PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 25 (1) 5-14 January 2021

Remediation of Crude Oil Contaminated Soil, Using Organic Supplement: Effects on Growth and Heavy Metal Uptake in Cassava (*Manihot esculenta* Crantz)

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ABSTRACT: Field experiments were conducted in 2016 and repeated in 2017 to evaluate the effectiveness of Oil Palm Bunch Ash (OPBA) and Dried Poultry manure (DPM) as organic supplement, applied singly and in combination on growth properties and heavy metal uptake of cassava cultivars grown in crude-oil contaminated soil of the Niger Delta Region, Nigeria. There were four different organic amendments viz: DPM, OPBA, OPBA +DPM and control using TMS 30572, NR8082 and local variety as test crops. Results were elaborated statistically with the use of three-by-four factor variance analysis with ANOVA. Effect of various application of amendment on morphometric parameters of cultivars revealed in 2017 that at 36 weeks after sprouting (WAS), DPM and OPBA +DPM significantly ($P \leq 0.05$) enhanced plant heights of TMS 3572 and LV but had no influence on number of nodes, stem girth and leaf area. However, the interactions resulted in significant ($P \leq 0.05$) uptake and concentration of the heavy metals Fe, Cu, and Pb in leaves and Fe in roots of TMS30572 and NR8082, indicating efficient metal removal by these varieties. Results of amendment also revealed remarkable increment in bacterial and fungal (from 10^4 to 10^6 and 10^3 to 10^4) populations respectively, correlating with reduction in soil total petroleum hydrocarbon (TPH) from 156.45 mg/kg to 146.73 mg/Kg. This is observed to diminish oil toxicity and improve soil status. The response of these cassava varieties to crude oil contamination appears to be optimistic. Consequently crop farmers are adviced against planting cassava and other arable crop at crude oil impacted soil for the risk of uptake of heavy metal in crop tissues.

DOI: https://dx.doi.org/10.4314/jasem.v25i1.1

Dates: Received: 02 October 2020; Revised: 26 November 2020; Accepted: 12 December 2020

Keywords: Remediation, Heavy metal, Oil-contaminated soil, Organic amendment, Growth properties, Cassava

In the Niger Delta region of Nigeria, cultivated arable land and available terrestrial ecosystem are characterized by the presence of high concentration of petroleum hydrocarbon. Harrison *et al.* (2018) posited that sources of the hydrocarbon such as crude oil explorations/exploitation activities, including leaks from corroded equipment and vandalisation through various transport mechanisms and exposure pathways, the pollutants get to receptors which include soil, sediments, surface and underground water source, plants, animals and man (Etuk *et al.,* 2013). When present in these matrices, the crude oil pollutant exhibit phenomenal toxicity that is related to the type of organism, nature of the system and concentration of the crude oil. In the soil matrix, crude oil adversely affect its fertility including nutrient status and soil enzymes (Udofia, 2018), other biophysical properties (Wang *et al.,* 2013), thus, presenting adverse effects on crops and vegetations (Ezeji *et al*., 2007 and Doelsch *et al*., 2006). Among the few remaining resilient crops in the region is cassava (*Manihot esculenta* Crantz). The crops is a dicotyledonous plant that belong to the family *Euphorbiaceae* (Aro *et al.,* 2010) grown over a large of climate and altitude on wide variety of soils. Cassava is a drought resistant crop that can adapt to wide variety of edaphic influences. The crop is grown by the almost every farming family in the Niger Delta region of the country, accounting for the daily calorie intake (Tewe and Lutaladio, 2004; FAO, 2006) and constituting one of the most important sources of carbohydrate for both humans and animal with about 93% of the produce being used as food (Okon *et al.*, 2012). Apart from food security threats due to crop yield and productivity decline, cassava grown in crude oil-impacted soils are known to accumulate heavy metals with a high consequence of transfer within the food web (Udousoro *et al.,* 2018). Heavy metals are often toxic to man and other organisms with human exposure coming through various routes including metal contaminated ground water, soil, dietary supplements and food (Ibrahim *et al.,* 2006). Heavy metals are known to be associated with crude oil such that metals are common in oil-impacted environments and pollution therefore is of global concern (Udofia,

