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Biodegradation of Gasoline Polluted Soil Using Goat Dung

***[1]EGBEJA, TI; [2]OGUCHE, JU; [2]BASHIR, AA**

[1]Department of Animal and Environmental Biology, [2]Department of Plant Science and Biotechnology, Faculty of Natural Sciences, Kogi State University, P.M.B 1008, Anyigba, Kogi State, Nigeria
**Corresponding Author Email: Egbejati@ksu.edu.ng; Tel: +2348160373127*

ABSTRACT Petroleum product leakages from underground storage tanks, distribution facilities and various industrial operations represent an important source of soil and aquifer contamination. This study was carried out to determine the effects of Goat Dung (GD) on Polycyclic Aromatic Hydrocarbon (PAH) degradation and microbiological composition. Top soil (0-15 cm depth) was collected from Nigerian National Petroleum Corporation Satellite Depot, Ejigbo, Lagos State. One kilogram of the gasoline polluted soil was measured into nine containers. The GD 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 PAH determination using standard methods. Collected data were subjected to descriptive and inferential statistics. The HUB species identified were *Bacillus, Staphylococcus, Klebsiella, Escherichia, Pseudomonas and Enterobacter*. The PAH (mg kg^{-1}) of the soil before GD application was 192.65. After the amendments at 0, 50 and 100 g kg^{-1}, this value reduced to 167.32±2.45, 107.11 ±1.88 and 75.10±3.65, respectively at 21 days and 134.26±1.59, 74.16 ±2.27 and 46.14.14±1.93, respectively at 42 days. Biodegradation efficiency of 76 % was recorded after 42 days in soil amended with 100 g kg^{-1} of GD. Application of 100 g kg^{-1} of GD was more effective in the remediation of PAH contaminated soil. Results demonstrated that GD could be used to enhance activities of the microbial hydrocarbon-degrading bacteria during bioremediation of gasoline polluted soil.

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Petroleum product leakages from underground storage tanks, distribution facilities and various industrial operations represent an important source of soil and aquifer contamination. Most of these petroleum products are complex mixture of normal, branched and cyclic alkanes, and aromatic compounds obtained from the middle–distillate fraction during petroleum separation (Gallego *et al.*, 2001). Among several clean–up techniques available to remove petroleum hydrocarbons from the soil, biodegradation processes are gaining ground due to their simplicity, higher efficiency and cost–effectiveness when compared to other technologies like mechanical, burying, evaporation, dispersion, and soil washing (Alexander, 1994). Biodegradation processes rely on the natural ability of microorganisms to carry out the mineralization of organic chemicals, leading ultimately to the formation of carbon dioxide, water and biomass (Akpoveta *et al*., 2011). Amendment of soil with organic or inorganic nitrogen-rich nutrients in a process known as biostimulation is an effective strategy to enhance the biodegradation process (Margesin *et al*., 2007). The potential use of organic wastes derived from plants and animals have been investigated by few researchers. Such wastes include rice husk and coconut shell (Nyankanga *et al*., 2012), plantain peels and cocoa pod husk (Agbor *et al*., 2012), *Moringa oleifera* and soya beans (Danjuma *et al*., 2012) and animal organic wastes like cow dung, pig dung, poultry manure and goat dung (Yakubu, 2007; Adesodun and Mbagwu, 2008; Agarry *et al*., 2010; Agarry and Ogunleye, 2012) as biostimulation strategies for petroleum hydrocarbon biodegradation in polluted environments. However, cost effective methods and environmentally friendly strategies of enhancing petroleum hydrocarbon biodegradation in soil necessitated this study.

MATERIALS AND METHODS

Samples Collection, Preparation and Experimental Design: Goat dung was collected from Goat 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.

Top soil (0-15 cm depth) was collected from Nigerian National Petroleum Corporation Satellite Depot,

Ejigbo, Lagos State using a soil auger. 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 measured into nine clean dry containers of three litres each.

