Organic Wastes Utilization for Enhanced Biodegradability of Total Petroleum Hydrocarbon in a Crude Oil Polluted Soil Environment

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Abstract: Designing sustainable and low-cost tools to restore crude oil-contaminated soil (COCS) is gaining global attention. This study was aimed at utilizing organic wastes products for enhanced biodegradability of total petroleum hydrocarbon in a crude oil polluted soil. A microcosm was set up in 3 containers, each having a surface area of 328cm^2 and a volume of 1651 cm^3. COCS (300 g) were weighed into sets A−C. Sets A had 150 g of rabbit manure (RM) while B contained 150 g of *Nypa fruticans* ash (NFA) and Set C was not amended to serve as control. Monitoring was done for 5weeks. The baseline total petroleum hydrocarbon (TPH) was 6706.76280 ppm. The effects of amendments on the cumulative percentage of degradation indicated that TOC gave 44.8 % (RM) and 18.4% (NFA). Nitrate gave 9.43% (RM) and 24.7% (NFA). THB/HUB ratio was 2.1% (RM) and 0.82% (NFA), while the THF/HUF recorded 1.82 % (RM) and 1.49 % for NFA. The phosphate in RM and NFA was 39 % and 42.3 % respectively. Moisture in RM and NFA were 51.8 % and 39.3%. This shows that TPH has been reduced in the amended treatments to a level where if bioremediation proceeds, it becomes economical. The TPH dropped from 6706.76280 to 2818.42039 ppm (RM) and to 4054.55278 ppm (NFA), representing 57.9% and 39.6% loss. The order of TPH biodegradability is given as A < B < C. *Corynebacterium, Staphylococcus, Pseudomonas, Klebsiella, Bacillus Flavobacterium, Candida, Saccharomyces, Aspergillus, Penicillium, Mucor, Neurospora,* and *Rhizopus* fungal genera isolated. There was a significant ($p<0.05$) TPH reduction after week 5, indicating TPH biodegradability and uptake.

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1. Introduction

The history of oil exploration and production in the Niger Delta region of Nigeria is a long, complex and often painful one that to date has become seemingly intractable in terms of its resolution and future direction (Hammer, 1993; Moffat and Linder, 2005, O'Reilly *et al.*, 2001). The modern trend toward increasing dependence on the use of petroleum hydrocarbons for energy needs has resulted in increased accidental discharges of oil (Awobajo, 1981; Aboribo, 2001; Akpahwe and Solomon, 2012) and its products into the environment notwithstanding technology driven precautions (Abu and Dike, 2008, Aboribo, 2001) thereby creating environmental and socio-economic problems (O' Reilly *et al*., 2001; Boele *et al.*, 2001, Boele *et al.*, 2001). It is a common stance that many farmers in the oil producing areas in developing countries such as Nigeria are experiencing tremendous difficulties in restoring the fertility of polluted soil (Solomon *et al.,* 2018a, b, c, d). They also lack knowledge of appropriate remediation procedures (Hammer, 1993). The level of oil contamination of ecological media (soil, water, air and biota) in Nigeria has been reported (Ifeadi and Nwankwo, 1980; UNEP, 2011, Onyema *et al.,* 2013) and this calls for urgent attention in planning the right clean-up and remediation strategy (Moffat and Linden, 2005; Boele *et al*., 2001). The findings in UNEP report underline that pollution has perhaps gone further and penetrated deeper than many may have previously supposed.

Odeyemi and Ogunseitan (1985) have earlier reported on the petroleum industry and its pollution potential in Nigeria. Joint action of the multi-national oil companies, Nigerian government and responsible partners in oil business is essential for effective implementation and sustenance of our productive and green ecosystem. The numerous hydrocarbons and chemicals present in oil represent a carcinogenic risk (Okpokwasili and Odokuma, 1990; Nessel, 1999).

There exists a pressing demand for the introduction of environmentally save technologies for

effective clean-up (Venosa *et al*., 1996; Skipper, 1999, SPDC, 2005; Margesin and Schinner, 2001, Greenwood *et al*., 2009; Dawson *et al*., 2007, Welander, 2005). In order to prevent significant health risks and the loss of biodiversity, and to prevent further contamination, enhanced bioremediation methods are necessary (Alkorta and Garbisu, 2001; SPDC, 2005; Chikere *et al*., 2011). Bioremediation can result in a speedy recovery of the environment from crude oil pollution.

