Degradation of total petroleum hydrocarbon in petroleum products-contaminated soil using pig dung

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Abstract

In This study determined effect of pig dung on the degradation of total petroleum hydrocarbon in petroleum products-contaminated soil. Top soil (0-15 cm depth) was collected from Teaching and Research Farm, Federal University of Agriculture Abeokuta, Nigeria. One kilogram of the soil was measured into nine containers and contaminated with 10 % mixture of gasoline and diesel. Pig Dung (PD) was mixed with the soil at the rate of 0, 50 and 100 g kg^{-1} soil in triplicate and the containers were arranged in a Completely Randomized Design. Soil samples were taken from each container at 21 and 42 days for Hydrocarbon Utilizing Bacteria (HUB) and Total Petroleum Hydrocarbon (TPH) determination using standard methods. Collected data were subjected to descriptive and inferential statistics. The HUB species identified were *Bacillus*, *Staphylococcus*, *Escherichia* and *Klebsiella*. The TPH (mg kg^{-1}) of the soil before PD application was 83.55±0.22. After the amendments (at 0, 50 and 100 g kg^{-1}), values were 63.24±0.25, 50.09±0.64, 39.56±0.15 and 49.72±1.30, 34.51±0.56, 16.89±0.36 for 21 and 42 days respectively. Pig dung enhanced degradation of total petroleum hydrocarbon in the petroleum products-contaminated soil.

Introduction

Soil pollution by crude oil and petroleum products has been a lingering challenge in Nigeria. The pollution occurs during oil exploration, exploitation, transportation, storage and broken oil pipelines (vandalization). Crude oil and petroleum products, on getting to the environment could lead to loss of lives and damage to farmlands. Oil contains Poly Aromatic Hydrocarbons (PAHs) that create a serious threat to the ecology of aquatic and terrestrial environments by disrupting the ecosystem functions, damage of infrastructures and contamination of landscapes Dorn *et al*., 1998; Wong *et al*., 1999; Van Gestel *et al*., 2001; Schafer *et al*., 2009). Petroleum products create a risk for human health because of their carcinogenic, mutagenic and teratogenic properties (Bamforth & Singleton, 2005; Grant *et al*., 2007). Since the contamination of soil and groundwater by uncontrolled releases of petroleum products has become a significant problem, a number of technologies have been tested to remediate polluted sites. The methods commonly used for soil remediation include mechanical, burying, evaporation, dispersion, and washing. However, these methods are expensive and not environment friendly (Das & Chandra, 2011). For these reasons, scientists have focused their researches on new strategies and technologies that are environmentally friendly and cost effective for the remediation of soil polluted by contaminant such as heavy metal and petroleum hydrocarbons. The use of biological materials has been put in place as an alternative to the conventional methods. Among these, biodegradation process which involves the use of microorganisms to detoxify

or degrade the contaminants has been found to be an environmentally-friendly, noninvasive and relatively cost-effective option (April *et al*., 2000).

Soil pollution by petrol and diesel do occur along pipe line areas in Nigeria as a result of vandalization and this has resulted to loss of soil fertility. Amendment of soil with organic or inorganic nitrogen-rich nutrients is an effective strategy to enhance the biodegradation process (Margesin *et al*., 2007; Abioye *et al*., 2009). Remediation of polluted soil with inorganic fertilizer is not sustainable because of its non-availability to most famers and the cost of the little one available (Agarry *et al*., 2010; Danjuma *et al*., 2012). Bioremediation of oil polluted soil using organic wastes derived from plants and animals have been investigated (Danjuma *et al*., 2012; Adesodun & Mbagwu, 2008; Agarry & Ogunleye, 2012). Pig dung management is one of the challenges faces by pig farmers. Much work has not been carried out on the bioremediation of petroleum products polluted soil using pig dung. The attempt to bridge the gap form the thrust of this study. Therefore, the objectives of this study were to assess effect of pig dung on the soil chemical properties and degradation of total petroleum hydrocarbon in petrol and diesel-contaminated soil.

Materials and Methods

Samples Collection, Preparation and Experimental Design

Pig dung was collected from Piggery Unit, Teaching and Research Farm, Federal University of Agriculture Abeokuta (FUNAAB), Nigeria. The manure was air dried, ground, mixed, sieved with a 2 mm sieve and stored in polythene bag.

The gasoline and diesel were purchased from a petroleum products marketing station in Abeokuta, Nigeria and mixed in ratio 1:1.