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2010; Liang *et al.,* 2011). The presence of crude oil alongside heavy metals in major ecosystem of the Niger Delta region has brought issues of environmental and public health to the front burner and the repercussions have drawn attention of researchers to further explore eco-friendly approaches toward mitigation and soil restoration efforts. Options under consideration in remediation are chemical, physical and biological (bioremediation) methods. Though there are several bioremediation techniques, however, bio-stimulation and phytoremediation have been commonly applied with much success (Vidali, 2001; Xu *et al.,* 2005; Udofia, 2018). Bio-stimulation is the addition of oxygen, electron, donor, electron acceptors or nutrients to coordinates sites in order to increase the population and activities of naturally occurring organisms for them to remedy sites of pollutants (Perfomo, 2007). Some of the materials that have been used locally in this direction are oil palm bunch ashes (OPBA) and poultry manure (DPM). These materials are considered important soil supplement that can improve both biophysical and chemical properties of impacted soils. Researchers on application of OPBA have presented several reports including reduction in acidity and increased pH (Odedina *et al.,* 2003; Ogbuehi, 2006) improved soil chemical properties and increased soil nutrients (Awodun *et al.,* 2007; Ezekiel *et at.,* 2009) and increased supply of Na, K, P, Ca and Mg (Oyeniyi *et al.,*2010). Similarly, application of bedding materials enriched with poultry droppings have been reported to improve soil physical properties by reducing soil bulk density, increasing total porosity and moisture content (Agbede *et al.,* 2008). Ibeawuchi *et al.* (2006) further reported that application of poultry manure increased residual soil N, K, Ca, Mg, P and organic matter. According to Mullens *et al.* (2002), poultry droppings contain considerable amount of nitrogenous materials and the carbonates. These amendments material apart from improving soil chemical and physical conditions also enhances growth and multiplication of autochthonous microbial population whose activities usually aid in elimination of pollutants and increase soil fertility. Following a devastating event of oil spill from corked wells in the study area in 2007 when a large volume of crude oil discharge spread over an extensive area of farmland, ponds and streams. This study was conducted to assess the persistence and residual effect of hydrocarbon on soil properties, plant growth and heavy metal removal efficiencies of cassava cultivars-a decade after the main pollution event.

MATERIALS AND METHODS

Site Description: Ikot Ada Udo, a Village in Ikot Abasi Local Government of Niger Delta is one of the on-shore locations where hydrocarbon was discovered in commercial quantities more than 50 years ago in Nigeria, but has remained untapped. During the years these wells remained corked, oil and gas have spilled from the corked wells several times (Udo, 2008). A major spill event occurred between August and November, 2007 with extensive land area and aquatics system pollution. Following this large-scale spill, surveys were conducted in 2008 (Udo, 2008) and in 2012 (Udo and Chukwu, 2014) to assess the effects of the pollution on land/soil, plants and aquatic lives and other components of the impacted environment. A field experiment was conducted under natural climatic and environmental conditions in 2016 and repeated in 2017 planting seasons.

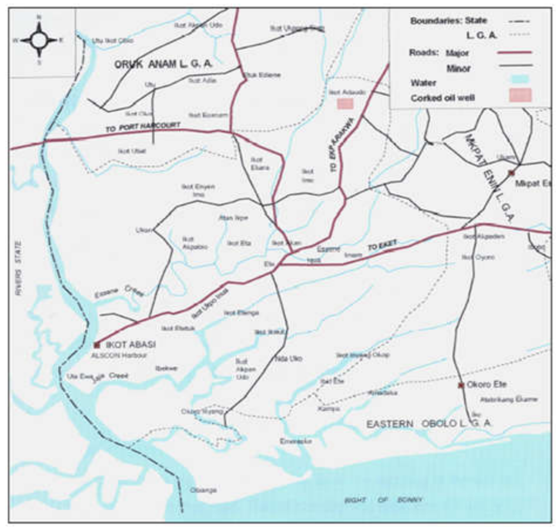

Fig 1: The location of the corked oil well at Ikot Ada Udo in Ikot Abasi L. G.A. of Akwa Ibom State, Nigeria
Source: (Udo, 2018)

Experimental Design and Field layout: This experiment was carried out (on the 2007 crude oil-impacted soil) during the 2016 and repeated in 2017 planting seasons. The experiment was a 3x4 factorial in randomized complete block design (RCBD) with three replications. Factor A was soil supplement, comprising of four levels viz: dried poultry manure (DPM), Oil Palm Bunch Ash (OPBA), OPBA+DPM and Control (No supplement) while Factor B was cassava cultivar, and three cultivars were studied namely: TMS 30572, NR - 8082 and Local Variety (Nsak Idaha). Thus, there were a total of twelve (12) treatment combinations replicated thrice to thirty six (36) observations. The experimental field which measured 42m x 19m (798m^2) was cleared, tilled and demarcated in line with the experimental design into three (3) blocks comprising of twelve (12) subplots each to give a total of thirty six (36) subplots measuring 3m x 3m ($9m^2$) with 1m path separating both the subplots and the blocks. (Osu, 2017).

Collection of Experimental Materials Oil Palm Bunch Ash (OPBA): Discarded oil palm bunches were obtained from palm oil processing mills in the study area. Fresh samples were cut into smaller pieces and dried in the oven (Galenkamp) preset at 65^0C for three days. The dry pieces of the oil palm bunch sample were further placed in crucible and ashed in the furnaces (Galenkamp-England) at 45^0C- 500^0C. The OPBA was stored in airtight containers for further use.

Dried Poultry Manure (DPM): Poultry droppings in bedding materials (Sawdust) was obtained from commercial poultry farms in an around the study area; air-dried on concrete surfaces to aid volatilization of some pungent components while the solvated ammonia become self-ionized to give ammonium ions (NH_4^+) and amide (NH_2^-).

Cassava cultivars: Cassava cuttings (TMS 30572 and NR8082 were sourced from Ikot Abasi Zone of Akwa Ibom Agricultural Development Programme (AKADEP). Local variety –"*Nsak Idaha"* generally grown by the indigenes was collected from the community.