Biological treatment offers the best environmentally, friendly method for remediating hydrocarbon and heavy metal contaminated soil because it utilized the capability of the indigenous microorganisms in the soil environment to break down the hydrocarbons and heavy metals into innocuous substances (El Fantroussi and Agathos, 2005; Okoh, 2006, Gradi, 1985). The long term aim of biological remediation is to present cost effective designs which reduces the pollutant level to a level referred to 'as low as reasonable and practicably possible' (ALARP). The use of widely available and ecologically friendly nutrients such as Nipa palm and Rabbit manure to enhance the microbial activities in mineralizing hydrocarbons in soil environment will contribute to superb management of the environment for sustainability and enhance healthy lifestyle across the globe. Using this method instead of excavation and mass change could diminish the emission of CO_2 during cleanup and remediation (Yerushalmi *et al.,* 2003; Greenwood *et al*., 2009). It would be especially important to study the effect of Nipa palm ash and rabbit manure on TPH biodegradation to ascertain their impact, as their usage pose no threat to soil ecosystem.

The issues of Nipa palm invasion on Niger Delta mangrove ecosystem health and biodiversity pose severe threat and should be tackled before these large productive wetlands and tidal mudflats are irreversibly damaged. Although report has it that over thirty craft items with materials sourced from Nipa palm have been designed and perfected in Nigeria (Osabor *et al*., 2008), there is little or no report on its use in the cleanup of environmentally contaminated soil (Fig. 3). This research, therefore, seeks to investigate the contribution and applicability of Nipa palm ash and rabbit manure on enhanced biodegradability of crude oil polluted soil in Yorla, Ogoniland.

2. Materials And Methods

2.1. Sampling and Analyses

The study site was Yorla farmland in Kpean Community, Khana Local Government Area of Rivers State. Its choice was informed by its heavy crude oil it received from a damaged pipeline in the flow station 15 years ago and to explore the option of enhancement which is cost effective. The topsoil (15cm depth) was sampled using a manual auger into a clean polythene bag. This was transported to the environmental microbiology laboratory for immediate physicochemical, gas chromatographic and microbiological examination within 24 h. Nipa Palm was collected from a swampy, wetland soil at Inter Wogba creek in Trans Amadi Industrial Layout, Port Harcourt and transported via sack bag to the University of Port Harcourt reference herbarium for identification. The Nipa palm fruits bunch were dehusked, crushed and dried in a Prime oven (GallenKamp BS, 250, England) at 60^{o}C for five days. It ash were used as bulking agent to enhanced rate of degradation of crude oil in polluted soil. Rabbit manure was collected from the faculty of Agriculture demonstration farm at Choba, University of Port Harcourt and transported in a polythene bag to the environmental laboratory. Samples were composted before used following Hussmann (1993) standard procedure.

2.2. Microcosm description

Three hundred grams (300g) of COCS) were weighed and placed in containers (A, B, and C), each having a volume of 1651 cm^2 and a surface area of 328 cm^2. Sets A and B were amended with 150g of NFA and RM respectively. Set C was not amended to serve as control. Monitoring was done for a period of five weeks (35 days) and all parameters were measured at interval of one week.

2.3. Bioremediation protocol

The experimental design consisted of three treatments sets designated as thus:

Set A: COCS (300g) + NFA (150g) +Tilling
Set B: COCS (300g) + RM (150g) +Tilling
Set C: COCS (300g) + un-amended + Tilling

All sets were moistened by the addition of 1litre of distilled water after an interval of two days and tilled to 15 cm depth. This was done periodically to mix nutrients with polluted soil properly and to enhance aeration and optimum microbial metabolism until the end of 35 days bioremediation.

2.4. Enumeration of total hydrocarbon utilizing bacteria and fungi

Vapour-phase transfer method was adopted. A modified MSM of Mills *et al*. (1978) was used as the base. It contained: $MgSO_4.7H_2O$, 0.40g; KCl, 0.28g; KH_2PO_4, 0.80g; Na_2HPO_4, 1.20g; NH_4NO_3, 0.40g, NaCl, 15g; agar No. 2, 20g in 1 liter of de-ionized water. The pH was adjusted to 7.1 and media autoclaved at 121^{0}C for 15 minutes.

Soil slurry was prepared by mixing 1g of wet soil with 9ml of sterile saline suspension. Crude oil was added by soaking a 9cm Whatman No. 1 filter paper with 10 ml of fresh Bonny light crude. The flooded filter paper was then placed on the lid of the agar plate

and incubated for 5 to 7 days at 28±2^{0}C in an inverted position. The filter papers supplied the hydrocarbons by vapour-phase transfer to inverted inoculums.

The filter paper saturated with crude oil served as a sole source of carbon (Abu and Ogiji, 1996). For total hydrocarbon utilizing fungi, the same procedure were followed except that 1ml of lactic acid was added for fungal media, Sabauraud Dextrose agar (SDA, Antech) to inhibit the growth of hydrocarbon utilizing bacteria, following the procedure by Obire *et al*. (2008).

