

## Original Research Article

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## A Comparative Study of Enhanced Crude Oil Degradation in Three Tropical Soils using Pig and Goat Manures as Organic Amendments

O. A. Ojo-Omoniyi<sup>1\*</sup>, N. Dike-Ekeh<sup>1</sup> and O. M. Owoeye<sup>2</sup>

<sup>1</sup>Department of Microbiology, Lagos State University, Ojo, P.M.B. 0001,  
LASU Post Office, Lagos-Nigeria

<sup>2</sup>Handsonlabs Software Academy, Lagos, Nigeria

\*Corresponding author

### ABSTRACT

The bio-recovery of three different oil-polluted tropical soils – RS (oil-polluted soil sample from River state), LS (soil sample from NNPC depot, Lagos) and POS (oil-polluted soil sample from Oriade L.G.A., Lagos) by manure amendment was studied for eight weeks. The rates of crude oil biodegradation after manure application as quantified by Gas chromatographic analysis of residual total petroleum hydrocarbon (TPH) showed the same trend of decrease in the total petroleum hydrocarbons in both the LS and RS series. The TPH for the LSC (non-oil polluted LS soil sample as Control) series at weeks 4 and 8 were  $1893.42 \pm 26.16$  mg/kg and  $1080.86 \pm 14.33$ mg/kg, this was significantly different from that of LSp<sub>g</sub> (pig manure-amended soil) which for the same weeks which were  $1107.19 \pm 18.41$ ,  $258.56 \pm 4.16$  mg/kg TPH respectively and from that of LSgt (goat manure-amended soil sample) which were  $1355.15 \pm 7.45$ ,  $491.24 \pm 20.82$  mg/kg TPH respectively. The results show that pig manure was a better organic amendment than goat manure in the LS series soil but at the long run not better than goat manure in the RS and POS soils. Microbiological counts showed a peak at week 6 with HUF (hydrocarbon - utilizing fungi) ( $34 \times 10^3$ cfu/g) for LSC; a peak at week 2 with HUB (hydrocarbon-utilizing bacteria) ( $252 \times 10^5$ cfu/g) for LSp<sub>g</sub> and a peak at week 6 for LSgt with HUB ( $91 \times 10^5$ cfu/g). Also, microbial counts revealed a peak in week 2 with HUB ( $206 \times 10^5$ cfu/g) for POSC; a peak at week 8 for POSp<sub>g</sub> (oil polluted + pig manure amended Oriade soil sample) with HUB ( $197 \times 10^5$ cfu/g) and a peak at week 8 for POSgt with HUB ( $138 \times 10^5$ cfu/g). Microbial counts also revealed peaks at week 4 ( $97 \times 10^5$  cfu/g),  $64 \times 10^5$  cfu/g and  $119 \times 10^5$  cfu/g for RSC (River state control soil sample), RSp<sub>g</sub> and RSgt samples respectively all with HUB. In terms of soil properties, in RS soils, pig manure added more ammonium, total organic matter, nitrate, phosphorus and potassium than goat manure; in POS soils, pig manure lowered pH,  $\text{NH}_4^+$ , Phosphorus more than goat manure; in LS soils, goat manure added more total organic matter, sodium and potassium while pig manure added more  $\text{NH}_4^+$ , nitrate and phosphorus.

### Keywords

Biodegradation,  
Bio-recovery,  
Organic  
amendments,  
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### Introduction

Biodegradation of organic waste is becoming an increasingly important method of waste treatment (Atlas, 1981). The advantages of this option include its being environment-friendly, cheap source of nutrient and its

simplicity. Although, crude oil contamination has some adverse effects on crops and other vegetation. Bioremediation and phytoremediation of crude oil contaminated soil is the most promising and environment-friendly for effective clean-up of crude oil contaminated soil (Ezeji *et al.*, 2007). Doran

and Parkin (1994) reported that soil quality is defined as the capacity of the soil to function within ecosystem boundaries, sustaining plant and animal health. Soil as a key component of natural ecosystems upon which environmental sustainability largely depends, therefore any pollution of the soil will undoubtedly impact the ecosystem and agricultural activities.

Crude oil pollution of soil provides an excess carbon that might be unavailable for microbial utilization, induces a limitation on soil Nitrogen and Phosphorus which may result in virtual eradication of some of the primary food chain components which in turn have major consequences on predator and consumer species (Doran and Parkin, 1994; Ezejiet *et al.*, 2005; Onuh *et al.*, 2008). Crude oil spillages into soils also lead to high accumulation of Aluminium and Manganese ions which are toxic to plant growth. Thus, soil fertility is compromised. It is also remarkable that even at low parts per billion (ppb) concentrations, oil spillages into sub soils are destructive, creating anoxic conditions in the rhizosphere which is unfavourable to most heterotrophic soil bacteria (Baker and Herson, 1994; Atlas, 1981). However, the presence of crude oil in the environment creates a niche for specially adapted indigenous petroleum-degrading microorganisms well represented in the soil and water environments. These organisms include bacteria of the genera; *Arthrobacter*, *Pseudomonas*, *Acinetobacter*, *Bacillus* *etc.*, while fungi such as *Candida*, *Rhodotorula*, *Mortierella*, *Aspergillus* as well as algae-*Protothecazopfi*, cyanobacteria, green and red alga (Ijah *et al.*, 2008; Ezejiet *et al.*, 2005).

