



Effect of Soil Amendments on Leaf Pigmentation and N₂ Status in Cassava (*Manihot esculenta* Crantz) grown in Crude Oil Contaminated Soil

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ABSTRACT: Growth studies on TMS 30572, NR8082 and Local cassava cultivars grown on previously crude oil polluted soil were conducted to assess effects of oil palm bunch ash (OPBA), dried poultry manure (DPM) on Leaf pigmentation and N₂ status of test crops. In addition, physical and chemical properties of soils were determined. The pH was raised from 5.62±1.50 in control soil to values that ranged between 6.97±1.23 to 9.24±1.89 in soil treated with organic amendment. The result of organic amendment ensures reduction of C: N ratio from 50:1 in control to critical threshold that ranged between 8:1 – 10:1 for improved decomposition and soil quality. SPAD measurement revealed no significance (P≤ 0.05) in readings for both treatments and control experiments. However, the interactive effect of both cassava variety and type of amendments were significant. Cultivar TMS 30572 was found to be brought to high productivity under treatment with combined OPBA + DPM and recorded highest chlorophyll content per unit area of leaf surface with Chl. a/Chl.b ratio of 2.13 and thicker leaf blade. While Leaf chlorophyll is directly associated with the efficiency and capacity of the photosynthetic apparatus and hence chlorophyll content in this study was found to be directly related to good health status of the test crops. Results revealed that quality of crude oil contaminated soil was greatly enhanced by organic amendment.

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Cassava (*Manihot esculenta* Crantz) has been identified as a major contributor towards global food security and is the third most important tropical food crop after rice and maize, which contribute directly to feeding the growing population under very challenging environmental condition (Hyman *et al.*, 2012). Cassava is an important staple food crop in the Niger Delta Region where crude oil pollution is common, and due to its adaptation to a wide agro-ecology, the crop is able to grow on polluted site depleted of soil nutrients. It can also tolerate water shortage, resist pests and diseases and produce high quantity of tubers per unit area (Chipeta, *et al.*, 2016). Cassava is grown by nearly every farming family in the Niger Delta and contributes to the daily calorie intake. Cassava cultivars (TMS-30572, NR-8082 and Local Variety (LV) commonly grown in the area can attain heights of 1 to 4 meters depending on the soil fertility and level of management (Harrison *et al.*, 2018). Poultry manure is an essential by-product of the poultry industry produced by the enrichment of the poultry bedding materials with poultry droppings. Dikinya and Mufwanzala (2010) reported that the need

and utilization of dried poultry manure has overtaken the use of other animal waste because of its high content of Nitrogen, Phosphorus and Potassium. While, Awodun *et al.* (2007) on the other hand posited that palm bunch ash is an effective fertilizer and liming material for improving soil fertility. Researchers reported Palm Bunch Ash to contain considerable amounts of plant nutrients that can improve the physical and chemical properties of soils (Adejei and Christian, 2013; Ibeawuchi *et al.*, 2006; Mensah, 2013; Ojeniyi *et al.*, 2010; Ezekiel *et al.*, 2009; Awodun *et al.*, 2007; Ogbuehi, 2016). Consequently, there is need to protect the environment from the menace by adopting a cost effective amendment technique in order to clean up the contaminated environments. Findings have revealed photosynthesis as the process which high energy is absorbed and with water molecule, converted into organic substance for the synthesis of organic matter in plants and overall formation of plant pigments (Chlorophyll – a, Chlorophyll – b and Carotenoids) (Pavlovic *et al.*, 2014). Taiza and Zeiger (2010) posited that chlorophyll is the most important pigment in leaves