Goat dung was applied at the rate of 0 (control), 50 and 100 g kg^{-1} soil in triplicate. The goat dung was thoroughly mixed with the soil and the nine containers were arranged in a Completely Randomized Design in a greenhouse. Soil samples were taken from each container at 21 and 42 days for pH, organic carbon, nitrogen, phosphorus, potassium, hydrocarbon degrading bacteria count, hydrocarbon utilizing bacteria and polycyclic aromatic hydrocarbon determination.

Soil Chemical Properties: The pH, organic carbon, total nitrogen, potassium and available phosphorus were determined in the soil samples using the methods described by Chopra and Kanwar (2011).

Cultural Characterization of Bacteria: Pure cultures of representative bacteria colonies were randomly picked from inoculated plates and were grouped on the basis of their colonial characteristics such as colony elevation, colour, size, opacity, shape, consistency, and edge (Barnett and Hunter, 1985).

Morphological Characterization of Bacteria: Cultural grouping was followed by microscopic examination of isolates for cellular morphology. Day-old cultures of the bacteria isolates were stained with cotton blue lacto-phenol blue and observed microscopically for cell shape, size and sporulation (Barnett and Hunter, 1985).

Biochemical Characterization of Bacteria: 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 carried out on mineral salt medium (MSM) agar as described by Balogun and Fagade (2010); and the isolated microorganisms were identified using Bergey's manual of systemic bacteriology (Krieg and Holt, 1984).

Determination of Polycyclic Aromatic 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. The procedure was repeated twice and the aliquots were 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.

The degradation of petroleum products was expressed as the percentage of petroleum products degraded in relation to the amount of the remaining fractions in the appropriate abiotic control samples (Equation 1). The biodegradation efficiency (BE) based on the decrease in the total concentration of hydrocarbons, was calculated using Equation 1 (Mohan *et al*., 2006).

$$BE = 100 - \left(\frac{As \times 100}{Aac}\right) \ldots\ldots\ldots\ldots 1$$

Where As = total area of peaks in each sample, Aac = total area of peaks in the appropriate abiotic control and BE (%) = biodegradation efficiency.

*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). Statistical Analysis System (SAS) software version 9.0 portable was used.

RESULTS AND DISCUSSION

Chemical Properties of Soils and Pig Dung: The soil pH, total nitrogen (N), available phosphorous (P), exchangeable potassium (K), Organic Carbon (OC) and Polycyclic Aromatic Hydrocarbon (PAH) before GD application were shown in Table 1.

Table 1: Chemical Properties of the Polluted Soil prior to Goat Dung Application

Parameters	Polluted soil
pH	6.70±0.11
Nitrogen (g kg^{-1})	1.21±0.21
Available phosphorus (mg kg^{-1})	29.25±2.59
Exchangeable potassium (Cmol kg^{-1})	0.31±0.11
Organic Carbon (g kg^{-1})	55.89±3.56
THDB (cfu g^{-1})	2.23 X 10^4±1.87.00 × 10^2
PAH (mg kg^{-1})	192.65±1.22

Values are means ± SD of three replicates.

The polycyclic aromatic hydrocarbons (PAH) compounds detected in the soil before goat dung application were sixteen (Table 2). The goat dung used in this study was high in organic carbon (47.86±2.37 g

kg^{-1}) and also contained total hydrocarbon-degrading bacteria of $7.35 \times 10^3 \pm 1.87 \times 10^3$ CFU g^{-1} while the polycyclic aromatic hydrocarbon compounds were below detection limit (Table 3).

Table 2: Concentration of Polycyclic Aromatic Hydrocarbon Compounds in the Polluted Soil before Amendment

S/N	Compounds	Concentration ($mgkg^{-1}$)
1	Naphthalene	1.58±0.42
2	Acenaphthylene	2.07±0.36
3	Acenaphthene	1.96±0.22
4	Fluorene	1.84±0.17
5	Phenanthrene	7.04±1.26
6	Anthracene	20.15±2.83
7	Fluoranthene	29.82±3.77
8	Pyrene	26.51±2.88
9	Benzo(a)anthracene	34.18±3.12
10	Benzo(b)fluoranthene	16.34±1.82
11	Chrysene	9.07±1.23
12	Benzo(k)fluoranthene	10.82±1.24
13	Benzo(a)pyrene	9.50±1.29
14	Indeno(1,2,3,cd)pyrene	12.87±1.36
15	Dibenz(a,h)anthracene	6.32±1.44
16	Benzo(g,h,I)perylene	4.56±0.66

Values are means ± SD of three replicates.

