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EFFECTS OF ORGANIC SUPPLEMENT ON GROWTH, LEAF CHLOROPHYLL AND NITROGEN INDEX OF CASSAVA (*Manihot esculenta Crantz*) CULTIVATED IN CRUDE-OIL- CONTAMINATED SOIL IN NIGERIA

[1]Ekanem*, J. O., [2]Osu, S. R. and [2]Harrison, U. E
[1]Department of Microbiology, University of Uyo, Akwa Ibom State, Nigeria.
[2]Department of Biology, Akwa Ibom State College of Education, Afaha Nsit,
P. M. B. 1019, Etinan, Nigeria.
***Correspondent's E-mail:** *johnnykoko01@yahoo.com;* **Phone:** +2348063011636

ABSTRACT
A field study was conducted in the 2015-2016 cropping season to investigate the effect of soil amendment on the growth, leaf chlorophyll and nitrogen index of cassava (Manihot esculenta Crantz) cultivated in crude oil contaminated soil located at Ikot Ada Udo, Ikot Abasi Local Government Area LGA) of Akwa Ibom State, Nigeria. The soil samples were amended with organic supplement (palm bunch ash, PBA and dried poultry litter, DPL) singly and in combination using three different cassava cultivars (TMS 30572, NR 8082 and Local variety) where the growth parameters, chlorophyll content and nitrogen index were monitored at eight (8) weeks intervals for twenty-four (24) weeks. The mean results obtained showed that plant height, leaf area and stem girth increased with increase in the number of weeks with PBA+DPL and DPL amended soil showing significant increase in plants height as compared to the PBA amended soil and the control soil. The same trend was observed with other growth parameters. For the plant varieties, growth in TMS 30572 was significantly higher than others except for the 8th week where the local variety was highest with NR 8082 being the least in all growth parameters. After 16 weeks of sprouting, plots treated with PBA had higher number of Nodes and Leaf Nitrogen Index while plots treated with DPL had higher Chlorophyll A and B contents. This study recommended that varieties TMS 30572 and the Local (Nsak Idaha) should be planted on a crude-oil-polluted site and the soil should be amended with organic nutrients to improve the crop growth performance.

Keywords: Soil Amendment, Leaf Chlorophyll, Nitrogen Index, *Manihot Esculenta* Crantz and Contaminated Soil

INTRODUCTION
Oil spills have become a global problem particularly in industrialized and developing countries. Attention has been focused on the marine environment, because oceans and estuaries have generally been the sites of the largest and most dramatic spills (Cooney, 1984). Apparently inevitable spillages, which occur during routine operations of crude oil production, refining, distribution and as a consequence of acute accidents, have generated continuous research interest in this field (Okoh, 2003).

Crude oil pollution can be defined as the introduction of crude oil or its derivatives with its associated gases into the environment (air, water and land). The availability and abundance of this product have led to increasing demands and prices for petroleum products. With the increasing demands for oil to meet the world's energy needs, more petroleum is transported to distance places, and in the process is spilled into the terrestrial and aquatic environments. Oil spillage in terrestrial environment affect the physicochemical properties of the soil thus endangering the growth performance of plants due to prevention of water and oxygen from reaching the plant, thereby resulting to either the suffocation or loss of plant viability (Tanee and Akonye, 2009). Petroleum hydrocarbon contamination may affect plants by retarding seed germination and reducing plant height, stem density, photosynthetic rate and biomass accumulation or resulting in complete mortality (Pezeshki, *et al*., 2000).

Petroleum (crude oil) pollution has been found to affect the cultivation and production of economic crops including cassava especially in the Niger Delta Area. There is therefore, need to remediate such polluted sites to improve plant performances. Environmental Biotechnologies have through decades of intensive study devised means of combating the problems of environmental pollution through a method known as bioremediation. The process of bioremediation involves the transformation and breakdown of complex organic molecules through Biostimulation and Bioaugmentation into simpler substances such as fatty acids, carbon-dioxide and water (Atlas, 1981). Biostimulation is a strategy that involves the addition of soil nutrients, trace minerals, electron acceptors, or electron donors in order to enhance the biotransformation of a wide range of soil contaminants (Li *et al.*, 2010). Biostimulation is relatively trouble-free and inexpensive, relative to other methods; it is the method of bioremediation most frequently used to mitigate soil contamination (Cunningham and Philip, 2000). Biostimulation causes a rapid depletion of the available pools of major hydrocarbons, is easy to maintain and is a cost effective treatment over large areas (Margesin and Schinner, 2001; Bento *et al*., 2005). In biostimulation, the activity of naturally-occurring microbes is stimulated by circulating water-based solutions through contaminated soils. Nutrients (nitrogen, phosphorus, carbon and others), oxygen, or other amendments, may be used to enhance bioremediation and contaminant desorption from subsurface materials (Van Deuren *et al*., 1997). It is possible that even if the native microorganism population is large enough, it does not have the ability to degrade components of high molecular weight or to emulsify insoluble compounds. This study attempts to investigate the effect of Palm Bunch Ash and Dry Poultry Litter singly and in combination as amendments on the Growth performance, Chlorophyll and Nitrogen index of three varieties of cassava (var. TMS 30572, NR 8082 and Local variety).

MATERIALS AND METHODS

The Study Area

The field research was carried out in an oil-impacted-farmland, located around the cork well at Ikot Ada Udo in Ikot Abasi LGA of Akwa Ibom State located in a geopolitical zone called the South-Southern Nigeria. It lies within latitudes 4° 30' and 5° 30' N and longitudes 7° 30' **E** and 8° 20' E. The climate is humid tropical, annual rainfall ranges from more than 3000 mm along the coast to about 2250 mm at the extreme north, with 1 – 3 dry months in the year. Mean annual temperature varies between 26 and 28 °C, while mean relative humidity is 80%. The original natural vegetation which comprised lowland rainforest, mangrove forest and coastal vegetation, has given way to a mosaic farmland/ oil palm forest, riparian forest and oil palm forest (Petters *et al.*, 1989).