Top soil (0-15 cm depth) was collected from FUNAAB Teaching and Research Farm (Latitude 7^{o} 9^{1}N and Longitude 30^{o} 21^{1}E) using a soil auger and this location had no pollution history. The soil was air dried in a clean, well ventilated laboratory, homogenized by crushing and sieved by passing through a 2 mm mesh sieve. One kilogram of soil was weighed into each of the nine containers. Ten percent spiking of petroleum products (mixture of gasoline and diesel) was adopted to achieve severe contamination based on the report of Osuji *et al*. (2005) that oil concentration in soil above 3 % affect both the soil biota and crop growth. The soil samples were then allowed to weather for a period of two weeks before the addition of pig dung at the rate of 0, 50 and 100 g kg^{-1} soil in triplicate. Application rate of pig dung was based on the 50 g chicken manure kg^{-1} soil recommended for the clean-up of polluted soil (Ijah *et al*., 2008). The pig dung was thoroughly mixed with the soil and the nine containers were arranged in a Completely Randomized Design. Soil samples were taken from each container at 0, 21 and 42 days for pH, organic carbon, nitrogen, phosphorus, potassium, hydrocarbon degrading bacteria count, hydrocarbon utilizing bacteria and total petroleum hydrocarbon determination.

Laboratory Analysis

Soil Chemical Properties and Proximate Analysis of Pig dung

Soil chemical properties were determined before and after contamination. The pH was

determined in distilled water according to Folson *et al*. (1981). Total nitrogen, potassium and available phosphorus were determined using Bremner (1996), Olsen *et al*. (1995) and Sankaram (1996) respectively. Proximate analysis of pig dung was carried out using the methods described in Chopra & Kanwar (2011).

Cultural Characterization

Serial dilution was carried out on the soil sample as 1 g of the soil was dispersed into 10 ml of sterile distilled water. This was thoroughly mixed after which 1 ml of the mixture was transferred into another 9 ml of sterile distilled water. The process was repeated till dilution factor 10^{-6} was obtained. Pour plate technique was thereafter used to isolate microorganisms in the soil sample. One ml of the dilution factor 10^{-6} was transferred into sterile petri dish after which sterile molten nutrient agar was poured and swirled. This was allowed to set and the plates were thereafter inverted in the incubator at 37˚C for 24 hours. After incubation, the cultural characteristics of the colonies such as colour, shape, elevation and opacity were determined (Sharma, 2009).

Morphological Characterization

The organisms were identified morphologically using Gram Stain Technique. A loopful of the organism was placed on a clean grease-free slide and smeared. This was thereafter heat fixed and the primary stain crystal violet was poured and left for 60 seconds after which it was washed with distilled water. The Mordant Iodine was thereafter poured on the slide for 60 seconds and alcohol was used as decolorizer. The secondary stain Safranin was eventually poured and left for 60 seconds before washing with distilled water. The slides were left to drain-dry and observed under the microscope for their size, shape and Gram stain (Cheesbrough, 2006).

Biochemical Characterization

A modified method of Cheesbrough (2006) was used for Gram staining, Catalase test, Urease test, Citrate utilization test, Indole test, Motility test, Coagulase test and Sugar fermentation test.

Determination of Total Hydrocarbon Utilizing Bacteria Count

Total Hydrocarbon Utilizing Bacteria Count was determined in both soil and pig dung. This was carried out on Mineral Salt Medium (MSM) agar as described by Balogun & Fagade (2010); and the isolated micro-organisms were identified using Bergey's manual of systemic bacteriology (Krieg & Holt, 1984).

Determination of Total Petroleum Hydrocarbon

Ten grams of the petroleum products polluted soil sample was weighed into a clean bottle and 25 ml of dichloromethane was added; the mixture was allowed to stand on a mechanical shaker for a period of 3- 4 hours at a speed of 350 rpm. The procedure was repeated twice and the aliquots was collected and mixed together in a beaker. The aliquots were concentrated on a steam bath reducing the extracts to about 5 ml. The concentrate was passed through a pipette packed with anhydrous sodium sulphate on top of a glass wool to remove moisture and other impurities. The final extract was analysed using a Hewlett-Packard 5890 series GC system coupled to a mass spectrophotometer VG TRIO 2000 to determine the quantity of total petroleum hydrocarbons. Concentration degraded and

percentage degradation was calculated using equations 1 and 2 respectively.

$$\text{Concentration degraded} = C_1 - C_2 \text{ ------ Equation 1}$$

$$\text{Degradation (\%)} = \frac{C1 - C2}{C1} \times 100 \text{ -------- Equation 2}$$

C_1 = Concentration after contamination
C_2 = Concentration after pig dung application (at 21 and 42 days)

Statistical Analysis

Data obtained were subjected to descriptive (mean and standard deviation) and inferential (ANOVA) statistics. Means were separated using Duncan Multiple Range Test (DMRT).

