutrient amendment. However, the application of NPK fertilizers has inherent negative environmental consequences. In addition, excessive nutrient concentrations can also inhibit the biodegradation activity	**Microbial population**: The population density of microbes is directly related to biodegradation
3.	**Toxicity**: Highly toxic hydrocarbon compound will require more resistant microbes	**Photo degradation**: Photo-oxidation increases the biodegradability of petroleum hydrocarbons by increasing its bioavailability and thus enhancing microbial activities	**Microbial diversity**: The more the variety of microbial population, the more efficient the biodegradation process

S/N	Target Compound (Petroleum Hydrocarbon)	Environmental Factor	Microorganism Factor
4.	**Polarity**: This factor facilitates solubility in polar solvents such as water, hence increases solubility and bioavailability for biodegradation	**Soil properties**: (i) Soil organic matter content: this readily absorbs hydrophobic compounds such as petroleum hydrocarbons. The major binding sites in soil organic matter are the soluble humic substances, in particular, humic and fulvic acids. (ii) Soil moisture: facilitates biodegradation of petroleum compounds because microbes thrive better in moist than in dry environments (iii) Soil pH: is a measure of soil acidity or alkalinity. The acidity (pH) of the soil is an important soil parameter. Soil pH can vary from 2.5 (highly acidic soils) to 11.0 (highly alkaline soils). Soil pH value affects microbial activity with moderate alkaline being the most favorable (iv) Soil aggregate: this increases bioavailability of the pollutant (v) Soil oxygen: little or no hydrocarbon metabolism occurs in strictly anoxic soil condition; hence, oxygen is a very important parameter for biodegradation	**Microbial enzyme activity, adaptability, reproduction potentials, and metabolic capabilities**: The more the enzyme activity, the better and faster the biodegradation process. Microorganisms that can easily adapt to different types of environments are more suited for bioremediation methods. Microbial species with high population turnover is more desirable The availability of microorganisms with appropriate metabolic capabilities is a major requirement for biodegradation of oil sample

Table 1. Description of basic factors that influence the success of a biodegradation process

The objectives of this study are (i) to present an overview of past and present practices in field-scale biodegradation procedures employed in the detoxification of petroleum hydrocarbon polluted soils in Nigeria and (ii) to demonstrate the efficacy of the novel, eco-safe and nano-technology based bioremediation technique (CNB-Tech) in the remediation and restoration of petroleum impacted soils to beneficial end products.

2. Research Methodology

In this study, the research methods used were literature review, field survey, screen house farming, pilot-scale bioremediation and standard laboratory techniques for relevant chemical and biological analyses.

2.1. Assessment of field-scale petroleum hydrocarbon biodegradation techniques utilized in Nigeria: past and present

Research tools used for this study were literature review and field survey. Formal and informal interactions with relevant stakeholders utilizing remediation procedures in petroleum industries and remediation project sites.

2.2. Pilot-scale biodegradation of hydrocarbons in petroleum impacted soil using novel and eco-safe CNB-Tech

Research method employed for this study was a practical pilot-scale remediation using a biodegradation process referred to as CNB-Tech, whose basic procedure has been described in [1]. However, there were modifications specific to the sample matrix used in this study. Permissions to procure petroleum impacted soil material consignments from the Shell Petroleum Development Company's remediation project site and to conduct the pilot-scale project were obtained from the appropriate authorities in the company. The spill site of about 15.6 hectares was situated between latitude 4°N and longitude 7° 7.5′E, in Eleme Local Government Area of Rivers state. This site was impacted by crude oil in 1969 as a result of damage by external device to Bomu-Bonny Trans Niger Pipeline (TNP) at Ejema and was accompanied by fire outburst. The hydrocarbon pollution was therefore up to 42 years long at the time study (ERMS, 2011). With the assistance of project site workers, clay soil sample bulk was collected in 2 x 200 L plastic drums, which were immediately conveyed to the pilot-scale remediation project site in Shell Industrial Area (Shell IA), Port Harcourt.

CNB-Tech biodegradation procedures were then applied to the samples. Untreated clay soil samples served as controls. Both controls and tests were replicated three times. Composite samples, collected under appropriate conditions and methods (before and after treatment) were sent to an ISO certified laboratory in the USA (by courier) and another in Nigeria for the analyses. Quality control and quality assurance protocols were strictly followed and parameters of interest were:

- Hydrocarbon compounds: Total petroleum hydrocarbon (TPH) and 17 polynuclear aromatic hydrocarbons (PAHs)
- Soil fertility parameters: pH, electrical conductivity and nitrogen (N), phosphorus (P), potassium (K)
- Heavy metals: Lead (Pb), mercury (Hg), arsenic (As), barium (Ba), copper (Cu), zinc (Zn), cobalt (Co), and nickel (Ni)
- Soil recovery and restoration indices: Reestablishment of microbial community and ability to sustain plant life investigated via microbial activity assessments at 48 h and 96 h periods (conducted only by the USA-based laboratory) and seed germination potential assessment conducted in Nigeria.

As a demonstration of the beneficial utility of the end product, the CNB-Tech remediated soils were used to grow indicator crops, namely *Zea mays L.*, (corn), *Telfairia Occidentalis* (fluted pumpkin), and *Manihot esculenta Crantz* (cassava) in screen house farming scheme but only the results of the second crop are reported in this presentation.

2.3. Comparative evaluation of growth performances for cassava crop grown in crude-oil-polluted soils remediated using biodegradation technique (RENA) adopted by a reputable oil company in Nigeria and the innovative CNB-Tech

In this study, soil samples from one of the rural communities in Rivers State, Nigeria, called Bomu (K-Dere) in Gokana, Ogoniland (Fig. 4), where crude-oil-impacted farm land area was remediated using RENA technique, were collected and used for this comparative evaluation. The major remediation technique adopted by one of Nigeria's leading international oil companies (the Shell Petroleum Development Company, Port Harcourt, Nigeria) for crude-oil-impacted soil, at the time of study, is referred to as RENA (Remediation by Enhanced Natural Attenuation). Permission to conduct the investigation was obtained from the designated authority of the oil company. Sample collection was supervised by (i) two representative staff of the oil company, (ii) a community relations officer (CRO) and (iii) some representatives of the community youth forum. Due to low literacy level, oral interviews were conducted on the community representatives to elicit information on factors such as (i) type of actions taken during the RENA remediation project, (ii) common utility of the land area prior to spill and (iii) experiences of farmers utilizing the remediated land area. Information was also obtained from the staff of the oil company on the mode of RENA remediation works carried out at the study site.