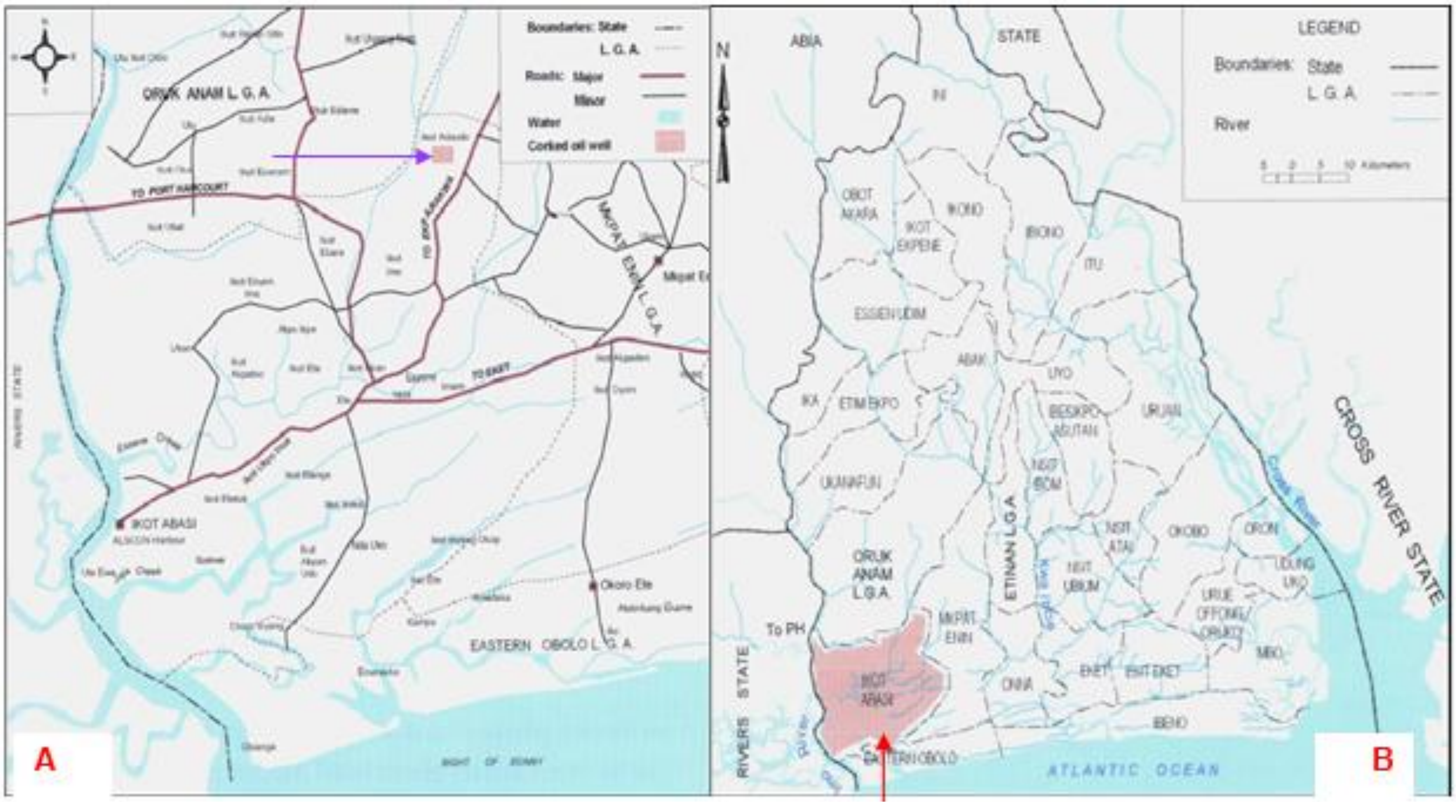

Fig. 1: [A] Map of Ikot Abasi LGA showing the Location of the Corked Oil Well (Ibibio 1) at Ikot Ada Udo
[B] Map of Akwa Ibom State showing Ikot Abasi LGA
Ikot Abasi LGA
Corked Oil Well (Ibibio 1)

Collection of Plant Materials for Cultivation

The cassava cultivars (TMS 30572, NR 8082 and local (Nsak Idaha) were identified by the AKADEP experts in-charge of Ikot Abasi zone and was obtained from Akwa Ibom Agricultural Development Programme (AKADEP) unit, Ikot Abasi zone, Nigeria. Palm Bunch Ash (PBA) was obtained from oil processing mills in Ikot Ada Udo in Ikot Abasi Local Government Area (L.G.A.), while Dried Poultry Litters (DPL) was bought from some poultry farms in Ikot Ada Udo, Ikot Abasi L.G.A. where the study was undertaken. The crude-oil-impacted-soil was obtained from the oil spill affected areas in Ikot Abasi L.G.A. of Akwa Ibom State. Whereas, the unimpacted soil (garden soil) used in this study was obtained from the neighboring L.G.A. about 5km away from Ikot Ada Udo in Ikot Abasi L.G.A.

Physicochemical Analysis of Soil Samples and Amendment

Soil samples were analyzed following the standard procedures outlined by the American Public Health Association (APHA, 1998). Soil pH were measured using Beckman's glass electrode pH meter, organic carbon by the Walkey Black wet oxidation method (Jackson, 1962), available phosphorus by Bray P-1 method (Jackson, 1962). The total nitrogen content was determined by Micro-Kjeldahl method (Jackobson, 1992). Soil particle size distribution was determined by the hydrometer method (Udo and Ogunwale, 1986) using mechanical shaker, and sodium hexametaphosphate as physical and chemical dispersant.

Experimental Design

The experimental site was cleared, tilled, marked and measured as 42 x19 m (198m^2) which is equivalent to 0.0798ha^{-1}. The field was demarcated into 4 major plots for the four treatments (control, palm bunch ash, dry poultry litter and combination of palm bunch ash and dry poultry litter). Each major plot was divided into 3 replicates; each replicate was subdivided into three (3) subplots for the three varieties of cassava, measuring 3m x 3m ($9m^2$) and was separated by 1m apart while the replicate was also separated by 1m apart for easy accessibility. Twenty-five kilograms (25 kg) of palm bunch ash (PBA), dry poultry litter (DPL) and mixture of 12.5 kg of PBA and 12.5 kg of DPL were incorporated into the soil in the randomized complete block design (RCBD). Twenty centimetres (20 cm) of cassava stem cuttings of three cultivars: TMS 3572, NR 8082 and local variety (Nsak Idaha) were planted slanting to make an angle of 45^0 in a row in each subplot at spacing 1m x 1m (Udo *et al*., 2005). Nine (9) stands of cassava were planted in each subplot, giving a total plant population of 324 stands for the 36 subplots.