*Effect of Goat Dung Application on the Soil Chemical Properties***:** Application of goat dung significantly ($p < 0.05$) increased pH of the contaminated soil compared to the control (without goat dung application) at 21 and 42 days (Table 3). The pH of soil before goat dung application was 7.90±0.10 while control, 50 and 100 g of goat dung were 6.8 ± 0.06 and 6.7 ± 0.20; 7.3 ± 0.10 and 7.2± 0.06; 7.4 ± 0.15 and 7.2 ± 0.06 for day 21 and 42 respectively indicating a downward trend.

Table 3: Proximate Analysis of the Goat Dung

Parameters	Cow dung
pH	6.70±0.20
Nitrogen ($g\ kg^{-1}$)	17.27±1.20
Phosphorus ($mg\ kg^{-1}$)	1.10±0.11
Potassium ($Cmol\ kg^{-1}$)	0.22±0.11
Organic Carbon ($g\ kg^{-1}$)	47.86±2.37
THDB (CFU g^{-1})	$7.35 \times 10^3 \pm 1.87 \times 10^3$
PAH ($mg\ kg^{-1}$)	BDL

Values are means ± SD of three replicates

Total N of the soil ($g\ kg^{-1}$) before goat dung application was 1.92 while control, 50 and 100 g of goat dung were 0.69 ± 0.21 and 0.42 ± 0.31, 1.19 ± 0.10 and 0.86 ± 0.30, 2.11 ± 0.10 and 2.11 ± 0.10 for 21 and 42 days respectively indicating a downward trend for the experimental. Significantly ($p < 0.05$) lower N, P, K and organic carbon was observed in 50 and 100 g goat dung kg^{-1} soil at 21 and 42 days respectively.

Table 4: Effects of Goat Dung Amendment on the Soil Chemical Properties

Goat dung level (g)	DDA	pH	Nitrogen ($g\ kg^{-1}$)	Phosphorus ($mg\ kg^{-1}$)	Potassium ($Cmol\ kg^{-1}$)	Organic carbon ($g\ kg^{-1}$)
0	21	6.8 ± 0.1^{bb}	0.69 ± 0.21^{e}	60.11 ± 2.10^{e}	0.28 ± 0.06^{cc}	32.24 ± 0.59^{c}
	42	6.7 ± 0.1^{b}	0.42 ± 0.31^{f}	47.23 ± 2.14^{f}	0.15 ± 0.10^{c}	27.02 ± 1.22^{d}
50	21	7.2 ± 0.10^{aa}	1.19 ± 0.10^{c}	134.15 ± 4.11^{c}	1.11 ± 0.02^{bb}	40.28 ± 3.11^{b}
	42	7.1 ± 0.2^{a}	0.86 ± 0.30^{d}	109.22 ± 1.27^{d}	0.72 ± 0.23^{b}	32.36 ± 0.11^{cc}
100	21	7.2 ± 0.1^{aa}	2.11 ± 0.10^{a}	198.56 ± 5.33^{a}	1.13 ± 0.04^{a}	52.10 ± 3.76^{a}
	42	7.1 ± 0.2^{aa}	1.07 ± 0.05^{b}	145.12 ± 4.56^{b}	1.11 ± 0.05^{bb}	38.22 ± 2.14^{cc}

Values are means ± SD of three replicates. Different superscript in the same column indicate significant difference at $p < 0.05$ (DMRT), DDA-Days after amendment

Effect of Goat Dung on the Hydrocarbon Degrading Bacteria Counts and Identification: The values of total hydrocarbon degrading bacteria decreased from 21 to 42 days in 0 (control), 50 and 100 g goat dung kg^{-1} soil (Table 5). The total hydrocarbon degrading bacteria were found to be higher in soil amended with goat dung than the control soil. Morphological characteristics of bacteria isolated from the polluted soil amended with Goat dung at 42 days are presented in Table 6.

