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Efficiency of Plantain Peels and Guinea Corn Shaft for Bioremediation of Crude Oil Polluted Soil

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Abstract The efficiency of some agro-waste samples - plantain peels (PP) and guinea corn shaft (GCS) as amendments for the bioremediation of crude oil polluted soil was studied for 56 days. The PP and GCS samples were found to contain nutrient, heterotrophic and crude oil degrading bacteria. The heterotrophic bacterial types and counts were found to be higher than the crude oil utilizing bacterial types and counts with amended samples having the highest values. The crude oil utilizing bacterial counts for the amended and non-amended samples ranged from 3.35±0.01 x 10^5 cfu/g to 15.10±0.01 x 10^5 cfu/g and 2.30±0.01 x 10^5 cfu/g to 4.90±0.01 x 10^5 cfu/g respectively. The agro-waste samples (PP and GCS) showed a total nitrogen content ranging from 0.44% to 0.55%, the potassium content ranged from 5.88 to 6.13 Meq/ 100g while the phosphorus contents ranged from 0.28 to 0.43mg/kg. The carbon, nitrogen and phosphorus content of the soil used for the bioremediation studies were 1.83%, 0.09% and 9.64mg/kg respectively. There were also appreciable levels of other trace elements. The soil samples used for the study were composed of 81.6% clay, 16.4 % sand and 2% silt. The pH of the amended samples during the period of study ranged from 7.03 to 8.62. The total petroleum hydrocarbon (TPH) in the samples decreased from day zero to day 56. The reduction in total petroleum hydrocarbon was highest in GCS amended samples when compared with the PP amended samples and controls. These findings showed that agro-wastes (PP and GCS) significantly improved the rate of petroleum hydrocarbon biodegradation in polluted soil.

Keywords Biodegradation, crude oil, Agro-waste, Bacteria

1. Introduction

Fast growing industrialization and urbanization over the past many decades has resulted in contamination of all the components of the environment that is the air, the water, the soils and even our food. Pollution generally has been defined as an undesirable change in physical, chemical and biological characteristics of all the components of the environment [1]. The precipitous discharge of large amount of hydrocarbons poses stress to the environment, thereby disrupting the economic life of the populace. Oil spillage as it is referred to have deleterious impact on flora, fauna and microbiota of the ecosystem. The most commonly found environmental contaminants are petroleum hydrocarbon, though they are not usually classified as hazardous wastes [2]. According to [3], hydrocarbon contains substances that are toxic to the flora and fauna found in the ecosystem, becoming serious threat to the ecology. The processing, distribution and use of petroleum and petro-chemical products, has lead to soil and water contamination [4].

The use of naturally occurring organisms to clean up hydrocarbon polluted sites by transforming toxic and other undesirable materials into more benign or volatile substances is gaining favorable publicity [5]. Such microorganisms are have been reported by several authors [6-8]. Biodegradation of crude oil in the natural environment is apparently a slow process and the major factor responsible for this is the nutritional imbalance created by oil spills. The rates of petroleum hydrocarbon degradation are limited by various factors including lack of essential nutrients such as nitrogen. Therefore, the addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process [9, 5].

To boost the natural degradation process, sufficient nitrogen and phosphorus are needed to balance the available hydrocarbons and enhance the microbial population that are present in the polluted environment which eventually leads to an increase in microbial growth and hydrocarbon reduction [10]. According to [11], nitrogen and phosphorus are the most important limiting nutrients that have been used to support microbial growth in biostimulation processes. Positive effects of nitrogen amendment on microbial activity and petroleum hydrocarbon degradation have been widely