Data collection

Four plant stands per subplot were randomly selected and tagged for data collection. **Plant height** was measured in centimeters from ground level to the highest point of canopy using tape. **Leaf area index** was obtained by measuring the length and width of four leaflets of the tagged leaves per plant and the product multiplied by a factor (0.45) and divided by plant spacing. **Number of nodes** was obtained by counting the number of functional nodes. **Stem girth** was obtained in centimeters by using a calliper to find the actual girth and the mean was calculated. Chlorophlly a and b were determined by spectrophotometric method (Onwuka, 2005).

A Minolta SPAD-502 leaf chlorophyll meter was used for non- destructive data collection on the chlorophyll and nitrogen status of cassava (*Manihot esculenta* Crantz) to qualitatively evaluate foliage quality. According to Richardson *et al*., (2002) three leaves from matured cassava plants (TMS, NR and LOCAL) were identified for measurement using SPAD 502 chlorophyll meter. Leave measurements were taken at three stages of crop development (Young leaves of 8 weeks old, fully expanded leaves of 16 weeks old and mature and pre-senescenting leaves of 24 weeks old). SPAD meter was pressed onto the leaf surface and a relative non-destructive greenness reading was taken in a few seconds (Peng *et al*., 1993). Triplicate SPAD readings were collected from each of the three (3) leaves of cassava species (TMS, NR and LOCAL) for chlorophyll a & b, and nitrogen concentration index.

With the provision of the USB cable, the chlorophyll meter was later connected to personal computer (PC) for downloading data for further analysis. The rapid test and data gathering capability was to surely provide a tremendous advantage to all types of research. This innovative pocket size instrument was designed to play an important role in improving crop yield and producing higher quality foods (Wang *et al*., 2004)

Statistical Analysis

The data collected on growth parameters (leaf chlorophyll and nitrogen index) were analyzed using Analysis of Variance (ANOVA) and the means were separated using least significant difference (LSD) at $P>0.05$ probability level.

RESULTS AND DISCUSSION

Physico-chemical Properties of Soil of the Experimental Site Prior to Treatment.

The physicochemical properties of soil of the experimental site prior to treatment are presented in Table 1. The soil texture of the experimental site was loamy sand with mean sand fraction of 82.3 %, silt fraction was 6.8 % while clay fraction was 10.9 %. The mean soil pH was moderately acid (6.0) with mean electrical conductivity of 0.03 ds/m indicating that the soil was non-saline. The mean organic carbon was very high (6.0 %), total N was moderate (0.2 %) and mean available P was very high (40.7 mg/kg). Mean exchangeable Ca (4.4 cmol/kg), Na (0.05 cmol/kg), K (0.2 cmol/kg) and ECEC (7.8 cmol/kg) were low while mean exchangeable Mg (1.6 cmol/kg) was moderate. Mean base saturation (80.7 %) and total hydrocarbon content (18.8 mg/kg) were high. The sand fraction, silt, soil pH, available P, Ca, Mg, Na ECEC, base saturation and total hydrocarbon were less variable (> 15 %) in the experimental site. Electrical conductivity (EC), organic carbon, total N and K were moderately variable (15 – 35 %) while clay content was highly variable (> 35 %) in distribution within the experimental site (Table 1).

Chemical Composition of Organic Amendment Used in the Study

The organic amendments used in the field experiment were Dried Poultry Litter (DPL), Palm Bunch Ash (PBA) singly and in combination as presented in Table 2. PBA+DPL recorded the highest pH, which was alkaline (11.5) while PBA had the least pH which was 8.7 and while DPL was mid-way in pH value. The most abundant of all minerals in the organic amendments were K, and Ca with values for DPL, PBA and PBA+DPL being 1107.6, 1117.4 and 1000.4mgkg^{-1}, respectively for K and 24047, 1389 and 27263mgkg^{-1}, respectively for Ca. the third most abundant of the minerals was Na with values for DPL, PBA and PBA+DPL being 757.8, 864.9 and 421.4mgkg^{-1}, respectively. PBA was significantly richer than DPL in Na, K, while DPL was richer in Mg, Ca contents (Table 2), while PBA+DPL were intermediate. However, values of N, phosphate and OC for PBA were higher than those for DPL.

Table 1: Physical and Chemical Properties of the Experimental Soil Prior to Treatment

Soil properties	Minimum	Maximum	Mean	CV (%)
Sand (%)	73.6	85.6	82.3	6.4
Silt (%)	5.2	7.4	6.8	13.0
Clay (%)	7.0	15.0	10.9	35.5
Texture			Loamy sand	
Ph	5.7	6.3	6.0	3.4
EC (ds/m)	0.02	0.05	0.03	32.9
Organic C (%)	5.2	10.5	7.6	26.7
Total N (%)	0.1	0.2	0.2	21.9
Aval. P (mg/kg)	35.0	46.8	40.7	10.1
Exch. Ca (cmol/kg)	3.6	5.2	4.4	13.8
Exch. Mg (cmol/kg)	1.4	1.8	1.6	11.4
Exch. Na (cmol/kg)	0.04	0.06	0.05	14.6
Exch. K (cmol/kg)	0.1	0.9	0.2	17.1
Exch. acidity (cmol/kg)	1.3	2.1	1.7	17.1
ECEC (cmol/kg)	7.1	8.9	7.8	9.6
Base sat. (%)	70.2	90.9	80.7	8.2
TPH (mg/kg)	17.6	20.1	18.8	5.3

Where: TPH-Total Petroleum Hydrocarbon, EC- Electrical conductivity, ECEC- Effective Cation Exchange Capacity, CV- Coefficient of Variation

Table 2: Chemical Composition of Dried Poultry Litter (DPL), Palm Bunch Ash (PBA) and the Combination of Dried Poultry Litter and Palm Bunch Ash (DPL + PBA)