However, since the discovery of petroleum in large volume, pollution of the environment occurred simultaneously, bioremediation and recovery approaches to remedying polluted environments became necessary. All the approaches to recovery are based on physical,

chemical and biological means. However, studies have shown that the biological approaches are the technologies of choice (Adedokun and Ataga, 2007). Since oil degradation is not limited by electron donor, that is, hydrocarbons but by supply of nutrients or oxidans (electron acceptors), to combat the plethora of environmental pollution in present day society, efficient and environmentally safe organic waste treatment technologies are needed by oil-producing countries. In consequence, enhanced crude oil degradation by soil microbiota in the presence of poultry manure in Nigerian polluted soils has been reported (Ibekwe *et al.*, 2006; Amadi and Uebari, 1992). Amadi *et al.*, (1993), Obire and Akinde (2006) reported that nutrient supplementation of oil-polluted soil with poultry droppings as organic nutrient source in particular is beneficial for maize growth and it also enhances both biodegradation of oil and soil recovery. Nwogu *et al.*, (2015) investigated bio-stimulant energies of *Capra aegarushircus* for processing of crude oil - polluted soil under certain tropical environment. Their findings revealed that *C. A. hircus* manure is an excellent bio-stimulant that enhances the performance of native hydrocarbonclastic bacteria respectively. Owabor and Yusuf (2010), Agarry *et al.*, (2013) both evaluated the blend of poultry, piggery, goat manures as well as chemical fertilizers (kerosene, diesel and gasoline) as bio-stimulant for over three weeks of processing. The results suggested that farm feed manures displayed higher levels of degradation and hydrocarbon reductions as well as being environment- friendly. Furthermore, diverse organic fertilizers like wood ash, pig, goat manure, oil palm kernels used as amendments were observed to provide better soil nutrients compared with those of nitrogenous manures (Moyin-Jesu, 2019). Meanwhile, Ukpaka *et al.*, (2020) reported the efficacy of Total Petroleum Hydrocarbon (TPH) mineralisation in loamy

soil enriched (within 90 days) with water which suggested promising result compared to other soil enrichment processes. Finally, Verma *et al.*, (2020) reported bio-gas as a good source of renewable energy and sustainable agriculture for rural areas in India using cattle dung and related wastes as recyclable sources of soil nutrients.

Although, there have been reports of laboratory investigations on the use of organic nutrients such as cow dung and poultry droppings in bioremediation of oil - polluted sites (Amadi and UeBari, 1992), there has not been investigations into the use of other organic nutrients such as pig and goat dung as biodegradation enhancing agents in southwest Nigeria. It is therefore necessary to carry out studies on the application of pig and goat droppings in bioremediation of crude oil-polluted soils.

## **Materials and Methods**

### **Sample collection**

Three different soil samples were used in the research-OS, RS and LS. The first 'OS' was randomly collected from an Agricultural farmland in Oriade L.G.A Lagos (that has no recorded experience of petroleum hydrocarbon pollution), at a soil depth of 15-20cm, using a clean soil auger. RS, soil samples polluted on site, were collected randomly at the same depth, from an Agip flow station in Ebocha, (Rivers State) into a sterile glass bottle before being transported to the laboratory. The last sample LS which was heavily flooded with crude oil at the sampling site (NNPC depot in Lagos) was collected and stored as described above. The organic manures used were collected from a local farm in Ebocha, Rivers State. Also, the crude oil type used (Ebocha Blend) was supplied by Nigeria Agip, Ebocha Flow Station, Rivers State.

### **Physicochemical characterisation of Soils and Manures**

Samples of soils and organic manures were weighed using Mettler PE 1600 chemical balance (Gallenkamp) and their physicochemical parameters were determined as well as their petroleum hydrocarbon profiles before the manure treatments. Soil treatments were done at the ratio of 5: 1, that is, 5 parts of soil to 1 part of manure.

### **Pollution of soil (code OS)**

Pollution of soil was done at the rate of 200mls of crude oil per 1000g of soil. The polluted soil was left at room temperature for 14 days during which its physicochemical parameters were determined, its microbiological properties were determined, before the manures were applied as reported above.

### **Evaluation of residual Total Petroleum Hydrocarbon (TPH)**

Residual petroleum was quantified using gas chromatographic analysis. Residual total petroleum was extracted once as follows: 10g of polluted soil was extracted with 10ml of Dichloromethane. After the solvent vented off, the residual TPH was dissolved in acetone and concentrated to 1ml. TPH concentrations in the acetone were determined using the Hewlett Packard 5890 Series gas chromatograph On- Column Injector type (column OV-101, thickness and width-80/100 mesh, stationary phase WHP 5%) equipped with Flame Ionization Detector (FID). The injector and detector temperatures were maintained at 200°C and 260° C respectively. The column temperature was programmed to rise to 230°C. The GC was programmed at an initial temperature of 70°C, this was held for 2min, then ramped at 10°C /min to 230°C and held for 10mins (Adebusoye *et al.*, 2007).