2.5. Enumeration of total culturable heterotrophic bacteria and fungi.

The total heterotrophic bacteria count was performed on nutrient agar (oxoid). It comprised: meat extract 1g, yeast extract 2g; peptone 5l, $NaCl_2$ 5g, agar 15, distilled water 1litre and final pH 7.4±37^{0}C. The spread plate method was used (Gradi, 1985). Soil slurry was prepared accordingly.

Serial ten-fold dilutions of the slurry were done up to 10^{-5} dilutions (Chikere *et al*., 2009). An amount of 0.1 ml of each dilution was spread-plated. Culture plates were then incubated at 28±2^{0}C for 48 hours. For hydrocarbon utilizing fungi, the same procedure was followed except that 1ml of lactic acid was added for fungal media (SDA, Antech) to inhibit the growth of hydrocarbon utilizing bacteria. Plates yielding counts between 30-300 colonies were enumerated and used for calculating the colony forming unit per gram of soil.

2.6. Determination of Colony-Forming Unit Per gram of soil

The average colony was counted and multiplied by the dilution factor of the specimen that the count represented and since 0.1ml was spread-plated, the number were again multiplied by factor 10 to obtain the number of bacteria per gram of soil. Enumeration was done using the formula by Ibiene *et al.* (2011):

$$\frac{\text{Average No. of colonies} \times \text{Dilution factor}}{\text{Volume plated (ml)}}$$

2.7. Identification of hydrocarbon utilizing bacteria and fungi isolates

Colonies of different hydrocarbon utilizing bacteria and fungi were picked randomly using a sterile inoculating wire loop and subculture to purify, by sub-utilizing on nutrient agar plates and Sabouraud dextrose agar plates respectively.

The plates were incubated at 30^{0}C for 24 hours at 28±2^{0}C for 3 days to obtain pure colonies. Pure fungal isolates was placed on clean and grease free slide and a drop of lactophenol was added. The preparation was covered with cover slip and slide observed under x10 and x40 objective lenses following method by Obire *at al.* (2008).

2.8. Determination of Physicochemical parameters

The parameters studied were the soil pH, concentration of phosphate, nitrate, moisture content, and total organic carbon (TOC). The TOC was determined by titration method using potassium permanganate as oxidant while moisture content was by procedure described by the Association of Analytical Chemists (1990) and method previously described by Stewart et al. (1974).

2.8. Extraction/Gas chromatography

Gas chromatographic analysis allowed us to estimate the degradation of TPH in the light (C_{12}-C_{23}) and the heavy fractions (C_{23}-C_{40}). The residual total petroleum hydrocarbons (TPH) were determined using a modified EPA 8015 technique. The soil sample was extracted with methylene chloride and an aliquot of the extract injected into a gas chromatograph (HP 5890, Hewlett Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID). The extractable petroleum hydrocarbons was quantified according to the method of ASTM (1998) and USEPA 8270B (TPI, 2007).

2.9. Statistical analysis of data

All data generated in this research work were subjected to statistical analysis to determine level of degradation of total petroleum hydrocarbons and significant difference among the different nutrient used to amend the crude oil-polluted soil resource using the students' "t" test. A value of ($p<0.01$) was accepted as significant and ($p>0.01$), considered not.

3. Results and Discussion

The Monitoring and optimization of enhanced bioremediation process took a period of five weeks (35 days). Week 1 was for the assessment of the baseline properties of the soil (set C). The result show that the concentration of total petroleum hydrocarbons in the crude oil polluted soil was 6706.76280ppm at 15cm depth. The nitrate, phosphate and total organic carbon (TOC) was 17.82mg/kg, 24.12mg/kg and 32.48mg/kg, while that of pH and moisture content was 5.34 and 13.43mg/kg (Table 1). The total culturable heterotrophic bacteria count (THB), total culturable fungal count (THF), total hydrocarbon utilizing bacteria (HUB) and total hydrocarbon utilizing fungal (HUF) counts were $1.76x10^5$ cfu/g, $1.54x10^4$ cfu/g, $2.93x10^3$cfu/g and $1.43x10^3$ cfu/g respectively from the baseline study. The percentage loss of nitrate was 16.8%, phosphate (12.4%), total organic carbon (6.2%) and moisture content (14.9%). The pH showed 18.7% reduction.

