

Biodegradation of Petroleum Hydrocarbons in a Tropical Ultisol Using Legume Plants and Organic Manure

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Abstract

Global emphasis on food security and soil health should consider rehabilitation of degraded lands, especially where oil contamination limits the use of such lands. Three legume plants (*Gliricidia sepium*, *Leucaena leucocephala* and *Calopogonium caerulean*) alone or along with 0.5% (w/w) (equivalent of 10 tons ha⁻¹) poultry manure, were used to treat the soil, in which the oil residuals were monitored for three years. Results showed that significantly high levels of residual total petroleum hydrocarbon content (RTPHs) persisted in the non-amended soil after 36 months. At 3 months, 43% of RTPHs was removed by *Gliricidia sepium* and *Leucaena leucocephala* along with poultry manure. Net loss of RTPHs after 12 months was 69% for *Gliricidia sepium* and *Leucaena leucocephala* combined with poultry manure and only 38% for A₅, explaining that, degradation of petroleum hydrocarbon by indigenous soil micro-organisms was very low. At 18 months when additional load of oil was applied, the degradation rate increased from 71.7 mg kg⁻¹ day⁻¹ to 142 mg kg day⁻¹ within 6 months for all the legume plants along with poultry manure. This explained increased in number of hydrocarbon-degrading micro-organisms due to increase in oil load. The oil inhibited germination and yield of maize crop completely. Treatments with legume plant along with poultry manure significantly ($p < 0.05$) increased both germination and yield of maize crop. The effects of legume plants combined with poultry manure were the better treatment than legume or poultry manure alone in improving the soil properties for growth and performance of maize crop.

Keywords: contaminated soil, residual hydrocarbons, degradation rate, poultry manure, legume plants

1. Introduction

Biodegradation of petroleum hydrocarbons is presumed to be based on the stimulation of microbial degradation in the rhizosphere. Effective soil treatment technologies require or suggest that residual total petroleum hydrocarbons (RTPHs) concentration in soils be reduced to 1000 mg kg⁻¹ or in some areas, below 100 mg kg⁻¹ (USEPA, 1991; Ram et al., 1993). The treatment technologies are often developed and evaluated in order to conform to the regulatory demands and International standards (Lee & Banks, 1993). However, many of the standard treatment methods available for cleaning-up soils contaminated with petroleum hydrocarbons have been limited in their application, are prohibitively expensive, or may be only partially effective (Merkl et al., 2005). The high cost associated with a number of these methods, has also been a major limitation to the sustainability of such methods (Kelly et al., 1992; Van Gestel et al., 1992). The excavation and soil washing methods are often extremely costly and insufficient risk-reducing (Van Gestel et al., 1992; Alloway & Ayres, 1997). Literature reports many examples in which bacterial strains and microbial systems have been successfully utilized to reduce and/or transform selected pollutants in petroleum-contaminated soils under laboratory conditions (Gallizia et al., 2003; Harayama et al., 2004).

Naturally occurring micro-organisms may be able to biodegrade hydrocarbons and other organic compounds in unsaturated soil if the level of contaminations is low and does not produce toxicity for the active bacteria (Molina-Barahona et al., 2004). Biodegradation of petroleum hydrocarbons in soil can be limited by many factors, for example: type of micro-organisms, nutrients, pH, temperature, moisture, oxygen and soil properties. The use of organic wastes, such as cow dung and pig droppings (Okurumeh & Okiemen, 1998) and rubber

processing sludge (C. O. Okiemen & F. E. Okiemen, 2002), have also been reported to give positive results in *in situ* remediation of oil-contaminated soils.

The indiscriminate discharge of petrol oils and grease into open vacant plots and farm lands is becoming an acute environmental problem in Nigeria, particularly, when large areas of agricultural lands are affected (Anoliefo & Vwioko, 1995). In most cases, the soils may remain unsuitable for crop growth for months or years, until oil is degraded to a tolerable level. Depletion in nutrient status, inhibition of microbial activities and reduced oxygen has been reported in spent-oil-contaminated soils (Kirk et al., 2005). Formation of waxy texture, reduced water affinity to soil aggregates and formation of hydrophobic micro-aggregates with clay surfaces are a few other problems associated with soils contaminated with petroleum hydrocarbons (Anoliefo & Vwioko, 1995). The oil succeeding water in the competition for pore spaces and reduction of water film thickness around macro-aggregates decreased both saturated and unsaturated hydraulic conductivity Rasiah et al., 1990; Amadi et al., 1993).