Results

Soil Chemical properties and Proximate analysis of pig dung

The soil chemical properties before and after contamination were presented in Table 1 while concentrations (mg kg^{-1}) of aliphatic and aromatic hydrocarbons were presented in Tables 2 and 3 respectively. The soil pH, total nitrogen (N), available phosphorous (P) and exchangeable potassium (K) decreased (after contamination with petroleum products) from 6.9 to 6.2, 1.92 to 1.87 g kg^{-1}, 15.65 to 10.35 mg kg^{-1} and 0.26 to 0.24 cmol kg^{-1} respectively (Table 1). Total Petroleum Hydrocarbon (TPH) increased from Below Detection Limit (BDL) to 83.55 mg kg^{-1}. The number of aliphatic and aromatic hydrocarbon compounds detected were fourteen (Table 2) and three (Table 3) respectively. Dodecane had highest concentration of 42.49 mg kg^{-1} among the aliphatic compounds detected while highest concentration of 0.97 mg kg^{-1} was observed in Fluoranthene of polycyclic aromatic hydrocarbon.

The pig dung contained total hydrocarbon-degrading bacteria of 8.0×10^3 CFU g^{-1} while the total petroleum hydrocarbon was below detection limit (Table 4).

Effect of pig dung application on the soil chemical properties

Pig Dung (PD) application at 50 and 100 g significantly ($p < 0.05$) increased the soil

TABLE 1
Soil chemical properties before and after contamination with petroleum products

Parameters	Before contamination	After contamination
pH	6.9±0.15	6.2±1.15
Nitrogen (g kg^{-1})	1.92±0.12	1.87±0.01
Available phosphorus (mg kg^{-1})	15.65±2.23	10.35±1.14
Exchangeable potassium (cmol kg^{-1})	0.26±0.20	0.24±0.02
THDB (cfu g^{-1})	2.98 X 10^3±1.15 x 10^3	2.09 X 10^4±1.01 x 10^3
TPH (mg kg^{-1})	BDL	83.55±0.22

Values are mean ± standard deviation

THDB = Total Hydrocarbon Degrading Bacteria

TPH = Total Petroleum Hydrocarbon

BDL = Below Detection Limit

TABLE 2
Mean concentration of aliphatic hydrocarbon compound in the contaminated soil

Compounds	**Concentration (mg kg^{-1})**
Nonane	0.042
Decane	0.790
Undecane	1.700
Dodecane	42.490
Tridecane	18.420
Tetradecane	12.958
Pentadecane	0.692
Hexadecane	1.664
Heptadecane	0.014
Pentadecane 2, 6, 10, 14 …	BDL
Octadecane	1.330
Nonadecane	0.038
Eicosane	0.038
Heneicosane	0.582
Docosane	1.290
Tricosane	BDL
Tetracosane	BDL
Pentacosane	BDL
Hexacosane	BDL
Heptacosane	BDL
Triacontane	BDL
Heneicosane	BDL

BDL = Below Detection Limit

TABLE 3
Mean concentration of polycyclic aromatic hydrocarbon compound in the contaminated soil

Compounds	**Concentration (mg kg^{-1})**
Napthalane	0.020
Biphenylene	0.578
Acenaphthene	BDL
Fluorene	BDL
Phenanthrene	BDL
5H-Indeno[1, 2 – b] pyridine	BDL
Fluoranthene	0.970
Pyrene	BDL
Triphenylene	BDL
Benz [e] acephenanthrylene	BDL
Benzo [k] fluoranthene	BDL

BDL = Below Detection Limit

pH compared to 0 (control) at 21 and 42 days (Table 5). Soil pH at 50 g PD was higher than control by 9 and 6 % at 21 and 42 days respectively while percentage increase of 9 and 7.5 % at 21 and 42 days respectively when compared 100 g PD with control. Highest pH value of 7.3±0.2 was observed at 50 and 100 g PD kg^{-1} soil at 21 days; and the soil pH decreased from 21 to 42 days. The N, P and K contents in the soil decreased from 21 to

TABLE 4
Proximate analysis of the pig dung

Parameter	**Concentration**
pH	7.9±0.10
Nitrogen (g kg^{-1})	16.4±0.03
Phosphorus (mg kg^{-1})	1.25±1.51
Potassium (cmol kg^{-1})	0.28±0.08
Moisture Content (%)	92.2±3.24
THDB (cfu g^{-1})	8.0 X 10^3±1.11 x 10^3
TPH (mg kg^{-1})	BDL

