Bio-Stimulation of a Spent Auto-Engine Oil Contaminated Sandy-Loam *Ultisol* Using Maize (*Zea mays*) As Test Crop

Henry Ahamefule[1] and Oyemisi Fawole[2]

Abstract— A study was conducted in the teaching and research farm of the University of Nigeria, Nsukka to evaluate the applicability of some organic materials as bio-stimulation agents. Spent engine oil (Sp) was the main plot treatment applied at 0 (control), 10,000, 20,000 and 30,000 mg/Kg ('Sp'/soil) in a single dose whereas sub-plot treatments were control (No Amendment = NA), Palm Oil Mill Effluent (PE), Palm Bunch Refuse (PR) and Cassava Peels (CS) applied at 12 Mg/ha each, per year. Treatments were arranged in a split-plot format in Randomized Complete Block Design (RCBD) with three replications. Results indicated that microbial population in amended plots followed the order PE > CS > PR > NA. The PE amended soils reached a peak of 11-fold reduction in total hydrocarbon content relative to un-amended equivalents. Bio-remediation efficiency reached 111.3 % (highest) in PE treatment compared to 51.3% (least) in PR treatment. Increased 'Sp' contamination inhibited maize germination.

Keywords—Maize germination, soil contamination, soil properties, spent engine oil

I. Introduction

THE indiscriminate disposal of spent engine oil by artisans and road-side auto-technicians pose serious environmental challenge in Nigeria. Nigeria was reported to produce more than 87 million litres of spent automobile engine oil annually [1], and adequate attention has not been given to its disposal [2]. Spent auto engine oil contains potentially phyto-toxic polycyclic aromatic hydrocarbons [3]. [4] and [5] noted that oil in soil have deleterious effects on biological, chemical and physical properties of the soil depending on the dose, type of the oil and other factors. [6] and [7] also reported that the microbiological components of soil were usually negatively affected following oil application to the soil. [8] observed a shift in carbon substrate utilization patterns in soil contaminated with oil and related it to the development of hydrocarbon utilizing bacterial community. The latter authors study further showed that *Pseudomonas* and *Bacillus* micro-organisms were prevalent in the oil contaminated sites, whereas dramatic reduction occurred in the total microbial community due to the additions of petroleum waste sludge. [9] reported that petroleum waste sludge adversely affected the microbial population by depleting essential inorganic nutrients and growth factors and lowering the pH immediately around negatively charged soil surfaces. According to [10], germination of seeds planted in crude oil polluted soil area is delayed while percentage germination is also significantly affected. [11] and [12] reported poorer germination response of cowpea with increasing dose of spent engine oil. The slow natural recovery of polluted soils, the vast area under pollution and expensive clean-up operations further compounds the problem. In this light bioremediation being a cheaper clean-up procedure may be a useful tool in such localities. Therefore this study was aimed at investigating the potentials of the abundant agricultural wastes available in the experimental location as nutrient base for the stimulation of the growth of indigenous micro-organisms in spent engine oil contaminated soil for the purpose of biodegradation of the soil contaminant.

Henry Ahamefule[1] is with the University of Ilorin, Ilorin, Kwara state, Nigeria (corresponding author's phone:+2348037763570, *+2347010614368* ; e-mail: flamepearls@yahoo.com, ahamefule.he@unilorin.edu.ng).

Oyemisi Fawole [2], is with University of Ilorin, Ilorin, Kwara state, Nigeria(e-mail:oyemisifawole@yahoo.com).

II. Materials And Methods

1.1.1 Site Description: The experiment was conducted between the years 2006 and 2008 in the University of Nigeria Nsukka campus teaching and research farm, located by latitude $05^0 52$'N and longitude $07^0 24$'E and at an elevation of 400 m above sea level. The mean annual maximum temperature in this location ranges between 27^0C and 32^0C in the period from March to May [13] – [14] and rainfall with mean annual total in excess of 1700 mm [15]. The soil is an *Ultisol*, [16] reported it as sandy-loam textured and acid in reaction.

1.1.2 Field Methods

1.1.2.1. Experimental design: The treatments were arranged in a split-plot format in Randomized complete Block Design (RCBD) with four (4) treatments in each main plot and four (4) in each sub plot. Each treatment was replicated three (3) times giving a total of 48 sub plots. The main plot treatments was comprised of four (4) levels (0, 1, 2, and 3 %) of spent auto-engine oil equivalent to 0, 10 000, 20000 and 30000 mg kg^{-1} of soil respectively. The subplots were treated with three types of organic amendments (oil palm bunch refuse, palm oil mill effluent and cassava peels) at 12 Mg/ha and un-contaminated – non-amended (control).

1.1.2.2. Experimental layout: A land area of 0.0256 ha was used for the study. The main plots measured 15.125 m^2 (5.50 m x 2.75 m) while the sub-plots measured 2.75 m^2 (2.75 m x 1.0 m).

http://dx.doi.org/10.15242/IICBE.C0215129

1.1.2.3. Field preparations: Soil samples were collected in a grid of 4 x 2 m, bulked and a composite sample taken for laboratory analyses to determine the initial physical and chemical properties of the site. Glyphosate, a post emergence herbicide (a.i ispropylamine) and butachlor, a pre-emergence herbicide (a.i.2-chloro-2, 6- diethyl – N (butoxy methyl) acetanilide) were used to control weeds. Plots were tilled in the first year of the experiment and were zero tilled in the subsequent two years. The nutrient supplements (organic amendments) were applied to both spent oil contaminated and uncontaminated plots 7 days after oil application. After organic amendment, the treated and untreated plots were left for two weeks before planting to allow for incubation. Oil treatment was applied in a single dose at the start of the experiment while the organic amendments were applied in repeated yearly doses. The test crop was maize (*Zea mays Var.Oba supper II*), grown for three planting seasons (2006, 2007 and 2008) with each planting season spanning from May to August. Sowing was done manually at the rate of two seeds per hole, to a depth of 2.5cm and spacing of 50 cm x 25 cm in double rows, and thinned down to one plant per stand after emergence. Each sub-plot contained 20 plants, giving a plant population of 80,000 plants ha^{-1}.