The microbial counts had percentage loss of 2.3%, 1.3%, 0.10% and 0.14% losses for THB, THF, HUB and HUF (Table 1). Values of loss of hydrocarbon due to natural attenuation processes of

chemical and photo-oxidation, evaporation, dispersion, sorption, transformation, biodegradation, dilution spreading and volatilization at various time intervals have been reported (Venosa *et al*., 1996; Alkorta and Garbisu, 2001, Ibiene *et al*., 2011; Agarry and Ogunleye, 2012; Oforibika *et al.,* 2018).

There was a minimal reduction of TPH from 6706.76280ppm to 6695.3828ppm by week 5 in the control (set C). This represents 0.17% reduction of total petroleum hydrocarbons in the un-amended control. Treatment prior to Nipa palm addition. The TPH reduced from 6706.76280ppm to 6667.09836ppm (week 2), representing 0.59% loss. The TPH further dropped from 6667.09836ppm to 4682.98663ppm in week 3, giving a 30.18% loss. By week 5, it had reduced from 4682.98663 to 4374.04667ppm (Table 2). This represents 34.78% loss.

On week 5, the TPH concentration had significantly ($p<0.01$) dropped by 39.6%, to 4054.55278ppm. After week 5, in the Nipa palm treatment, a cumulative reduction of 39.6% in TPH was estimated (Table 4). The total organic carbon (TOC), nitrate, phosphate, moisture content and pH reduced from 32.48 to 31.45mg/kg, 17.82 to 16.61mg/kg; 24.12 to 22.41mg/kg, 13.43 to 12.85mg/kg and 5.34 to 5.18(week 2), representing 3.2%, 6.8%; 7.1%, 4.3%; and 2.9% in the Nipa palm option.

By week 3, the total heterotrophic bacteria, hydrocarbon utilizing bacteria, total heterotrophic fungi and hydrocarbon utilizing fungal counts increased from $1.93x10^3$ to $2.62\ x10^4$ cfu/g, $1.76x10^5$ to $2.68\ x10^4$ cfu/g; $1.54x10^4$ to $2.99\ x10^5$ cfu/g and 1.43x103 to $3.92\ x10^4$ cfu/g.

Three Weeks after enhanced bioremediation process, the total organic carbon (TOC), nitrate, phosphate, moisture content and pH value decreased drastically from 31.45 to 30.51mg/kg (6.1%), 16.61 to 16.03mg/kg (10%); 22.41 to 20.67mg/kg (14.3%), 12.85 to 10.34mg/kg (23.2%); and 5.18 to 5.08 (4.9%).

Thus, the nitrogen and phosphate reduction as the TPH reduced is a clear indication that the nitrogen and phosphorus concentration can be used as fertilizers to the microorganisms that breakdown the total petroleum hydrocarbon in polluted soil. There was a significant ($p<0.01$) increase in the populations of total heterotrophic bacteria, hydrocarbon utilizing bacteria, total heterotrophic fungi and hydrocarbon utilizing fungi, after Week 3 from $2.62\ x10^4$ to $3.32\ x10^5$ cfu/g, $2.68\ x10^4$ to $4.35\ x10^4$ cfu/g; $2.99\ x10^5$ to $4.43\ x10^5$ cfu/g and $3.92\ x10^4$ to $4.32\ x10^4$ cfu/g (Table 2).

This suggests that the indigenous microorganisms degrading the crude oil within the second and third weeks produce significant results. This may probably be due to nutrient availability and utility by the microorganisms. However, by Week 4, the microbial populations decreased drastically from $3.32\ x10^5$ to $2.56\ x10^5$ cfu/g, $4.35\ x\ 10^4$ to $4.35x10^4$ cfu/g; $4.43\ x10^5$ to $4.09\ x10^5$ cfu/g and $4.32\ x10^4$ to $3.54\ x10^3$cfu/g. The total organic carbon (TOC), nitrate, phosphate, moisture content and pH after Week 4 reduced from 30.51 to 28.45mg/kg (12.4%), 16.03 to 15.05mg/kg (15.6%); 20.67 to 18.97mg/kg (21.4%), 10.34 to 9.44mg/kg (29.7%) and 5.08 to 4.65 (12.9%).