Major report on the use of plants to clean-up soils contaminated with petrol oils and grease has been concentrated on heavy metal removal (Gallizia et al., 2003; Harayama et al., 2004). Whereas, the use of legume plants, or along with organic manure to remove petroleum hydrocarbons in soils, is not well known (Mager & Hernandez-Valencia, 2003; Revera-Cruz et al., 2004). The method is effective, economical and ecologically acceptable. It is also rapidly deployable in a wide range of physical settings (Catallo & Portier, 1992; Kelly et al., 1992; Ram et al., 1993). Plants can enhance microbial degradation of hydrocarbons by providing oxygen in the root area along root channels and loosened soil aggregates. Legumes plants are especially promising in this method, because they are nitrogen independence, which is of significance in oil-contaminated soils (Yeung et al., 1997; Revera-Cruz et al., 2004). The use of some tropical legume plants to remove petroleum hydrocarbon from contaminated sites with a view to making it available for crop production is of research interest. Therefore, the concern in this study was to bridge the gap in knowledge regarding the use of tropical legume plant species and poultry manure for *in situ* degradation of petroleum hydrocarbons. It will provide valuable data on the effectiveness of *Gliricidia sepium* and *Leucaena leucocephala* and *Galapogonium caerulean* along with or without poultry manure in cleaning up soil contaminated with petroleum hydrocarbons.

2. Materials and Methods

2.1 Site Description and Application of Treatments

The study was carried out on 45 plots (each measuring 2.5 × 1.5 m) at the University of Nigeria, Nsukka, Research Farm (Lat. 06°52'N, and Long. 07°24'E). The soil is sandy loam (*Typic Kandiuistult*) derived from False-Bedded sand stone (Nwadialo, 1989). The average slope of the site is < 5%. Mean annual rainfall is more than 1700 mm with maximum temperature of 32 °C (Inyang, 1978).

2.2 Application of Oil and Treatments

The plots were impacted with 5% (w/w) (equivalent of 50,000 mg kg⁻¹) mono- and multi-grade crankcase oils from petrol and diesel engines, together with gear oils and transmission fluids in a single dose, each for two years. By the second year, oil contaminated plots had spent oil application load of 100,000 mg kg⁻¹, representing a total oil load of 10% (w/w). Three (3) legumes: Biodegradation was enhanced using three (3) legume plants: *Gliricidia sepium*, *Leucaena leucocephala*, and *Galapogonim caerulean* alone or combinations with 0.5% (w/w) (equivalent of 10 tons ha⁻¹) of poultry manure. The legume plants used in the experiment have the ability to grow fast, generate high biomass, nitrogen-independent and encourage high population of hydrocarbon-degrading micro-organisms in the rhizosphere (Anderson et al., 1993). The experiment was arranged as a Randomized complete block design (RCBD), with nine (9) treatments in five replications viz:

Treatments:	Designation:
- Uncontaminated soil (Control)	C
- Spent oil only	A ₅
- Spent oil + <i>Calapogonium spp</i>	A ₅ + Ca
- Spent oil + <i>Gliricidia spp</i>	A ₅ + Gl
- Spent oil + <i>Leucaena spp</i>	A ₅ + Le
- Spent oil + poultry manure	A ₅ + Pm
- Spent oil+ <i>Calapogonium</i> + poultry manure	A ₅ + Ca +Pm
- Spent oil + <i>Gliricidia</i> + poultry manure	A ₅ + Gl +Pm

- Spent oil+*Leucaena* +poultry manure A₅ + Le + Pm

The legume plants along with poultry manure were introduced to the plots seven days after oil contamination during the early rains to allow for incubation. The second applications of 5% (w/w) spent oil was carried out at 360 days after the first application.

2.3 Planting

The *Calapogonium caerulean* was planted at 30 × 90 cm spacing, (density of 37,000 plants ha⁻¹), *Gliricidia sepium* and *Leucaena leucocephala* was planted at 1 m × 90 cm spacing (density of 11,100 plants ha⁻¹). Maize variety, (FASR-W) used as test crop was planted at 30 cm × 75 cm spacing.