1.1.3 Data Collection: Percent emergence (germination) was taken 8 days after planting as the number of emerged seedlings expressed as a percentage of the total number of seeds planted per plot. Disturbed and undisturbed core soil samples were collected from 0 – 20 cm and 20 – 40 cm depths at 0 months (before treatment) and 3, 6, 12, 18, 24, 30, 36 months after application of treatment for determination of viable microbial count and total hydrocarbon content. All data collected were subjected to statistical analysis to determine treatment effects and interactions using SPSS version 16.0 computer statistical package while significant treatment means were separated at 5 % probability level with Fisher's Least Significant Difference (F-LSD) approach.

1.1.4 Laboratory Studies

1.1.4.1 Viable microbial count: In this procedure, 10 g of the soil sample was mixed with 90 ml of sterile Ringers saline to get the stock solution. Then 1ml of the stock solution was taken and serial dilution done using tenfold dilution. A ml aliquot was plated out with 19mls of sterile nutrient agar, allowed to gel and incubated for 48 hrs at 37^0C. The count of viable micro-organisms was taken using a colony counter and results expressed as Colony Forming Units ($CFUg^{-1}$ soil).

1.1.4.2 Total hydrocarbon content (THC): Total hydrocarbon content at each sampling date was determined gravimetrically by toluene extraction (cold extraction) method as described by [17].

1.1.4.3 Remediation efficiency (R.E): The remediation efficiency which shows (in percentage) the effectiveness of the organic amendments (nutrient source) relative to the un-amended plots in reducing the total hydrocarbon content (THC) of plots treated with same quantity of spent engine oil was calculated thus:

$$R.E = \frac{THCci - THCti}{THCci}.100$$

Where:

THC_{ci} = total hydrocarbon content in control plot under a given oil loading.

THC_{ti} = total hydrocarbon content in an amended plot under a given oil loading.

III. Results And Discussion

1.1.5.1 Initial properties of the experimental site and organic materials: Some characteristics of the top (0 – 20 cm) soil of the experimental site at the beginning of the experiment and the organic amendments used are presented in Table 1. The result showed that the texture of the experimental site was sandy-loam, characteristic of soils from false bedded sandstone origin [18] whereas the organic carbon content, pH, P, and ECEC were generally low to very low which is typical of degraded *Ultisols*. The nutrient status of the organic amendments indicated PE > PR > CS whereas their C/N ratio followed the order PR > CS > PE, been more than 3 times higher in PR and CS compared to PE. Going by results in Table 1 the PE amendment indicated a richer nutrient (N, P and K) source compared to the other organic amendments.

TABLE I
SOME CHARACTERISTICS OF THE TOP SOIL (0 – 30 CM DEPTH) OF THE EXPERIMENTAL SITE AND THE ORGANIC MATERIALS

Parameter	Soil	PE	CS	PR
Sand %	67	-	-	-
Silt %	15	-	-	-
Clay %	18	-	-	-
Textural class	Sandy- loam	-	-	-
pH (1:2.5 H_2O)	4.7	-	-	-
pH (0.01MKcl)	3.8	-	-	-
Organic Carbon (%)	1.32	36.4	48.7	60.0
Total N (%)	0.085	2.7	1.0	1.1
C/N ratio	15.5	13.5	48.7	54.6
Av.P($mgkg^{-1}$)*/ % P^a	8.67^*	1.2^a	0.7^a	1.1^a
Exchangeable bases ($cmolkg^{-1}$)*				
Na	0.55	-	-	-
K^*/ % K^a	0.02^*	2.5^a	1.1^a	1.5^a
Ca	1.14	-	-	-
Mg	3.10	-	-	-
Exchangeable acidity ($cmolkg^{-1}$)				
Al^{+3}	1.20	-	-	-
H^+	2.40	-	-	-
ECEC ($cmolkg^{-1}$)	8.41	-	-	-

[a]unit in %, PE = palm oil mill effluent, CS = cassava peels and PR = palm bunch refuse.

1.1.5.2 Effects of spent auto-engine oil and organic amendment on microbial population: The studies revealed an initially lower population of viable micro-organisms following contamination, indicating that the contaminant was lethal at impact to some of the indigenous microbes. However, the population grew subsequently along the path of increasing spent auto-engine oil dose (Table 2). The populations which were represented in colony forming units per gram soil ranged between 1.3 x 10^4 – 2.3 x $10^7 cfug^{-1}$ in 3% spent auto-engine oil treated plots, 1.2 x 10^4 – 1.1 x 10^7 $Cfug^{-1}$ in 2 % oil treated plots, 1.1 x 10^4 – 2.0 x 10^6 $Cfug^{-1}$ in plots under 1 % oil and 1.4 x 10^4 - 3.0 x 10^4 $Cfug^{-1}$ in the uncontaminated plots following post-contamination sampling (3 – 36 months). Uncontaminated plots showed significantly lower microbial growth relative to the contaminated plots. This indicates that the presence of spent auto-engine oil either attracted hydrocarbon degrading organisms or served as substrate for the multiplication of some indigenous hydrocarbon degrading microbes. This was corroborated by [19] and [20]. [17] also observed that the presence of gasoline (a hydrocarbon) in the soil resulted in significant increase in microbial population and metabolic activities. [17] further reported that the number of hydrocarbon utilizing organisms were most abundant in oil polluted sites than in the unpolluted sites.