Determination of physical and chemical properties of soil and organic amendments: Soil data were obtained before field experiment and also determined during the course of the field work. The data were those of microbiological, physicochemical and heavy metal contents of soil, DPM, OPBA and DPM + OPBA.

Microbiological Analysis of Samples: Soil samples were collected from five locations within the area of the corked well where massive crude oil spillage had occurred in 2007. Samples were collected at a depth of 0-15cm (surface topsoil) with the aid of soil auger and pooled to have a composite sample. Other samples were those of DPM and OPBA. Ten grams of samples were weighed out and suspended in 90ml of sterile physiological saline and shaken vigorously for about I minute using a vortex shaker. Samples were then centrifuged at 100rpm X5g for 5 minutes to dislodge microorganisms, disrupt flocculent materials and randomly distribute the microbial cells and the supernatant drawn for serial dilutions. Ten-fold serial dilution was carried out before enumeration of densities of microbial groups. Density determination was done by the pour plate methods (Harrigan and Micance, 1990). Total heterophic bacterial counts in samples were enumerated on nutrient agar, while total heterotrophic fungal counts were on sabouraud dextrose agar (SDA). Inoculated nutrient agar plates were incubated for 24 hours at room temperature (28± 2^0C) while the SDA plates were incubated at room temperature for between 5 and 7 days before enumeration.

Physical and chemical analysis of soil samples: Soil pH was measured with 1:2.5 soil-water suspension and read using Beckman's glass electrode pH meter, organic carbon was determined by the Walkey Black wet oxidation method (Jackson, 1962), available phosphorus was measured by Bray P-1 method (Jackson, 1962). The total nitrogen content was determined by micro-Kjeldahl method (Jackobson, 1992). Soil particle size distributions were determined by the hydrometer method (Udo and Ogunwale, 1986) using sodiumhexametaphosphate as dispersant. Exchange acidity was determined by titration with 1N KCl (Kramprath, 1967). Exchangeable bases were determined by extraction with 1M NH_4OAc (One molar ammonium acetate solution), after which Ca and Mg were determined by EDTA titration method while sodium and potassium were determined by photometry method. The effective cation exchange capacity (ECEC) was calculated by the summation method (that is summing up of the exchangeable bases and exchangeable acidity. Base saturation was calculated by dividing total exchangeable bases by ECEC and multiplied by 100.

Determination of heavy metals in soil, leaf and tuber samples: Soil, leaf and tuber samples collected in each of the groups from the two seasons (2016 and 2017) were processed and air dried to remove the moisture and water content simultaneously. They were then dried to constant weight in an oven maintained at 105ºC, and ground to fine powder using a laboratory grinder. The ground leaf and tuber were collected into well labeled polythene bags and placed in desiccators. Three grams (3.0g) of each sample was carefully weighed into clean platinum crucible and ashed at 450-500ºC then cooled to room temperature in desiccators. The ash was dissolved in 5ml of 20% hydrochloric acid and the solution was carefully transferred into a 100ml volumetric flask. The crucible was well rinsed with distilled water and transferred to the flask, made up to the mark with distilled water and shaken to mix well. The resulting sample solutions were then taken for the determination of the heavy metal (Fe, Cr, Cd, Cu, Ni and Pb) concentrations using Atomic Absorption Spectrophotometer (AAS). According to the procedures of the Association of Official Analytical Chemist (Williams 2000).

Field Experimentation: The OPBA and DPM were individually incorporated into designated plots at a rate of 25 tonnes / hecter. Approximately 12.5 tonnes / hectar of thoroughly mixed OPBA and DPM were similarly incorporated as OPBA + DPM into appropriate subplots. Control plots were chosen without any amendment. After this land preparation, cassava cultivars TNS30572, NR8082 and the local variety were planted slanting at 45º at a spacing of Im X Im, nutured by watering and weeding at interval of two weeks for a period of 36 weeks (Udo *et al.,* 2005).

Determination of Morphometric Parameters in Plants: The following growth parameters were measured: plant height (cm), number of nodes per plant, stem girth (cm) and leaf area (cm^2). Growth parameters were randomly measured on four plants per subplot from which the mean was determined. Plant height was measured from ground level to the highest point of canopy using a measuring tape. The numbers of nodes from the tagged plants were counted. Stem girth was measured using a caliper. To find the leaf area, the length and width of four leaflets of the tagged leaf per plant was measured and the product multiplied by a factor (0.45) as described by Ramanujam and Indira (1978).

Statistical Analysis: The experimental data obtained were processed and subjected to analysis of variance (ANOVA) and significant means were separated using the least significant difference (LSD) within and between groups according to the procedure of statistical analysis system (SAS, 1999). Probability limit was set at 95% level of significance ($P<0.05$) as described by Adepoju and Adebanjo (2011).