Values are mean ± standard deviation

THDB = Total Hydrocarbon Degrading Bacteria

TPH = Total petroleum hydrocarbon

BDL = Below Detection Limit

TABLE 5
Effects of pig dung application on the soil chemical properties

Pig dung level (g)	Days after amendment	pH	Nitrogen (g kg^{-1})	Phosphorus (mg kg^{-1})	Potassium (cmol kg^{-1})
0	21	6.7 ± 0.15^{b}	1.51 ± 0.33^{bc}	112.22 ± 2.23^{e}	0.22 ± 0.03^{c}
	42	6.7 ± 0.20^{b}	1.11 ± 0.07^{cd}	102.49 ± 3.96^{f}	0.20 ± 0.02^{c}
50	21	7.3 ± 0.20^{a}	1.37 ± 0.11bcd	146.59 ± 1.57^{c}	0.74 ± 0.02^{b}
	42	7.1 ± 0.06^{a}	1.07 ± 0.10d	124.60 ± 4.51^{d}	0.46 ± 0.37^{c}
100	21	7.3 ± 0.20^{a}	2.72 ± 0.18^{a}	176.51 ± 4.12^{a}	1.35 ± 0.10^{a}
	42	7.2 ± 0.15^{a}	1.70 ± 0.43^{b}	156.80 ± 4.82^{b}	1.10 ± 0.05^{a}

Values are mean ± SD of three replicates.

Different superscript in the same column indicate significant difference at $p < 0.05$ (DMRT)

TABLE 6
Total hydrocarbon degrading bacteria of the contaminated soil amended with pig dung

Pig dung level	Days after amendment	THDB (cfu g^{-1})
0	21	$1.67 \times 10^4 \pm 1.15 \times 103^{bc}$
	24	$1.33 \times 10^4 \pm 1.04 \times 103^{c}$
50	21	$1.96 \times 10^4 \pm 1.46 \times 103^{ab}$
	24	$1.62 \times 10^4 \pm 7.11 \times 103^{bc}$
100	21	$2.50 \times 10^4 \pm 2.12 \times 103^{a}$
	24	$1.70 \times 10^4 \pm 1.91 \times 103^{bc}$

Values are mean ± SD of three replicates. Different superscript in the same column indicate significant difference at $p < 0.05$ (DMRT)

42 days at every PD level. Significantly ($p < 0.05$) higher N, P and K of 2.72 g kg^{-1}, 176.51 mg kg^{-1} and 1.35 cmol kg^{-1} respectively were observed in 100 g PD kg^{-1} soil at 21 days.

Effect of pig dung on the hydrocarbon degrading bacteria counts and identification

Total Hydrocarbon Degrading Bacteria (THDB) decreased from 21 to 42 days in 0 (control), 50 and 100 g PD kg^{-1} soil (Table 6). The THDB were found to be higher in soil amended with PD than the control soil. Significantly ($p < 0.05$) higher THDB was observed in the soil amended with 50 and 100 g PD at 21 days. The THDB in soil amended with 100 g PD was 27.55% higher the THDB in soil amended with 50g PD.

The size of the bacteria ranged between

1 – 5 mm; and most of the bacteria were irregular in shape, grey-white in colour, wet consistency, smooth edges, flat elevation and opaque opacity (Table 7). The kinds and relative abundance of microbial communities in microcosms due to natural attenuation and biostimulation treatment methods in the contaminated soil was presented in Table 8. Four hydrocarbon utilizing bacteria were identified from the contaminated soil. The hydrocarbon degrading bacteria identified belong to the genera Bacillus, Staphylococcus, Escherichia and Klebsiella. Bacillus species were the most predominant isolated bacterial species.

Biodegradation and kinetics of total petroleum hydrocarbon in the contaminated soil

Among the fourteen aliphatic compounds detected, Heptadecane concentration was below detection limit at 100 g pig dung kg^{-1} soil at 21 and 42 days after amendment (Table 9). Pig dung application had no effect on the degradation of Nonadecane and Eicosane. Highest percentage degradation was observed in all the compounds at 100 g pig dung kg^{-1} soil 42 days after amendment except Pentadecane (at 50 g pig dung kg^{-1} soil, 42 days after amendment). However, Table 10 shows effect of pig dung application on the polycyclic aromatic hydrocarbon compounds detected in

TABLE 7
Morphological characteristics of bacteria isolated from the contaminated soil