### **Isolation of petroleum utilizing bacteria and fungi**

The mineral medium described by Kastner *et al.*, (1994) was used. The medium contains per litre Na<sub>2</sub>HPO<sub>4</sub>, 2.13g; KH<sub>2</sub>PO<sub>4</sub>, 1.30g; NH<sub>4</sub>Cl, 0.50g, MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.20g; Agar-agar, 10.0g. The pH of the medium for bacteria was adjusted to 7.2 and fortified with Nystatin and Nalidixic acid at 50ug/l to suppress fungal growth. The medium for fungi was fortified with 0.05g of Yeast extract to encourage fungal growth and with 0.5ml of streptomycin to suppress bacterial growth. Trace elements solution (1ml/litre) described by Bauchop and Elsdon (1960) was sterilized separately and added aseptically to the medium. Contaminated and uncontaminated soil samples (1.0g) were serially diluted in sterile distilled water while 0.2ml of this serially-diluted aliquots were inoculated (with a sterile Hockey stick) on Minimal Salt Agar (MSA) plates to which crude oil was fed using crude oil-soaked sterile filter paper in vapour - phase transfer technique. These were then incubated at room temperature for 4 and 6 days for bacterial and fungal isolates respectively. Several of the colonies that appeared were further purified by sub-culturing on Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA) plates to which crude oil was introduced by the same technique and Luria Bertani (LB) plates. The ability to degrade crude oil was confirmed by inoculating NA grown pure cultures (20h) into fresh MSA plates supplemented with crude oil. The purified isolates were then maintained on LB agar slants for further identification (Raymond *et al.*, 1976).

### **Microbial total count**

Aseptically 1ml of the serially diluted samples were inoculated by pour-plate method on solid SDA plates and NA plates for the enumeration of total saprophytic fungi

and total heterotrophic bacterial counts respectively. The inoculated plates were incubated at room temperature for 72 and 48 hours respectively. Colonies which appeared on the plates were counted and expressed as cfu/g of soil.

### **Substrate Specificity**

The ability of two of the bacterial isolates to grow on crude oil was further evaluated in 20ml of liquid media fortified with 0.2ml crude oil. Incubation was at room temperature for 32days. Degradation was monitored by TVC and visual observation for turbidity. The extent of crude oil utilization was determined using GC analysis.

### **Identification and Characterization of Isolates**

Pure cultures of bacterial isolates were identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics. Pure Fungal cultures were observed while still on plates and after wet mount in lacto-phenol blue on slides under the compound microscope. The observed characteristics were recorded and compared with the established identification key of Malloch (1997).

### **Agricultural evaluation of bioremediated soils**

In order to evaluate the extent of remedy accomplished in the soils, one type of white corn bought from two different markets in Lagos state; Okokomaiko and Iyana-Iba were planted on the bioremediated soils at a depth of 1.5cm, maintained at 60% of their water holding capacities and also on the control soils that were not amended with organic manures. The dates of the emergence of plumules of the maize seeds so planted, stem and leaf measurements of the grown plants

were used in the evaluation of the soils extent of recovery for agricultural purposes.

## Results and Discussion

### Isolation of petroleum utilizing bacteria and fungi

Petroleum-hydrocarbon utilizing microorganisms identified using standard and conventional methods include; *Bacillus coagulans*, *Corynebacterium sp.*, *Micrococcus vairians*, *Acinetobacter mallei*, *Bacillus polymyxa*, *Bacillus megaterium* and *Micrococcus roseus* while the fungal isolates include; *Aspergillus tamari*, *Aspergillus flavus*, *Aspergillus candidus* and *Penicillium viridicatum*.

### Enhancement of Biodegradation of Polluted soils Amended with Organic manures

The growth kinetics of hydrocarbon-utilizing bacteria and fungi (HUB and HUF) following the addition of manures as represented in Fig.1 showed that at week 2, 4 and 6 in the LSp<sub>g</sub> soil, HUB was more at bioremediation than HUF, but by week 8, HUF was slightly more in terms of population. The same trend was noticed in LS<sub>g</sub>t soils. In LSC, HUF was higher in number than HUB only at week 4, there were slight variations in the other weeks. In LSp<sub>g</sub>; HUB was higher in count in week 1, peaked in week 2 and then proceeded to decrease in number. HUF peaked in week 1. However, at week 8 HUF was higher than HUB. TSC peaked at week 4 and became highest in number among the others by week 8. THC peaked at week 4, and slightly increased by week 8. The peak of the bioremediation was by week 2 when HUB count was highest (Fig. 1c and 1d).

In Figure 2, for the RS<sub>g</sub>t soil samples, HUB was highest in week 1, peaked again in week

4, before finally reducing, leaving HUF to pick up. The same trend was observed in RSC samples. For RSp<sub>g</sub> samples, HUB was more until week 6 when it gave way for HUF. As shown in Figure 2b for RSC, the Peak of the bioremediation was in week 4 with HUB, by week 8 fungi took over and the Hydrocarbon utilizers became the lowest in number. Similarly, in RSp<sub>g</sub> the peak was at week 4 with HUB, when TPH dropped between weeks 6 and 8, fungi took over (Fig. 2c and 2d).

The results for POS samples were shown in Figure 3. In POSC, HUF was highest in week 1, and then alternated within the weeks. HUB peaked in week 2, decreased and peaked again in week 8. The peak was in week 2 with HUB doing more (Fig. 3b). POS<sub>g</sub>; the peaks were in weeks 1 and 8 with HUB doing more. Here the pig manure was more of a source of HUB than HUF (Fig. 3c and 3d).