The size of the bacteria ranged between 1 – 5 mm. Most of the bacteria were irregular in shape, grey-white in colour, wet consistency, smooth edges, flat elevation and opaque.

The types and relative abundance of microbial communities in microcosms due to natural attenuation and biostimulation treatment methods observed in the contaminated soil are presented in Table 7. Six hydrocarbon degrading bacteria were identified from the polluted soil. The hydrocarbon degrading bacteria identified belong to the genera *Bacillus, Staphylococcus, Klebsiella, Escherichia, Pseudomonas and Enterobacter*. *Bacillus species* were the most predominantly isolated bacterial species.

Table 5: Total Hydrocarbon Degrading Bacteria Count of the Polluted Soil Amended with Goat Dung

Goat dung level	DDA	THDB (CFU g^{-1})
0	21	$1.10 \times 10^4 \pm 2.00 \times 10^{2d}$
	42	$0.97 \times 10^4 \pm 4.00 \times 10^{2e}$
50	21	$1.38 \times 10^4 \pm 2.00 \times 10^{2c}$
	42	$1.10 \times 10^4 \pm 3.00 \times 10^{2cd}$
100	21	$2.14 \times 10^4 \pm 5.00 \times 10^{2a}$
	42	$1.37 \times 10^4 \pm 3.00 \times 10^{2b}$

Values are means ± SD of three replicates. Different superscript in the same column indicate significant difference at $p < 0.05$ (DMRT); DDA-Days after amendment

Table 6: Morphological Characteristics of Bacteria Isolated from the Polluted Soil

Isolate Code	Size (mm)	Shape	Colour	Consistency	Edges	Elevation	Opacity
0 g GD	2-3	Irregular	Grey-white	Wet	Rough	Raised	Opaque
	3-4	Smooth	Yellow	Dry	Smooth	Slightly raised	Opaque
	3-4	Round	Grey-white	Wet	Rough	Flat	Opaque
50 g GD	3-5	Round	White	Wet	Smooth	Flat	Opaque
	1-2	Irregular	Green	Wet	Smooth	Raised	Opaque
	3-4	Round	Grey-white	Wet	Smooth	Raised	Opaque
100 g GD	1-2	Round	White	Dry	Rough	Flat	Opaque
	3-5	Smooth	Yellow	Wet	Smooth	Flat	Opaque
	3-4	Irregular	Grey-white	Wet	Smooth	Flat	Opaque

Table 7: Types and relative abundance of micro-organisms in the polluted soil

Isolate code	GR	SP	CP	CA	CO	MO	IN	OX	CI	UR	MR	VP	G	L	M	Probable organism
0 g GD	GPB	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*
	GPB	+	+	+	-	+	-	-	-	-	+	_	A	-	-	*Bacillus subtilis*
	GNB	-	-	+	-	+	-	-	+	-	+	-	A	A	-	*Enterobacter sp*
50 g GD	GNB	-	-	+	-	+	+	-	-	-	+	-	A	A	-	*Escherichia coli*
	GNB	-	-	+	-	+	-	+	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
	GNB	-	-	+	-	-	-	-	+	+	-	+	A	A	-	*Klebsiella sp.*
100 g GD	GPB	+	+	+	-	+	-	-	-	-	+	-	A	A	-	*Bacillus Subtilis*
	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, OX- Oxidase, CI- citrate, UR- Urea, MR- Methyl-red, VP- Vogesproskeur, G- Glucose, L- lactose, S- Sucrose, M- Mannitol, A-Acid production, CD = cow dung, g = gram, - =Absent, + = Present, A = Abundant

Table 8: Rate of Change of Polycyclic Aromatic Hydrocarbon during Biodegradation of the Polluted oil

Goat dung level (g)	DAA	PAH (mg kg $^{-1}$)	PAH Degraded (mg kg $^{-1}$)	Degradation (%)
0	21	167.32±1.71^{a}	25.33	13.15
	42	134.26±1.59^{b}	58.39	30.31
50	21	107.11 ±1.88^{c}	85.54	44.40
	42	74.16 ±2.27^{d}	118.49	61.50
100	21	75.10±3.65^{e}	117.55	61.01
	42	46.14±1.93^{f}	146.51	76.05