Parameters	DPL	%	PBA	%	DPL +PBA	%
pH	10.4	-	8.7	-	11.5	-
Organic matter (%)	22.5	-	42.0	-	2.4	-
Total N (%)	1.12	-	2.10	-	0.12	-
Available P (mg/kg)	30	0.003	76	0.0076	53	0.0053
Ca (mg/kg)	24047	2.4	1389	0.14	27263	2.73
K (mg/kg)	1107.6	0.11	1117.4	0.11	1000.4	0.1
Na (mgl/kg)	757.8	0.076	864.9	0.086	421.4	0.042
Mg (mg/kg)	478.1	0.048	462.7	0.046	452.8	0.046
Exch. Acidity (meq/100g)	0.2	-	0.2	-	0.2	-
Base saturation (%)	99.7	-	99.6	-	99.9	-

Where; PBA – Palm Bunch Ash; DPL – Dried Poultry Litters

Effect of Soil Amendment and Cassava Variety on Plant Height (cm)

The effect of soil amendment and cassava variety on plant height (cm) is presented in Table 3. Plant height increased with age. Eight (8) weeks after sprouting, the mean plant height of experimental plot treated with dry poultry litter (DPL) (62.7 cm) was significantly higher ($P < 0.05$) than the plot treated with palm bunch ash (PBA) (43.4 cm) but not significantly different from the control (53.2 cm) and plot treated with combination of palm bunch ash and dry poultry litter PBA+DPL (60.7 cm). There was no significant difference in plant heights of local variety (58.4 cm) and TMS 30572 (56.2cm) but they were significantly higher ($P < 0.05$) than that of NR 8082 (50.4 cm). The interaction of local variety with DPL (64.9cm) had the highest plant height while interaction of NR 8082 with PBA (37.1 cm) had the least. After 16 weeks of sprouting, the mean plant height of experimental plot with PBA+DPL (115.5cm) and DPL (111.9cm) were significantly higher ($P < 0.05$) than the plot with palm bunch ash (PBA) (79.7) and control (91.8 cm). There was no significant different ($P < 0.05$) among the varieties after 16 weeks of sprouting. The interaction of TMS 30572 with PBA+DPL (122.8 cm) had the highest plant height while interaction of NR 8082 with PBA (71.2 cm) had the least. After 24 weeks of sprouting, there was no significant difference ($P < 0.05$) in height of plants treated with DPL and PBA+DPL but they were significantly different from height of plants treated with PBA as well as the Control. The plant height of TMS 30572 (158.6 cm) was significantly higher ($P < 0.05$) than that of NR 8082 (125.6 cm) but not different from that of the local variety (154.1 cm). The interaction of local variety with PBA+DPL (182.1 cm) had the highest plant height while interaction of NR 8082 with Control (127.4 cm) had the least.

Effect of Soil Amendment and Cassava Variety on Leaf Area Index

The effect of soil amendment and cassava variety on leaf area index is presented in Table 4. Eight (8) weeks after sprouting, the mean leaf area index of experimental plot treated with DPL (310.2) was significantly higher ($P < 0.05$) than the plot with combination of palm bunch ash and dried poultry litter (PBA+DPL) (300.8) and control (303.1) but not different from plot treated with PBA (309.7). The mean leaf area index of TMS 30572 variety (337.9) was significantly higher ($P < 0.05$) than that of local (300.3) and NR 8082 (279.8). The interaction of TMS 30572 variety with DPL (355.0) had the highest leaf area index while interaction of local variety with DPL (261.8) had the least. After 16 weeks of sprouting, the mean leaf area index of experimental plot with DPL (333.7) was significantly higher ($P < 0.05$) than the Control (306.4) but not significantly different from plot with combination of palm bunch ash and dried poultry litter (PBA+DPL) (323.1) and plot with PBA (315.2). The mean leaf area index of TMS 30572 variety (346.7) was significantly higher ($P < 0.05$)

than that of local (318.8) and NR 8082 (293.4). The interaction of TMS 30572 variety with PBA+DPL (361.3) had the highest plant leaf area while interaction of local and NR 8082 with DPL (285.1) had the least. After 24 weeks of sprouting, there was no significant different (P < 0.05) in leaf area among the bio-stimulants under consideration and control. The leaf area index of TMS 30572 variety (350.3) was significantly higher (P < 0.05) than that of NR 8082 (297.3) but not different from that of local (322.4). The interaction of TMS 30572 variety with PBA+DPL (362.1) had the highest plant leaf area while interaction of NR 8082 with DPL (290.2) had the least.

Table 3: Effect of Soil Amendment and Cassava Variety on Plant Height (cm)

Soil x Cultivar	**8WAS**				**16WAS**				**24WAS**			
	Local	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**
PBA	48.1	37.1	44.9	**43.4**	82.3	71.2	85.6	**79.7**	142.4	137.6	146.1	**141.0**
DPL	64.9	59.4	63.7	**62.7**	116.7	100.4	118.6	**111.9**	168.2	131.7	171.5	**157.1**
PBA+DPL	61.4	57.2	63.7	**60.7**	117.0	106.8	122.8	**115.5**	182.1	128.7	181.5	**164.1**
Control	59.2	47.9	52.6	**53.2**	95.0	87.6	92.9	**91.8**	123.6	127.4	135.2	**128.7**
Mean	**58.4**	**50.4**	**51.5**		**102.8**	**91.5**	**105.0**		**154.1**	**125.6**	**158.6**	
LSD (0.05	Amendment	7.9			17.9				37.7			
	Cultivar	6.9			15.5				32.7			
	Interaction	13.7			31.1				65.3			

TMS – Tropical Manihot Specie; NR – New Release 8082; LV – Local variety (Nsak Idaha)
PBA – Palm Bunch Ash; DPL – Dried Poultry Litter;
LSD – least significant difference; WAS - weeks after sprouting