When these contaminated plots were subjected to yearly organic amendments, it was observed that there was a consequent significant bloom in hydrocarbon degrading microbial population. It was also observed that viable count of spent auto-engine oil degrading micro-organisms in amended plots was significantly ($P < 0.01$) higher than that in un-amended plots in the order PE > CS > PR > NA. The viable counts for plots under PE through the 36 months of this experiment ranged between $1.5 \times 10^4 – 2.5 \times 10^7$ cfug^{-1} and that in CS treated plots was from $1.3 \times 10^4 – 9.7 \times 10^6$ cfug^{-1} while PR plots was between $1.4 \times 10^4 – 1.7 \times 10^6$ cfug^{-1} and NA ranged between $1.1 \times 10^4 – 4.2 \times 10^4$ cfug^{-1}. The viable count of micro-organisms in the un-amended plots (NA) 36 months after oil contamination (4.2×10^4 cfug^{-1}) was less than that in the amended plots (1.5×10^5, 6.4×10^4 and 6.0×10^4 cfug^{-1} respectively, for PE, CS and PR) in 12 months. This showed that the decomposition and mineralization of the organic amendments provided nutrients which supported the rapid growth of hydrocarbon degrading micro-organisms. [21] reported that "counts of hydrocarbon degraders are usually higher in soil with addition of nitrogen and phosphorus sources", which these organic amendments typifies.

TABLE II
VARIATION IN VIABLE MICROBIAL COUNT (CFU G^{-1}) OF THE TOPSOIL OF A SPENT ENGINE OIL CONTAMINATED *ULTISOL* UNDER ORGANIC AMENDMENT

Oil conc.(%)	Months 0	3	6	12	24	36
0	1.2×10^4	1.4×10^4	1.3×10^4	1.5×10^4	2.1×10^4	3.0×10^4
1	1.2×10^4	1.1×10^4	3.0×10^4	7.3×10^4	2.3×10^5	2.0×10^6
2	1.2×10^4	1.2×10^4	4.3×10^4	8.2×10^4	3.2×10^5	1.1×10^7
3	1.2×10^4	1.3×10^4	5.3×10^4	1.3×10^5	1.4×10^6	2.3×10^7
$LSD_{0.05}$	n.s	421.8	580.2	835.0	1000.8	1686.0
Organic amendment						
NA	1.2×10^4	1.1×10^4	1.5×10^4	1.8×10^4	3.2×10^4	4.2×10^4
PE	1.2×10^4	1.5×10^4	6.2×10^4	1.5×10^5	1.6×10^6	2.5×10^7
CS	1.2×10^4	1.3×10^4	3.3×10^4	6.4×10^4	2.2×10^5	9.7×10^6
PR	1.2×10^4	1.4×10^4	3.1×10^4	6.0×10^4	1.0×10^5	1.7×10^6
$LSD_{0.05}$	n.s	401.5	611.0	762.5	918.0	2188.0
Interaction	n.s	**	**	**	**	**

NA = control, PE = palm oil mill effluent, CS = cassava peels, PR = palm bunch
refuse, ns = non significant, ** and* = significant at 1 and 5 % probability level

Samples of the sub (20 – 40 cm) soil (Table 3) yielded no growth of hydrocarbon degrading micro-organisms in the first 3 months following contamination and amendment of the top 0 – 20 cm soil.

TABLE III
VARIATION IN VIABLE MICROBIAL COUNT (CFU G^{-1}) OF THE SUBSOIL OF A SPENT ENGINE OIL CONTAMINATED *ULTISOL* UNDER ORGANIC AMENDMENT

Oil conc.(%)	Months 0	3	6	12	24	36
0	-	-	-	-	1.0×10^4	1.1×10^4
1	-	-	1.3×10^4	1.1×10^4	2.2×10^4	2.5×10^5
2	-	-	1.3×10^4	1.1×10^4	3.3×10^4	5.3×10^5
3	-	-	1.3×10^4	1.3×10^5	4.5×10^4	6.0×10^5
$LSD_{0.05}$	n.s	n.s	816.6	807.0	846.0	925.4
Organic amendment						
NA	-	-	-	1.0×10^4	1.3×10^4	1.5×10^4
PE	-	-	1.1×10^4	1.5×10^5	6.0×10^4	7.2×10^5
CS	-	-	1.0×10^4	1.2×10^4	2.5×10^4	3.4×10^5
PR	-	-	1.0×10^4	1.3×10^4	1.9×10^4	4.2×10^5
$LSD_{0.05}$	n.s	n.s	820.2	812.0	830.0	908.5
Interaction	n.s	n.s	*	*	**	**

NA = control, PE = palm oil mill effluent, CS = cassava peels, PR = palm bunch
refuse, ns = non significant, ** and* = significant at 1 and 5 % probability level