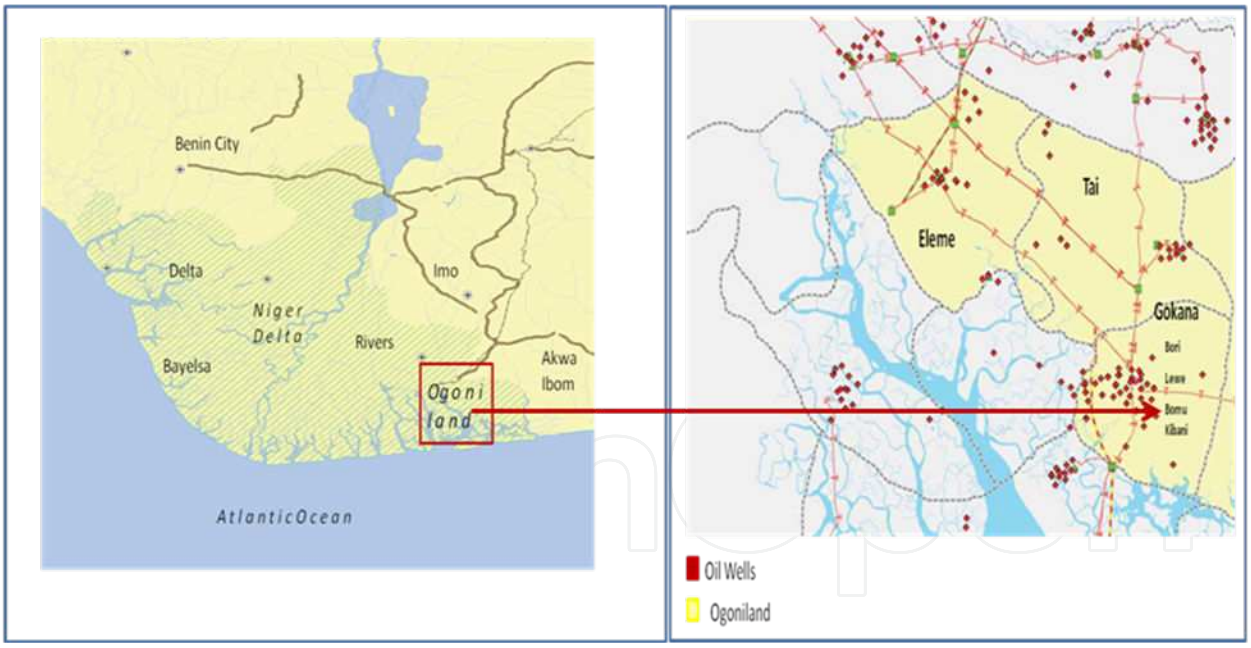

Figure 4. Map showing the location of Bomu in Ogoniland, Nigeria; sourced from [25].

Soil sample collection and analysis: Due to the heterogeneity found at the site, the land area was delineated into three zones (Subsite A, Subsite B, and Subsite C), which are briefly described as follows: (i) Subsite A stood for RENA remediated land area where no food crop was grown. Bulk sample from this site was denoted as IMS. (ii) Subsite B stood for RENA remediated land area used by natives for crop production. Only one type of food crop (Cassava:

Manihot esculenta Crantz) was grown in this subsite. Bulk soil sample collected from this site was denoted as RMS. (iii) Subsite C stood for agricultural area/farm soil, which the locals claimed did not experience crude oil spill. Samples collected from Subsite C were denoted as AGS. This subsite served as control. Following delineation exercise, soil samples were randomly collected from 14 places in a given subsite at depth 0–0.30 m. Samples collected from these places were mixed to give a composite of approximate weight of 56 kg. Bulk soil sample from each subsite was differently stored in sacks and conveyed to project site within 60–90 min.

On arrival at the pilot-scale remediation project site in Port Harcourt, the three different sample bulks of 56 kg each were homogenized, spread out on blue PVC sheets (in order not to contaminate the surrounding environment), air dried in the laboratory and then sieved through a 2 mm mesh size. Grid templates of 12 cells were then created for each sample bulk as shown in Fig. 5. Approximately 2 kg soil was collected from each of the12 subcells, mixed together to give the final composite of 24 Kg soil for a subsite. This was repeated four more times to give five replicate samples for each subsite. All together, 15 samples (n = 15) were obtained for the three subsites in the study area. The 15 soil samples were contained in properly labeled sample bottles, transferred into thermostated, ice-packed boxes and sent to a Chemical laboratory (Laser Engineering and Resources Consultants Limited, Port Harcourt, Nigeria) certified by the National regulatory body. The 15 parameters analyzed for in each soil sample were: temperature, pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (N), soil organic matter (SOM), total petroleum hydrocarbons (TPH), potassium (K), sodium (Na), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), nickel (Ni) and zinc (Zn) using standard methods.

Figure 5. The three different sample bulks showing grid template of 12 cells created on each sample, from which samples were collected for physicochemical analyses

Preassessment of RENA remediated soils for crop production: After sample collection for physicochemical analyses, the homogenized and gridded soil samples for each subsite were remixed and transferred to labeled and designated evaluation pots at 4 Kg per pot and at three replicates per site, giving a total of nine experimental pots in all. All the pots were then arranged in completely randomized block design. The soil in each pot was thereafter watered and allowed 14 days to stabilize. No hole was made in the pots (to avoid loss of matter) but to forestall flooding; soils were watered at about 60% of approximate field capacity, determined against gravity. Cassava (*Manihot esculenta Cranzt*) was selected as indicator crop. A long stem of cassava was obtained from an indigenous farmer who was harvesting from his farm at Subsite C during this study at K-Dere. This was done to ensure utilization of same cassava crop type for (i) the investigation and (ii) the actual farming by the locals. The cassava stem was then professionally cut to uniform pieces of 15 cm (length) for each; to allow for planting in the experimental pots. Complete burial planting method was adopted and stems were planted one per pot. On germination, agronomical parameters monitored during the period of growth were (i) plant height, (ii) stem girth and (iii) plant leaf number. Germinated crops were allowed to grow for 91 days before harvest.