Table 4: Effect of Soil Amendment on Leaf Area

Soil x Cultivar	**8WAS**				**16WAS**				**24WAS**			
	Local	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**
PBA	294.7	295.1	339.4	**309.7**	302.1	298.4	345.0	**315.2**	303.2	298.5	346.2	**315.9**
DPL	313.9	261.8	355.0	**310.2**	360.0	285.1	356.2	**333.7**	360.0	290.2	361.4	**337.2**
PBA+DPL	295.2	272.0	335.4	**300.8**	312.4	295.7	361.3	**323.1**	325.2	305.4	362.1	**330.9**
Control	297.3	290.1	322.0	**303.1**	300.7	294.4	324.2	**306.4**	301.2	295.1	331.4	**309.2**
Mean	**300.3**	**279.8**	**337.9**		**318.8**	**293.4**	**346.7**		**322.4**	**297.3**	**350.3**	
LSD (0.05	Amendment	47.0			54.4				64.9			
	Cultivar	40.7			47.1				56.2			
	Interaction	81.5			94.2				112.4			

TMS – Tropical Manihot Specie; NR – New Release 8082; LV – Local variety (Nsak Idaha)
PBA – Palm Bunch Ash; DPL – Dry Poultry Litter;
LSD – least significant difference; WAS - weeks after sprouting

Effect of Soil Amendment and Cassava Variety on Stem Girth (Cm)

The effect of soil amendment and cassava variety on stem girth is presented in Table 5. Stem girth increased with age. Eight (8) weeks after sprouting, the mean stem girth of experimental plot treated with PBA+DPL (4.2 cm) was significantly higher (P < 0.05) than the plot treated with PBA (3.5 cm) but not different from the plot treated with DPL (4.1) and control (3.8 cm). The mean stem girth of TMS 30572 variety (4.3 cm) was significantly higher (P < 0.05) than that of local (3.9 cm) and NR

8082 (3.6 cm). The interaction of TMS 30572 variety with PBA+DPL (4.9 cm) had the highest stem girth while interaction of local variety with PBA (3.3 cm) had the least. After 16 weeks of sprouting, the mean stem girth of experimental plot with PBA+DPL (5.5 cm) and DPL (5.5 cm) was significantly higher ($P < 0.05$) than the plot with PBA (4.0 cm) and control (4.6 cm). The mean stem girth of TMS 30572 variety (5.2 cm) was significantly higher ($P < 0.05$) than that of NR 8082 (4.4 cm) but not different from the local variety (5.2 cm). The interaction of TMS 30572 variety with PBA+DPL (6.4cm) had the highest stem girth while interaction of NR 8082 variety with PBA (3.7 cm) had the least. After 24 weeks of sprouting, there was no significant difference ($P < 0.05$) in stem girth among the bio-stimulants under consideration except for Control. The mean stem girth of TMS 30572 variety (8.1 cm) was significantly higher ($P < 0.05$) than that of NR 8082 (5.8 cm) but not different from the local variety (7.2 cm). The interaction of TMS 30572 variety with PBA+DPL (9.2 cm) had the highest stem girth while interaction of NR 8082 variety with Control (5.1 cm) had the least

Table 5: Effect of Soil Amendment on Stem Girth (cm)

Soil x Cultivar	8WAS				16WAS				24WAS			
	Local	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**
PBA	3.3	3.5	3.6	**3.5**	4.4	3.7	4.0	**4.0**	7.1	6.5	7.3	**7.0**
DPL	4.1	3.8	4.5	**4.1**	5.9	5.0	5.5	**5.5**	7.5	5.8	9.0	**7.4**
PBA+DPL	4.1	3.7	4.9	**4.2**	5.3	4.9	6.4	**5.5**	7.7	5.8	9.2	**7.6**
Control	4.0	3.4	4.1	**3.8**	5.1	4.0	4.7	**4.6**	6.3	5.1	6.8	**6.1**
Mean	**3.9**	**3.6**	**4.3**		**5.2**	**4.4**	**5.2**		**7.2**	**5.8**	**8.1**	
LSD (0.05	Amendment	0.5			0.6				2.1			
	Cultivar	0.4			0.5				1.8			
	Interaction	0.8			1.1				3.7			

TMS – Tropical Manihot Specie; NR – New Release 8082; LV – Local variety (Nsak Idaha)
PBA – Palm Bunch Ash; DPL – Dry Poultry Litter;
LSD – least significant difference; WAS - weeks after sprouting

Effect of Soil Amendment and Cassava Variety on Number of Nodes

The effect of soil amendment and cassava variety on number of nodes is presented in Table 6. Number of nodes increased with age. Eight weeks after sprouting, the mean number of nodes of experimental plot treated with PBA (26.3) was significantly higher ($P < 0.05$) than the plot treated with combination of palm bunch ash (PBA) and dried poultry litter (DPL) (23.8) but not different from plot with DPL (25.9) and control (25.0). There was no significant difference ($P < 0.05$) in number of nodes among the varieties under consideration. The interaction of TMS 30572 variety with PBA (27.8) had the highest number of nodes while interaction of TMS 30572 variety with DPL+PBA (22.7) had the least. After 16 weeks of sprouting, the mean number of nodes of experimental plot with PBA (51.0) was significantly higher ($P < 0.05$) than the plot with combination of palm bunch ash (PBA) and dried poultry litter (DPL) (44.9) but not different from plot with DPL (49.7) and control (46.1). There was no significant difference ($P < 0.05$) in number of nodes among the varieties under consideration. The interaction of TMS 30572 variety with PBA (53.7) had the highest number of nodes while interaction of TMS 30572 variety with DPL+PBA (41.6) had the least. After 24 weeks of sprouting, there was no significant difference ($P < 0.05$) in number of nodes among the bio-stimulants under consideration. The mean number of nodes of local variety (104.2) was significantly higher ($P < 0.05$) than that of NR 8082 (85.6) but not different from the TMS 30572 variety (92.5). The interaction of local variety with DPL (112.5) had the highest number of nodes while interaction of local variety with DPL+PBA (93.9) had the least.