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demonstrated [13, 7, 14, 15, 16, 17, 8]. In most soil bioremediation studies, inorganic chemical fertilizers have been widely used as biostimulating agent, however, it is relatively scarce and costly as well as not sufficient for agriculture due to high demand, let alone for cleaning oil spills [7, 18]. Therefore, the search for cheaper and environmentally friendly options of enhancing petroleum hydrocarbon degradation through biostimulation has been the focus of research in recent times [7, 18, 19]. One of such option is the use of organic wastes derived from plant and animals. The potentials of these organic waste - rice husk and coconut shell [19], *Molinga oleifera* and soya beans [18] and animal organic wastes like cow dung, pig dung, poultry manure and goat dung [20, 21] as biostimulating agents in the cleanup of soil contaminated with petroleum hydrocarbons were investigated and found to show positive influence on petroleum hydrocarbon biodegradation in a polluted environment. Besides nutrients and other environmental factors, the genetic makeup of the organisms have been demonstrated to play a role in biodegradation processes [22, 23]. Nevertheless, the search for cost effective and environmentally friendly methods of enhancing petroleum hydrocarbon biodegradation in soil is still ongoing.

The aim of this study was to determine the efficiency of plantain peels and guinea corn shaft as stimulants for the biodegradation of crude oil in crude oil polluted soil. Plantain peels and guinea corn shafts are agro-waste (organic materials) readily available in the Niger Delta Region of Nigeria where crude oil pollution of the soil is a regular occurrence.

2. Materials and Methods

2.1. Sources/Collection of Samples

Nigerian crude oil (Bonny Light Oil) was collected from N.N.P.C Benin City, Nigeria. Plantain peels were obtained locally in Ekpoma from roasted plantain sellers and those that produce plantain flour while guinea corn shafts were equally obtained locally from those that process guinea corn into Ogi and other fermented food stuff from corn. Surface soil samples were collected from farmland near students' hostels in Ambrose Alli University, Ekpoma.

The modified mineral salt medium was used [24, 25]. Bacteriological agar (oxoid) was added to obtain a solid medium at a rate of 1.5 (%) when necessary. The general purpose media used included commercial preparations of oxoid nutrient agar, nutrient broth, MacConkey agar, peptone water, urease agar and citrate agar.

Media were sterilized by autoclaving at 121°C for 15 min. Crude oil used for biodegradation studies was filter - sterilized using sterile 0.22 µm pore size membrane (Type: MILLEX-GS Millipore Corporation, Bedford, MA01730 Rev. 9/94 12172). Glass wares were sterilized at 160°C for one hour using hot air oven.

2.2. Processing of Samples/experimental Set up

Plantain peels and guinea corn shaft (agro-wastes) used for this study were sun-dried for 5days. The wastes were milled into semi fine particles (using Corn Mill dx-2200, China) and sieved using 2mm mesh. Two kilograms (2kg) of soil samples each were introduced into nine (9) different plastic buckets (PB) labeled A to I. The plastic buckets A and B were polluted with 5% crude oil and amended with 100g each of plantain peels and guinea corn shaft respectively. C and D were polluted with 10% crude oil and amended with 100g each of plantain peels and guinea corn shaft respectively, while E and F were polluted with 15% crude oil and amended with 100g each of plantain peels and guinea corn shaft respectively. Plastic buckets G, H and I served as the controls for the experimental set up containing 2kg of soil sample polluted with 5%, 10% and 15% crude oil respectively without any amendment. Periodic sampling from each PB was carried out at 14days intervals for 56days. The samples were then analyzed for changes in pH, total petroleum hydrocarbon as well as for their bacterial types and numbers.

2.3. Determination of Total Heterotrophic and Total Hydrocarbon Utilizing Bacterial Numbers and Types

Bacterial Enumeration: The total heterotrophic bacterial counts of the samples were determined by making ten-fold serial dilution of the samples on normal saline (0.85% w/v) sterile NaCl. Then 1 mL of the appropriate dilution was pour plated in duplicates on the surface of the appropriate media. The plates were then incubated for 24-48 h at a temperature of 37°C. Thereafter emerging colonies were counted. Also, mineral salt agar medium was used for the enumeration of hydrocarbon utilizing bacterial species. Nigerian crude oil (Bonny Light Oil) collected from N.N.P.C Benin soaked in sterile 9 cm Whatman (No.1) filter paper and placed in dish cover served as carbon source. Thus the hydrocarbon was supplied to the inoculums by vapour-phase transfer. The media was made selective for bacteria by adding nizoral (100mg/L). After incubation at room temperature for 1-5days, emerging colonies were counted.