Comparative evaluation of RENA and CNB-Tech remediated soils for crop production: Following the preassessment of RENA remediated soils and based on crop performance, only the soils collected from Subsite B (RMS) and Subsite C (AGS) were used for this comparative evaluation. Procedures previously described for preassessment of RENA remediated soils for crop production were adopted but due to project time constraint, the crops were grown for 37 days.

2.4. Statistical analysis

Data obtained in this study were subjected to relevant statistical analysis using SPSS 17.0 for Windows Evaluation Version. Descriptive statistics were used to obtain means and deviations, Pearson linear correlations were useful for the establishment of relationships and means were compared by Analysis of variance (ANOVA).

3. Results and Discussion

3.1. Review of biodegradation procedures employed for detoxification of petroleum-hydrocarbon-polluted soils in Nigeria

Information from literature review showed that most researchers focused on two major factors: (i) isolation of potential hydrocarbon degrading microbial strains and biostimulation via nutrient augmentation. For instance, [17] isolated about 15 hydrocarbon-degrading bacterial and fungal species from three bitumen deposits believed to be of relevance in biodegradation of petroleum (kerosene and diesel) contaminated systems in Nigeria. [9] carried out an experiment involving biostimulation with agricultural fertilizers to evaluate the biodegradation of hydrocarbon compounds found in a crude-oil-polluted agricultural soil at different levels of soil water. Petroleum pollution of an agricultural soil was simulated on the field by pouring crude oil on the cells from perforated cans. Biostimulation options were (i) introduction of mineral fertilizers and (ii) periodic application of different amounts of water. Results showed an increase in the total heterotrophic bacterial (THB) counts and a corresponding reduction in soil organic carbon and total hydrocarbon content (THC) at the end of the six-week remediation period. The implication is that by manipulating soil water content and nutrient levels (via inorganic fertilizer application), microbial population and activity were stimulated, suggesting that the level of water in the soil is a major factor that affects biodegradation rate. The use of isolated microbial strains to biodegrade petroleum hydrocarbon has not been successfully applied at the field scale for the remediation and restoration of crude-oil-polluted soils. Most of these works are still at the laboratory scale.

In practice, oil companies in Nigeria contract out bioremediation projects to certified vendors who then apply approved technologies under the supervision of the particular oil company and National Regulatory Agencies. The most commonly practiced bioremediation is land farming, a process believed to utilize indigenous microorganisms to biodegrade petroleum hydrocarbon pollutants under specified conditions.

This is a type of biodegradation by enhanced natural attenuation, which goes by different names for different companies such as RENA for the Shell Petroleum Development Company, Nigeria [25]. Limitations of in situ biodegradation via land farming where environmental controls are not put in place are highlighted in Table 2.

The issues highlighted in Table 2 clearly show that in situ biodegradation via land farming without the necessary environmental control measures, as often practiced, do not achieve legislative compliance and do not meet best management practices locally or internationally and constitute risk to the environment and public health.

S/N	Environmental Issues	Implication
1.	Impact of rainfall/precipitation	When rain falls on the project site, due to lack of critical environmental controls, there will be leaching of hydrocarbons from the windrows and runoffs will be generated
2.	Effect of temperature	This results in evaporation of hydrocarbons with associated occupational hazards to on-site workers and endangered health of neighboring communities
3.	Fate of runoffs	Runoffs emanating from impact of rainfalls on the windrows, constructed during land farming, will endanger nearby farms, communities, swamps, water bodies (ponds, lakes, streams, rivers, and groundwater). Runoffs have the potential to increase polluted land area
4.	Air pollution	Increased temperature such as is experienced in Nigeria will enhance the presence of volatile hydrocarbons in the atmosphere, resulting in air pollution. Most often, air pollution is not monitored during the remediation projects
5.	Vertical infiltration of pollutant	During the in situ biodegradation via land farming, the absence of impervious barriers allows for vertical penetration of oil/pollutants, thus resulting in the pollution of subsoil and groundwater

Table 2. Limitations of in situ biodegradation via land farming

3.2. Results on biodegradation of petroleum hydrocarbons in crude-oil-impacted clay soils using CNB-Tech

Biodegradation of total petroleum hydrocarbons: the initial concentration of total petroleum hydrocarbon (TPH) contained in the crude-oil-impacted clay soil was 33600 ± 245 mg/kg, as provided by the USA-based laboratory. On first dose application of CNB-Tech procedure, the concentration was reduced to 4193 ± 344 mg/kg, corresponding to 87.52% biodegradation. Second dose application further reduced the concentration to 293 ± 20 mg/kg, corresponding to 99% reduction relative to the initial concentration of TPH. The guideline for TPH level in soil stipulated by the Nigeria regulatory body is 5000 mg/kg [11, 21], implying that the CNB-Tech-treated soils contained hydrocarbons within the acceptable range.

Biodegradation of polynuclear aromatic hydrocarbons: Out of the 17 PAH compounds analyzed for in the petroleum-polluted clay soils, only 5 were detected. The individual components of the PAHs found in the petroleum-hydrocarbon-contaminated soil and their respective concentrations in this study were: (i) benzo (k) fluoranthene (8.84 ± 0.71 mg/kg), (ii) benzo (a) pyrene (15.33 ± 3.79 mg/kg), (iii) dibenzo (a,h) anthracene (18.51 ± 9.68 mg/kg), (iv) benzo (g,h,i) perylene (25.02 ± 6.10 mg/kg) and (v) indeno (1,2,3-cd) pyrene (24.69 ± 9.30 mg/kg). Total concentrations summed up to 92.39 ± 26.82 mg/kg. The percentage composition of these PAH compounds relative to the total concentration is presented in Fig. 6.