A culture of the 6th month sample showed significant ($P < 0.05$) growth of hydrocarbon degrading micro-organisms in the sub-soils of all contaminated top-soils, with sub-soils under PE amendment showing significantly higher growth relative to other amendments while there was no growth in sub-soils with un-amended (NA) top-soils. The growth of hydrocarbon degraders was significantly observed in most of the treated sub-soils from the 12th – 36th month. Comparing results obtained in the top (0 – 20 cm) and sub (20 – 40 cm) soil showed that viable counts of micro-organisms declined with increasing soil depth. The highest count of viable micro-organisms in the 20 – 40 cm soil depth was 1.5×10^6 cfug^{-1} compared to 5.7×10^7 cfug^{-1} in the 0 – 20 cm soil depth. The same interaction (PE × 3 % oil) produced these results. The results obtained are supported by those of [22], [8] and [9].These researchers reported that microbial population decreased with soil depth. This phenomenon is due to increasing bulk density with soil depth which determines pore space through which water and air can move [23]. Pore size affects the rate of growth of soil organisms [24].Growth of *Escherichia coli* was found to be reduced in smaller pores, possibly due to a restriction of bacterial cell division [25].

1.1.5.3 Effects of spent auto-engine oil and organic amendment on total hydrocarbon content (THC) of soil: The results obtained (Table 4) showed that THC in the top (0 – 20 cm) soil significantly ($P < 0.05$) increased with increasing rate of spent auto-engine oil application. The THC in plots treated with 0, 1, 2 and 3 % spent auto-engine oil ranged from 954 - 998 mg kg^{-1}, 7455 - 1325 mg kg^{-1}, 15889 - 6362 mg kg^{-1} and 24429 - 11550 mg kg^{-1} respectively through the 36 months of this experiment.

The sub-plot results showed that only those (0 – 20 cm soil) treated with PE exhibited significantly ($P < 0.05$) lower THC compared to control (NA) and the other organic amendments in the 3rd month. In the 6th month PE and CS treated plots showed significant reduction in THC values compared to those under PR and control. From the 12th – 36th month a clear order of reduction ($P < 0.05$) in the THC was established by the organic amendments thus: PE > CS > PR > NA. The mean THC values in the PE amended plots fell from 9553 mg kg^{-1} in 3 months to 1997 mg kg^{-1} in 36 months, CS: from 12047 to 3712 mg kg^{-1}, PR: from 13426 to 6373 mg kg^{-1} and NA: from 13697 to 8152 mg kg^{-1}. In an experiment to determine the rate of leaf and fine root decomposition, [26], reported a high correlation between C/N ratio and rate of decomposition of plant residues.

TABLE IV
VARIATION IN TOTAL HYDROCARBON CONTENT (MG KG^{-1}) OF THE TOPSOIL OF A SPENT ENGINE OIL CONTAMINATED *ULTISOL* UNDER ORGANIC AMENDMENT

Oil conc.(%)	Months 0	3	6	12	24	36
0	905	954	987	919	957	998
1	905	7455	5963	4615	3560	1325
2	905	15889	14034	12010	8541	6362
3	905	24429	21166	20910	17223	11550
$LSD_{0.05}$	n.s	5100	4850	3012	2138.3	761.9
Organic amendment						
NA	905	13697	12992	12042	10821	8152
PE	905	9553	7121	6538	3195	1997
CS	905	12047	9794	8919	6819	3712
PR	905	13426	12244	10955	9447	6373
$LSD_{0.05}$	n.s	1127.3	1006.8	951.0	712.5	539.0
Interaction	n.s	**	**	**	*	*

NA = control, PE = palm oil mill effluent, CS = cassava peels, PR = palm bunch refuse, ns = non significant, ** and* = significant at 1 and 5 % probability level.

Therefore the behaviour of these organic amendments with respect to THC reduction may be connected to their C/N ratios (Tables 1). The amendments with comparatively lower C/N ratio decomposed and mineralised faster, hence releasing the needed nutrients for enhancement of rapid microbial multiplication and consequent degradation of the hydrocarbon. The nutrients N and P were reported to be most critical in bioremediation [27], [28], [20]. The order of reduction of THC by these organic amendments was also observed to reflect their N and P contents.

A test for the presence of the hydrocarbon (spent auto-engine oil) in the 20 – 40 cm soil depth, 3 months following contamination of the top 0 - 20 cm, yielded negative results (Table 5). However, 6 months later, significant quantity of oil was observed in sub (20 – 40 cm) soils of plots under 3 % and 2 % oil treatment and all the amended and control but the PE plots. The observation in the PE amended plots showed that a comparatively higher rate of bioremediation of the oil took place in it, such that oil seepage down-wards was much hampered, this was confirmed by the higher remediation efficiency of PE treatment compared to CS and PR as shown in Table 6.

TABLE V
VARIATION IN TOTAL HYDROCARBON CONTENT (MG KG^{-1}) OF THE SUBSOIL OF A SPENT ENGINE OIL CONTAMINATED *ULTISOL* UNDER ORGANIC AMENDMENT

Oil conc.(%)	Months 0	3	6	12	24	36
0	801	801	800	801	801	801
1	801	801	801	1410	1543	1542
2	801	801	861	1670	3194	3407
3	801	801	887	2358	3844	4248
$LSD_{0.05}$	n.s	n.s	57.8	120.6	319.0	811.0
Organic amendment						
NA	801	801	850	3016	4115	4562
PE	801	801	801	903	1157	1227
CS	801	801	849	1062	1521	1588
PR	801	801	850	1401	2590	2622
$LSD_{0.05}$	n.s	n.s	48.0	108.3	251.6	385.9
Interaction	n.s	n.s	n.s	*	*	*