Bacterial Characterisation and Identification: The phenotypic and biochemical characteristics used to characterize and identify bacterial isolates included Gram staining, colonial appearance, motility, urease, catalase, indole, oxidase, citrate, methyl red, voges proskaeur and sugar fermentation. These tests were performed and emerging colonies identified using standard methods [26-28]

Determination of physicochemical properties of samples: Methods for the determination of physicochemical properties of samples (plantain peels guinea corn shaft crude oil polluted and non polluted soil) were used as outlined by APHA [29]. The pH meter used was pocket-sized HANA pHep + HI 98108 with automatic temperature compensation. Conductivity values were determined using conductivity meter (Jenway 4010 UK) and temperatures were measured

using standard mercury thermometer. Total organic carbon was determined by the modified dichromate wet oxidation method [30, 38]. Nitrate content was determined using the macro Kjeldahl digestion method [31] and available phosphorus, calcium and magnesium were determined using standard methods [31, 32]. Sulphate was determined using the turbidometric method, while oil and grease were determined by the partition gravimetric method. Sodium and potassium were determined using flame photometric method. The metal contents were determined using an atomic absorption spectrophotometer (AAS) (Perkin Elmer AA Unit Model: 3100 Serial Number: 148157)

2.4. Determination of Total Petroleum Hydrocarbon (TPH)

The TPH in the samples was determined as previously described [20]. Crude oil polluted soil sample (5g) was suspended in 25ml of hexane and shaken for 20 minutes using a mechanical shaker. The solution was filtered using a whatman (No.1) filter paper and the filtrate diluted by taking 1ml of the extract into 50ml of hexane. The absorbance of this solution was read at 460nm with HACH DR/2010 Spectrophotometer using n-hexane as blank. Total petroleum hydrocarbon was determined at weekly intervals for eight weeks. The actual TPH concentration (mg/kg) was deduced as follows;

$$\text{TPH} = \frac{\text{Instrument reading (Conc. obtained from calibration) x Volume of extract (mL)}}{\text{Weight of sample (kg)}} \text{ x DF}$$

Where TPH = Total petroleum hydrocarbon
DF = Dilution factor
Conc. = Concentration

2.5. Statistical Analysis

Results were analyzed using analysis of variance (ANOVA) and means were compared for significance at $p \leq 0.05$ using Duncan's multiple range analysis.

3. Results and Discussions

3.1. Bacterial Isolates and Counts

The distribution of heterotrophic and crude oil utilizing bacterial isolates from the various samples is summarized in Table 1. A total of 48 bacterial isolates from fourteen (14) different genera (5- Gram positive, 9- Gram negative) were obtained from the study. Of the 48 bacterial isolates, 30 were heterotrophic while 18 were crude oil utilizing. Non polluted soil sample had the highest number of isolates. The most common occurring bacterial isolate is *Bacillus subtilis* followed by *Bacillus cereus* and *Proteus vulgaris* while *Alcaligenes faecalis* had the least occurrence. The crude oil utilizing bacterial species isolated from the soil, plantain peels and guinea corn shaft in this study include *Bacillus subtilis, Klebsiella aerogenes, Staphylococcus epidermidis, Klebsiella pneumoniae, Actinomyces bovis, Aeromonas hydrophilia, Pseudomonas fluorescens, Pseudomonas aeruginosa, Corynebacterium* spp*, Escherichia coli, Acinetobacter calcoaccticus, Chryseomonas luteola, Bacillus cereus, Streptococcus faecalis, Proteus vulgaris* and *Serratia marcescens*. Similar organisms have been implicated in crude oil biodegradation by other authors [33, 12]. Also the presence of these different bacterial genera from these samples aligns with the widely documented fact that microbes are present in almost any ecological niche [6]. The higher occurrence of Gram negative over Gram positive bacteria in this study is similar to those earlier reported [34] that both Gram positive and Gram negative bacteria are encountered in the degradation of contaminants with Gram negative bacteria dominating. These findings also correlates the report of previous workers [35, 8] who isolated more of Gram negative organisms suggesting that they are better degraders of crude oil when compared with their Gram positive counterparts. The higher ability of Gram negative bacteria to utilize crude may not be unconnected with the possession of porins in their cell wall which helps in the uptake of certain substances by the cell or extrusion of others which may be harmful [36]. Plasmid – borne or chromosomal genes involved in hydrocarbon degradation in the bacterial species may also account for the ability of isolates to degrade crude oil as has been reported earlier [37, 22, 23].