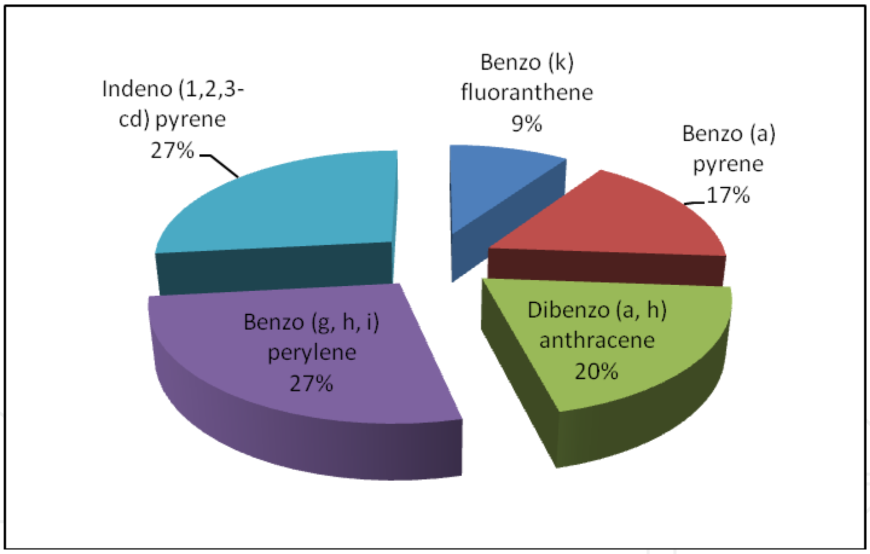

Figure 6. Percent composition of PAH compounds relative to total PAH found in the petroleum-impacted clay soil

Amazingly but very reassuring, none was detected in the CNB-Tech treated samples. Results from the Nigeria-based laboratory showed that by the application of CNB-Tech remediation procedures to the petroleum-hydrocarbon-polluted clay soils, the five PAHs were completely degraded, resulting in 100% reduction in concentration.

Reduction in soil heavy metal concentration: Concentrations of the metals found in the treated soil samples fell below the acceptable levels (Table 3). Relative to the initial concen-

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trations in the polluted soils, heavy metal concentrations were reduced by 2.38 to 100% for the different metals presented in Fig. 7.

S/N	Parameter	Mean ± SD (Nigeria)	Mean ± SD (USA)	n	DPR Intervention Value
1.	pH	7.47 ± 0.06 (7.40–7.50)	9.06 ± 0.12 (9.00–9.20)	3	NA
2.	Cd (mg/kg)	7.05 ± 0.60 (6.40–7.65)	ND	3	12
3.	Cu (mg/kg)	9.37 ± 0.53 (7.70–9.85)	12.30 ± 0.69 (11.50–12.70)	3	190
4.	Pb (mg/kg)	BDL	5.79 ± 0.66 (5.10–6.41)	3	530
5.	Ni (mg/kg)	4.55 ± 1.34 (3.10–5.75)	3.39 ± 0.58 (2.96–4.05)	3	210
6.	Zn (mg/kg)	122.86 ± 4.20 (120–128)	51.73 ± 19.50 (12.90–74.40)	3	720
7.	Co (mg/kg)	BDL	ND	3	240
8.	As (mg/kg)	BDL	ND	3	55
9.	Cr (mg/kg)	11.13 ± 1.17 (10.10–12.40)	ND	3	380
10.	Hg (mg/kg)	4.83 ± 0.50 (3.90–5.60)	0.02 ± 0.01 (BDL -0.03)	3	10
11.	Ba (mg/kg)	ND	437.33 ± 66.71 (263–4920	3	625

DPR = Department of Petroleum Resources, BDL = below detection limit, ND = not determined, n = sample population and NA = not available, values in parenthesis stand for minimum–maximum

Table 3. Selected properties (pH and heavy metal levels) of CNB-Tech treated soil samples

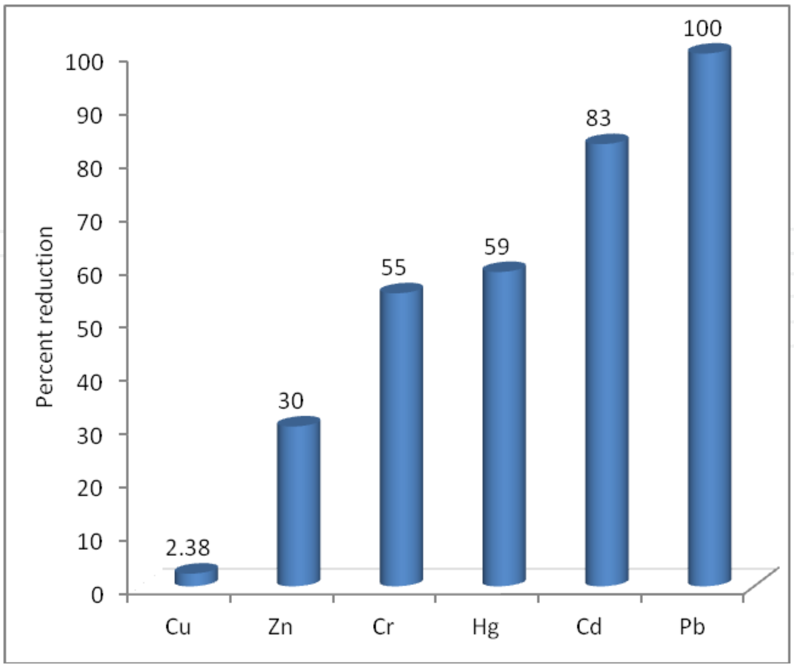

Figure 7. Reduction in soil heavy metal concentrations in CNB-Tech-treated soil samples

Soil material physical appearance: The outlook of the crude-oil-impacted clay soils before and after treatment is presented in Fig. 8. Before treatment, contaminated soil was characterized by strong hydrocarbon odour and was lumpy/pasty in nature. It also exhibited strong oil sheen in soil–water suspension. However, after treatment, there was complete disappearance of hydrocarbon odour and soil texture transformed to nonpasty. The colour changed from dark brown to black, characteristic of humus soil.