RESULTS AND DISCUSSION

Physical and chemical properties of soils of study Area: Some properties of the crude oil contaminated soil prior to the amelioration treatment in 2016 and 2017 are presented in Table 1. In the two planting seasons, sand fraction dominated the particle size distribution of the soil with the texture of the soil being loamy sand. The soil had a high bulk density of 1.65 and 1.62 Mg m^{-3} in 2016 and 2017, respectively and a corresponding low total porosity 37.74 and 38.90, respectively as well as hydraulic conductivity of 2.16 and 1.67 cm h^{-1}, respectively.

Table 1: Mean values of Soil Properties under the Effect of Crude Oil as Determined in 2008, 2012, 2016 and 2017

Soil Properties	2008 *	2012 *	2016 **	2017 **	Max: Tolerable Limit (EIA, 2018)
Sand (g/kg^{-1})	905	895.00	872.00	843.50	Nil
Silk (g/kg^{-1})	68	30.00	36.98	40.60	Nil
Clay (g/kg^{-1})	140	105.50	91.02	115.90	Nil
Texture	Loamy Sand	Loamy Sand	Loamy Sand	Loamy Sand	
Bulk density (Mgm^{-3})	2.45	1.85	1.65	1.62	ND
Total porosity (%)	25.5	30.50	37.74	38.90	ND
Ksat (cmh^{-1})	-	-	2.16	1.67	
PH	6.8	5.5	5.08	5.60	6.8-7.5
Ec (dsm^{-1})	-	-	0.43	0.50	2 – 4.0
Org. C(g/kg^{-1})	201	105	30.67	32.87	20.0
Total N(g/kg^{-1})	5.7	0.7	0.63	0.69	2.0
Av.P(mg/kg^{-1})	6.1	17.0	13.23	15.05	20.0
Ca (cmol/ kg^{-1})	2.20	2.21	3.00	3.23	10-20.0
Mg (cmol/ kg^{-1})	1.1	1.90	1.05	1.36	2.0-8.0
K (cmol/ kg^{-1})	0.08	0.20	0.53	0.37	0.6-1.2
Na (cmol/ kg^{-1})	0.04	0.09	0.11	0.14	0.7-1.2
EA (cmol/ kg^{-1})	1.32	1.55	1.64	1.75	2.1-4.1
ECEC (cmol/ kg^{-1})	4.96	5.72	6.15	8.92	10.0
Base sat (%)	68.5	71.33	80.00	80.50	60-80
TPH (mg/ kg^{-1})	3,294.9	2,150.00	1,56.45	1,47.93	-
Fe (mg/ kg^{-1})	23,086.8	18,500.00	9,500.00	3,200.00	10,000-100,000
Cr (mg/ kg^{-1})	7.00	4.50	1.50	0.20	5,.0-1,000.0
Cd (mg/ kg^{-1})	4.20	2.54	0.90	0.42	20.0-500.0
Cu (mg/ kg^{-1})	29.1	20.55	18.50	15.00	2.0-100.0
Ni (mg/ kg^{-1})	45.50	31.35	21.25	12.00	100.0-1,000.0
Pb (mg/ kg^{-1})	2,316.00	1,200.00	520.00	150.00	10.0-300.0
THB (CFU/g^{-1})	-	-	3.2x10^4	3.1x10^6	
THF (CFU/g^{-1})	-	-	5.9x10^3	3.1x10^4	

**Data source: Udo and Chukwu, (2014), ** Data for present study: Ksat – Saturated hydraulic conductivity, Ec-Electrical conductivity, Org. c-organic: Carbon, Total N-Total Nitrogen, Av.P-Available phosphorus, EA- Exchangeable acidity, ECEC- Effective Cation exchange capacity, Base sat.-Base saturation, TPH-total Petroluem Hydrocarbon, THB-Total heterotrophic bacteria, THF-Total Heteretrophic fungi.*

The soil pH in 2016 was 5.08 and in 2017 the value was 5.60. In both years, (2016 and 2017) organic carbon recorded 30.67gkg^{-1} and 32.87gkg^{-1}; Available Phosphorus (13.23 mgkg^{-1} and 15.02 mgkg^{-1}); Total Nitrogen (0.63 gkg^{-1} and 0.69 gkg^{-1}). Values for Effective cation Exchange capacity were 6.15Cmol kg^{-1} and 5.72Cmol kg^{-1} with Total Petroleum Hydrocarbon content of 156. 45 mgkg $^{-1}$ and 147.93 mgkg^{-1} for 2016 and 2017 respectively. Mean values of soils physical and chemical properties and metal contents as determined in 2008 varied from values determined for the same soils in 2016 and 2017.

Parameters that decrease in values were sand, silk, clay, bulky density, pH, organic carbon, total nitrogen, TPH and the metals. Other parameters have their mean values increased with time including AV.P. Cartions, EA, ECEC, THB and THF. The TPH content (3, 294.90 mg/kg) in 2008 was very high, plausibly from the spill event of the previous year. However there was a remarkable reduction in concentration in 2017. A similar trend was observed for the heavy metals. Evidently, crude oil pollution had an enhanced effect on the values of some properties while depressing other. The metals Fe and Pb concentrations of 23, 086.8 mg/kg and 2,316.00 mg/kg in soil were noticeably high following spills of 2001, but exhibited remarkable decline to 3,200.00 mg/kg and 150.00 mg/kg for Fe and Pb respectively after treatment in 2017. Population of bacteria (THB was observed to increase from 10^4 to 10^6 CFU/g soil and fungi (THF) population also increased from 10^3 to 10^4 CFU/g soil.