The presence of crude oil utilizing bacteria in the amendments (Plantain peels and guinea corn shaft) suggested that they played the role of biostimulation and bioaugmentation in the biodegradation process.

Counts of heterotrophic bacteria (table 2) during the 56 days of study were higher in the amended samples than the non-amended (control) samples and the counts of heterotrophic bacteria in the amended and non-amended (control) samples ranged from $43.00\pm0.01 \times 10^6$ cfu/g to $264.00\pm0.03 \times 10^6$ cfu/g and $1.30\pm0.01 \times 10^6$ cfu/g to $63.00\pm0.00 \times 10^6$ cfu/g respectively. There was decrease in the heterotrophic counts for all samples on the second week of study. For the same period of study the crude oil utilizing bacterial (Table 3) were also higher in the amended samples than in the non-amended (control) samples and ranged from $3.35\pm0.01 \times 10^5$ cfu/g to $15.10\pm0.01 \times 10^5$ cfu/g and $2.30\pm0.01 \times 10^5$ cfu/g to $4.90\pm0.01 \times 10^5$ cfu/g respectively. The heterotrophic bacterial counts were found to be higher than the crude oil utilizing bacterial counts in all the samples. This could be as a result of nutrient limitation in the enumeration media for crude oil utilizers. The counts of crude oil utilizing bacteria in all the soil amended with agro-wastes were appreciably higher compared to those of non-amended control soil. The reason for higher counts of bacteria in amended soil might be as a result of presence of appreciable quantities of nitrogen and phosphorus in the agro-wastes, which are necessary nutrients for bacterial biodegradative activities. The agro-wastes may have also served as bulking agent which helped to loosen the compactness of the soil making sufficient aeration available for the indigenous bacteria present in the soil, thereby

enhancing their metabolic activities in the contaminated soil [39, 40, 41]. The highest crude oil utilizing bacterial count was recorded with 5% crude oil polluted soil sample amended with Guinea Corn Shaft (GCS). These justify the fact that higher concentration of the pollutant decreases the rate of biodegradation. These findings also showed that GCS had a better ability to neutralize the toxic effects of the oil on the microbial population by rapid improvement of the soil physicochemical properties. This is in line with earlier findings [36]. The initial decrease in the heterotrophic bacterial count could be as a result of adaptation to the polluted environment as well as the toxic effect of crude oil on the microbial population. This has been asserted earlier [42, 43].

3.2. Physicochemical Properties

The results of the physicochemical properties (Table 4) of the agro-wastes samples (plantain peels and guinea corn shaft) showed a total nitrogen content ranging from 0.44% to 0.55%, the potassium content ranged from 5.88 to 6.13 Meq/100g while the phosphorus contents ranged from 0.28 to 0.43mg/kg. The carbon, nitrogen and phosphorus content of the soil used for the bioremediation studies were 1.83%, 0.09% and 9.64mg/kg respectively. There were also appreciable levels of other trace elements. The soil samples used for the study were composed of 81.6% clay, 16.4 % sand and 2% silt. The pH of the amended samples during the period of study ranged from 7.03 to 8.62 as shown in Table 5. The low level of carbon, nitrogen and phosphorus (C, N and P) in the garden soil samples could have been caused by leaching or erosion. The presence of these limiting nutrients (C, N and P) in the agro-waste samples analysed in this study is in consonance with the earlier reports of [44, 45, 46, 16, 14, 17, 8]. They noted that the addition of these limiting nutrients is a key factor in achieving effective biodegradation of hydrocarbons. The pH range of the experimental samples (7.03 to 8.62) observed in this study is within the favourable range for biodegradation of crude oil in polluted soil. Similar observations have been documented [47, 15].