A cross section of hydrocarbon contaminated clay soil at site before treatment

Soil after treatment using CNB-tech products

Figure 8. Appearance of the crude-oil-impacted clay soil before and after CNB-Tech treatment

Microbial activity assessment: The digital captures of microbial population in two replicates (RS01 and RS02) of CNB-Tech-treated soil samples and the polluted, untreated crude-oil-impacted clay soil (CTS) are shown in Fig. 9a, while the quantitative counts are presented in Fig. 9b. For ease of presentation, all the values presented in Fig. 9b were raised to 10^7.The mean microbial population for 48 h microbial activity assessment for CNB-Tech-treated samples was 1.98 x 10^7CFU/mL and for 96 h activity assessment, the mean microbial population was 3.56 x 10^7 CFU/mL. The polluted, untreated crude-oil-impacted clay soil samples gave 1.52 x 10^6 CFU/mL and 1.84 x 10^6CFU/mL for 48 h and 96 h activity assessments, respectively.

At 48 h and 96 h assessments, the microbial activity found in the CNB-Tech treated soils exceeded that found in the polluted samples by approximate factors of 13 and 19, respectively. Results indicate that the polluted clay soils did not totally inhibit microbial growth, unlike what was obtainable for polluted oil-based mud [1]. CNB-Tech treatment replenished the microbial community. When soil is fully recovered and administration of treatment terminated, microbial population gradually adjusts back to normal population in the habitat [1].

Utility of CNB-Tech biodegradation end product: The success story of CNB-Tech procedures is not only in the reduction of concentrations of total petroleum hydrocarbons, polynuclear aromatic hydrocarbons and heavy metals but also in the utility of the treated substrates. CNB-Tech biodegradation procedures convert the petroleum impacted soil to "clean" reusable substrate. In this study, the petroleum-impacted clay soil was converted to arable soil suitable

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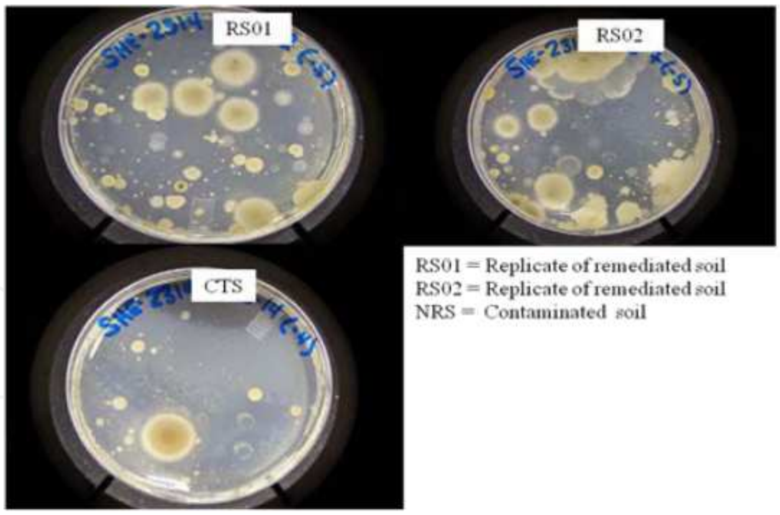

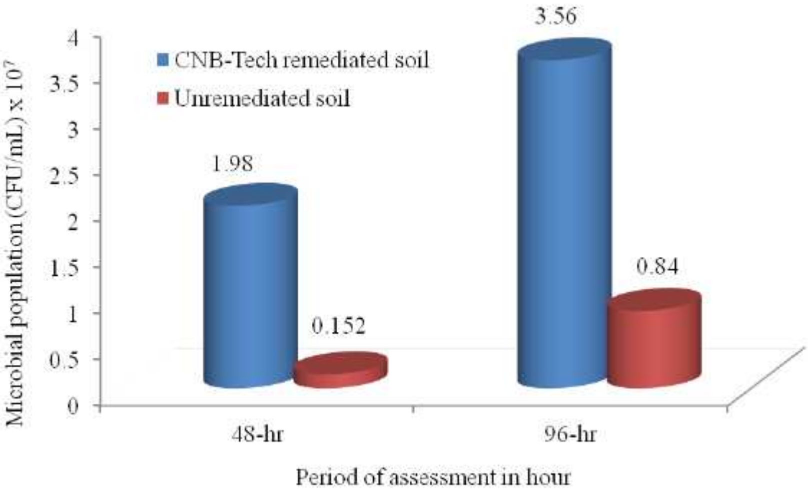

Figure 9. a: Qualitative microbial population in contaminated soil sample (CTS) and samples undergoing remediation (RS01 and RS02) as obtained by the USA-based laboratory. b: Quantitative representation of the microbial population in contaminated soil sample (CTS) and samples undergoing remediation (RS01 and RS02) as obtained by the USA-based laboratory

for crop production. The utility of CNB-Tech-treated soils in crop production was demonstrated in Fig. 10, in which the appearance of green leafy vegetable (Fluted pumpkin: *Telfairia Occidentalis*) grown in CNB-Tech-treated soil [(a) and (b)] was compared with that grown in a real farm (c) in an area not impacted by crude oil within Rivers State, Nigeria.

In terms of crop growth, the CNB-Tech remediated soils gave excellent support to both germination and growth of the vegetable crop. A mean plant height of 207 ± 10 cm was recorded for crops grown in CNB-Tech-treated soils, which excelled over crop performance (171 ± 8 cm) of vegetable crops grown in the control (farm soil) by 21%. On the other hand, petroleum-impacted clay soils used in this study did not support germination or growth of the vegetable, giving 100% inhibition to plant growth. The aim of remediation is to restore polluted site/land area to its previous use or modified beneficial use. The common land use in the Niger Delta region of Nigeria is crop production. Results have shown that CNB-Tech biodegradation remediation protocol achieved detoxification and restoration of petroleum-hydrocarbon-polluted soil to original land use. Results are in line with the findings reported by [1] for the treatment of polluted-oil-based mud using CNB-Tech. The enhanced crop growth performance of CNB-Tech treated soils could be attributed to increased fertility of the treated soils as supported by data on NPK status obtained in this study. Nitrogen was increased from 0.026% to 0.431%. Phosphorus was raised from mean values of 0.003 to 2.530% and potassium was raised from 0.082% to 0.481% (results from Nigerian laboratory). This is further strengthened by favorable pH status (which has the potential to enhance plant nutrient uptake and soil microbial activity) and reduction of heavy metal concentration (Fig. 7) thereby reducing their potential phytotoxicity.