2.4 Sampling

Disturbed soil samples were collected from the 0-30 cm depth using a soil auger at 3, 6, 12, 18, 24 and 36 months after oil-contamination for laboratory studies. The implications of the oil and treatments on maize performance were evaluated using germination index at 2-4 weeks after planting and grain yield at maturity

2.5 Determination of total Hydrocarbon

Total Hydrocarbon (TH) at each sampling period was determined gravimetrically by toluene extraction (cold extraction) method described by Odu et al. (1989). The liquid phase of the cold extract was measured with a spectrophotometer and fitted into a standard curve derived from fresh spent oil treated with toluene.

2.5 Biodegradation Rate of Total Hydrocarbon Loss

Average biodegradation rates (mg kg⁻¹ day⁻¹) of hydrocarbons were calculated according to the method of Yeung et al. (1997) as:

$$\Delta HL = HC_{ini} - HC_{end} / Time_{inc} \quad (1)$$

Where: ΔHL is the average hydrocarbon content in the soil (mg kg⁻¹), HC_{ini} is the initial hydrocarbon content in the soil (mg kg⁻¹), HC_{end} is the hydrocarbon content when the experiment ended (mg kg⁻¹), and $Time_{inc}$ is the degradation time (d).

2.6 Measurement of Unsaturated Hydraulic Conductivity

Unsaturated hydraulic conductivity, $K_{(o)}$ was predicted from saturated hydraulic conductivity, (K_{sat}) and soil moisture retention characteristics data as proposed by Campbell (1974), and confirmed to be reliable by Rasiah et al. (1990), in this procedure, the pressure potential H_o is related to the relative saturation water content (Q_v/Q_s) by a power function equation:

$$H_o = c [Q_v/Q_s]^{-b} \quad (2)$$

and

$$K_{(o)} = K_{sat} [Q_v/Q_s]^{(2b+3)} \quad (3)$$

Where: b and c are fitting parameters, and H_o , $K_{(o)}$, Q_v , Q_s and K_{sat} are the pressure potential, unsaturated hydraulic conductivity (cm hr⁻¹), volumetric moisture content at any specific matrix potential (cm³ cm⁻³), volumetric moisture content at saturation (cm³ cm⁻³), and saturated hydraulic conductivity (cm hr⁻¹) respectively. Soil water matrix potential of -6 Kpa (60 cm water suction or tension), representing field capacity which drained pores > 50 μ m equivalent cylindrical diameter was used. The b estimate obtained in Equation (2) was used in Equation (3) to predict $K_{(o)}$ for the soil. Soil moisture retention was measured by the hanging column method as described by Galganov et al. (1993). Saturated hydraulic conductivity (K_{sat}) was determined by the constant-head permeameter technique (Klute & Dirksen, 1986). Volume of water draining out was measured overtime, until flow was constant, at which the final flow rate was determined from the equation

$$K_{sat} = Q/AT \times L/\Delta H \quad (4)$$

Where Q is the volume of water (cm³) that flows through across-sectional area A (cm²) in time T (sec.), ΔH is the hydraulic head difference, and L is the length of core sample.

2.7 Data Analysis

Statistical analyses were carried out using the SAS Software (SAS Institute, 2001). Means were separated using the LSD (Fishers protected test) (K. A. Gomez & A. A. Gomez, 1984).

3. Results and Discussions

The soil data before the experiment showed that the soil is sandy loam. The sand, silt and clay contents of the top soil are 820, 60 and 120 g kg⁻¹ respectively (Table 1). The low clay and silt content is an indication of the highly

weathered, low fertility soils of the southern Nigeria with characteristic low buffering capacity to attenuate contaminants (Alloway & Ayres, 1997). The soil also has low C:N ratio and low water holding capacity (Table 1). The spent oil and poultry manure (Pm) have Organic carbon content in spent oil and poultry manure was 31.5 and 28.6 g kg⁻¹ respectively. The pH of the soil was 4.7 and that of poultry manure was 6.5.