Table 1. Distribution of heterotrophic and hydrocarbon utilizing bacteria isolated from the samples

Samples	Bacterial Isolates	
	Heterotrophic	**Crude Oil Utilizing**
Plantain Peels	*Staphylococcus aureus* *Bacillus subtilis* *Bacillus cereus* *Proteus vulgaris* *Corynebacterium* spp *Klebsiella aerogenes* *Staphylococcus epidermidis*	*Bacillus subtilis,* *Klebsiella aerogenes,* *Staphylococcus epidermidis*
Guinea Corn Shaft	*Escherichia coli* *Streptococcus faecalis* *Proteus vulgaris* *Bacillus subtilis* *Klebsiella pneumoniae,* *Actinomyces bovis* *Aeromonas hydrophilia* *Bacillus cereus* *Staphylococcus saprophyticus*	*Bacillus subtilis,* *Klebsiella pneumoniae,* *Actinomyces bovis,* *Aeromonas hydrophilia*
Non-Polluted Soil Sample	*Bacillus cereus,* *Streptococcus faecalis,* *Proteus vulgaris,* *Staphylococcus saprophyticus,* *Staphylococcus aureus,* *Alcaligenes faecalis Serratia marcescens* *Pseudomonas fluorescens,* *Bacillus subtillis,* *Pseudomonas aeruginosa,* *Corynebacterium* spp *Escherichia coli,* *Acinetobacter calcoaccticus,* *Chryseomonas luteola,*	*Pseudomonas fluorescens,* *Bacillus subtillis,* *Pseudomonas aeruginosa,* *Corynebacterium* spp, *Escherichia coli,* *Acinetobacter calcoaccticus,* *Chryseomonas luteola,* *Bacillus cereus,* *Streptococcus faecalis,* *Proteus vulgaris* *Serratia marcescens*

Table 2. Viable Heterotrophic Bacteria Enumerated in 5%, 10%, and 15% Crude Oil Polluted Soil Amended with PP and GCS

	Sampling Time (Day)	Mean Bacterial Count (cfu/g ± SD) × 10^6		
		PP	GCS	Control
5% crude oil Polluted Soil	0	77.00±0.04^{g}	84.00±0.01^{h}	25.00±0.03^{b}
	14	63.00±0.01^{e}	75.00±0.00^{f}	1.90±0.01^{a}
	28	95.00±0.03^{i}	129.00±0.00^{j}	26.00±0.01^{c}
	42	134.00±0.01^{k}	196.00±0.01^{m}	45.00±0.01^{d}
	56	186.00±0.00^{l}	264.00±0.03^{n}	63.00±0.00^{e}
10% crude oil Polluted Soil	0	69.70±0.00^{i}	69.00±0.03^{h}	24.80±0.01^{c}
	14	54.00±0.03^{e}	57.00±0.01^{g}	1.60±0.01^{a}
	28	75.00±0.01^{j}	102.00±0.01^{k}	22.50±0.00^{b}
	42	116.00±0.03^{l}	146.00±0.00^{n}	39.50±0.01^{d}
	56	140.00±0.01^{m}	190.00±0.01^{o}	55.10±0.00^{f}
15% crude oil Polluted Soil	0	69.00±0.04^{j}	65.00±0.00^{h}	24.50±0.00^{c}
	14	43.00±0.01^{e}	55.00±0.04^{g}	1.30±0.01^{a}
	28	67.00±0.03^{i}	100.00±0.01^{l}	20.50±0.01^{b}
	42	91.00±0.01^{k}	137.00±0.02^{n}	33.30±0.01^{d}
	56	108.00±0.04^{m}	189.00±0.01^{o}	49.90±0.01^{f}

SD, standard deviation; cfu, colony forming unit; PP, plantain peel; GCS, guinea corn shaft; Control, soil + crude oil
For each microbial group, Rows and Columns with the same superscript are not significantly different ($p \leq 0.05$).