Values are means ± SD of three replicates. Different superscript in the same column indicate significant difference at p < 0.05 (DMRT); DDA-Days after amendment

*Effects of Goat Dung on Polycyclic Aromatic Hydrocarbon in the polluted Soil***:** At 42nd day after goat dung amendment, 146.51mg kg^{-1} (76.05 %) reduction in polycyclic aromatic hydrocarbon was observed at 100 g kg^{-1}of GD while 118.49mg kg^{-1} (61.50 %) reduction in polycyclic aromatic hydrocarbon was observed at 50 g kg^{-1}of GD from an initial concentration of 192.65 mg kg^{-1}. The polycyclic aromatic hydrocarbon concentrations decreased from 21 to 42 days (Table 8).

Biodegradation increased significantly ($p < 0.05$) within the first three weeks with 117.55mg kg^{-1}(61.01 %) reduction in polycyclic aromatic hydrocarbon at 100 gkg^{-1} of GD and 85.54mg kg^{-1} (44.40 %) reduction in polycyclic aromatic hydrocarbon at 50 gkg^{-1} of GD (Table 8). In the control soil, 25.33 mg kg^{-1} (13.15 %) reduction in polycyclic aromatic hydrocarbon was observed in the first three weeks while 58.39mg kg^{-1}(30.31 %) reduction was observed on the sixth week (forty second day). The polycyclic aromatic hydrocarbon concentrations decreased from 21 to 42 days. This study was carried out to determine the effects of Goat Dung (GD) on Polycyclic Aromatic Hydrocarbon (PAH) degradation and microbiological composition. There was significantly ($p < 0.05$) increase in pH on application of GD to the polluted soils compared to the control at 21 and 42 days. In an experiment conducted by Vidali and Yakubu (2001), they observed that a pH range between 6.9 and 7.5 is good for most hydrocarbon degrading bacteria. In this study, there was gradual decrease in pH as biodegradation progressed. This decrease was connected to the biodegradation process which removed the contaminant and introduced some salts and ions from goat dung (Akpoveta *et al*., 2011). The decrease in soil N, P, K and organic carbon content from 21 to 42 days at every goat dung level might be due to their high demand by microorganisms for sugar phosphorylation, nucleic acid synthesis and other cellular processes (Andrew and Jackson, 1996). It has 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 and Jackson, 1996). There was reduction in population of total hydrocarbon degrading bacteria from 21 to 42 days in

0 (control), 50 and 100 g cow dung kg^{-1} soil. This reduction in population of total hydrocarbon degrading bacteria was connected to the fact that mineralization of hydrocarbons could have possibly resulted in the production of toxic metabolites which on introduction into the system reduces the growth phase of the microbes (Akpoveta *et al*., 2011). Microorganisms generally require mineral nutrients sources for growth (Andrew and 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. In this study, *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 goat 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 degrading bacteria isolated from this study have previously been implicated in hydrocarbon biodegradation, though from different sources (Ijah and Antai, 2003; Yakubu, 2007).

Degradation of polycyclic aromatic hydrocarbon in the contaminated soil amended with goat dung might be due to the bacterial consortium in the goat dung that attacked and degraded the components of the hydrocarbon (Yakubu, 2007; Adesodun and Mbagwu 2008). Significantly ($p < 0.05$) higher concentration of polycyclic aromatic hydrocarbon was observed in the soil without goat dung amendment. Biostimulation has been reported as an important factor that enhance soil bioremediation (Cardona and Iturbe, 2003; Gallego *et al*., 2010). Egbeja *et al*. (2019) in their study of in situ bioremediation techniques reported that it is possible to degrade up to 99 % of hydrocarbon pollutant, during biostimulation.

*Conclusion***:** This study determined the effects of GD on Polycyclic Aromatic Hydrocarbon (PAH) degradation and microbiological composition. After 42 days of incubation, approximately 76 % of PAH removal was observed in microcosms with 100 g $kg^{-1}$of GD compared to only 30 % of PAH removal in microcosms without amendments. Results indicate that GD is effective in hydrocarbon biodegradation when conditions such as pH requirement and nutrient availability are taken into consideration.

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