The rates of crude oil biodegradation after manure application as quantified by GC Fig.4. The results showed that pig manure was a better remediation-agent than goat manure in the LS series soil (Fig. 4). In eight weeks, there was no significant difference in enhanced bioremediation between pig manure-amended and goat manure-amended soils (Fig. 5). Also, in the case of RS soils; at week 4 pig manure-amended soil (79.3 mg/kg TPH) did better than goat manure-amended soil (114.43 TPH), but by the eight week, there was no significant difference between their performances (Fig. 6).

The tropical soil samples were all slightly acidic, low in moisture and they were rich in organic matter as well as minerals (Table 1). The results showed that the rates of hydrocarbon biodegradation were highest in the first four weeks for all the samples except for LSC and POSC, after which they declined in all except in the two mentioned above. This

finding agreed with that of Obire and Nwaubeta, (2001). Pig manure was a better remediation-agent than goat manure in the LS series soil (Figure 4) since it seemed to harbour certain physicochemical constituents and biological properties that favored bioremediation of LS soils more than goat manure.

In the absence of manures, there was biodegradation, though at a slower rate than in those amended with manures. In the POS series, something out of the trend was observed between week 2 and 4 in the control, the TPH became slightly increased. This may be attributed to experimental bias or to the migration of hydrocarbons from the atmosphere into the soil samples. But for the manure - amended soils, POSpg at week 4 (69.46mg/kg TPH) was better bioremediated than POSgt (147.29 mg/kg TPH).

However, by the eight week, there was no significant difference in enhanced bioremediation between pig manure -

amended and goat manure-amended soils (Figure 5). Also, in the case of RS soils, at week 4 pig manure (79.3 mg/kg TPH) did better than goat manure (114.43 TPH), but by the eight week, there was no significant difference between their performances (Figure 6). This could mean that in the long run, goat and pig manures were equally good bioremediation-agents, though pig manure does so faster.

In terms of percentage petroleum hydrocarbon loss, a total of 56.44% loss was seen with LSC, 89.67% and 80.38% losses in LSp<sub>g</sub> and LS<sub>g</sub>t samples respectively over the eight weeks period. Ijah *et al.*, (2008) recorded a 56.3% crude oil loss in un-amended polluted tropical soil and a 75% loss in chicken dropping- amended soil. A total percentage loss of 99.79 % and 99.76% respectively for the pig manure-amended and goat manure-amended RS soils was observed against 61.45% loss in the un-amended control soil (Table 2–5).

**Table.1A** Determination of physico-chemical parameters of soils before manure amendment

Soil code	Physico-chemical parameters							
	pH	Moisture %	TOM %	Na mg/kg	K mg/kg	NH <sub>4</sub> -N mg/kg	NO <sup>-3</sup> mg	Phosphorus mg/kg
<b>RS</b>	6.6	2.61	75.87	1.64	57.46	ND	55.34	42.88
<b>LS</b>	6.5	9.15	96.66	2.36	82.59	ND	55.34	49.74
<b>OS</b>	6.8	1.64	94.12	6.81	29.10	ND	23.85	75.07

KEY:RS- River state, LS- Lagos state, OS- Oriade LGA (soil with no previous history of oil contamination)  
TOM- Total organic matter

**Table.1B** Physicochemical properties of soils before manure amendment

Code	Parameters							
	pH	Moisture (%)	TOM	Sodium	Potassium	NH <sub>4</sub> -N	Nitrate	Available phosphorus
<b>RS</b>	6.6	2.61	75.87	164.09	57.46	ND	55.34	42.88
<b>LS</b>	6.5	9.15	96.66	236.11	82.59	ND	55.34	49.74
<b>OS</b>	6.8	1.64	94.12	681.05	29.1	ND	23.85	75.07
<b>POS</b>	5.9	9.69	82.85	336.01	52.46	6.06	25.2	ND
<b>POSC</b>	6.3	3.4	90.65	657	9.5	ND	ND	43.44

**Table.2** Physicochemical properties of manure-amended and Un-amended polluted soils at (week 10)

Sample	pH	Moisture	TOM	Na	K	NH <sub>4</sub> -N	NO <sup>3-</sup>	P
POSC	6.0±0.17a,b	0.98±0.03c	4.99±0.02c	367.59±0.05c	9.6±0.01b	1.9±0.04c	3.9±0.20a	5.62±0.04b
POSp <sub>g</sub>	5.7±0.02b	1.45±0.03a	8.70±0.01a	454.91±0.07a	10.15±0.03a	2.2±0.06b	3.7±0.05a	6.20±0.04a
POS <sub>g</sub> t	6.1±0.02a	1.11±0.06b	8.00±0.01b	396.06±0.07b	8.8±0.05c	2.4±0.04a	2.9±0.08b	6.17±0.02a
<b>RSC</b>	<b>7.9±0.10a</b>	<b>1.50±0.02c</b>	<b>9.4±0.03c</b>	<b>450.00±0.14a</b>	<b>6.5±0.02c</b>	<b>1.6±0.03b</b>	<b>4.1±0.03a</b>	<b>6.7±0.05a</b>
RS <sub>g</sub> p <sub>g</sub>	7.0±0.5c	i.82±0.03b	11.53±0.07a	370.22±0.03b	40.7±0.05a	1.8±0.07a	3.5±0.12b	6.24±0.06b
RS <sub>g</sub> t	7.5±0.10b	6.80±0.04a	9.68±0.07b	304.65±0.05c	11.9±0.16b	1.9±0.04a	2.6±0.03c	5.84±0.08c
<b>LSC</b>	<b>6.7±0.3a</b>	<b>1.9±0.14c</b>	<b>7.5±0.09c</b>	<b>399.26±0.64b</b>	<b>4.7±0.11c</b>	<b>1.12±0.05b</b>	<b>2.9±0.11b</b>	<b>6.3±0.03b</b>
LS <sub>g</sub> p <sub>g</sub>	6.5±0.1a	3.93±0.04b	20.11±0.02b	395.85±0.14c	11.7±0.16b	2.1±0.13a	4.7±0.05a	8.4±0.08a
LS <sub>g</sub> t	6.9±0.2a	5.11±0.02a	22.12±0.05a	454.94±0.09a	34.5±0.11a	1.17±0.03b	2.2±0.07c	6.06±0.13c