NA = control, PE = palm oil mill effluent, CS = cassava peels, PR = palm bunch refuse, ns = non significant, * = significant at 5 % probability level

Results presented in Table 6 further indicate that at the end of the 12th month spent auto-engine oil contaminated plots (3, 2 and 1%) amended with PE lost 35.7, 65.0 and 76.1 % of its total hydrocarbon content (THC) to microbial degradation and 1.01, 0.51 and 0.00 % by gravity/seepage to the 20 – 40 cm soil depth with remediation efficiencies of 19.1, 53.8 and 61.0 % respectively. Microbial degraded THC values in CS treated plots were 26.9, 47.7 and 83.7 % and loss to gravity were 2.4, 1.5 and 0.29 % while the remediation efficiencies were 9.8, 32.1 and 73.9 % respectively. In PR amended soils THC loss due to microbial degradation were 18.0, 35.2 and 49.0 % while loss due to gravity were 4.8, 2.9 and 3.8 % with 1.4, 17.4 and 23.0 % as remediation efficiencies respectively. By the 36th month of this study, the THC lost under PE treatment due to microbial degradation were 85.2, 91.2 and 100.9 % whereas that lost to gravity were 3.8, 2.8 and 0.0 % for 3, 2 and 1 % oil contaminations respectively with remediation efficiencies of 81.7, 88.3 and 111.3 %. The CS amended plots under 3, 2 and 1 % oil contaminations lost 64.6, 84.6 and 95.5 % to microbial degradation and 7.7, 3.8 and 0.5 % oil to gravity respectively whereas the remediation efficiencies of CS amended plots were 54.0, 77.3 and 49.6 % under 3, 2 and 1 % oil treatments. Plots under same oil contaminations, amended with PR, lost 48.7, 42.5 and 86.4 % to microbial degradation and 8.2, 17.2 and 6.7 % oil to gravity; while remediation efficiencies were 28.4, 21.4 and 2.8 % respectively. The results obtained also showed that the degradation rate of spent auto-engine oil increased with increase in the quantity of oil applied as also reported by [25] and with increased nutrient amendments. This suggests that the microbial population which degraded oil increased as its substrate (oil) increased with a corresponding increase in the nutrient supply which sustained them. This was confirmed by the results of the viable count of hydrocarbon degrading micro-organisms (Tables 2 and 3). [29] and [20] reported similar findings. The result obtained with PE amendment was a reflection of its higher nutrient composition compared to CS and PR. It was therefore not surprising that PE gave the highest remediation efficiency among the organic amendments. The results showed that oil contamination reduced in two ways: by gravity (downward seepage) and by microbial degradation, the faster of the two determined the extent of damage done to soil physical, chemical and biological properties, and to what depth. With optimum supply of nutrients, microbial degradation of oil out-runs the downward seepage, hence reducing 'collateral damage' to the soil. This was evident in the comparatively low oil (shown by low THC values) reaching the lower 20 - 40 cm of soils amended with PE and hence close to the initial properties of the

TABLE VI
EFFECT OF SOIL AMENDMENT ON TOTAL HYDROCARBON CONTENT OF SPENT AUTO-ENGINE OIL CONTAMINATED *ULTISOL*

Treatments	Spent oil Loading ($mgkg^{-1}$)	Residual THC ($mgkg^{-1}$)	Total loss in THC (%)	Loss by gravity (%)	Loss due to microbial activity (%)	R.E* (%)
			12th Month			
SP_3NA	30000	23502	21.6	12.5	9.1	-
SP_3PE	30000	19019	36.7	1.01	35.7	19.1
SP_3CS	30000	21203	29.3	2.4	26.9	9.8
SP_3PR	30000	23175	22.8	4.8	18.0	1.4
SP_2NA	20000	14971	25.2	15.4	9.8	-
SP_2PE	20000	6906	65.5	0.51	65.0	53.8
SP_2CS	20000	10169	49.2	1.5	47.7	32.1
SP_2PR	20000	12374	38.1	2.9	35.2	17.4
SP_1NA	10000	6129	38.7	10.2	28.5	-
SP_1PE	10000	2391	76.1	0.00	76.1	61.0
SP_1CS	10000	1602	84.0	0.29	83.7	73.9
SP_1PR	10000	4717	52.8	3.8	49.0	23.0
			24th Month			
SP_3NA	30000	21739	27.5	20.6	6.9	-
SP_3PE	30000	5453	81.8	3.0	78.8	74.9
SP_3CS	30000	17909	40.3	6.5	33.8	17.6
SP_3PR	30000	20170	32.8	7.1	25.7	7.2
SP_2NA	20000	12734	36.3	24.6	11.7	-
SP_2PE	20000	3448	82.8	2.4	80.4	72.9
SP_2CS	20000	4920	75.4	4.1	71	61.4
SP_2PR	20000	9443	52.8	16.8	36.0	25.8
SP_1NA	10000	5212	47.9	21.7	26.2	-
SP_1PE	10000	311	96.9	0.3	96.6	94.0
SP_1CS	10000	623	93.8	1.0	92.8	88.1
SP_1PR	10000	4475	55.3	6.7	48.6	14.1
			36th Month			
SP_3NA	30000	18048	39.8	24.0	15.8	-
SP_3PE	30000	3310	89.0	3.8	85.2	81.7
SP_3CS	30000	8304	72.3	7.7	64.6	54.0
SP_3PR	30000	12918	56.9	8.2	48.7	28.4
SP_2NA	20000	10247	48.8	28.3	20.5	-
SP_2PE	20000	1204	94.0	2.8	91.2	88.3
SP_2CS	20000	2322	88.4	3.8	84.6	77.3
SP_2PR	20000	8053	59.7	17.2	42.5	21.4
SP_1NA	10000	710	92.9	21.9	71.0	-
SP_1PE	10000	-80	101.1	0.0	100.9	111.3
SP_1CS	10000	358	96.4	0.9	95.5	49.6
SP_1PR	10000	690	93.1	6.7	86.4	2.8