Properties of organic supplements used: Organic amendments used in this study were dried poultry manure (DPM), oil palm bunch ash (OPBA) and a combination of DPM and OPBA (OPBA+DPM). Table 2 presents some properties of these organic amendments. For the two seasons the pH of dried poultry manure (DPM), Oil palm bunch ash (OPBA) and the combination of DPM and OPBA (OPBA+DPM) were neutral, strongly alkaline and moderately alkaline, respectively. Organic carbon, total nitrogen and phosphorus concentrations of both organic supplements were generally high, in addition to high concentrations of basic cations (Ca, Mg, K and Na). The C:N ratio of the organic amendments ranged between 9.35 – 15.28. This ratio influences microbial reactions in soils.

Table 2: Chemical Composition of Dried Poultry Manure (DPM), Oil Palm Bunch Ash (OPBA) and Combination of OPBA+DPM used for the Study

Parameters	2016 Planting Season			2017 Planting Season		
	DPM	OPBA	OPBA+DPM	DPM	OPBA	OPBA+DPM
pH	6.88	8.98	8.12	6.97	9.24	8.19
EC (dS m^{-1})	4.26	6.01	5.44	3.56	5.67	4.54
OC (g kg^{-1})	302.3	145	201.7	202.54	167.26	189.56
N (g kg^{-1})	19.79	14.69	16.53	21.67	12.93	14.78
C/N Ratio	15:1	9.8:1	12.2:1	9.3:1	12.94:1	12.8:1
P(mg kg^{-1})	6300.5	3500.68	4300.62	5639.84	3290.98	4170.43
Ca (mg kg^{-1})	8540.45	7440.56	8223.42	7865.21	5698.78	5983.01
Mg (mg kg^{-1})	677.37	1089.32	4435.82	875.43	1167.32	2354.83
K (mg kg^{-1})	1946.47	3883.68	2657.28	2378.34	4537.67	3257.65
Na (mg kg^{-1})	1456.97	1965.24	1891.19	1573.21	1383.21	163.98

DPM – Dried poultry manure; OPBA – oil palm bunch ash; EC – electrical conductivity

Effects of cassava variety and organic amendment on growth performance of test crop grown in crude oil-impacted soils: Result presented in Table 3 revealed that soils treated with DPM in 2016 enhance the following morphometric parameters of the test cassava cultivars. Local variety (Nsak idaha) attained the highest height of 215.11cm, NR 8082 displayed highest number of nodes (125) while TMS 30572 exhibited the broadest leaf area of 549.52cm^2. However, for the stem girth, the treatment of OPBA and DPM in combination enhanced the wildest girth of 9.23cm in TMS 30572 varieties when compared with the control (6.99cm). On the other hand NR 8082 grew to the height of 239.63cm after treatment with DPM. Amendment of soils with composite OPBA and DPM ensures 113 nodes for NR 8082 and 514.07cm^2 of leaf area for Local variety during 2017 planting season. This results revealed mild variability, therefore, the interactive effect of cassava variety and organic amendments has no significant ($P \leq 0.05$) influence on plant height, number of nodes, stem girth and leaf area. Effect of cassava variety and organic amendment on heavy metal uptake in leaf and roots of cassava grown in crude oil-impacted soil: Heavy metal uptake in leaves and roots of test cassava varieties grown in organically amended crude oil-impacted soil during the 2016 and 2017 planting season is presented in Table 4. Results of heavy metal uptake pattern in leaves and roots of test cassava varieties grown in organically amended crude oil-impacted soil is presented in table 4. Comparing metals removed by test crops for the two seasons, results revealed higher metal uptake in 2016 than 2017. Furthermore, comparison of heavy metal removal efficiency between plant organs showed that while leaves effectively accumulated Fe, Cu and Pb, the roots accumulated only Fe at concentrations above WHO permissible limits. The residual concentrations of heavy metals in test soils were Fe(9500.00mg/kg), Cu(18.50 mg/kg) and Pb (520.00 mg/kg) for 2016 and the results of interactive effects of plant variety, plant organ and type of amendment for that year on uptake of heavy metals in test crops shows that leaves of TMS 30572 cultivar exposed to DPM treatment accumulated 140.86 mg/kg Fe and 2.19 mg/kg Pb, while those treated with combined OPBA+DPM accumulated 21.63 mg/kg Cu. Similarly, NR 8082 accumulated 154.60 mg/kg of Fe and 17.80 mg/kg of Cu when treated with OPBA.