Table 6: Effect of Soil Amendment on Number of Nodes

Soil x Cultivar	8WAS				16WAS				24WAS			
	Local	NR	TMS	Mean	Local	NR	TMS	Mean	Local	NR	TMS	Mean
PBA	25.8	25.3	27.8	**26.3**	55.1	44.2	53.7	**51.0**	105.1	96.1	101.6	**100.9**
DPL	25.8	25.6	26.1	**25.9**	53.4	47.7	48.1	**49.7**	112.5	79.4	93.4	**95.1**
DPL+PBA	23.0	25.9	22.7	**23.8**	50.0	43.0	41.6	**44.9**	93.9	79.7	79.0	**84.2**
Control	27.8	24.0	23.3	**25.0**	49.7	43.9	44.8	**46.1**	105.6	87.3	96.0	**96.3**
Mean	**25.6**	**25.2**	**25.0**		**52.1**	**44.7**	**47.0**		**104.2**	**85.6**	**92.5**	
LSD (0.05	Amendment	2.2			5.7				21.0			
	Cultivar	1.9			4.9				18.2			
	Interaction	3.7			9.9				36.4			

TMS – Tropical Manihot Specie; NR – New Release 8082; LV – Local variety (Nsak Idaha)
PBA – Palm Bunch Ash; DPL – Dry Poultry Litter;
LSD – least significant difference; WAS - weeks after sprouting

Effect of Soil Amendment and Cassava Variety on Chlorophyll – a ($C_{55}H_{7705}N_4Mg$)

The effect of soil amendment and cassava variety on percentage concentration of chlorophyll-a is presented in Table 7. Eight (8) weeks after sprouting, the mean percentage concentration of chlorophyll -a form experimental plot treated with control (26.3 %) was significantly higher ($P < 0.05$) than the plot treated with (DPL) (23.8 %) but not different from the plot treated with combination of palm bunch ash (PBA) and dried poultry litter DPL (25.9 %) and PBA (25.0 %). There was no significant difference ($P < 0.05$) in percentage concentration of chlorophyll- an among the varieties under consideration. After 16 weeks of sprouting, the mean percentage concentration of chlorophyll an of experimental plot with PBA+DPL (36.0 %) was significantly higher ($P < 0.05$) than PBA (33.4 %) but not different from plot with Control (35.4 %) and plot with (DPL) (35.1%). The mean percentage concentration of chlorophyll a form experimental plot with TMS 30572 (36.8 %) was significantly higher ($P < 0.05$) than the plot with NR 8082 (33.5 %) and local (34.5 %). The interaction of TMS 30572 variety with PBA+DPL (40.5 %) had the highest percentage concentration of chlorophyll- a while interaction of TMS 30572 variety with DPL (33.1 %) had the least. After 24 weeks of sprouting, the mean percentage concentration of chlorophyll a form experimental plot with PBA+DPL (35.2 %) was significantly higher ($P < 0.05$) than the plot with (DPL) (32.2%) but not different from plot with Control (33.1 %) and PBA (34.8 %). The mean percentage concentration of chlorophyll a form experimental plot with TMS 30572 (36.0 %) was significantly higher ($P < 0.05$) than the plot with NR 8082 (33.0 %) and local (32.4 %). The interaction of TMS 30572 variety with PBA+DPL (37.8 %) had the highest percentage concentration of chlorophyll- a while interaction of local variety with DPL (29.2 %) had the least.

Effect of Soil Amendment and Cassava Variety on Chlorophyll – b ($C_{55}H_{7006}N_4Mg$)

The effect of soil amendment and cassava variety on percentage concentration of chlorophyll-b is presented in Table 8. Eight (8) weeks after sprouting, the mean percentage concentration of chlorophyll -b from experimental plot treated with Control (4.2 %) was significantly higher ($P < 0.05$) than the plot treated with (DPL) (3.5 %) but not different from plot treated with PBA+DPL (4.1 %) and PBA (3.8 %). The mean percentage concentration of chlorophyll–b from experimental plot with TMS 30572 (4.3 %) was significantly higher ($P < 0.05$) than the plot with NR 8082 (3.6

%) and local (3.8 %). The interaction of TMS 30572 variety with PBA+DPL (4.9 %) had the highest percentage concentration of chlorophyll- b while interaction of local variety with DPL (3.3 %) had the least. After 16 weeks of sprouting, the mean percentage concentration of chlorophyll -b of experimental plot with PBA+DPL (24.0 %) was significantly higher ($P < 0.05$) than PBA (22.2 %) but not different from plot with the control (23.6 %) and plot with (DPL) (23.4 %). The mean percentage concentration of chlorophyll–b from experimental plot with TMS 30572 (24.6 %) was significantly higher ($P < 0.05$) than the plot with NR 8082 (22.4 %) and local (23.0 %). The interaction of TMS 30572 variety with PBA+DPL (27.0 %) had the highest percentage concentration of chlorophyll- b while interaction of local variety with PBA (20.9 %) had the least. After 24 weeks of sprouting, the mean percentage concentration of chlorophyll -b from experimental plot with PBA+DPL (23.4 %) was significantly higher ($P < 0.05$) than the plot with the control (19.6 %) but not different from the plot with (DPL) (21.8 %) and PBA (23.1 %). There was no significant different ($P < 0.05$) in percentage concentration of chlorophyll a among the varieties under consideration. The interaction of TMS 30572 variety with PBA+DPL (25.1 %) had the highest percentage concentration of chlorophyll- b while interaction of TMS 3572 variety with the control (15.9 %) had the least.

Effect of Soil Amendment and Cassava Variety on Leaf Nitrogen Index

The effect of soil amendment and cassava variety on mean leaf N index is presented in Table 10. Eight (8) weeks after sprouting, there was no significant different ($P < 0.05$) in leaf Nitrogen index in experimental plots with different cassava varieties and different stimulants under consideration and control. After 16 weeks of sprouting, the mean leaf N index of experimental plot with the control (0.31 %) was significantly higher ($P < 0.05$) than the PBA (0.26 %) but not different from plot treated with PBA+DPL (0.29 %) and plot treated with (DPL) (0.28 %). There was no significant difference ($P < 0.05$) in leaf nitrogen index in plots with different cassava varieties under consideration. The interaction of local variety with the control (0.34 %) had the highest mean leaf N index while interaction of local variety with PBA (0.24 %) had the least. After 24 weeks of sprouting, there was no significant difference ($P < 0.05$) in leaf nitrogen index in experimental plots with different stimulants and control. The mean leaf N index of experimental plot with TMS 30572 (0.31 %) was significantly higher ($P < 0.05$) than that of NR 8082 (0.26 %) and local (0.26 %). The interaction of TMS 3572 variety with PBA+DPL (0.30 %) had the highest mean leaf N index while interaction of local variety with DPL (0.25 %) had the least.