Table 1: Physicochemical and Microbial Population Changes during 35 day Nipa palm (*Nypa fruticans*) Enhanced Bioremediation of crude oil in contaminated soil

***Nipa fruticans* Ash (NFA)**

Period	TPH	TOC	NO_3	PO_4	pH	Moisture	THB	HUB	THF	HUF
Week1	6706.76280	32.48	17.82	24.12	5.34	13.43	$1.93x10^3$	$1.76x10^5$	$1.54x10^4$	$1.43x10^3$
Week2	6667.09836	31.45	16.61	22.41	5.18	12.85	$2.62\ x10^4$	$2.68\ x10^4$	$2.99\ x10^5$	$3.92\ x10^4$
Week3	4682.98663	30.51	16.03	20.67	5.08	10.34	$3.32\ x10^5$	$4.35\ x10^4$	$4.43\ x10^5$	$4.32\ x10^4$
Wkee4	4374.04667	28.45	15.04	18.97	4.65	9.44	$2.56\ x10^5$	$3.20\ x10^4$	$4.09\ x10^5$	$3.54\ x10^3$
Week5	4054.55278	26.50	13.42	13.92	4.60	8.15	$0.48x10^3$	$1.42\ x10^4$	$0.84\ x10^4$	$0.51\ x10^3$

The microorganisms make use of the nitrate and phosphate in the degradation of the oil (Kim *et al.*, 2005) but the nutrients may have been used up or depleted by the microbes therein. The total heterotrophic bacteria, total hydrocarbon utilizing bacteria, total heterotrophic fungi and total hydrocarbon utilizing fungi finally reduced to $0.48x10^3$ cfu/g, $1.42\ x10^4$ cfu/g; $0.84\ x10^4$ cfu/g and $0.51\ x10^3$ cfu/g, with a corresponding reduction of the total organic carbon (TOC), nitrate, phosphate, moisture content and pH to 26.50mg/kg (18.4%), 13.42mg/kg (24.7%); 13.92mg/kg (42.3%), 8.15mg/kg (39.3%) and 4.60mg/kg (13.9%).

The cumulative percentage of the total organic carbon (TOC), nitrate, phosphate, moisture content and pH in the Nipa palm enhanced bioremediation option were 18.4%, 24.7%; 42.3%, 39.4% and 13.9%. This is indicative of the increased biodegradation observed. There were observable reductions in the

TPH value during the 35 days bioremediation, indicating degradation by autochthonous microorganisms. Crude oil-degrading microorganisms were stimulated by labile hydrocarbon sources (probably linear and open-chain hydrocarbons) that induced a high percentage of degradation. Indigenous microorganisms are well adapted to their own environment.

An immediate increase in the population density of these microorganisms has been known to ensure rapid degradation of the pollutant (Skipper, 1999; Juhash *et al*., 2000). Abioye *et al.* (2009) demonstrated the positive effect of enhanced bioremediation of used motor oil using organic wastes that comprised banana skin; brewery spent grain, and spent mushroom compost. Table 3 shows results of addition of rabbit manure in set B. The TPH reduced from 6706.76280 to 3732.06703ppm (week 2).

The TPH concentration dropped by 44.4%. After week 3, the TPH was reduced from 3732.06703 to 3565.98333ppm. It dropped by 46.8%. However, by week 4 of the remediation process, the TPH was further degraded by autochthonous microbes from 3565.98333 to 3422.92211ppm (week 4), giving a percentage reduction of 48.9%. By the fifth week, the TPH concentration had reduced from 3422.92211 to 2818.42039ppm, corresponding to 57.9% loss in TPH from the soil environment.

The cumulative TPH reduction was 57.9%. This loses may be attributable to biodegradability of the crude oil by indigenous microbes in the soil. (Chikere *et al.,* 2011; Greenwood *et l.,* 2009, Odokuma and Akpanah, 2010). The results also indicated that nitrate, phosphate and TOC concentration increased to 21.41mg/kg, 22.31mg/kg and 35.45mg/kg by week 2. By week 3, the concentration of nitrate, phosphate and TOC decreased from 21.41mg/kg to 20.19mg/kg, 22.31mg/kg to 19.24mg/kg and 35.45mg/kg to 29.50mg/kg respectively.

In week 4, it further decreased to 18.24mg/kg, 17.62mg/kg and 23.52mg/kg and finally dropped in week5, from 18.24mg/kg to 16.14mg/kg, 17.62mg/kg to 14.71mg/kg and 23.52mg/kg to 22.41mg/kg for nitrate, phosphate and TOC. The concentration of nitrate and phosphate decreased as the remediation process progressed. This is in agreement with the work of Onifade and Abubaker (2007). The pH value decreased to 5.22 in week 2 and 6.5 in week 3.

The pH further dropped from 5.13 to 5.21 and then to 6.42 in week 5. The pH values at pre-amendment were in acidic range. There was further drop to high acidity after 35 days of bioremediation. However, there was a shift towards neutrality (6.42) at week 3 (Table 2). The shift may be probably due to increase in the number of oil degrading organisms which thought to have deposited their waste product of metabolism in the soil.