Table 3: Effects Cassava Variety and Organic Supplements on Growth Properties of Cassava Grown in Crude Oil-Impacted Soil

Cassava Variety	Organic Supplements	Plant Height			No. of Nodes/plant			Stem Girth			Leaf Area		
		12 WAS	24 WAS	36 WAS	12 WAS	24 WAS	36 WAS	12 WAS	24 WAS	36 WAS	12 WAS	24 WAS	36 WAS
						2016 Planting Season							
TMS	PBA	47.94	100.39	164.89	23.33	46.89	99.22	4.10	5.08	9.16	191.04	359.59	455.47
	DPM	57.19	115.82	120.78	26.11	52.67	93.44	4.53	5.70	9.02	195.17	391.52	549.52
	PBA+DPM	62.81	117.04	158.00	27.78	53.67	101.56	4.89	6.46	9.23	190.89	379.59	529.07
	Control	37.14	71.98	83.22	10.78	23.11	45.11	2.99	3.42	3.79	74.46	133.76	348.21
NR	PBA	52.60	93.33	159.22	27.78	49.67	105.56	3.99	4.97	6.36	175.38	352.36	473.27
	DPM	64.88	111.06	131.67	25.89	56.89	125.33	4.40	5.57	9.08	187.83	374.19	423.24
	PBA+DPM	61.41	90.78	114.89	25.44	55.11	115.00	4.03	4.74	8.51	195.48	377.17	468.79
	Control	44.84	82.46	90.56	13.00	20.00	73.78	7.33	3.42	3.99	130.91	219.51	357.12
LV	PBA	59.28	104.96	143.56	24.00	43.89	87.44	3.43	4.02	7.13	180.03	341.24	453.80
	DPM	59.37	129.07	215.11	25.67	47.67	79.44	3.82	5.03	5.79	188.86	363.76	416.90
	PBA+DPM	57.12	119.73	205.56	25.33	44.22	81.89	3.71	4.81	4.82	186.17	339.81	401.60
	Control	18.12	72.33	87.44	12.33	40.78	43.78	1.06	2.90	3.43	117.23	230.13	284.77
	LSD $_{(0.05)}$	ns	ns	Ns	Ns	Ns	ns	ns	Ns	ns	Ns	Ns	ns
						2017 Planting Season							
TMS	PBA	55.97	106.16	188.61	25.22	49.67	104.06	3.46	4.58	5.89	185.38	362.36	483.27
	DPM	55.77	113.68	174.07	26.11	52.44	99.17	3.69	5.05	6.72	197.83	384.19	433.24
	PBA+DPM	73.00	131.52	210.52	28.89	55.72	108.22	3.97	5.03	7.05	205.48	387.17	478.79
	Control	37.00	42.99	64.60	14.33	25.50	47.44	1.68	3.72	4.64	130.91	229.51	367.12
NR	PBA	49.61	96.28	188.53	26.44	48.39	102.89	3.81	**5.27**	7.42	175.03	**336.24**	448.80
	DPM	55.74	107.94	239.68	24.00	47.89	105.00	4.36	6.18	7.29	183.86	377.84	411.90
	PBA+DPM	68.00	113.39	238.83	28.00	53.11	113.33	4.73	6.36	8.08	181.17	351.50	396.60
	Control	44.84	66.07	48.01	20.22	36.28	92.00	2.47	3.95	4.60	116.23	170.13	180.77
LV	PBA	64.28	116.76	175.89	21.67	44.94	93.28	4.14	5.65	8.58	176.04	344.59	440.47
	DPM	69.81	132.12	173.77	20.67	47.78	94.33	4.53	6.25	8.46	180.17	357.01	447.97
	PBA+DPM	76.19	136.06	170.83	23.44	48.39	99.28	4.89	6.89	8.53	175.89	347.54	514.07
	Control	43.12	54.29	93.35	10.67	20.22	40.33	1.47	2.81	5.28	120.46	190.76	333.21
	LSD $_{(0.05)}$	ns	ns	Ns	Ns	Ns	ns	ns	Ns	ns	Ns	Ns	ns

TMS – Tropical Manihot Species, NR– New Released, LV – Local variety, OPBA – oil palm bunch ash, DPM – Dried poultry manure, LSD – least significant difference, WAS – weeks after sprouting, ns – not significant

Table 4: Effect of Cassava Variety and Organic Amendment on Heavy Metal Uptake in Leaves and Roots of Cassava Grown in Crude Oil-Impacted Soil