Isolated code	Size (mm)	Shape	Colour	Consistency	Edges	Elevation	Opacity
0 g pig dung	2-3	Round	White	Wet	Smooth	Flat	Opaque
	2-3	Round	White	wet/mucoid	Smooth	Raised	Opaque
	3-5	Irregular	Grey-white	Dry	Rough	Flat	Opaque
50 g pig dung	3-5	Irregular	Grey-white	Dry	Rough	Flat	Opaque
	1-2	Smooth	Yellow	Wet	Smooth	Slightly raised	Opaque
	3-5	Irregular	Grey-white	Dry	Rough	Flat	Opaque
100 g pig dung	2-3	Round	White	Wet	Smooth	Flat	Opaque
	1-2	Smooth	Yellow	Wet	Smooth	Slightly raised	Opaque
	3-5	Irregular	Grey-white	Dry	Rough	Flat	Opaque

TABLE 8
Types and relative abundance of microorganism in the contaminated soil

Isolate Code	GR	SP	CP	CA	CO	MO	IN	OX	CI	UR	MR	VP	G	L	M	Probable organism
0 g PD	GNB	-	-	+	-	+	+	-	-	-	+	-	A	A	-	*Escherichia coli*
	GNB	-	-	+	-	-	-	-	+	+	-	+	A	A	-	*Klebsiella sp.*
	GPB	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*
50 g PD	GPB	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*
	GPC	-	-	+	+	-	-	-	-	-	-	+	A	A	A	*Staph. aureus*
	GPB	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*
100 g PD	GNB	-	-	+	-	+	+	-	-	-	+	-	A	A	-	*Escherichia coli*
	GPC	-	-	+	+	-	-	-	-	-	-	+	A	A	A	*Staph. aureus*
	GPB	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*

Keys : GR-Gram Staining, SP- Spore Staining, CA- Capsule Staining, CT- Catalase, MO-Motility, IN- Indole, OX- Oxidase, CI- Citrate, IN- Indole, UR- Urea, MR- Methyl-red, VP- Vogesproskeur, G- Glucose, L- lactose, S- Sucrose, M- Mannitol, A-Acid production, PD = Pig Dung, - = Absent, + = Present, A = Abundant

TABLE 9
Effect of pig dung on the concentrations of aliphatic hydrocarbon compounds in the contaminated soil

Compounds	Pig dung (g kg^{-1})	Time (DAA)	Concentration (mg kg^{-1})	Concentration Degraded (mg kg^{-1})	Degradation (%)
Nonane	0	21	0.040	0.002	4.76
		42	0.020	0.022	52.38
	50	21	0.036	0.006	14.29
		42	0.020	0.022	52.38
	100	21	0.020	0.022	52.30
		42	0.002	0.040	95.24
Decane	0	21	0.644	0.146	18.48
		42	0.506	0.284	35.95
	50	21	0.578	0.212	26.84
		42	0.336	0.454	57.47
	100	21	0.442	0.348	44.05
		42	0.166	0.624	78.99
Undecane	0	21	1.762	0.028	1.65
		42	1.366	0.334	19.65
	50	21	1.592	0.108	6.35
		42	1.122	0.578	34.00
	100	21	1.484	0.216	12.71
		42	0.690	1.010	59.41
Dodecane	0	21	35.804	6.686	15.74
		42	27.878	14.612	34.39
	50	21	26.690	15.800	37.19
		42	19.642	22.848	53.77
	100	21	23.308	19.182	45.14
		42	10.698	31.792	74.82
Tridecane	0	21	12.244	6.176	33.53
		42	9.880	8.540	46.36
	50	21	10.066	8.354	45.35
		42	7.112	11.308	61.39
	100	21	5.932	12.488	67.80
		42	2.504	15.916	86.41
Tetradecane	0	21	7.184	5.774	44.56
		42	6.072	6.886	53.14
	50	21	6.076	6.882	53.11
		42	4.078	8.88	68.53
	100	21	4.310	8.648	66.74
		42	1.634	11.324	87.39
Pentadecane	0	21	0.468	0.224	32.37
		42	0.314	0.378	54.62
	50	21	0.376	0.316	45.66
		42	0.102	0.59	85.26
	100	21	0.454	0.238	34.39
		42	0.108	0.584	84.39