Table 1. Some characteristics of the site, poultry manure and spent oil used in the experiment

Parameter	Unit	Soil	Poultry Manure	Spent Oil
Sand (2000-50 µm)	g kg ⁻¹	820	-	-
Silt (50-2 µm)	g kg ⁻¹	60	-	-
Clay (< 2µm)	-	120	-	-
Texture	g kg ⁻¹	Sandy loam	-	-
Organic carbon	g kg ⁻¹	6.84	28.6	31.5
Total N	g kg ⁻¹	0.76	4.5	2.79
C:N	-	9	6	11
p ^H (H ₂ O)	-	4.7	6.5	-
Na	C mol kg ⁻¹	0.10	1.94	-
Exchangeable acidity	C mol kg ⁻¹	2.6	-	-
Saturated hydraulic conductivity	cm hr ⁻¹	20.44	-	-
Bulk density	g cm ⁻³	1.52	-	-
Water holding capacity	cm ³ cm ⁻³	0.31	-	-
Total porosity	%	51.	-	-

3.1 Residual Total Hydrocarbons

The distributions of residual total hydrocarbons in the soil as modified by the treatments are shown in Tables 2 and 3. Very high amount of residual total hydrocarbon (RTHs) (35064 mg kg⁻¹) persisted in the contaminated soil after 36 months (Table 2). In 12 months, the RTH due to the effects of A₅ + Gl + Pm, A₅ + Le + Pm and A₅ + Ca + Pm, were 15471, 15549 and 15816 mg kg⁻¹ respectively, representing 69%, 69% and 68% reductions in compared to high amount of 30648 mg kg⁻¹ found in the A₅ soil. When additional 5% (w/w) load of spent oil was added to the soil after 12 months, residual RTPHs in the soil at 24 month were significantly low (p < 0.05), in A₅ + Gl + Pm, A₅ + Le + Pm and A₅ + Ca + Pm soils. Treatment with only poultry manure (Pm) or legume plants alone did not show significant reductions in RTPHs (Table 2). This implied that biodegradation of RTPHs was higher when the *Gliricidia spp*, *Leucaena spp* and *Calapogonium spp* were used along with 10 tons ha⁻¹ of poultry manure. It could be inferred that the significant reductions in total hydrocarbon found in soils amended with the legume plants along with poultry manure may have been possible due to enhanced positive changes in the physico-chemical conditions of the soils by the legume plants. It is also believed that the plants may have participated in biodegradation of hydrocarbons via support of symbiotic root-associated micro-organisms which was earlier reported by Stamps et al. (1994) and Ensley et al. (1997). The *Gliricidia* and *Leucaena* showed better promise than *Galapogonium* in reducing RTPHs in the soil, indicating that different species of plants have varying effects on rhizosphere micro-organisms and on hydrocarbon degradation activities in the soil (Merkl et al., 2005). As low as 14,936 mg kg⁻¹ total hydrocarbons, representing 30% was degraded naturally by the native soil microorganisms after 36 months (Table 2).

Table 2. Changes in total hydrocarbon content (THC) of the soil as affected by treatment after 36 months

Treatments	THC (mg kg ⁻¹)					
	Months after oil application					
	3	12	18	24	36	Mean
A ₅	35492	30648	41033	36416	31731	35064
A ₅ + Gl	34784	17742	36617	29011	20619	27755
A ₅ + Le	34652	17886	36214	29930	21174	27971
A ₅ + Ca	33964	17421	36347	30662	24366	28552
A ₅ + Pm	34011	16638	39118	31457	28694	29984
A ₅ + Gl + Pm	28413	15471	35473	21974	20416	24349
A ₅ + Le + Pm	28519	15549	35718	22603	20544	24587
A ₅ + Ca + Pm	28944	15816	35736	23146	20712	24871
C	2390	2075	1964	19103	19004	2048
Mean	29018	16583	33136	25224	21128	-

Note. Total loss (%) = [Spent oil loading - Residual THC (Treatment)/Spent oil loading] × 100; LSD (0.05): Treatment = 9646, Month = 125.318, T × M = 594.437.

3.2 Quantification of Total Hydrocarbon Degradation Rate

The net loss and degradation rate of the RTPHs is shown in Table 3. At 3 months, net loss of RTPHs was 14% and degradation rate of 240 mg kg⁻¹ day⁻¹ for *Gliricidia* and *Leucaena spp* along with 10 tons ha⁻¹ poultry manure. When net degradation rate of RTPH was 240 mg kg⁻¹ day⁻¹, then by calculations, 50,000 mg kg⁻¹ total petroleum hydrocarbon would have been completely removed from the soil in 208 days, if the soil is to be treated with *Gliricidia* or *Leucaena spp* supplemented with 10 tons ha⁻¹ poultry manure. There were progressive reductions in residual total hydrocarbons, particularly in the amended soils. Degradation rates decreased during 12 months, because, the numbers of hydrocarbon utilizing micro-organisms have usually been found to be high in soil immediately and a few months after oil application (Molina-Barahona et al., 2004). Degradation rate under natural conditions (A₅), was 161 mg kg⁻¹ day⁻¹ in the first 3 months and drop to 72 mg kg⁻¹ day⁻¹ at 12 months (Table 3). This implies by calculations that it will take more than 674 days for 50,000 mg kg⁻¹ total petroleum hydrocarbon to be degraded naturally from the top soil. This further confirmed that some naturally occurring indigenous micro-organisms which degrade petroleum hydrocarbons by nature exist in some tropical soils environments as reported earlier by Wang and Bartha (1990); Wilson and Jones (1992).