A	B	Fe	Cr	Cu	Cd	Ni	Pb	Fe	Cr	Cu	Cd	Ni	Pb
		Leaf						Root					
						2016 Planting Season							
TMS	OPBA	128.39	0.92	13.16	0.40	2.39	1.54	185.72	0.32	4.93	0.10	3.48	0.49
	DPM	140.86	0.51	15.79	0.21	2.65	2.19	169.81	0.25	4.30	0.01	4.05	0.63
	OPBA+DPM	116.43	0.58	21.63	0.40	1.50	1.97	178.67	0.27	4.20	0.01	3.80	0.89
	Control	220.11	1.01	17.83	0.48	5.22	1.88	210.06	0.38	6.36	0.01	6.37	0.90
NR	OPBA	154.60	0.98	10.51	0.36	2.36	1.36	171.41	0.28	4.81	0.11	3.78	0.62
	DPM	117.43	0.68	13.16	0.30	4.21	1.75	146.27	0.26	4.78	0.09	1.53	0.41
	OPBA+DPM	119.95	0.89	17.80	0.31	1.64	0.89	155.14	0.30	4.82	0.01	1.21	0.51
	Control	225.71	1.12	24.37	0.48	4.40	1.90	196.19	0.40	6.01	0.04	4.89	0.94
LV	OPBA	105.87	0.70	9.22	0.38	0.96	1.77	153.28	0.26	3.44	0.11	3.81	0.90
	DPM	131.32	0.92	14.80	0.21	4.11	1.66	172.72	0.29	4.99	0.10	2.84	0.53
	OPBA+DPM	115.04	0.56	15.51	0.17	2.18	2.31	131.32	0.28	4.42	0.01	2.09	0.75
	Control	264.24	0.99	22.19	0.65	3.46	1.92	197.12	0.47	7.73	0.03	5.30	0.95
	LSD(0.05)	8.67	0.07	4.53	0.05	Ns	0.96	10.67	0.05	1.05	ns	0.88	0.56
						2017 Planting Season							
TMS	OPBA	107.3	0.45	14.47	0.16	2.03	2.15	122.48	0.26	4.12	0.01	1.95	0.7
	DPM	122.48	0.86	13.8	0.2	3.83	1.55	161.10	0.27	4.65	0.09	2.65	0.49
	OPBA+DPM	98.75	0.65	8.6	0.35	0.9	1.65	142.97	0.24	3.21	0.1	3.55	0.84
	Control	205.3	0.94	16.63	0.45	4.87	1.75	195.93	0.35	5.93	0.01	5.94	0.84
NR	OPBA	111.88	0.83	16.6	0.29	1.53	0.83	144.70	0.28	4.5	0.01	1.13	0.48
	DPM	109.53	0.63	12.27	0.28	3.93	1.63	136.43	0.24	4.46	0.08	1.43	0.38
	OPBA+DPM	179.88	0.91	9.8	0.34	2.2	1.27	159.88	0.26	4.49	0.1	3.53	0.58
	Control	210.52	1.04	22.73	0.45	4.1	1.77	182.99	0.37	5.61	0.04	4.03	0.88
LV	OPBA	108.6	0.54	20.17	0.37	1.4	1.84	166.65	0.25	3.92	0.01	3.54	0.83
	DPM	131.38	0.48	14.73	0.2	2.47	2.04	158.38	0.23	4.01	0.01	2.44	0.59
	OPBA+DPM	119.75	0.86	12.27	0.37	2.23	1.44	173.22	0.3	4.6	0.09	3.25	0.46
	Control	246.46	0.92	20.7	0.61	3.23	1.79	183.86	0.44	7.21	0.03	4.94	0.89
	LSD(0.05)	6.66	0.06	Ns	0.07	Ns	0.59	5.9	0.05	2.56	ns	0.71	0.72
WHO Permissible Limit		20.00	1.30	10.00	0.02	10.00	2.00	20.00	1.30	10.00	0.02	10.00	2.00

TMS – Tropical *Manihot* Species, NR – New Released, LV – Local variety, OPBA – oil palm bunch ash, DPM – Dried poultry manure, LSD – least significant difference, ns – not significant