Table 7: Effect of Soil Amendment on Chlorophyll –a ($C_{55}H_{7705}N_4Mg$)

Soil x Cultivar	**8WAS**				**16WAS**				**24WAS**			
	Local	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**
PBA	27.8	24.0	23.3	**25.0**	31.3	32.9	35.8	**33.4**	32.6	34.0	37.7	**34.8**
DPL	23.0	25.9	22.7	**23.8**	39.1	33.2	33.1	**35.1**	29.2	33.6	33.7	**32.2**
PBA+DPL	25.8	25.6	26.1	**25.9**	34.4	33.1	40.5	**36.0**	34.4	33.3	37.8	**35.2**
Control	25.8	25.3	27.8	**26.3**	33.2	35.0	37.9	**35.4**	33.2	31.2	34.9	**33.1**
Mean	**25.6**	**25.2**	**25.0**		**34.5**	**33.5**	**36.8**		**32.4**	**33.0**	**36.0**	
LSD (0.05	Amendment	2.2			1.6				2.5			
	Cultivar	1.9			1.4				2.1			
	Interaction	3.7			2.8				4.3			

TMS – Tropical Manihot Specie; NR – New Release 8082; LV – Local variety (Nsak Idaha)
PBA – Palm Bunch Ash; DPL – Dry Poultry Litter;
LSD – least significant difference; WAS - weeks after sprouting

Table 8: Effect of Soil Amendment on Chlorophyll –b ($C_{55}H_{7006}N_4Mg$)

Soil x Cultivar	8WAS				16WAS				24WAS			
	Local	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**
PBA	4.0	3.4	4.1	**3.8**	20.9	22.0	23.9	**22.2**	21.7	22.6	25.1	**23.1**
DPL	3.3	3.5	3.6	**3.5**	26.1	22.1	22.1	**23.4**	20.5	22.3	22.4	**21.8**
PBA+DPL	4.1	3.8	4.5	**4.1**	22.9	22.0	27.0	**24.0**	22.9	22.2	25.1	**23.4**
Control	4.1	3.7	4.9	**4.2**	22.2	23.4	25.3	**23.6**	22.1	20.8	15.9	**19.6**
Mean	**3.8**	**3.6**	**4.3**		**23.0**	**22.4**	**24.6**		**21.8**	**22.0**	**22.1**	
LSD (0.05	Amendment	0.5			1.1				3.4			
	Cultivar	0.4			0.9				2.9			
	Interaction	0.7			1.8				5.9			

TMS – Tropical Manihot Specie; NR – New Release 8082; LV – Local variety (Nsak Idaha)
PBA – Palm Bunch Ash; DPL – Dry Poultry Litter;
LSD – least significant difference; WAS - weeks after sprouting

Table 10: Effect of Soil Amendment on Leaf Nitrogen Index

Soil x Cultivar	8WAS				16WAS				24WAS			
	Local	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**	**Local**	**NR**	**TMS**	**Mean**
PBA	20.4	22.5	21.0	**21.3**	0.24	0.26	0.29	**0.26**	0.26	0.27	0.30	**0.28**
DPL	21.2	20.9	22.1	**21.4**	0.31	0.27	0.27	**0.28**	0.25	0.27	0.27	**0.26**
PBA+DPL	21.6	21.3	22.2	**21.7**	0.27	0.27	0.32	**0.29**	0.27	0.27	0.30	**0.28**
Control	22.1	19.5	22.1	**21.3**	0.34	0.28	0.30	**0.31**	0.26	0.25	0.28	**0.26**
Mean	**21.3**	**21.0**	**21.9**		**0.29**	**0.27**	**0.29**		**0.26**	**0.26**	**0.29**	
LSD (0.05	Amendment	2.1			0.04				0.02			
	Cultivar	1.8			0.04				0.01			
	interaction	3.6			0.07				0.03			

TMS – Tropical Manihot Specie; NR – New Release 8082; LV – Local variety (Nsak Idaha)
PBA – Palm Bunch Ash; DPL – Dry Poultry Litter;
LSD – least significant difference; WAS - weeks after sprouting

DISCUSSION

From the results, 8 weeks after spouting, cassava experimental plots treated with palm bunch ash (PBA+DPL) had higher plant height, leaf area index and stem girth while PBA was higher in the number of nodes, chlorophyll –a and b compared to others. After 16 weeks, the trend was sustained for plant height, leaf area index and stem girth with PBA+DPL being highest while plots treated with PBA had higher number of nodes and leaf Nitrogen index with plots treated with DPL (dried poultry litter) having the highest plant chlorophyll a and b content compared to others. The trend in superiority of growth indices was as follow: plots treated with PBA+DPL > plots treated with DPL > plots treated with PBA > plots treated with no soil amendment (control). The increase in plant height, leaf area index, stem girth, number of nodes, chlorophyll –a and b in plots treated with PBA+ DPL compared to others could be attributed to the effect of soil amendment (PBA and DPL), which stimulated the establishment and growth of beneficial micro-organisms and also humic and fulvic acids content of the bio-stimulants (Pinton *et al*., 2009). Phytohormones like auxins, cytokinins, gibberellins and ethylene are synthesized by beneficial micro-organisms which promotes cell division and elongation, apical dominance, root initiation, differentiation of vascular tissue, ethylene biosynthesis and the expression of specific genes (Idris *et al*., 2007; Dodd *et al*., 2010). Among the varieties under consideration, TMS 30572 had the highest leaf area index, stem girth, chlorophyll- a and b, while local variety had highest plant height, number of nodes. NR 8082 was the least in all the parameters under consideration. The varietal difference among the cultivars could be attributed to genotypic differences between them, indicating that TMS 30572 had high root/shoot ratio and more tolerance to crude oil contaminated soil, followed by local variety while NR 8082 had the least (Ikeorgu and Ogbanna, 2009). The result is also in line with the studies of Essien *et al*., (1998) and Esenowo *et al*., 2012 who reported that oil contaminated soil treated with organic supplements was less toxic to corn, wheat and cassava root and shoot elongation.