Rapid decomposition of wastes and residues is usually in the range of 6.5─8.5. The degradation of TPH was relatively rapid for rabbit manure treatment, indicating that the pH values recorded during the study period were suitable for enhanced biodegradation (Ijah and Abioye, 2003). The moisture content reduced from 13.43% to 12.50% in week 2 and further dropped to 8.32% in week 3. On the fourth week, the moisture content had reduced from 8.32% to 9.42% and finally to 6.48% in week 5.

Moisture content is an important limiting factor in biodegradation and the cumulative amount of 51.8% is sufficient to maintain the soil pH in close neutrality. There was a significant ($p<0.01$) increase in the microbial population from $1.76x10^5$ to 4.32x105cfu/g; $1.54x10^4$ cfu/g to 3.64x105cfu/g; $2.93x10^3$cfu/g to 4.56x104 cfu/g and $1.43x10^3$ cfu/g to 3.53x106 cfu/g for total heterotrophic bacteria, total culturable fungal, total hydrocarbon utilizing bacteria and total hydrocarbon utilizing fungal respectively.

After three weeks of remediation process, there was a continued upward rise to $4.25x10^5$ cfu/g, $2.75x10^5$ cfu/g, $4.13x10^4$ cfu/g and $3.05x10^4$ cfu/g for total heterotrophic bacteria, total culturable fungal, total hydrocarbon utilizing bacteria and total hydrocarbon utilizing fungal in soil. This is in agreement with the findings of Odokuma and Akpana (2010) who reported the positive effect of nutrient supplementation on bioremediation.

By week 4, the THB, THF, HUB and HUF population had increased from $4.25x10^5$ cfu/g to $5.43x10^4$ cfu/g, $2.75x10^5$ cfu/g to $1.42x10^5$ cfu/g, $4.13x10^4$ cfu/g to $3.56x10^4$ cfu/g and $3.05x10^4$ cfu/g to $2.42x10^3$ cfu/g. However, five weeks after bioremediation, the total heterotrophic bacteria, total fungal, total hydrocarbon utilizing bacteria and total hydrocarbon utilizing fungal population decreased to $1.35x10^4$ cfu/g, $0.86x10^3$ cfu/g; $1.55x10^3$ cfu/g and $0.69x10^3$ cfu/g.

Table 2: Physicochemical and Microbial Population Changes during 35 day Rabbit Manure Enhanced Bioremediation of crude oil in contaminated soil in Yorla, Ogoniland

Rabbit Manure (RM)										
Period	**TPH**	**TOC**	**NO_3**	**PO_4**	**pH**	**Moisture**	**THB**	**HUB**	**THF**	**HUF**
Week1	6706.76280	32.48	17.82	24.12	5.34	13.43	$3.76x10^5$	$2.93x10^3$	$1.54x10^4$	$1.43x10^3$
Week2	3732.06703	35.45	21.41	22.31	5.22	12.50	$4.32x10^5$	$4.56 x10^4$	$3.64 x10^5$	$3.53 x10^6$
Week3	3565.98333	29.50	20.19	19.24	6.42	9.42	$4.25 x10^5$	$4.13 x10^4$	$2.75 x10^5$	$3.05 x10^4$
Week4	3422.92211	23.52	18.24	17.62	5.21	8.32	$5.43 x10^4$	$3.56 x10^4$	$1.42 x10^5$	$2.42 x10^3$
Week5	2818.42039	22.41	16.14	14.71	4.74	6.48	$1.35 x10^4$	$1.55 x10^3$	$0.86 x10^3$	$0.69 x10^3$

The HUB and HUF counts reduced both in number and proportion to the increase in the THB and THF population. The HUB and HUF population finally reduced to $1.35x10^3$ cfu/g, and $7.40x10^3$ cfu/g, representing 47.1% of an increasing THB population of $1.63x10^5$ cfu/g, giving 92.3% and THF population of $1.45x10^4$ cfu/g, representing 94.4%.

Ibiene *et al.* (2011) have demonstrated the positive effect of other organic wastes (spent mushroom, cow dung and poultry droppings) on the bioremediation of hydrocarbon contaminated soil on a 28 days study period. Researchers seek the combination of organic wastes that will definitely increase rate of crude oil biodegradation within a short period (Yakubu (2007; Agarry *et al*. (2010; Solomon *et al.,* 2017). Agarry and Owabor (2011) have demonstrated the positive effect of pig manure on enhanced bioremediation of petroleum hydrocarbons. The rabbit manure amended sample in our study showed a high cumulative value of 57.9% TPH reduction and more activities in terms of peak heights and concentrations.