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and responsible for their greenness. Another pigment, carotenoids consisting of Xanthophylls and carotenes is one of the most essential classes of plant pigments which play a crucial role in defining the quality parameters of fruits and vegetables. Leaf chlorophyll content can be used as a nitrogen status indicator because it is an essential element in photosynthetic protein synthesis. Nitrogen (N₂) occupies a conspicuous place in plant metabolism. Due to confirmed high- correlation between leaf pigments and N₂ contents, plant optical properties are now used to measure N₂ status in plants. (Pavlovic *et al.*, 2014). Determination of optical activity and content of photosynthetic pigments in leaves is one of the key techniques in studying the process of photosynthesis and measurement of plant productivity. The procedure is utilized for measuring effect of deficits of macro and micro element in mineral nutrition of plant resulting in changes in chlorophyll content, plant biomass and N₂ content (Chao – Yi and Der – Ming, 2008; Jarrahi *et al.*, 2013). Chlorophyll contents of plants are known to be affected by plant growth stage, cultivars and deficiency of nutrients other than N₂. In the Niger Delta Region of Nigeria, most lands are under threats of petroleum hydrocarbon pollution arising from large scale hydrocarbon development. Crop plants facing oil pollution challenges manifest in poor growth, development and retarded yield. However some varieties of cassava (*Manihot esculenta* Crantz), a known stress resistant plant according to Aro *et al.*, (2010) are highly adapted to a wide varieties of edaphic and environmental influences. To this end, some cultivars are used for bioassay to evaluate effects of organic amendments (oil palm bunch ash – OPBA and dried poultry manure – DPM) singly and in combination on pigmentation and N₂ status of test plants grown in crude oil polluted soil. The focus of the study is to determine the effects of variety and different amendments on crop performance and productivity with intent of producing useful information about photosynthetic potential and primary production of cultivars under crude oil stress for land managers and eco-physiologist in the Niger Delta Region, Nigeria.

MATERIALS AND METHODS

Description of Study Area: This study was conducted under natural climatic conditions at Ikot Ada Udo Village located in Ikot Abasi Local Government Area of Akwa Ibom State, in the Niger Delta Region of Nigeria (Figure 1.). The climate of the area is humid tropical with heavy rainfall having a mean value of 400mm. The soils are formed on Tertiary Coastal Plain sands that are deep with loamy sand to sandy loam surface over loam to sandy clay subsoil (Petters *et al.*, 1998). Because of the study nature, the soils are fragile

and highly susceptible to erosion. The mean annual temperature varies between 26°C-28°C with relative humidity above 75% (Udo, 2008). A major crude oil spill event occurred in the area between August and November, 2007 with extensive damage to Land area and aquatic system and following this spills studies were conducted by Udo, (2008); Udo and Chukwu, (2014) and Osu (2017) to assess the effect of residual oil pollutants on land/ soil, plants, aquatic lives and other components of the contaminated environment.

Experimental Design: The study was conducted during the rainy/planting season of 2017, to evaluate effects of organic amendment and cultivars on chlorophyll a, chlorophyll b, carotenoids contents and N₂ status of leaves of cassava a grown in soil contaminated by crude oil in the year 2007. The experiment was laid out in a 3x4 factorial arrangement fitted into a randomized complete block design (RCBD) in three replicates. The factors were three cassava varieties; TMS 30572, NR 8082 and local Variety (LV – Nsaka Idaha). The four treatments in the study were dried poultry manure – DPM, Oil palm bunch ash – OPBA, DPM + OPBA and control (without treatment). This amounted to 12 treatment combinations laid out in 3 blocks to give a total of 36 treatment combinations.

Land Preparation and Field Experimental Layout: The area of land on which the experiment was carried out was manually cleared using machete and available stumps removed. The soil was tilled and homogenized so that lumps were broken to very fine particles with shovel and rake. The essence of the tilling and homogenization was to uniformly distribute the petroleum contaminants and break up the soil lumps to fine particles thereby increasing the surface area. The field was marked and measured as 42m x 19m (798m²) which is equivalent to 0.0798ha⁻¹. The field was demarcated into twelve plots of which each plot was replicated three times giving a total of thirty six (36) sub-plots. Each sub-plot was measured 3m x 3m (9m²) and was separated from one another by a 1 meter path while the blocks were also separated by a 1 meter path for easy accessibility. Each sub-plot carried nine (9) stands of cassava, giving a total population of three hundred and twenty four (324) viable stands within the layout. (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°C 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^oC- 500^oC. 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 and 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 (NH₄⁺) and amide (NH₂⁻).