CONCLUSION

Among the bio-stimulants under consideration, PBA+DPL were superior in biodegradation of hydrocarbon and enhance plant nutrition, growth and health, followed by DPL while PBA was the least. Among the varieties under consideration, TMS 30572 had high root/shoot ratio and more tolerance to crude oil contaminated soil, followed by local variety while NR 8082 had the least. It was also observed that there were differences in the way crude oil pollution affected the different plant varieties as it was less toxic to TMS 30572 and the Local variety compared to NR 8082. Thus, amended plots increased the soil physical properties and better yields than the control and it is recommended that farmers should amend their soil with organic supplements and use improved plant varieties such as TMS 30572 to remediate crude oil contaminated soil.

REFERENCES

American Public Health Association, APHA. (1998). Standard Methods for the Examination of Water and Waste Water. 16th Ed. Washington, D C.: America Public Health Association p. 65.

Atlas RM (1981). Microbial degradation of petroleum hydrocarbons: An environmental perspective. *Microbiology Reviews*, 45: 180-200.

Bento, F. M., Carmago, F.O.A., Okeke, B.C., Frankenberger, W.T (2005). Comparative bioremediation of soil contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*, 96:1049-1055.

Cooney, J. J. (1984). *The fate of petroleum pollutants in freshwater ecosystems*, In R. M. Atlas (ed.), Petroleum microbiology (p. 399-434). New York. Macmillan Publishing Co.

Cunningham, C. J. and Philp, J. C. 2000. Comparison of bioaugmentation and biostimulation in *ex situ* treatment of diesel contaminated soil. *Land Contamination and Reclamation* 8: 261-269.

Dodd, I. C., Zinovkina, N. K., Safronova, V. I. and Belimov, A. A. (2010). Rehizobacterial mediation of plant homone stately. *Ann. Appl. Biol*. 157: 361-379.

Esenowo, G. J., Okon, J. E., and Umoh, N. S (2012). Effect of Crude Oil Pollution of Soil and Amelioration Treatment on the Growth of Eight Varieties of *Manihot Esculenta* Crantz *International Journal of Chemical, Environmental and Pharmaceutical Research* Vol. 3, No.2, 163-169

Essien, J. P., Ubom, R. M. and Udosen, E. D. (1998). Bioremediation of Petroleum Contaminated Soil, Effect on the Population Dynamics and Degrading Capabilities of Hydrocarbonoclastic Bacteria. *West African Journal of Biological and Applied Chemistry*, Vol. 3, pp. 22-28.

Idris, E. E., Iglesias, E. J., Talon, M. And Borriss, R. (2007). Tryptophan-dependent production of Indole-3-acetic acid (IAA) affects level of plant growth promotion by

Bacillus amyloliquefaciens FZB42. *Nol. Plant-microbe Interact* 20: 619-626.

Ikeorgu, J. G. and Ogbanna, M. R. (2009). Varietal response of 7 hybrid white yam (D. rotundata) Genotypes to minic tuber production. In: Proceedings of the 43rd annual conference of the Agricultural Society of Nigeria. Abuja 2009: 160-163.

Jackobson, ST (1992). Chemical Reactions and Air Change during Decomposition of Organic Matter. *Research Conservation and Recycle*, 6, 259 - 266.

Jackson, MI. (1962). *Soil Chemical Analysis*. New Jersey: Prentice-Hall Inc. Englewood Cliffs. pp. 498.

Li, C.H, Wong, Y. S, Tam, N. F.(2010). Anaerobic biodegradation of polycyclic aromatic hydrocarbons with amendment of iron(III) in mangrove sediment slurry. *Bioresource Technology* 101: 8083−8092.

Margesin, R., and Schinner, F. (2001). Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an Alpine glacier skiing area. Applied and *Environmental Microbiology*, 67, 3127–3133.

Okoh, A. I. (2003). Biodegradation of bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria, *African Biotech*, 2:140

Onuwuka, G. I. (2005). Food Analysis and Instrumentation (Theory & Practice). Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Umuahia, Nigeria: 63-84.

Peng, S., Garcia, F. V., Laza, R. C., Cassman, K. G. (1993). Adjustment for Specific Leaf Weight Improves Chlorophyll Meter's Estimate of Rice Leaf Nitrogen Concentration. *Agron. J*. 85. 987-900.

Pezeshki, S.R., Hester, M.W., Lin,Q. And Nyman, J.A. (2000). The effects of oil spill and clean-up on dominant US Gulf coast march microphytes*; a review*. *Environmental pollution* 108: 129-139.

Pinton, R., Cesco, S., Iacoletting, G., Astolfi, S. and Varanino, Z. (2009). Modulation plasma membrane H^+ ATPase. *Plant Soil 215:* 155-161.

Richardson AD, Duigan SP, Berlyn GP (2002). An evaluation of non-invasive method to estimate foliar chlorophyll content. *New Phytologist* 153:185-194.

Tanee, F.B.G. and Akonye, L.A. (2009). Effectiveness of *Vigna unguiculata* as a phytoremediation plant in the remediation of crude oil polluted soil for cassava (*Manihot esculenta*,Crantz) cultivation. *J. Apl. Sci. Environ. Manage*., 13(1): 43-47.

Udo E. J., Ogunwale T. A. (1986). Laboratory manual for the analysis of soil, plant and water samples, 2nd ed., University Press, Ibadan Nigeria, pp.10-11.

Udo, D. J., Ndon, B. A., Asuquo, P. E. and Ndaeyo, N. U. (2005). Crop Production Techniques for the tropics. Concept Publication Limited, Lagos: 188-195.

Van Deuren, J., Wang, Y and Ledbetter, J (1997*). Remediation Technologies Screening Matrix and Reference Guide.* Tercera edition. Technology Innovation Office, EPA. http://www.epa.gov/tla/remed.htm.

Wang Q. B., Chen M.J., and Li Y.C., 2004. Non-destructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. *J. Plant Nutr.* 27: 557 – 569.