This may be as a result of the nitrogen (2.4%), phosphate (1.4%) and potash (0.6%) content of the manure. Table 4 shows the summarized effects of the bioremediation treatments using Nipa palm and rabbit manure on the cumulative percentage of degradation of TPH, TOC, nitrate, phosphate, moisture content, pH, THB, HUB, THF and HUF during the 35 days enhanced bioremediation of COCS in Yorla, Ogoniland. Total organic carbon (TOC) gave 44.8% for rabbit manure (RM), and 18.4% for Nipa palm (NFA).

Nitrate gave 9.43% (RM), and 24.7% for Nipa palm (NFA). This was also evidence in the THB/HUB and THF/HUF ratio in which THB/HUB gave 2.1% for rabbit manure (RM) and 0.82% for Nipa palm (NFA). Also, THF/HUF recorded 1.82% for rabbit manure (RM) and 1.49% for Nipa palm (NFA). The percentage changes of phosphate for RM and NFA was 39% and 42.3% while that of pH was 11.2% and 13.9% for RM and NFA option. On the same vein, the % moisture for RM, and NFA was calculated as 51.8% and 39.3% respectively.

These results shows an ALARP (As Low as Reasonable and practicably possible) condition for TPH which explains that TPH has been reduced to a level where if bioremediation proceeds, it becomes economical and sustainable. It was obvious that after 5 Weeks of optimization and incubation, the greatest percentage of degradation of total petroleum hydrocarbons (TPH) was observed in the rabbit manure (57.9%), followed by *Nypa fruticans* ash (39.6%).

The order of biostimulation effectiveness among the two amendments agents studied were as thus: Rabbit manure (RM) < *Nypa fruticans* ash (NFA). Hydrocarbon utilizing bacteria isolated were identified to the generic level. These included *Corynebacterium, Staphylococcus, Pseudomonas, Achromobacter, Klebsiella, Serratia, Escherichia, Bacillus, Proteus, Lactobacter, Micrococcus, Clostridium, Acinetobacter, Flavobacterium Citrobacter* and *Alcaligenes.*

Their presence in polluted soil encourages the development of adaptive features such as plasmid which support hydrocarbon co-metabolism. Several studies have indicated that crude oil polluted soils contained oil degrading microorganisms (Bento *et al.*, 2005; Lynch *et al.*, 2004; Abu and Dike, 2008, Chikere *et al.*, 2009) including bacteria and fungi capable of utilizing oil as their source of carbon and energy. Although some studies have showed that, oil-polluted soils are dominated by Gram negative bacteria (Macnaughton *et al*., 1999; Kaplan and Kitz, 2009, Chikere *et al.,* 2009), the dominant culturable hydrocarbon utilizing bacteria from our experimental sets were made up of Gram positive bacteria of the genera *Corynebacterium, Staphylococcus, Lactobacter, Micrococcus and Serratia.*

This corroborates the findings of other workers (Chikere and Okpokwasili (2003, 2004; Humamura *et al*., 2006, Chikere and Chijioke-Osuji, 2006, Chikere *et al*., 2009, 2011, Okerentugba and Ezeronye, 2003). These researchers have isolated Actinobacteria, including stains of *Rhodococcus, Norcardia, Corynebacterium, Staphylococcus,* and *Lactobacter* as the dominant hydrocarbon degraders from various hydrocarbon-contaminated media and shoreline.

The prevalence has been reported by other workers in Nigeria (Atlas, 1981, Obire *et al.*, 1988; 2008, Onifade and Abubakar, 2007; Obiukwu and

Abu, 2003). A total of four Gram positive isolates (*Corynebacterium*, *Staphylococcus*, *Lactobacter* and *Micrococcus*) were isolated. The Gram negative bacteria isolated from crude oil polluted soil in Yorla farmland included members of the genera: *Pseudomonas*, *Achromobacter*, *Klebsiella*, *Serratia*, *Escherichia*, *Bacillus* and *Proteus.*

Majority of these bacterial isolates found in COCS in Yorla has also been isolated from aquatic habitat, and predominate in Shrimp (*Palaemonetes* sp.) by Solomon and Ibe, (2012) and their antibiotic resistance profiles have been determined (Solomon *et al.*, 2013). The present study shows that these isolates have the advantages of being well-adapted to the crude oil contaminated soil environment, leading to efficient biodegradation oil contaminants in the soil. Yeasts are fungi that are known to show primarily unicellular mode of growth. Numerous species of yeasts have been described (Mbakwem-Aniebo and Wokoma, 2007; Sullivan and Coleman, 1998). Of these, approximately two dozen are of clinical importance (Mbakwem-Aniebo, 2010; Atlas, 1981).