SP_1, SP_2 and SP_3 = 1, 2 and 3% spent oil, NA = no amendment, PE = palm oil mill effluent, CS = cassava peels,PR = palm bunch refuse.
*Remediation Efficiency

lower soil exhibited under PE treated plots. The complete remediation achieved in 36 months only in plots contaminated with 1 % oil and amended with PE with almost no loss of oil to the lower (20 - 40 cm) soils indicate that the quantity of the amendments should be increased beyond 12 Mg ha^{-1} for all levels of the contaminations (1, 2 and 3%) for complete and faster remediation in this soil. According to [20] the speed and efficiency of biodegradation of a soil contaminated with petroleum and petroleum products depend on the number of indigenous hydrocarbon-degrading micro-organisms. They further noted that the most important factors for the growth of these micro-organisms are temperature, oxygen, pH, nutrient status (N and P), hydrocarbon class and their effective concentration. [30] and [31] added that the degree and rate of bioremediation are influenced by the type of soil in which the process occurs.

Generally, it is important to have information on the rates of contaminant/pollutant biodegradation under specified environmental conditions to be able to assess the potential fate of the compounds [32], to evaluate the efficacy of the *in-situ* biodegradation treatment and to assign appropriate approaches to enhance the degradation rates [33]. However when *in-situ* bioremediation experiments are conducted and conclusions on bioremediation rate of non-volatile petroleum hydrocarbons drawn from observations in the top-soil without recourse to the THC of the sub-soil, such conclusions are bound to be superfluous and hence projections made with such results may be misleading. The peculiarity of this work is that THC reduction is shown to be achieved not only by biodegradation, as commonly reported by most researchers conducting similar experiments, but by its combination with loss due to gravity (downward seepage).This finding was due to the examination of the sub-soil over time, which showed that plots under no amendment and organic amendments with low remediation efficiency will lose comparatively more portion of the hydrocarbon to deeper soil layers. This portion lost by gravity is often-times erroneously reported as part of that lost by biodegradation, especially in field experiments. Though there was no significant loss of the hydrocarbon to gravity in 1 % oil contaminated plots under PE amendment which may also be observed in any other experiment depending on the bioremediation efficiency of the organic amendment and other factors earlier stated, nevertheless the sub-soils of treated plots should always be examined and tested in such experiments to account for loss due to seepage or at least to eliminate the fact that there was loss via seepage and hence ensure accurate reportage. This becomes even more necessary when spent auto-engine oil contamination is up to 2 %, which was observed in this experiment to seep down the sub-soil in 6 months. The remediation efficiency (Table 6) evaluated the efficacy of organic amendments as tools for bioremediation of soils contaminated with petroleum hydrocarbons. Differences in remediation efficiency values indicated comparative advantage of an organic amendment over another.

1.1.5.4 Effects of spent auto-engine oil and organic amendment on maize germination: Increased oil contamination inhibited the germination of maize seeds (Table 7). In the first year (2006), 3 % and 2 % oil contaminations reduced maize germination from 86 % (control) to 41 and 50 % respectively while plots under 1 % oil treatment did not show significant reduction in germination compared to the control. The germination count of maize improved over the years (2007 - 2008). The germination count in plots under 3 % oil treatment improved from 51 % in 2008 to 60 % in 2009, while that in 2

% oil treatment improved from 55 to 64 %. Germination in plots under 1 % oil treatment did not vary ($P < 0.05$) from the uncontaminated plots (0 %). The germination count in amended plots was significantly higher than that in un-amended plots. In the first year, germination in plots under PE (72 %) was significantly ($P < 0.05$) higher compared to that in CS (69 %), PR (63 %) and NA (58 %). The germination count in PR plots did not differ ($P < 0.05$) from that in NA. The effect of the organic amendments on germination of maize in the second year followed the order: PE > CS = PR > NA while in the third year it was PE = CS > PR > NA. The non-significant difference in germination in the 0 and 1 % spent auto-engine oil contaminated soils in the first year following contamination indicate that a threshold value of 1 % (10,000mgkg^{-1})

spent auto-engine oil contamination has to be exceeded for a significant decline in maize germination to occur. [34] reported stimulation of germination at 1 % w/w spent auto-engine oil in soil for *Ricinus communis* seedlings while germination in higher concentration (2, 3, 4, 5 and 6 % w/w) exhibited depression. In the same vein [35] and [3] separately reported growth enhancement (fertilizer effect) at 1 % spent auto-engine oil contamination when compared to the control, for various plant species. Above the 1 % spent auto-engine oil contamination used in this work, a range of adverse soil conditions may have developed. These may include increased soil temperature (above optimum) due to the imparted dark colour of contaminated soils resulting in increased heat absorption, build-up of toxicity from the spent auto-engine oil and due to anaerobic conditions [4] and reduced soil water necessary for seed germination. [10] working on maize attributed depressed emergence under spent engine oil contamination to oil coating on seed surfaces which affected physiological functions within the seed. [2] reported that upon drying, the soils contaminated with spent engine oil became too hard to allow germination.