Table 3. Crude Oil Utilizing Bacteria Enumerated in 5%, 10%, and 15% Crude Oil Polluted Soil Amended with PP and GCS

	Sampling Time (Day)	Mean Oil Utilizing Bacterial Count (cfu/g ± SD) × 10^5		
		PP	GCS	Control
5% crude oil Polluted Soil	0	3.90±0.03^{e}	4.00±0.00^{f}	2.40±0.03^{a}
	14	5.70±0.01^{h}	6.10±0.03^{i}	2.60±0.01^{b}
	28	7.10±0.00^{j}	7.20±0.01^{k}	2.90±0.03^{c}
	42	9.20±0.04^{l}	9.50±0.03^{m}	3.70±0.00^{d}
	56	11.70±0.00^{n}	15.10±0.01^{o}	4.90±0.01^{g}
10% crude oil Polluted Soil	0	3.50±0.03^{e}	3.70±0.04^{f}	2.32±0.01^{a}
	14	3.70±0.00^{f}	3.90±0.01^{h}	2.52±0.00^{b}
	28	4.60±0.01^{i}	4.70±0.00^{j}	2.73±0.01^{c}
	42	6.50±0.03^{k}	6.70±0.03^{l}	2.95±0.00^{d}
	56	9.10±0.03^{m}	9.20±0.01^{n}	3.80±0.01^{g}
15% crude oil Polluted Soil	0	3.40±0.00^{g}	3.35±0.01^{f}	2.30±0.01^{a}
	14	3.62±0.01^{i}	3.52±0.00^{h}	2.40±0.03^{b}
	28	4.45±0.00^{k}	4.00±0.00^{j}	2.60±0.01^{c}
	42	6.20±0.01^{m}	5.50±0.03^{l}	2.74±0.01^{d}
	56	7.70±0.01^{o}	6.80±0.01^{n}	3.30±0.04^{e}

SD, standard deviation; cfu, colony forming unit; PP, plantain peel; GCS, guinea corn shaft; Control, soil + crude oil
For each microbial group, Rows and Columns with the same superscript are not significantly different ($p \leq 0.05$).

Table 4. Physiological Analysis of Soil Samples

Parameters		Non-Polluted soil sample	Polluted Soil Sample	Plantain Peels	Guinea Corn Shaft
Electrical Conductivity	(us/cm)	314	250	ND	ND
Organic Carbon	(%)	1.83	4.12	1.48	2.51
Organic matter	(%)	4.61	10.03	ND	ND
Total Nitrogen	(%)	0.09	0.149	0.44	0.55
Exchangeable Anion	(Meq/100g)	0.3	0.4	NA	NA
Sodium	(Meq/100g)	1.07	1.50	0.41	0.53
Potassium	(Meq/100g)	0.64	0.90	6.13	5.88
Calcium	(Meq/100g)	3.45	5.04	3.10	2.91
Magnesium	(Meq/100g)	1.09	1.14	2.27	1.51
Chlorine	(Meq/100g)	15.4	16.5	ND	ND
Phosphorus	(Mg/Kg)	9.64	3.71	0.43	0.28
Aammonical nitrogen (NH_4N)	(Mg/Kg)	6.13	8.51	ND	ND
Nitrogen dioxide (NO_2)	(Mg/Kg)	5.20	6.18	ND	ND
Nitrate (NO_3)	(Mg/Kg)	7.81	10.2	ND	ND
Sulfate (SO_4)	(Mg/Kg)	7.36	8.90	ND	ND
Fluorine	(Mg/Kg)	64.2	26.6	ND	ND
Manganese	(Mg/Kg)	1.71	1.99	ND	ND
Zinc	(Mg/Kg)	32.6	15.2	ND	ND
Copper		10.7	11.3	ND	ND
Cadmium	(Mg/Kg)	1.43	6.61	ND	ND
Chromium	(Mg/Kg)	2.24	6.20	ND	ND
Nickel	(Mg/Kg)	2.81	6.76	ND	ND
Lead	(Mg/Kg)	1.51	7.03	ND	ND
Vanadium	(Mg/Kg)	2.10	5.89	ND	ND
Total Hydrocarbon	(Mg/Kg)	9.40	3650.17	ND	ND
Clay	(%)	81.6	ND	NA	NA
Sand	(%)	16.4	ND	NA	NA
Silt	(%)	2	ND	NA	NA

Key
Meq. = Milli Equivalent,
EA = Exchangeable Acid
ND = Not Determined
NA = Not Applicable