The hydrocarbon utilizing fungi genera obtained are *Candida, Cryptococcus, Rhodotorula, Saccharomyces, Trichosporium, Geotrichum, Aspergillus, Penicillium, Mucor, Fusarium, Cladosporium, Cephalosorium, Monosporium, Neurospora, Rhizopus* and *Microsporium*. These correspond with the genera isolated by other workers (Obire *et al.*, 2008, Okpokwasili and Amanchukwu, 1988, Chikere *et al.*, 2009) in Nigeria.

Yeasts reproduce asexually by budding, bud fission. When buds (blastoconidia) remain attached to the mother cell, a filament (pseudohypha) is formed. Candida and Rhodotorula species have been reported to be members of the normal flora of the human gastrointestinal tract and the skin (McGinnis, 1980).

Human infections with yeasts are very common, particularly as a result of the increased use of broad-spectrum antibiotics and immunosuppressive drugs (Mbakwem-Aniebo, 2010, McGinnis, 1980). More of these fungal genera were got from rabit manure amended treatment which also shows the best rate of TPH degradation. Nigerian soils have been reported to habour hydrocarbon degraders that have been exposed to hydrocarbons as a result of the increased multifarious activities of the oil industries in the Niger Delta region of Nigeria.

The results are consistent with that of Nweke and Okpokwasili (2004), Obire *et al*. (2008) and Lynch *et al.* (2004). Results of their cultural characteristics indicated that four out of the six fungal genera (*Candida*, *Cryptococcus*, *Rhodotorula*, and *Saccharomyces*) selected grows best at 37^0C. This follows the observation of Pfeiffer and Ellis (1992). Only *Candida* has germ tube, chlamydo-conidia and produced capsules. *Trichosporon* and *Geotrichum* can grow at variable temperature.

Three of the genera including *Candida, Trichosporon* and *Geotrichum* have pseudo and /or true hyphae. However, all the hydrocarbon degrading fungal genera have blasto-conidia except *Geotrichum.* However, *Trichosporon* and *Geotrichum* contained arthro-conidia, which enhance their biodegradation ability. The role of Yeasts in human disease has been reported by various researchers (Umechuru and Elenwo, 1996; Sullivan and Coleman, 1998, Mbakwem-Aniebo, 2007, 2010). *Candida* is a normal body flora but also found in water.

Some species have been implicated in systemic infections and mild to severe infections of skin, nails, and mucous membranes (*C. albicans*), endocarditis, pyelonephritis, arthritis (*C. parapsilosis,* and *C. tropicalis, C. guilliermondii*). *Cryptococcus* sp. is found in soil, dust, milk, pigeon droppings and also a normal body flora of animal (Pfeiffer and Ellis, 1992). They have been involved in pulmonary infection, meningitis, abscesses in the lungs, brain, lymph nodes and skin (*C. neoformans,* is a pathogenic species). *Rhodotorula* is found on human skin and in the environment and is responsible for transient blood stream invasion and meningitis (Gyaurgieva *et al.,* 1996).

4. Conclusion

Organic waste utilization is currently receiving great research attention globally and the findings in this research work demonstrated the application of two organic wastes (rabbit manure and Nypa palm) in enhanced bioremediation of crude oil polluted soil in Yorla farmland, Ogoniland. The research has unveiled the diverse group of autochthonous hydrocarbonoclastic fungi and bacteria domicile in crude oil polluted soil.

Combinations of different enhancement approach are recommended during enhanced bioremediation. This will further enhance better understanding of the interactions of environmental factors on the chemicals of concerns (COCs) and soil amendment agents. It is our hope that the finding can catalyze not only significant environmental and social improvements, but a strategic policy on how the oil industry in the Niger Delta will function in a way that truly benefits the livelihoods of these communities now and in the nearest future.

5. Recommendations

It is important to state here that more research attentions are required on the pilot scale and ex-situ utilization of rabbit droppings and Nipa palm. Adequate baseline data should be generated for use in field trial. Further molecular studies are needed to

decipher the catabolic genes resident in these oil-degrading microorganisms and their hydrocarbon specificities. This will, invariably assist in developing economically acceptable, ecologically friendly, cost effective and efficient bioremediation protocol for the restoration of the negative impact of crude oil pollution in Nigeria.

More research is needed, which will encourage the sustainable utilization of Nypa palm and reduce their incidences in the coastal States of Nigeria. Future research could focus more on bacterial and fungal degradation abilities than on bacterial and fungal species, and qPCR would be the tool for this. It is our strong believe that this study can provide a firm foundation upon which all the stakeholders concerned can, if they so wish, draw up a response to the findings presented here and the gross environmental abuse by multi-national oil companies operating in Ogoniland, particularly and Niger Delta region of Nigeria, generally.

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