\*values are Means ±Standard Deviations of triplicate results

Means ±SD with similar superscripts in the same column are not significantly different from each other at P>0.05 for the different series studied.

\*\* TOM- Total organic matter and Moisture are in %, others with the exception of pH are in mg/kg

**Table.3** Physicochemical parameters of manures used

Manure code	pH	Nitrogen (%)	Phosphorus(mg/kg)	Potassium(mg/kg)
<b>Gt</b>	<b>5.9±0.02</b>	<b>3.73±0.09</b>	<b>26.78±0.09</b>	<b>12.90±0.09</b>
<b>Pg</b>	<b>5.8±0.11</b>	<b>2.14±0.04</b>	<b>12.98±0.09</b>	<b>14.70±0.03</b>

\*Values are means± Standard deviations of duplicate results; ‘pg’ represents pig manure, while ‘gt’ represents goat manure

**Table.4** Maize growth characteristics at 10 DAP (biomass 16 DAP) at week 10 of manure application on unpolluted, polluted and amended soils

Sample	stem length	leaf height	biomass	germination rate	no of days to germination
OS	5.10±0.53b	19.53±1.74a	1.45±0.0b	3	3
POSC	3.55±0.58c	12.60±0.60b	0.27±0.10c	2	3
POSp <sub>g</sub>	6.20±0.47a	21.50±1.05a	2.41±0.10a	2	3
POS <sub>g</sub> t	5.50±0.96a,b	21.50±3.65a	1.64±0.17b	1	3
<b>RSC</b>	0	0	0	2	16
RS <sub>g</sub> p <sub>g</sub>	3.00±1.0a	10.05±3.55a	1.37±0.18b	1	5
RS <sub>g</sub> t	1.70±0.10b	3.6±0.10b	1.76±0.37a	1	6
<b>LSC</b>	0	0	0.34±0.33b	2	16
LS <sub>g</sub> p <sub>g</sub>	4.46±0.15a	20.36±0.75a	1.97±0.42a	2	4
LS <sub>g</sub> t	4.50±0.3a	18.00±0.10a	2.03±0.56a	1	3

\*Means ± SD of values with similar superscripts in the same column are not significantly different at P>0.05 for the OS, POSC series together, RS series and for the LS series differently

**Table.5** Biochemical and morphological characterization of bacterial isolates

Isolate code	Gram Reaction	Cellular	Catalase test	Oxidase test	Indole test	Motility test	Methyl red test	Voges Proskauer	Citrate test	Urease test	Starch hydrolysis	Gelatin hydrolysis	Nitrate reductase	Coagulase test	Spore test	Glucose	Xylose	Lactose	Sucrose	Fructose	Raffinose	Mannitol	Maltose	Arabinose	Galactose	Pigmentation	Probable identity
RS1	+	R	+	+	-	+	-	+	-	-	+	-	+	-	+	+	+	+	+	-	+	-	+	-	-	Cream	<i>Bacillus coagulans</i>
RS3	+	C/R	+	-	-	-	-	+	-	+	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	Yellow	<i>Corynebacterium sp.</i>
LS3	+	C	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	-	-	-	+	+	+	Yellow	<i>Micrococcus varians</i>
LS4	-	R	+	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	Cream	<i>Acinetobacter mallei</i>
PO S3	+	R	+	+	-	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	Cream	<i>Bacillus polymyxa</i>
PO S2	+	R	+	+	-	+	-	-	+	-	+	+	-	-	+	+	+	-	+	-	-	+	-	+	-	Cream	<i>Bacillus megaterium</i>
PG 1	+	C	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	Pink	<i>Micrococcus seus</i>
GT 1	+	R	+	+	-	+	-	+	+	-	+	+	+	-	+	+	-	+	+	-	-	+	-	+	+	Cream	<i>Bacillus subtilis</i>

Key:

+ = Positive

- = Negative

C= Cocci

R= Rods



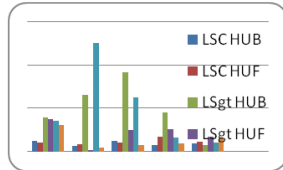


Fig 1a: Counts of Petroleum Utilizing bacteria and fungi after manure application in LS

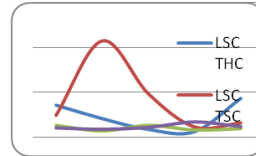


Fig1b: Total microbial counts in LSC over the eight weeks experimental period.