TABLE VII
EFFECT OF SPENT AUTO-ENGINE OIL CONTAMINATION AND ORGANIC AMENDMENT ON GERMINATION COUNT OF MAIZE (%)

Year	Oil concentration (A) %	Organic amendments (B) NA	PE	CS	PR	Mean
	0	86	82	89	85	86
2006	1	82	90	88	88	87
	2	32	61	54	50	50
	3	33	55	45	29	41
	Mean	58	72	69	63	
$LSD_{0.05}$	A = 9.5, B = 8.4, A× B = **					
	0	95	100	95	100	98
2007	1	81	92	90	89	88
	2	36	70	62	50	55
	3	36	58	56	52	51
	Mean	62	80	76	83	
$LSD_{0.05}$	A = 10.2, B = 3.5, A× B = *					
	0	100	100	98	92	98
2008	1	88	96	97	92	93
	2	48	78	70	60	64
	3	41	69	68	60	60
	Mean	69	86	83	76	
$LSD_{0.05}$	A = 9.0, B = 5.1, A × B = *					

NA = control, PE = palm oil mill effluent, CS = cassava peels, PR = palm bunch refuse, ns = non significant, ** and* = significant at 1 and 5 % probability level

IV. CONCLUSIONS

It can be concluded from this study that:

1. Spent auto-engine oil contamination of soil increased the total hydrocarbon content and reduced the macro-porosity of treated soils, however the quantity of the spent auto-engine oil and the type of organic amendment applied determined the speed of the remediation process and the depth to which the effects were observed.
2. Among the three organic amendments used, palm oil mill effluent gave the most significant results as a bio-stimulant.
3. Spent auto-engine oil toxicity on maize germination was observed above 1 % concentration while there was an initial depression of microbial activities following impact, however, with organic amendment, there was a significant increase in hydrocarbon degrading microbes which led to faster bioremediation of spent auto-engine oil in amended plots.

REFERENCES

[1] Anon.Collection and Disposal of oily wastes in Nigeria. Report prepared by RRI (Nig.) Ltd. for the petroleum oil wastes Disposal committees, 22nd April 1985.

[2] G.O. Anoliefo and D. E.Vwioko. Effect of spent lubricating oil on the growth of C*apsicum annum* I. and *Lycopersicon esculentum* mill. Environ. Pollution 99:361-364,1995. http://dx.doi.org/10.1016/0269-7491(95)93451-5

[3] M. Sharifi, Y. Sadeghi and M. Akharpour. Germination and growth of six plant species on contaminated soil with spent oil. Inter. J. Environ. Sci. Technol. 4(4): 463 – 470, 2007 http://dx.doi.org/10.1007/BF03325982

[4] E.I. Atuanya. Effect of Waste engine oil pollution on physical and chemical properties of soil. a case study of Delta soil in Bendel State. Niger. J. Appli. Sci. 5:115-176,1987.

[5] O.M. Agbogidi and O.R. Ejemete. An assessment of the effects of crude oil pollution on soil properties, germination and growth of *Gambaya albida* (L.).uniswa, Afri. J. Food Sci. Technol. Vol. 1(1) pp. 125-131, 2005.

[6] M.O. Benka-Coker and J.A. Ekundayo, Effect of an oil spill on soil physico-chemical properties of a spill site in the Niger Delta area of Nigeria. Environmental Monitoring and Assessment 36:93-104, 1995. http://dx.doi.org/10.1007/BF00546783

[7] M.O. Benka-Coker and J.A. Ekundayo, Applicability of evaluating the ability of microbes isolated from an oil spill site to degrade oil. Environ. Monitor. Assess. 45:259-272, 1997. http://dx.doi.org/10.1023/A:1005774602112

[8] L. Avidano, E. Gamalero, G.P. Cossa and E. Carraro, Characterization of Soil health in an Italian Polluted site by using micro-organisms as bioindicators. Appl. Soil Ecol. 30:21- 33, 2005. http://dx.doi.org/10.1016/j.apsoil.2005.01.003

[9] E. Katsivela, E.R.B. Moore, D. Maroukli, C. Strompl. D. Pieper, and N. Kalogerakis, Bacterial community dynamics during in-Situ bioremediation of petroleum waste sludge in land farming sites. Biodegradation 16:160-180, 2005. http://dx.doi.org/10.1007/s10532-004-4883-y

[10] E.O. Ekundayo, T.O. Emede and D.I. Osayande. Effects of Crude oil spillage on growth and yield of maize (*Zea mays* L.) in soils of Mid-western Nigeria. Plant Foods Hum. Nutri., 56:313-324, 2001. http://dx.doi.org/10.1023/A:1011806706658

[11] O.M. Agbogidi, Response of six cultivars of cowpea (*Vigna unguiculata L. Walp*) to spent engine oil. Afri. J. Food Sci. Technol. Vol. 1(6) pp. 139-142, 2010.

[12] E.H. Ahamafule, E.M. Obi, S.M. Amana, P.C. Peter, E.K. Eifediyi, and C.C. Nwokocha. Response of cowpea (*Vigna unguiculata L.Walp*) to changes in hydraulic properties of a spent crank-case oil contaminated *Ultisol* under biostimulation. Nig. J. Agric., Food and Env. 10(4):99 – 105, 2014.