Figure 10. Digital capture, showing cross sections of a green, leafy vegetable crop (Fluted pumpkin: *Telfairia occidentalis*) grown in CNB-Tech-treated clay soils [(a) and (b)] and the same crop grown in a real farm (c); in an area not impacted by crude oil within Rivers State.

The safety of crops grown in CNB-Tech treated soils for animal and human consumption is presently under intensive investigation. The crops are being assessed for hydrocarbon and metal contents in addition to other phytotoxicological parameters. Results of these investigations will soon be published.

3.3. Comparative evaluation of CNB-Tech and RENA remediated soils for crop production

Results on comparative evaluation of CNB-Tech and RENA remediated soils for crop production are presented and discussed. Data are provided on (i) plant height, (ii) stem girth and (iii) leaf number.

3.3.1. Results from preassessment of RENA remediated soils

Plant height: Data obtained for the preassessment of RENA remediated soils' potential to support crop growth are presented in Fig. 11-13. Results showed that the cassava heights for the three subsites A, B, and C increased with growth period. The best height was obtained for soils collected from the agricultural land area (Subsite C: AGS). Correlations with growth period gave coefficients of 0.897 (p = 0.001) for Subsite A, 0.987 (p < 0.001) for Subsite B, and 0.963 (p < 0.001) for Subsite C. This indicates that crop growth increased with time. After harvest, crop height for Subsite C (unpolluted farm soil: AGS), which served as the control, was 55.73 ± 8.75 cm. Relative to this, the mean height for cassava crop grown in RENA remediated soil/Subsite C (RMS) and Subsite A (IMS) were 30.87 ± 9.07 cm, representing 44.61% reduction and those grown in Subsite A (IMS) gave a mean height of 28.18 ± 3.05 cm, corresponding to 49.43% reduction [relative to the control (Subsite C: AGS)].

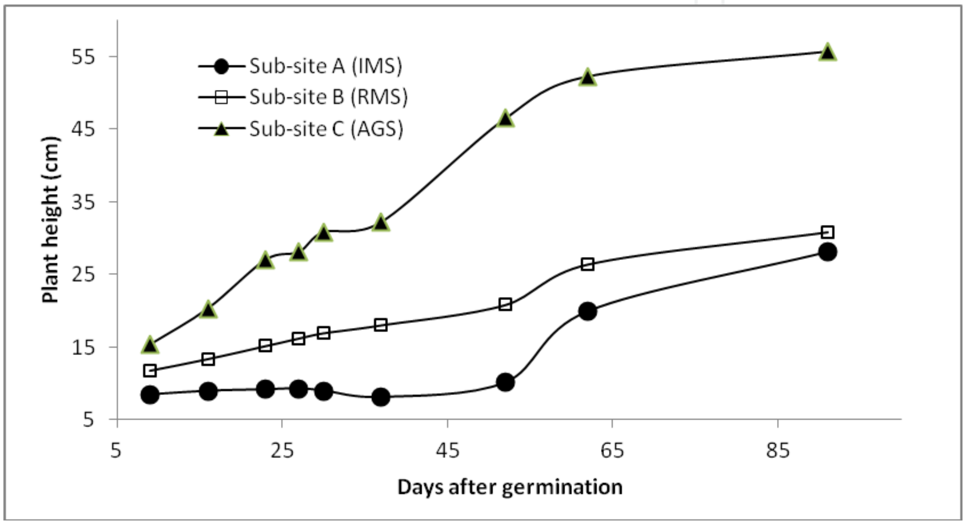

Figure 11. Variance of height of cassava crop grown in certain RENA remediated soils with time

Stem girth and leaf number: Graph of changes in stem girth relative to growth period is shown in Fig. 12. Results also showed that, similar to plant height, stem girth also increased with growth period (Fig. 12). Correlations with the growth period gave coefficients of 0.950 (p <0.001) for Subsite A (IMS), 0.868 (p = 0.002) for Subsite B (RMS), and 0.865 (p = 0.003) for Subsite C (AGS).

The cassava grown in the control (AGS) produced mean stem girth of 2.20± 0.01. Relative to this, the crop grown in RENA remediated soil (RMS) manifested 48.64% reduction in stem girth, having a mean stem girth of 1.13± 0.06 while that grown in IMS experienced 53.18% reduction; having stem girth of 1.03 ± 0.01. Graph of changes in leaf number relative to growth period is shown in Fig.13. The coefficient of correlation for leaf number versus growth period was 0.871 (p < 0.002) for IMS, 0.774 (p = 0.014) for RMS, and 0.903 (p = 0.001) for AGS. The mean leaf number of cassava grown in the control (AGS) was 55 ± 1. Using the performance of cassava in AGS as reference, cassava crops grown in RENA remediated soils (RMS and IMS) experienced 36.36% and 49.09% reductions in leaf number, respectively; having leaf numbers of 28 ± 6 and 35 ± 6, respectively. Generally, results showed that irrespective of the agronomical parameter, the best performance was obtained in this order: AGS (Subsite C) > RMS (Subsite B) > IMS (Subsite A).