TABLE 9 *Continued*

Compounds	Pig dung (g kg^{-1})	Time (DAA)	Concentration (mg kg^{-1})	Concentration Degraded (mg kg^{-1})	Degradation (%)
Hexadecane	0	21	0.920	0.744	44.71
		42	0.658	1.006	60.46
	50	21	0.552	1.112	66.83
		42	0.292	1.372	82.45
	100	21	0.168	1.496	89.90
		42	0.010	1.654	99.40
Heptadecane	0	21	0.014	0.000	0.00
		42	0.012	0.002	14.29
	50	21	0.010	0.004	28.57
		42	0.002	0.012	85.71
	100	21	BDL	BDL	BDL
		42	BDL	BDL	BDL
Octadecane	0	21	1.328	0.002	15.04
		42	0.682	0.648	48.72
	50	21	1.148	0.182	13.68
		42	0.328	1.002	75.34
	100	21	1.108	0.222	16.69
		42	0.038	1.292	97.14
Nonadecane	0	21	0.038	0.000	0.00
		42	0.038	0.000	0.00
	50	21	0.038	0.000	0.00
		42	0.038	0.000	0.00
	100	21	0.038	0.000	0.00
		42	0.038	0.000	0.00
Eicosane	0	21	0.038	0.000	0.00
		42	0.038	0.000	0.00
	50	21	0.038	0.000	0.00
		42	0.038	0.000	0.00
	100	21	0.038	0.000	0.00
		42	0.038	0.000	0.00
Heneicosane	0	21	0.552	0.030	5.15
		42	0.522	0.060	10.31
	50	21	0.508	0.074	12.71
		42	0.240	0.342	58.76
	100	21	0.478	0.104	17.87
		42	0.152	0.430	73.88
Docosane	0	21	1.234	0.056	4.34
		42	0.862	0.428	33.18
	50	21	1.106	0.184	14.26
		42	0.284	1.006	77.98
	100	21	1.010	0.280	21.71
		42	0.152	1.138	88.22

BDL = Below Detection Limit, DAA = Days After Amendment

the contaminated soil. There was no difference in the Napthalane concentration at 0 g pig dung kg^{-1} soil (control) 21 days after amendment compared to the initial concentration of 0.02 mg kg^{-1} in Table 2. Highest degradation (%) of 60, 92.39 and 49.28 were observed in Napthalane, Biphenylene and Fluoranthene respectively at 100 g pig dung kg^{-1} soil 42 days after amendment (Table 10)

Significantly ($p < 0.05$) higher concentration of Total Petroleum Hydrocarbon (TPH) was observed in 0 g PD kg^{-1} soil (control) at 21 days while the least significant ($p < 0.05$) value was observed in 100 g PD kg^{-1} soil at 42 days (Table 11). At 42 days after the amendment, highest TPH reduction of 66.66 mg kg^{-1}

TABLE 10
Polycyclic aromatic hydrocarbon components of the contaminated soil

Compounds	Pig dung (g kg^{-1})	Time (DAA)	Concentration (mg kg^{-1})	Concentration Degraded (mg kg^{-1})	Degradation (%)
Napthalane	0	21	0.020	0.000	0.00
		42	0.014	0.006	30.00
	50	21	0.014	0.006	30.00
		42	0.010	0.010	50.00
	100	21	0.012	0.008	40.00
		42	0.008	0.012	60.00
Biphenylene	0	21	0.468	0.110	19.03
		42	0.314	0.264	45.67
	50	21	0.376	0.202	34.95
		42	0.102	0.476	82.35
	100	21	0.074	0.504	87.20
		42	0.044	0.534	92.39
Fluoranthene	0	21	0.912	0.058	5.980
		42	0.872	0.098	10.10
	50	21	0.818	0.152	15.67
		42	0.732	0.238	24.54
	100	21	0.770	0.200	20.62
		42	0.492	0.478	49.28

DAA = Days After Amendment

TABLE 11
Rate of change of TPH during the biodegradation of the contaminated soil

Pig dung level (g kg-1)	Time (days)	TPH (mg kg -1)	In TPH	TPH Degraded (mg kg -1)	Degradation (%)
0	21	63.24±0.25a	4.1470	20.31	24.31
	42	49.72±1.30c	3.9064	33.83	40.50
50	21	50.09±0.64b	3.9138	33.46	40.05
	42	34.51±0.56e	3.5412	49.04	58.70
100	21	39.56±0.15d	3.6778	43.99	52.65
	42	16.89±0.36f	2.8267	66.66	79.78

Values are mean ± SD of three replicates. Different superscript in the same column indicate significant difference at $p < 0.05$ (DMRT)

(79.78 %) was observed in 100 g PD kg^{-1} soil followed by 49.04 (58.70 %) in 50 g PD kg^{-1} soil (Table 11) from an initial concentration of 83.55 mg kg^{-1} (Table 1).