Table 5. pH of the samples during the period of study

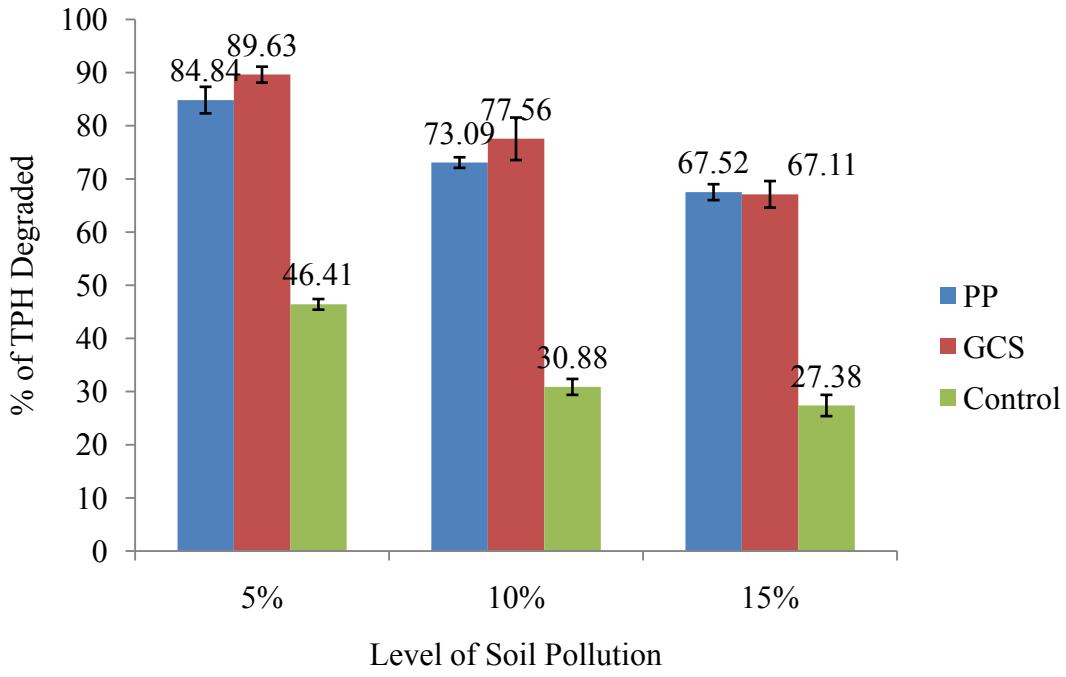
	Polluted soil samples amended with								
Sample Period (Days)	PP 5%	PP 10%	PP 15%	GCS 5%	GCS 10%	GCS 15%	5% Control 1	10% Control 2	15% Control 3
0	7.03	7.23	7.36	6.91	7.64	7.49	7.13	7.35	7.11
14	8.12	8.03	8.07	7.53	8.62	8.19	7.16	8.01	8.04
28	8.31	8.09	8.11	8.60	8.40	8.20	7.13	8.08	8.09
42	8.01	8.04	8.18	8.21	8.01	8.23	7.11	8.00	8.01
56	8.00	8.11	8.24	8.14	8.27	8.39	7.09	8.18	8.23

Legend:
PP = Plantain Peels
GCS = Guinea corn shaft
Control 1, 2, 3 = Soil + Crude oil only

Table 6. Total Petroleum Hydrocarbon (TPH) Recovered from the samples during the period of study