Treatment with *Gliricidia sepium* along with poultry manure increased the RTPHs degradation rates from 127.9 mg kg⁻¹ in 12 months to 443.5 mg kg⁻¹ day⁻¹ in 24 months and 442.1 mg kg⁻¹ in 36 months. This was not surprising, because the legume plant residues may have acted as bacterial biomass suppliers, which supported the high removal of petroleum hydrocarbons from the soil. Improvement in intrinsic properties of the soil, such as the unsaturated hydraulic conductivity (Table 4), by the legumes and poultry may have enhanced rapid biodegradation of the RTPHs.

3.3 Unsaturated Hydraulic Conductivity

The predicted unsaturated hydraulic conductivity in the A₅, at the vicinity of saturated water content was in the order of magnitude less than in the uncontaminated plots (C) (Table 4). The decrease in unsaturated hydraulic conductivity may have been due to the formation of oily scum or coatings on soil aggregates which acted as a barrier to water flow in the soil. Reduction of water film thickness around the macro-aggregates which lead to retarded movement of water into and out of macro-aggregates may lead to low unsaturated hydraulic conductivity observed in the A₅ soil. The A₅ + Gl + Pm treatment significantly (P < 0.05) increased the unsaturated hydraulic conductivity K_(o) of the soils from 71% in 3 months, to 602% in 36 months after oil contamination compared with the (A₅). Generally, treatments improved the unsaturated hydraulic conductivity of the soil in the order of A₅ + Gl + Pm > A₅ + Ca + Pm > A₅ + Le + Pm > A₅ + Gl > A₅ + Pm > A₅ + Ca > A₅ + Le.

Table 3. Degradation rate of residual total petroleum hydrocarbons (RTPH_s) soil as influenced by treatments

Treatments	Net Loss of TPHs (%)					TPHs Degradation Rate (mg kg ⁻¹ day ⁻¹)				
	Months after application					Months after application				
	3	12	15	24	36	3	12	18	24	36
A ₅	-	-	-	-	-	131.2	71.7	327.6	353.2	379.3
A ₅ + Gl	1.4	26.1	4.4	7.4	7.1	169.1	109.5	352.1	394.4	441.0
A ₅ + Le	1.7	25.8	4.8	6.5	10.5	170.5	118.9	354.4	389.3	437.9
A ₅ + Ca	3.1	26.8	4.7	5.7	7.3	178.5	120.7	353.6	385.2	420.2
A ₅ + Pm	3.0	28.3	1.9	4.9	3.0	177.7	123.6	338.2	380.8	396.2
A ₅ + Gl + Pm	14.2	30.7	5.5	14.4	11.3	239.9	127.9	358.5	443.5	442.1
A ₅ + Le + Pm	14.0	30.5	5.3	13.8	11.2	238.7	127.6	357.1	430.0	441.4
A ₅ + Ca + Pm	13.1	30.0	5.3	13.3	11.0	234.0	126.6	357.0	427.0	440.5

Note. LSD (0.05): Treatment = 1.642, Months = 19.971, T × M = 10.442; Net loss of TPHs = % loss in TPHs (Treatment) - % loss in oil (Control); TPHs Degradation = initial TPHs - TPHs at the end; TPHs = total petroleum hydrocarbons.