Concentration of TPH in the soil and their natural logarithm were plotted against time as shown in Figures 1 and 2 in order to analyze the kinetics for the biodegradation process. The biodegradation process followed first order kinetics since a plot of TPH concentration in soil against time had an exponential curve and ln of TPH concentration against time was linear. Rate constant was found to be 0.038 day^{-1} for soil amended with 100 g PD kg-1 soil and 0.021 day^{-1} for soil amended with 50 g PD kg^{-1} soil. Correlation analysis (r) for the petroleum products biodegradation kinetics process was 0.998 for soil amended with 100 g PD kg^{-1}

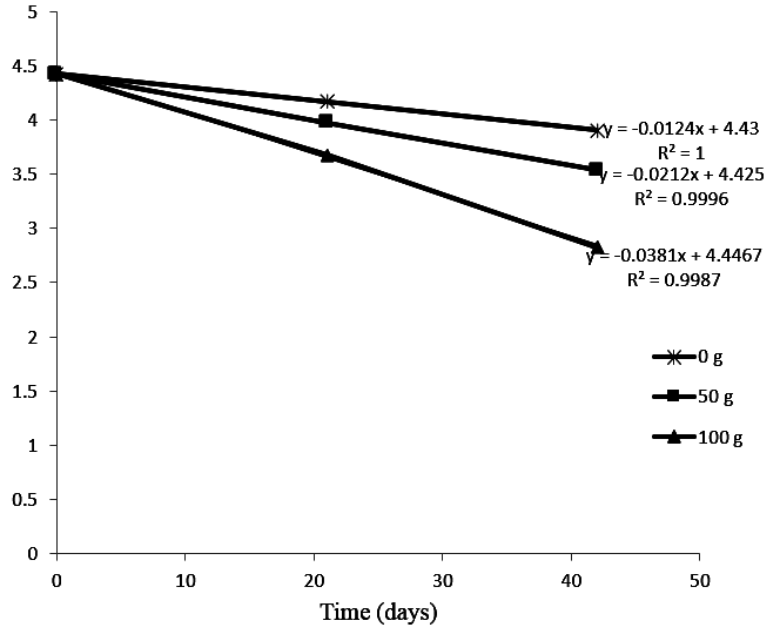

Fig. 1 ln TPH against time for biodegradation of the contaminated soil amended with 0, 50 and 100 g PD kg^{-1} soil

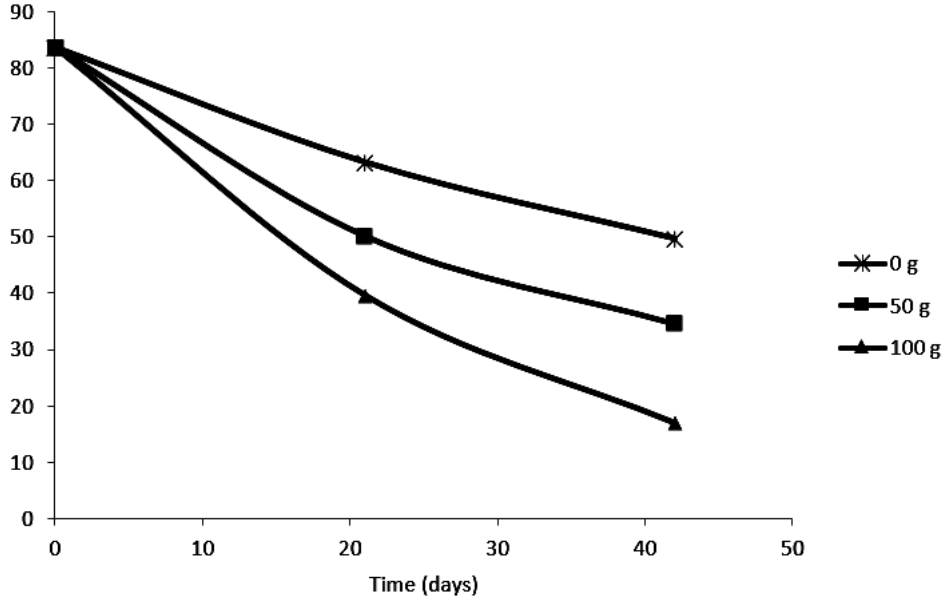

Fig. 2 First order profile for biodegradation of the contaminated soil amended with 0, 50 and 100 g PD kg^{-1} soil

soil and 0.999 for soil amended with 50 g PD kg^{-1} soil, indicating linearity and positive correlations for the decrease in concentration as a function of time. It was observed from the result that the rate of biodegradation within the amendment levels vary significantly ($p = 0.05$) at each sampling time.