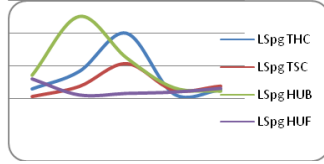


Fig 1c: Total microbial count after manure application in LSpG

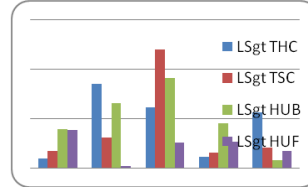


Fig 1d: Total microbial count after manure application in LSgt

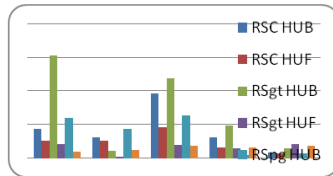


Fig 2a: Counts of Petroleum Utilizing bacteria and fungi after manure application in RS

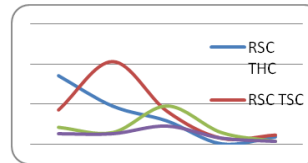


Fig.2b: Total microbial count over the eight weeks experimental period in RSC

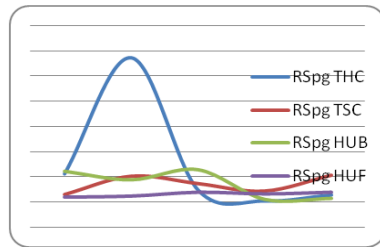


Fig. 2c: Total microbial count after manure application in RSpG

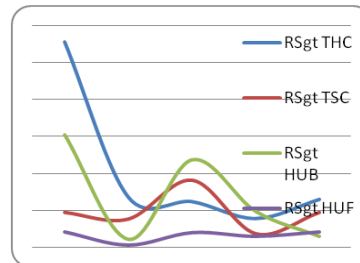


Fig. 2d: Total microbial count after manure application in RSgt

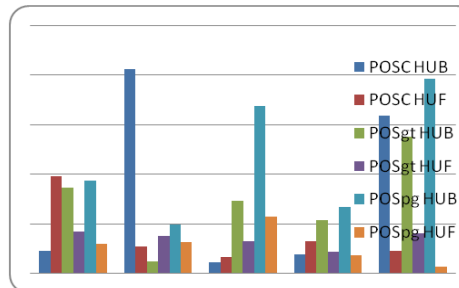


Fig.3a: Counts of Petroleum Utilizing bacteria and fungi after manure application in POS

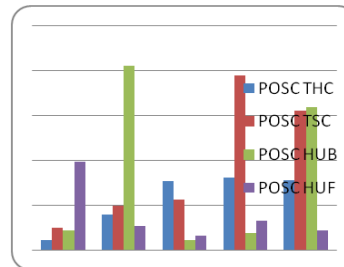


Fig. 3b: Total microbial counts over the eight weeks experimental period in POSC

**\*\* POSC is the code for the control sample of the POS soils without manure amendment, any code having 'pg' have been amended with pig manure, any with 'gt' have been amended with goat manure.**

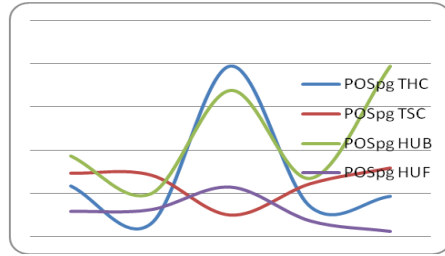


Fig.3c: Total microbial count after manure application in POSpg

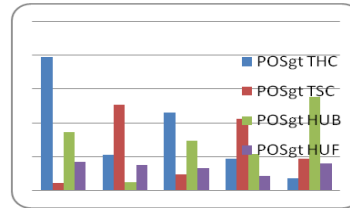


Fig. 3d: Total microbial count after manure application in POSgt

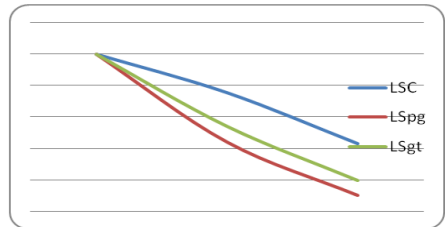


Fig 4: Total Residual Petroleum Hydrocarbon in LS Manure amended and Un-amended Soils

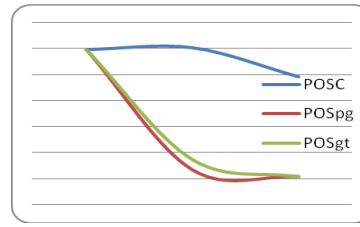


Fig. 5: Total Residual Petroleum Hydrocarbon in POS Manure amended and Un-amended Soils

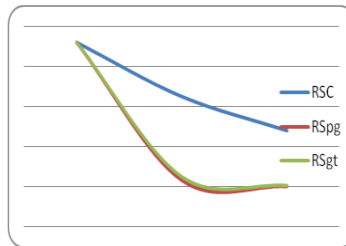


Fig.6: Total Residual Petroleum Hydrocarbon in RS Manure amended and Un-amended Soils

Similar results were obtained for the pig manure-amended and goat manure-amended POS soil samples except for the un-amended polluted control that registered only a 21.14% loss in total petroleum hydrocarbon. This may be attributed to the soil type and its characteristics that reduced the onset of biodegradation.