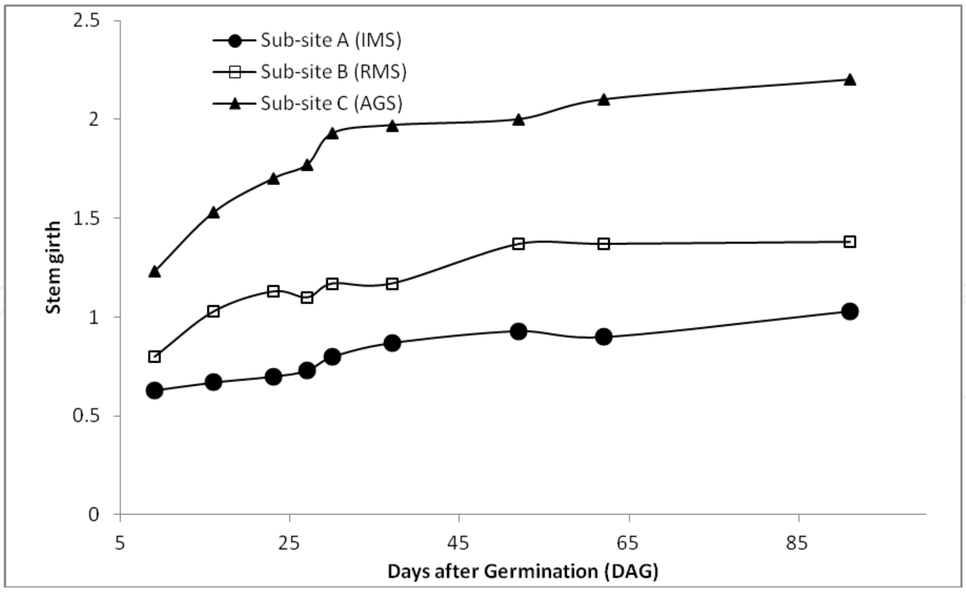

Figure 12. Variance of stem girth of cassava crop grown in certain RENA remediated soils with time

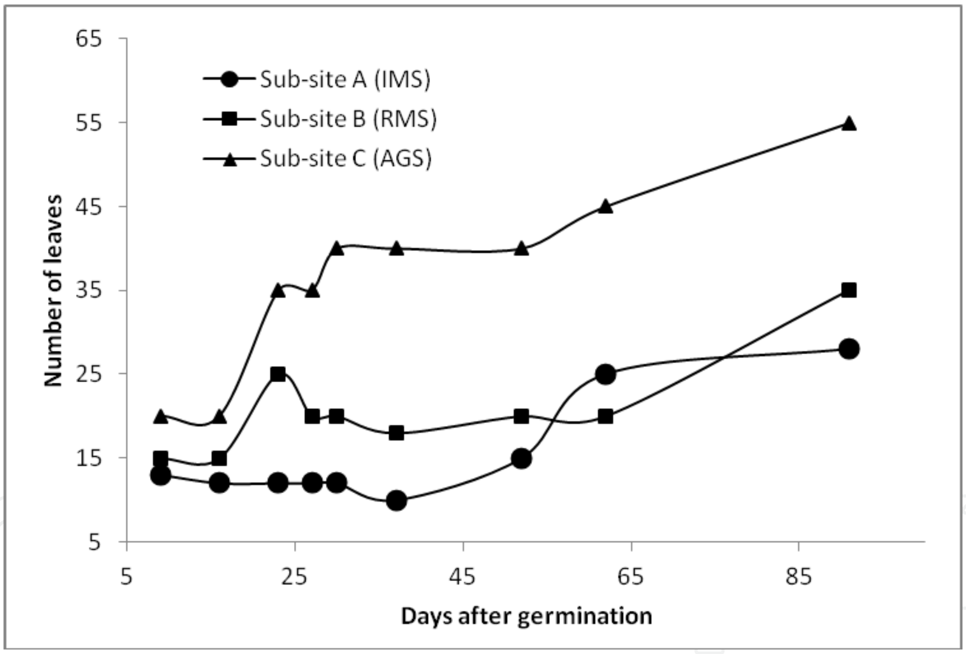

Figure 13. Variance of leaf number of cassava crop grown in certain RENA remediated soils with time

The very poor performance of crops grown in IMS (Subsite A) in comparison to the crops grown in RMS (Subsite B) and AGS (Subsite C) was attributed largely to an observation made at the site. This is briefly explained thus; after a heavy rainfall, the soil surface appeared to be coated with water but underneath was very dry, as illustrated in Fig. 14. This indicates severe soil hydrophobicity; which is a situation where water content of soil is extremely low. By contrast, the soil found at the agricultural site (AGS) after the same rainfall demonstrated satisfactory water penetration into the soil. The causative factor to this observation is not well-understood but it could have been due to crude oil effect. The release of crude oil into the soil environment often leads to alteration of normal activities of the soil medium. It adversely impacts soil's physical, chemical, and biological characteristics [14]. This perhaps explains why the local farmers did not use Subsite A (IMS) for crop production.

Figure 14. Reduced water infiltration in Subsite B (RMS) relative to the agricultural farm soil (Subsite C: AGS)

3.3.2. CNB-Tech versus RENA remediated soils for crop performance

Highlights of results from comparative analysis between the performances of RENA remediated soil (RMS) and CNB-Tech remediated soils (CRMS) are shown in Fig. 15. ME02 stands for the name of the indicator crop and its replicate number (*Manihot esculenta Crantz*, pot No.2) and DAG stands for day after germination. Fig.15a shows that on the sixth day of growth (DAG-6), the height of cassava crop grown in CNB-Tech remediated soils height was 15.60 cm. The equivalence of this height was found for the crop grown in RENA remediated soil at 37th day of growth (DAG-37). By implication, the performance of the crop grown in CNB-Tech remediated soil excelled that grown in RENA remediated soil by 3.31%. Furthermore, from Fig. 15b, on the 21st day of growth (DAG-21) for cassava grown in CRMS (CNB-Tech remediated soil), the height was 29.20 cm, excelling over the height (19. 60 cm) of cassava in RMS (RENA remediated soil) at DAG-52 by 48.98%.