Discussions

The soil nutrient decreased after contamination with petroleum products. Akpoveta *et al*. (2011) observed significant reduction in pH and available nutrients in soil on simulation of soil with kerosene. Increase in soil acidity as a result of petroleum hydrocarbon in soil is a concern for agricultural soil because low pH values are associated with adverse soil condition which includes reduced availability of plant nutrients, reduced microbial activity and increased availability and toxicity of heavy metals (Akpoveta *et al*., 2011).

Application of pig dung significantly ($p < 0.05$) increased pH of the contaminated soil compared to the control at 21 and 42 days. Soil pH range between 6.9 and 7.5 is good for most hydrocarbon utilizing bacteria (Vidali, 2001; Yakubu, 2007). The soil pH decreased from 21 to 42 days. The gradual decrease in pH as biodegradation progressed was not unconnected to the biodegradation process which removed the contaminant and introduced some salts and ions from pig dung (Akpoveta *et al*., 2011). Soil N, P and K contents decreased from 21 to 42 days at every pig dung level. The decrease might be due to their high demand by microorganisms for sugar phosphorylation, nucleic acid synthesis and other cellular processes (Andrew & Jackson, 1996). It has also been reported that petroleum hydrocarbon contaminants could destroy inorganic nutrient sources by reacting with them along with other substances present in soil (Teal *et al*., 1992; Andrew & Jackson, 1996).

Total Hydrocarbon Degrading Bacteria (THDB) decreased from 21 to 42 days in 0 (control), 50 and 100 g PD kg^{-1} soil. The reduction in population of THDB might be due to the fact that the microorganisms had exhausted the available nutrient supplies present in the system. Microorganisms generally require mineral nutrients sources for growth (Andrew & Jackson, 1996). If any of the required nutrients is lacking or becomes limiting, particularly the macro-mineral elements, microbial population will decrease (Giordani *et al*., 1998; Lehtola *et al*., 1998; Vidali, 2001). Akpoveta *et al*. (2011) also reported a decline in bacterial population as the biodegradation progressed.

The hydrocarbon degrading bacteria identified belong to the genera *Bacillus*, *Staphylococcus*, *Escherichia* and *Klebsiella*. *Bacillus* species were the most predominant isolated bacterial species, its prevalence could be attributed to the fact that it forms spores, which help microorganisms to withstand harsh conditions. Isolation of *Bacillus* species from hydrocarbon contaminated soil amended with pig dung could also be attributed to its ubiquitous distribution in nature. Mansour *et al*. (1999) reported the isolation of *Bacillus*, *Acinetobacter*, *Staphylococcus* and *Enterobacter* among other bacteria from hydrocarbon contaminated soil. The oil-utilizing bacteria isolated from this study have previously been implicated in hydrocarbon biodegradation, though from different sources (Ijah & Antai, 2003; Yakubu, 2007).

Percentage degradation was higher in the

aliphatic compound compared to aromatic compound. This might be due to the ring of atoms in aromatic compound. Degradation of total petroleum hydrocarbon in the contaminated soil amended with pig dung might be due to the bacterial consortium in the pig dung that attacked and degraded the components of the hydrocarbon (Yakubu, 2007; Adesodun & Mbagwu, 2008).

Significantly ($p < 0.05$) higher concentration of Total Petroleum Hydrocarbon (TPH) was observed in the soil without pig dung applications while the least significant ($p < 0.05$) value was observed in 100 g pig dung kg^{-1} soil. Biostimulation has been reported as an important factor to enhance soil bioremediation (Cardona & Iturbe, 2003; Gallego *et al*., 2010). Gallego *et al*. (2010) evaluated in situ bioremediation techniques and reported that it is possible to degrade up 90 % of hydrocarbon pollutant, during biostimulation. The TPH concentrations decreased from 21 to 42 days. It could be that mineral elements in pig dung contributed to the enhanced biodegradation. The degradation process followed first order kinetics because as concentrations of the contaminant in the soil continue to decrease with time, the microbial population also decreased (Peijun *et al*., 2004; Akpoveta *et al*., 2011).

Conclusion

This study determined effect of pig dung on the degradation of total petroleum hydrocarbon in petroleum products-contaminated soil. After 42 days of incubation, approximately 80 % of TPH removal was observed in microcosms with 100 g kg^{-1} of pig dung compared to only 40 % of TPH removal in microcosms without pig dung (Control). Pig dung increased phosphorus and potassium contents of petroleum products-contaminated soil. It also enhanced degradation of both aliphatic and aromatic hydrocarbons in the petroleum products-contaminated soil except Nonadecane and Eicosane. Therefore, pig dung, which is a waste from piggery, can be utilized effectively to reclaim soil contaminated with petroleum products.

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