### Effect of Manure Amendment on soil physicochemical parameters

The effect of manure amendment on soil physicochemical properties; Apart from potassium, goat manure is higher than pig manure in all the other parameters studied. In RS samples, pH increase was higher in the control RSC than in the RSgt and RSpG- this

showed that the pig manure was better in maintaining the pH range for optimal bioremediation in the soil studied; moisture increased in RSgt than in the other two; supported the growth of both petroleum hydrocarbon utilizers and non-users. This is in line with the findings of Okolo *et al.*, (2005). HUB alternated in number between the weeks and stabilized in the last 2 weeks. A similar trend was seen in HUF. The peak of the work seemed to be in week 6 with HUF doing more (Fig. 1b). It might mean that the heterotrophs and saprophytes generated certain metabolites that in some weeks reduced the number of the petroleum hydrocarbon users, until week 6 when the condition favoured the growth of HUF which completed the biodegradation process. Hence, future research would have to

both detect and evaluate the presence of some of these metabolites limiting the onset of biodegradation in a mixed population.

In LSp<sub>g</sub>; the first two weeks HUB and HUF were actively biodegrading but by week 8, when the TPH had drastically reduced, the saprophytic fungi had more or less taken over the soil. This suggested that petroleum-polluted soils generally support the growth of organisms which use the petroleum components as sources of carbon and energy (Atlas, 1977; Leahy and Colwell, 1990). LS<sub>g</sub>; The results showed that the hydrocarbon users began equally well by the first week, with the HUB being more at the biodegradation until the final week when the HUF took over. The peak of the bioremediation was at week 6 when the HC users were more than the total count of both bacteria and fungi (Fig. 1d). Total organic matter and sodium decreased in all, but was lowest in the control and highest in POS<sub>g</sub>; potassium slightly increased in all except POS<sub>g</sub>. In RS samples, pH increase was higher in the control RSC than in the RS<sub>g</sub>t and RS<sub>g</sub>p- this showed that the pig manure was better in maintaining the range of pH for optimal bioremediation in the soil studied; moisture increased in RS<sub>g</sub>t than in the other two; total organic matter decreased but more in RSC, least in RS<sub>g</sub>p, this showed that the manures added organic matter to the soil; sodium increased but more in RSC, least in RS<sub>g</sub>t, this might suggest that some organisms in the manures were making use of the sodium for their cellular activities; potassium decreased but most in RSC, least in RS<sub>g</sub>p; ammonium increased but most in RS<sub>g</sub>p and RS<sub>g</sub>t; nitrate decreased most in RS<sub>g</sub>t, least in RSC; phosphorus decreased most in RS<sub>g</sub>t, least in RSC.

In LS samples, there was no significant change in pH between the three. These results showed that apart from improved soil fertility

brought about by the addition of these organic nutrients to soil, the addition of goat dung and pig droppings to oil-polluted soils will result in an increase in the population of total saprophytic fungi, total heterotrophic bacteria and an increase in the population of petroleum-utilizing microorganisms in soil.

In conclusion the organic manure is an excellent bio-stimulant that enhances the performance of native hydrocarbonclastic bacteria provided the physicochemical parameters are optimum in a tropical environment and it is environment-friendly.

## References

- Adebusoye, S. A., Ilori, M. O., Amund, O. O., Teniola, O. D. and Olatope, S. O. (2007). Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World Journal of Microbiology and Biotechnology* 23: 1149- 1159.
- Adedokun O.M., and Ataga, A.E. (2007). Effects of amendments and bio-augmentation of soil polluted with crude oil, automotive gasoline oil, and spent engine oil on the growth of cowpea (*Vigna unguiculata* L. Walp). *Scientific Research and Essay*2(5):147-149.
- Agarry, S. E.Aremu, M. O. and Aworanti, O. A.(2013) Kinetic Modelling and Half-Life Study on Enhanced Soil Bioremediation of Bonny Light Crude Oil Amended with Crop and Animal-Derived Organic Wastes, *Journal of Petroleum & Environmental Biotechnology* 4:2 <http://dx.doi.org/10.4172/2157-7463.1000137>
- Amadi, A., and Uebari,Y. (1992). Use of poultry manure for amendment of oil polluted soils in relation to growth of maize (*Zea mays* L). *Environment International*18:521-527.
- Amadi A., Dickson, A. A. and Maate, G.O. (1993). Remediation of oil - polluted soils; Effect of organic and inorganic nutrient supplements on the performance