Table 4. Unsaturated hydraulic conductivity of the oil contaminated soil as influenced by the treatments

Treatment	K _(o) (cm hr ⁻¹)							
	Months after oil application							
	3	6	12	18	24	30	36	
A ₅	9.38	4.32	2.51	2.19	3.76	2.83	2.87	
A ₅ + Gl	12.09	10.49	17.47	14.23	17.93	18.82	19.56	
A ₅ + Le	9.11	13.05	12.48	12.67	15.17	16.92	17.12	
A ₅ + Ca	10.28	12.00	14.66	16.18	17.06	19.06	17.69	
A ₅ + Pm	12.88	16.18	17.55	20.78	18.07	19.29	19.77	
A ₅ + Gl + Pm	15.99	15.58	18.85	17.06	19.55	20.57	20.16	
A ₅ + Le + Pm	14.81	16.33	18.74	15.26	19.44	19.72	19.88	
A ₅ + Ca + Pm	16.05	16.97	16.63	18.62	20.16	18.09	20.41	
C	17.13	10.69	10.49	14.12	9.87	11.18	11.26	
LSD (0.05)	1.98	1.88	1.97	1.76	1.99	1.64	1.86	

Note. K_(o) = Unsaturated hydraulic conductivity.

The legume plants along with poultry manure significantly ($P < 0.05$), increased unsaturated hydraulic conductivity, an indication that soil water characteristics and availability to plant was enhanced. This way, water stress to plants is usually associated with oil contaminated soil was reduced. This further supports the assertion of Joner and Leyval (2004), that micro-organisms in the rhizosphere may have reduced plant stress through an increase in water availability which further enhanced oxidative enzyme production, and increased the volume of soil being remediated.

3.4 Effects on Germination Index and Yield of Maize

The germination index was adversely inhibited in the non-amended soil by the oil through the 3-year planting (Table 5). The few maize plants that germinated died after a few weeks. Hence, no yield was obtained in A₅. Several factors may have contributed to the death of the maize plants, among which may include: lack of adequate oxygen, decreased in water availability, crusting and other unsatisfactorily soil conditions due to the oil. Similar observations were reported by Molina-Barahona et al. (2004) on maize and sugar cane. Significantly higher percent germination were found in the plots treated with the legume plants along with poultry manure. At 36 months, (corresponding to third planting season), germination counts rose to 93% and 90% for *Gliricidia* and

Leuceana with poultry mamure respectively. Maize yield were 4.91, 8.25 and 6.46 tons ha⁻¹ respectively during the first, second and third planting seasons for A₅ + Gl + Pm. There was no yield in A₅ and Control soil in the three planting seasons, most probably caused by either nutrient deficiencies or adverse effects of the hydrocarbons on the soil physical properties.

Table 5. Effects of treatments on germination and grain yield of maize

Treatment	Maize grain yield (tons ha ⁻¹)			Germination count (%)		
	1 st Planting	2 nd Planting	3 rd Planting	1 st Planting	2 nd Planting	3 rd Planting
A ₅	0.0 ^a	0.0 ^a	0.0 ^b	41 ^a	36 ^b	34 ^a
A ₅ +Gl	3.06 ^c	5.16 ^b	4.18 ^a	57 ^b	53 ^a	67 ^a
A ₅ +Le	2.32 ^a	5.05 ^b	4.12 ^a	55 ^b	50 ^d	65 ^a
A ₅ +Ca	1.87 ^a	4.76 ^b	3.47 ^a	60 ^b	63 ^c	89 ^b
A ₅ +Pm	4.01 ^b	6.23 ^c	4.50 ^b	58 ^b	63 ^c	63 ^a
A ₅ +Gl+Pm	4.91 ^c	8.25 ^b	6.46 ^a	62 ^b	80 ^d	93 ^b
A ₅ +Le+Pm	3.91 ^b	6.90 ^c	5.03 ^b	63 ^b	81 ^d	90 ^b
A ₅ +Ca+Pm	4.16 ^b	7.02 ^a	6.21 ^a	66 ^b	79 ^d	88 ^b
C	0.0 ^a	0.0 ^a	0.0 ^b	78 ^c	70 ^d	78 ^c

Note. Yield and germination count followed by different letters within the years are significantly different at P < 0.05.

4. Conclusion

Conclusions drawn from this study are that: Contamination of soil with spent oils and grease increased the total and residual hydrocarbons in soil. Hydraulic conductivity and water affinity to the soil aggregates were reduced which adversely affected maize germination and growth. High amounts of petroleum hydrocarbons were found in the non-amended soils after 36 months of oil contamination. By comparing the degradation effect, the legume plants combined with poultry manure were the better treatment than legume or poultry manure alone. Degradation effect by indigenous micro-organism was negligible. The *Gliricidia sepium* and *Leucaena leucocephala* are more promising species than *Calapogonium caerulean* in bioremediation of soils contaminated with petroleum products.

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