	Sampling Time (Day)	Total Petroleum Hydrocarbon (mg/kg) ± SD		
		PP	GCS	Control
5% crude oil Polluted Soil	0	20485.02±0.01^{m}	20485.04±0.01^{m}	20485.23±0.01^{n}
	14	19005.00±0.28^{k}	18615.00±0.42^{j}	19173.00±0.28^{l}
	28	15170.00±0.28^{h}	14505.00±0.14^{g}	17120.00±0.42^{i}
	42	8870.00±0.14^{d}	8860.00±0.28^{c}	14333.00±0.14^{f}
	56	3105.00±0.28^{b}	2125.00±0.42^{a}	10979.00±0.28^{e}
10% crude oil Polluted Soil	0	21368.05±0.01^{m}	21368.07±0.01^{m}	21368.32±0.01^{n}
	14	20380.00±0.42^{l}	19452.00±0.28^{j}	20342.00±0.28^{k}
	28	16495.00±0.14^{g}	15380.00±0.42^{f}	18986.00±0.14^{i}
	42	9180.00±0.28^{c}	9545.00±0.14^{d}	16563.00±0.42^{h}
	56	5750.00±0.42^{b}	4795.00±0.28^{a}	14770.00±0.28^{e}
15% crude oil Polluted Soil	0	22350.03±0.01^{m}	22350.01±0.01^{m}	22350.36±0.01^{n}
	14	20999.00±0.14^{j}	21022.00±0.28^{k}	21570.00±0.28^{l}
	28	17505.00±0.28^{f}	17720.00±0.42^{g}	19842.00±0.14^{i}
	42	11230.00±0.14^{c}	11690.00±0.14^{d}	17789.00±0.42^{h}
	56	7260.00±0.42^{a}	7350.00±0.42^{b}	16231.00±0.28^{e}

SD, standard deviation; PP, plantain peel; GCS, guinea corn shaft, Control, soil + crude oil. Rows and Columns with the same superscript are not significantly different ($p \leq 0.05$)



Key: PP = Crude oil polluted soil sample amended with Plantain Peels
GCS = Crude oil polluted soil sample amended with Guinea Corn Shaft
Control = Crude oil polluted soil sample without amendment

Figure 1. Percentage of TPH degraded after 56 days of bioremediation studies

3.3. Total Petroleum Hydrocarbon (TPH)

The results in Table 6 and Figure 1 showed the TPH remaining in the samples and the percentage hydrocarbon degraded respectively. The TPH in the samples decreased from day zero to day 56 at the various pollution levels (5%, 10%, 15%). The highest reduction in TPH was in the 5% crude oil polluted soil sample amended with GCS (89.63%) while the least TPH reduction was in the 15% polluted control sample (27.38%). These results showed a marked significant decrease in the TPH content of the amended samples relative to the non-amended samples at the various levels of pollution. The high hydrocarbon loss in these agro-waste amended samples is in line with previous reports [48, 49, 16, 50]. They independently noted a significant loss in TPH in crude oil polluted soil amended with various organic manures. The agro-waste amendments used in this study could have enhanced biodegradation by supplying nutrient to the microbial community which was evidenced by the increased microbial count with increasing days of degradation studied. The low percentage of crude oil degraded in the control samples showed the possibility of natural degradation which occurs rather slowly. This is at variance with the work of another worker [16] whose non-amended (control) samples performed extremely well paralleling the amended samples in percentage crude oil degraded. This study also revealed that higher concentration of the pollutant (crude oil) in the soil reduced the rate of biodegradation because such high concentration could pose serious challenge to the metabolic activities of soil microorganisms. This correlates the findings of an earlier worker [14] who observed higher percentage of crude oil loss in the 5% used motor oil polluted soil sample amended with organic waste when compared with that of 15%.

4. Conclusions

The present investigation confirmed that the use of agro-waste such as plantain peels (PP) and guinea corn shaft (GCS) significantly improved the rate of petroleum hydrocarbon biodegradation in polluted soil. The results also showed that hydrocarbon degrading bacteria were present not only in the soil but also in the agro waste samples used for amendment (PP and GCS). Hence the agro-waste samples were supplying not only nutrient but also hydrocarbon degrading bacteria to the polluted environment. The reduction in total petroleum hydrocarbon was highest in GCS amended samples when compared with the PP amended samples and controls. Also the study has demonstrated that increasing concentration of crude oil in soil, slows down biodegradation as biodegradation rate have been shown to follow the order 5% > 10% > 15% pollution. Furthermore, the obvious increase in bacterial population in the amended soils suggests that the supplementation with the agro-wastes may enhance degradation of petroleum hydrocarbon in nutrient – poor soils. The microorganisms identified in this study when produced in large numbers can be used for bioaugmentation in hydrocarbon biodegradation process.

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