Keeping day of growth constant (Fig. 15a), and c at DAG-37 (37th day of growth), height of cassava grown in RENA remediated soil was 15.10 cm and that grown CNB-Tech remediated soil (CRMS) was 43.40cm, showing an enhanced performance by CNB-Tech relative to RENA by 187.42%. The growth of crop height per day, presented in Fig. 16, gave 0.31 cm for cassava grown in RENA remediated soil, 0.57 cm per day for that grown in farm soil (AGS), and 0.90 cm for the crop grown in CNB-Tech remediated soil. The improved performance of crops

Figure 15. Digital capture, illustrating growth of cassava crop grown in RENA remediated soil (RMS) and CNB-Tech remediated soil (CRMS), where DAG stands for day after germination, H stands for height, SG stands for stem girth, and ME02 stands for *Manihot esculanta Crantz* (Cassava).

grown in CNB-Tech treated soils over those grown in RENA treated soils was attributed to positive modification of soil properties such as pH, temperature, water dynamics, electrical conductivity, and enhanced plant nutrient bioavailability for easy plant nutrient [2, 3, 6, 7]. CNB-Tech products, which are biodegradable and eco-friendly, are also sources of natural plant and soil-beneficial mixed microbial consortia. CNB-Tech procedures do not involve the use of genetically engineered microorganisms and as a result of in situ generation of microorganisms, eliminates the daunting task of isolating specific microorganisms needed to remove specific contaminant.

According to [15], most remediation/biodegradation guidelines for detoxification of petroleum hydrocarbons are developed mainly for TPH or total mineral oil concentration but the spill of crude oil into the soil could cause varying degrees of toxicity, phytotoxicity, mutagenicity and carcinogenicity actions. Ecotoxicity bioassays should therefore be incorporated as supplementary tools for monitoring treatment effects. In a situation where, for instance, the end-use of the land is farming, using reduction of petroleum hydrocarbon concentrations as the only or major index for closeout of remediation projects without recourse to other ecological and socioenvironmental factors poses some threats to the environment in terms of soil quality, food security, food safety and means of livelihood for the populace. These in turn could stimulate poverty, endanger public health and impact negatively on national security.

In comparison with other works, the result obtained in this study on TPH reduction was higher than 7.42 ±1.02% reduction obtained by [18] when poultry manure alone and in combination

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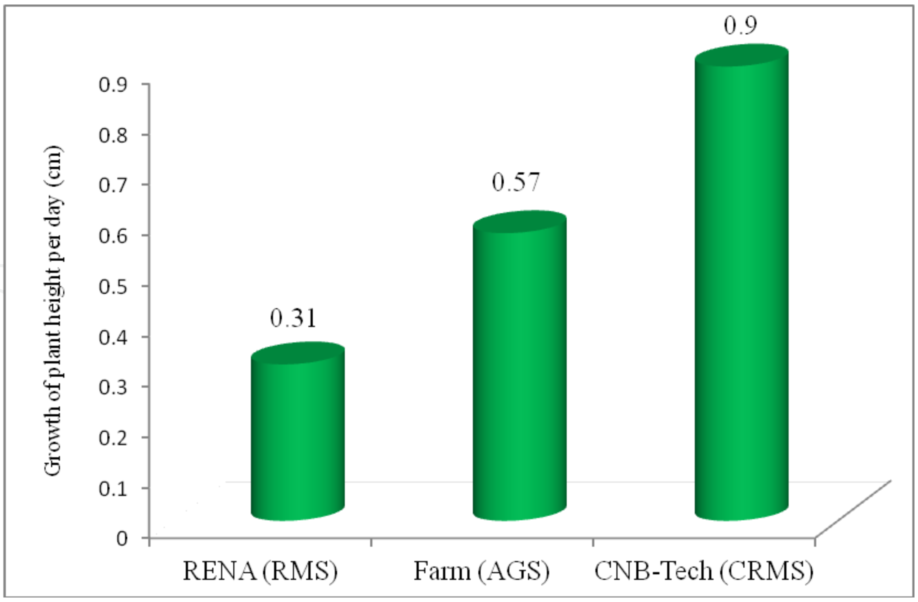

Figure 16. Growth in height of cassava crop per day for RENA remediated soil (RMS), CNB-Tech remediated soil (CRMS), and farm soil (AGS: control)

with glucose was applied to crude-oil-contaminated soil. Comparing the results obtained in this study with related investigations in other parts of the globe, [8] carried out bioremediation on sand samples contaminated with oil spill, which were collected from Pensacola beach (Gulf of Mexico) using isolated fungal diversity associated with beach sands. They investigated the ability of isolated fungi for crude oil biodegradation. Results from their study gave 4.7–7.9% biodegradation. [10] obtained 24.0–57.1% reduction in TPH by applying a biological treatment to crude-oil-contaminated soil in Russia. They used composting system, enhanced by nutrient (NPK fertilizer) addition and inoculation of *Rodococcus*–biosurfactant complexes.

In China, [20] conducted an investigation on two bioremediation technologies (bioremediation by augmentation and conventional composting using crude manure and straw) as treatment options for oily sludge and oil-polluted soil in which the total hydrocarbon content (THC) varied from 327.7 to 371.2 g/kg (327700 to 371200 mg/kg) for dry sludge and 151.0 g/kg (151000 mg/kg) for soil for a period of 56 days; after three times of biopreparation application, THC decreased by 46–53% in the oily sludge and soil. Note that the results (88–99% degradation in TPH) obtained from this present study was from only one dose application of CNB-Tech products. As stated earlier, repeated application of CNB-Tech products by two to three dose applications will achieve 100% degradation of TPH.

[13] carried out bioremediation of petroleum-hydrocarbon-–contaminated soil by composting in biopiles and recorded mineral oil decrease from 2400 to 700 mg/kg, corresponding to 70% reduction after 5 months. Majority of remediation works carried out in other parts of the globe took a period of 3 months to over 12 months to achieve between 75 and 98% reduction in TPH in hydrocarbon-contaminated soils (SGBP, 2007; [16]. CNB-Tech achieves a faster cleanup/TPH reduction, since projects can be completed in days/weeks instead of months/years.

4. Conclusions

CNB-Tech is an innovative, time-effective, cost-effective and eco-friendly remediation technique developed for the detoxification and restoration of crude-oil-impacted environmental matrices polluted with petroleum hydrocarbons, incorporating biodegradation process. This study revealed that it compares and has the potential to excel over some existing biodegradation procedures employed by many oil industries, especially in developing countries. Presently, a mini field-scale project sponsored by National Tertiary Education Trust Fund (TETFUND) is ongoing, focusing on optimization of the CNB-Tech in readiness for field-scale applications for industrial operations and safety assessments of different crops grown in the treated soils.

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