- of maize (*Zea mays* L). *Water, Air and Soil Pollution*66:59-76.
- Atlas, R. M. (1981). Microbial degradation of petroleum hydrocarbons: *An environment perspective. Microbiological Reviews*45:180-208.
- Baker, K. H. and Herson, D. (1994). Microbiology and biodegradation. In: *Bioremediation* (eds.) Baker K. & Herson D. McGraw Hill Inc., New York, Pp. 9 - 60.
- Bauchop, T., Elsdon, S.R. (1960). The growth of microorganisms in relation to their energy. *Journal of General Microbiology*23:457- 459.
- Carmichael, L. M., and Pfaender, F.K. (1997). The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. *Biodegradation*8: 1-13.
- Datta, R., Meena, R., Pathan, S., and Ceccherini, M. (2019). Biochar and Organic Amendments for Sustainable Soil Carbon and Soil Health. In: *Carbon and Nitrogen Cycling in Soil. Earth and Environmental Science* Springer, Singapore DOI, <https://doi.org/10.1007/978-981-13-7264-3-3>,
- Doran, J.W., and Parkin, T. B. (1994). Defining and Assessing Soil Quality. In: *Defining Soil Quality for a Sustainable Environment*. Soil Science Society of America special publication 35: 3 - 21.
- Ezeji, E.U., Anyanwu, B. N. Onyeze, G.O.C., and Ibekwe V.I. (2005). Studies on the utilization of petroleum hydrocarbons by microorganisms isolated from oil polluted soil. *International Journal of Natural and Applied Sciences*.1(2):122-128.
- Ezeji, U.E., Anyadoh, S. O. and Ibekwe, V.I. (2007): Clean up of crude oil-contaminated soil. *Terrestrial and aquatic Environmental Toxicology* 1(2): 54-59.
- Global Science Books. Ibekwe, V.I.K., Ubochi, C. and Ezeji, E.U., (2005). Effect of organic nutrient on microbial utilization of hydrocarbons on crude oil contaminated soil. *African Journal of Biotechnology*5 (10):983 - 986.
- Ijah, U. J. J., Safiyahu, H., and Abioye, O. P. (2008). Comparative Study of Biodegradation of Crude Oil amended with Chicken Droppings and NPK Fertilizer. *Science World Journal*, 3(2):63 - 67.
- Kastner, M., Breuer-Jammali, M., and Mahro, B. (1994). Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons. *Applied Microbiology and Biotechnology*, 41: 264 - 273.
- Leahy, J. G., and Colwell, R. R. (1990). Microbial Degradation of Hydrocarbons in the Environment. *Microbiological Reviews*5(3):305-315.
- Malloch, D. (1997). Moulds Isolation, Cultivation and Identification, Department of Botany, University of Toronto, Toronto USA.
- Moyin-Jesu, E. I. (2019). Comparative Evaluation of Different Organic Fertilizers on The Soil Chemical Composition, Growth, Leaf and Fruit Yields of Fluted Pumpkin (*Telfaria occidentalis* L.): *American Journal of Research Communication*, 7(3).
- Nwogu, T. P., Azubuike, C.C. and Ogugbue, C. J. (2015). Enhanced Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbons after Amendment with *Capra aegagrus hircus* (Goat) Manure, Hindawi Publishing Corporation *Biotechnology Research International* Article ID 657349, 7p. <http://dx.doi.org/10.1155/2015/657349>.
- Obire, O., and Nwaubeta, O. (2001). Biodegradation of Refined Petroleum Hydrocarbons in Soil. *Journal of Applied Sciences and Environmental Management*.5 (1):43 - 46.
- Obire, O., Anyanwu, E. C. and Okigbo, R.N. (2008). Saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bioremediating agents. *Journal of Agricultural Technology*4 (2): 81 - 89.

- Obire, O, and Akinde, S.B. (2006). Comparative study of the efficiency of cow dung and poultry manure as nutrient alternative sources in bioremediation of oil polluted soil. *Niger Delta Biologia* 5(2): 82 - 91.
- Okoh, A. I. (2006). Biodegradation alternative in the clean-up of petroleum hydrocarbon pollutants. *Biotechnology and Molecular Biology Review*1 (2): 38 - 50.
- Okolo, J. C., Amadi, E. N. and Odu, C. T. I. (2005). Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loamy soil. *Applied Ecology and Environmental Research*3(1): 47-53.
- Onuh, M.O., Ohazurike, N. C. and Madukwe, D. K. (2008). Interaction of Crude Oil and Manure Treatments and Its Effects on the Agronomic Characteristics of Maize (*Zea mays* L.) *Science World Journal*3 (2):107 - 111.
- Onwurah, I. N. E., Ogugua, V. N., Onyike, N. B., Ochonogor, A. E., and Otitoju, O. F. (2007). Crude oil spills in the Environment, Effects and Some Innovative Clean-up Biotechnologies. *International journal of Environmental Research*. 1(4):307320.
- Owabor, C. N. and Yusuf, R. O. (2010). Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbon Oil Mixtures: *Evaluation of the Use of Animal Manure and Chemical Fertilizer*, (Ed. S. E. Agarry), P. 189-195 | Published online: 06 Nov 2010, <https://doi.org/10.1080/10889868.2010.514965>.
- Raymond, R. L., Hudson, J. O. and Jamison, V. W. (1976). Oil degradation in soil. *Applied and Environmental Microbiology*31: 522-535.
- Sabate, J., Vinas, M., and Solanas, A. M. (2004). Laboratory-scale bioremediation experiments on hydrocarbon Contaminated soils. *International journal of Biodeterioration and Biodegradation* 54: 19-25.
- Verma, M. L., Saini, R., Sharma S., Rani V., Jana A. K. (2020) Suitability of the Lantana Weed as a Substrate for Biogas Production. In: Srivastava N., Srivastava M., Mishra P., Gupta V. (eds) *Substrate Analysis for Effective Biofuels Production*. Clean Energy Production Technologies. *Earth and Environmental Science* Springer, Singapore DOI [https://doi.org/10.1007/978-981-32-9607-7\\_3](https://doi.org/10.1007/978-981-32-9607-7_3), Print ISBN 978-981-32-9606-0

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