[13] P.E.B. Inyang. Climate Regime In: Nigeria in Maps. Eastern States. (ed.). Ofomata, G.E.K., Ethiope Publ. House. Benin city, Nigeria Pp. 27-29, 1978.

[14] C.L.A. Asadu, A comparative characterization of two foot slopes in Nsukka area of Eastern Nigeria. Soil Sci.150:527 – 534, 1990. http://dx.doi.org/10.1097/00010694-199008000-00006

[15] FORMECU (The Forestry Management, Evaluation and Co-ordination Unit). An Assessment of Vegetation and Land use Changes in Nigeria. Pp.44, 1998.

[16] E. Ahamefule and P.C. Peter. Cowpea (*Vigna unguiculata L.Walp*) response to Phosphorus fertilizer under two tillage and mulch treatments. Soil and Tillage Res.136:70 – 75, 2014. http://dx.doi.org/10.1016/j.still.2013.09.012

[17] C.T.I. Odu, L.C. Nwoboshi, S.O. Fagade, and P.E. Awani, Post impact study of SPDC's Nun River 8" delivery line oil spillage. Final report. SPDC, Nig. Pp. 95, 1989.

[18] F.O.R. Akamigbo and C.L.A. Asadu, Influence of parent materials on the soils of south eastern Nigeria .East. African Forst.J.48:81- 91, 1983.

[19] W. Minas and W. Gunkel. Oil pollution in the North sea. A microbiological point of view. Helgol Meeres 49: 143 – 158, 1995. http://dx.doi.org/10.1007/BF02368345

[20] S.M, Jelena, P. B. Vladimic, V.I. Mila, A.M. Samira. G.D. Goigic-Cvijouic and M.V. Miroslav. Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium. J. Serb. Chem. Soc. 74(4) 455- 460, 2009. http://dx.doi.org/10.2298/JSC0904455M

[21] M.H. Huesemann, and K.O. Moore. Compositional changes during land farming of weathered Michigan crude oil-contaminated soil. J. Soil Contam. 2:245-264, 1993. http://dx.doi.org/10.1080/15320389309383442

[22] I.D. Bossert and G.C. Compeau. Clean up of petroleum hydrocarbon contamination in soil. In: Microbiology Transformation and Degradation of Toxic organic chemicals (eds) L.Y. Young and C.E. Cernigha. Wiley-Liss, New York. Pp 77-125, 1995.

[23] S.B. Hornick, R.H. Fisher and P.A. Paolini. Petroleum wastes. In LandTreatment of Hazardous Wastes. Parr,J.F., Marsh,P.B., and Kla,J.M.,Eds.Noyes Data Corp., Park Ridge,NJ.pp.321 – 337, 1983.

[24] M.J. McInerney,E.W. Weirick,P.K. Sharma and R.M. Knapp. Noninvasive methodology to study the kinetics of microbial growth and metabolism in sudsurface porous materials.Dev.Petrol. Sci.(Microbial Enhancement of Oil Recovery:Recent Advances) 39:151 – 157, 1993.

[25] Eve Riser-Roberts. Remediation of Petroleum Contaminated Soils. Lewis Publishers,U.S.A. 542p, 1998.

[26] H. Nahrawi, M.H.A. Husni, R. Othman and A. Bah. Decomposition of leaf and fine root residues of three different crop species in tropical peat under controlled condition.Malaysian J. Soil.Sc.15:63 – 74, 2011.

[27] P. Morgan and R.J. Watkinson, R. Hydrocarbon degradation in soils and methods for soil biotreatment. Crit.Rev.Biotechnol .8:305 – 333, 1989. http://dx.doi.org/10.3109/07388558909148196

[28] R. Margesin and F. Schinner. Bioremediation (Natural Attenuation and Biostimulation) of diesel-oil contaminated soil in an alpine glacier skiing area. Applied and Environ. Microbiol.vol.67.no.7:Pp 3127 – 3133, 2001. http://dx.doi.org/10.1128/AEM.67.7.3127-3133.2001

[29] D. Delille, B. Delille, E. Pelletier. Effectiveness of bioremediation of crude oil contaminated subantarctic intertidal sediment: The microbial response. Microb. Ecol. 44:118-126, 2002. http://dx.doi.org/10.1007/s00248-001-1047-z

[30] B.E. Rittman and P.L. McCarty. Environmental Biotechnology: principles and applications, McGraw-Hill, New York P. 695, 2001.

[31] J. D. Van Hamme, A. Singh and O.P. Ward. Microbial: Mol. Biol. Rev. 67 p.503, 2003. http://dx.doi.org/10.1128/MMBR.67.4.503-549.2003

[32] E. K. Pfaender and J. V. Klump. Assessment of environmental biodegradation of organic pollutants. Abstr. Annu. mtg. Am. Soc. microbiol. P. 214, 1991.

[33] C. Fu, S. Pfanstiel, C. Gao, X. Yan, R. Govind and H.H. Tabak. Studies on contaminant biodegradation in slurry, water, and compacted soil tube reactors. Environ. Sci.Technol. 30(3):743 – 750, 1996. http://dx.doi.org/10.1021/es940581q

[34] D. E. Vwioko and D.S. Fashemi. Growth response of *Ricinus cummunis L* in spent lubricating oil polluted soil. J. App. Sci. Environ. Mgt. 9(2): 73 – 79, 2005.

[35] G. O. Anoliefo and D. E. Vwioko. Tolerance of *Chromolaen odorata* (L) K. and R. grown in soil contaminated with spent lubricating oil. J. Trop. Biosci. 1(1): 20 - 24, 2005.