Nsak idaha LV) on the other hand accumulated less metals of 131.38 mg/kg (Fe), 15.51 mg/kg (Cu) and 2.31 mg/kg (Pb) when exposed to DPM treatment. In 2017, heavy metal concentrations in amended soil reduces to 3,200.00 mg/kg for Fe, 15.00 mg/kg for Cu and 150.00 mg/kg for Pb. Results of metal uptake in plant parts under treatment for that year showed that leaves of TMS 30.572 under OPBA treatment accumulated 14.47 mg/kg and 2.15 mg/kg of Cu and Pb respectively. The cultivar further accumulated 122.48mg/kg of Fe under treatment. Moreover, the variety NR-8082 accumulated 16.60 mg/kg of Fe when exposed to OPBA and DPM+OPBA treatments respectively. Finally, results of metal uptake pattern in LV showed that leaves of "Nsak idaha" accumulated 131.38 mg/kg if Fe and 20.04 mg/kg of pb when the soil was treated with DPM, while 20.17 mg/kg of cu was accumulated in leaves when soil was treated with OPBA. Result also revealed that roots of the various varieties accumulated only Fe at concentrations far exceeding permissible limits. Roots of TMS30572 and NR-8082 accumulated 185.72 mg/kg and 171.41 mg/kg of Fe when planted in crude oil contaminated soil treated with OPBA, while LV accumulated172.72 mg/kg of Fe but under DPM treatment in 2016. However, in 2017, DPM treatment resulted in enhanced uptake of Fe (161.10 mg/kg) in roots of TMS 30572, whereas 159.88 mg/kg and 173.22 mg/kg of Fe accumulated in roots of NR-8082 and LV respectively. Several studies have confirmed that crude oil pollution always alter the chemical, physical and biological properties of soil and subsequently degrade its fertility (Baker, 1976; Amadi *et al.,* 2005; Osuji *et al*., 2005), but under certain natural and treatment conditions such alterations can be reversed. The magnitude of the differences in values of some soil properties in the study area (Ikot Ada Udo) analyzed in 2008 (when oil pollution was fresh) and 2017 (9 years after) is demonstrated in this study. The pH of soil in 2008 for example recorded values near neutrality (6.8) but became reduced to 5.08 and 5.60 in 2016 and 2017 respectively. Although soils derived from beach ridge sands are known to be acidic (Unyienyin, 2010), results of the present study show that crude oil pollution had impacted the pH value of the site by about 1.24 x10^2 magnitude. Values of organic carbon and total Nitrogen were higher in soils immediately following the spills, while available phosphorus was lowered in 2008 when the effect of the oil spills was strong. The result showed that oil pollution brings about increase in soil carbon and nitrogen content but with reduction in available P, probably resulting from formation of insoluble phosphorus compounds in soil solution. It was also observed that values for TPH and heavy metals recorded marginal reduction. Under natural attenuating conditions in 2008 and 2012 (Table 1), but experienced remarkable reduction in 2016 and 2017 after treatment of soil with organic residues. There were also remarkable increments in the population of soil bacteria and fungi in addition to improvement in other soil fertility indices. This results presents evidence of ameliorating potentials of organic amendments on these cassava varieties grown in crude oil-impacted soil. Based on the agronomic data obtained after treatment of soil and enhanced microbial load, the result revealed the presence of natural microbial populations in soil that can withstand and survive onslaughts of oil pollution. This result is an agreement with the recent findings of Udofia (2018) who isolated cultures with strong ability for ameliorating crude oil toxity and with remarkable capability in improving crop (*Zea mays*) performance when cultivated in crude oil polluted soil. These isolates were able to remove target contaminant molecule (Strond, Paton and Semple, 2007) and observed to effectively attenuate and return crude oil-contaminated soil to a near pristine state, while restoring soil fertility stature to optimism level. Results of the effect of the various application of organic amendment on the morphometric parameters of the different cassava cultivars revealed in 2017 that at 36 WAS, DPM and OPBA + DPM significantly ($P \leq 0.05$) influence on number of nodes, stem girth and leaf area. Interestingly however, the interaction of the three cassava varieties with organic amendment resulted in significant ($P \leq 0.05$) uptake and concentration of the heavy metals Fe, Cu and Pb in leaves and Fe in roots. This result indicates that heavy metal removal from contaminated soil can become accentuated when such soils are treated with DPM and OPBA. Although DPM contain sawdust which is known to be less readily degradable in soil, nevertheless, its presence maintain favorable air conditions in contaminated soil for a longer period, thus enhancing microbial activities which is of great importance in aerobic biodegradation of pollutants, enzyme activity of soil and for plant growth with the poultry droppings preventing nitrogen deficit in soil, thereby producing a beneficial effect on the unbalanced carbon – to – nitrogen ratio. Increase in organic matter content and other chemical components is an indication that poultry manure has high potential of gradual nutrient release to soil and hence help improve fertility of degraded soil thereby boosting growth and sustaining crop production (Mensah, 2013). Apart from boosting growth, OPBA and DPM in this study offers veritable means of enhancing uptake of heavy metal in crop tissue. Though Angelova *et al.* (2010) in their studies found application of organic amendment to decrease heavy metals contents in potato peel and tubers, it could be argued that the effect of organic supplements on heavy metal concentration in plant tissues however, depend on the nature of organic matter, microbial consortia, pH and redox potential as well as on the particular soil type and heavy metals (Walker *et al.,* 2004). In this study, the effectiveness of oil palm bunch ash (OPBA) and dried Poultry manure (DPM) as single and in combination as ameliorating agents on growth performance and heavy metal uptake (for removal purposes) potentials for certain varieties of cassava grown in crude oil-impacted soil in the Niger Delta region of Nigeria is evident. Recommendations: The application of OPBA+DPM and DPM is strongly advocated for the amelioration of crude oil-impacted soil, considering their nutrient contents and their ability to restore crude oil polluted soils. Organic amendment application is advocated for rapid uptake of heavy metal in cassava leaves and roots grown in crude oil-impacted soils. Further researches should be conducted to further find ways of reducing concentrations of metals in contaminated soils.

Conclusion: The most important and direct test for soil contamination and remediation is plant assay, which in this study has proved unambiguously that crude oil is strongly toxic to cassava crops. The crops were drastically inhibited in the control set up, and these adverse effects were effectively reversed by the application of oil palm bunch ash and dried poultry manure to soils under treatments. Thus, the influence of crude oil on second cropping (2017) was much weaker. The application of organic amendments aided the cassava varieties to effectively remove the heavy metals Fe, Cu and Pb from crude oil-polluted soil and enhance uptake in plant leaves. The response of these cassava varieties to crude oil contamination appears to be optimistic, and so does the alleviating influence of oil palm bunch ash and dried poultry manure application on cassava grown in the soils strongly polluted with crude oil.

Acknowledgements: This research was financially supported by a grant during 2018/2019 Session (TETFUND/DASTD/COE/AKWAIBOM STATE/CA/2018/VOL.2) from the Tertiary Trust Fund (**TET-FUND**) Abuja, Nigeria.

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