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.

Field experimentation: The OPBA and DPM were separately incorporated into designated plots at a rate of 25 Kg/hectar to form the first two treatments. The third treatment had approximately 12.5 Kg/hectar each of OPBA and DPM, thoroughly mixed (to give 25 Kg/ha.) and similarly incorporated as OPBA + DPM (Combined) into appropriate subplots. The fourth plot (ie the control) was chosen but without any application of amendment. After these exercises, test cassava cultivars were planted slantingly at 45^o at a spacing of 1m x 1m. Nurtured by watering and weeding at intervals of two weeks for a period of 36 weeks (Udo *et al.*, 2005).

Determination Of Chlorophylls A, B, Carotenoids And N₂ Status In Cassava Leaves Using SPAD – 502 Meter: A Minolta Soil – Plant Analyses Development (SPAD – 502) leaf chlorophyll meter was used to collect data on chlorophyll content and nitrogen (N₂) status of *Manihot esculenta* grown in amended crude oil contaminated soil to quantitatively evaluate foliar quality and health status of plant (Richardson, Durgan and Berlyn, 2002). Cassava plants (TMS – 30572, NR – 8082 and local Variety) were identified from appropriate sub-plots for measurements. A leaf section of the identified plants was enclosed in a small chamber of the meter and exposed to two light sources: (1) a red light – 640mm and (2) an infrared light – 940mm positioned just above the leaf. Light filtering through the leaf is captured sequentially by sensors below the leaf blade. The difference in transmission of the filtered wavelength is recorded as chlorophyll content per unit leaf area and displayed on indicator (Demotes – Mainards *et al.*, 2008). Leaf measurements were taken at three stages of crop development (young leaves of 12 weeks old, expanded leaves at 24 weeks and leaves on old plant at 36 weeks old) (Peng, Garcia, Laza and Cassma, 1992). USB cable from SPAD – 502 meter during data collection was linked to personal

computer for data storage and for statistical analyses. SPAD readings reported in relative units (µgcm⁻²).

Estimation of Leaf Blade Thickness/Health Status of Plant: Values of chlorophyll a and chlorophyll b from SPAD – 502 meter reading of a particular plant leaf was used to assess plant health by determining the ratio of chlorophyll a/chlorophyll b. value of this ration is a good indicator of plant health.

Determination of Some Physiochemical Properties of Soils under Study: Samples of control and soils under treatments were analyzed at the end of the experimental period (36 weeks) following standard procedure outlined by APHA (1998) for pH, electrical conductivity organic carbon, Available nitrogen, water soluble phosphate, calcium magnesium, potassium and sodium. The pH was measured in 1:1 water supplement ratio using Beck Man’s glass electrode. Electrical conductivity (dsm⁻¹) was determined according to AOAC (2011). Total Nitrogen (gkg⁻¹) was estimated by the micro Kjeldahl method and the value obtained multiplied by the factor 2.25 to obtain crude protein content. The total organic carbon (gkg⁻¹) was evaluated by the Walkey – Black method, while PO₄²⁻, Ca²⁺, Mg²⁺, K and Na were determined calorimetrically (Jacobson, 1992).

Statistical Analysis: Data obtained were subjected to analysis of Variance (ANOVA) and the Least Significant Difference (LSD) was employed to separate means according to SAS (1999).

RESULTS AND DISCUSSION

The results of some properties of soil at the end of experimental period of 36 weeks are presented in Table 1. The soil was found to be highly acidic at 5.62±1.50 with very high organic carbon content (402.20±3.45 gkg⁻¹) but low nitrogen with a mean value of 7.95gkg⁻¹. However, soils amended with DPM, OPBA and OPBA+DPM had pH values at slightly acidic 6.97± 1.23, moderately alkaline 9.24±1.89 and near neutral at 7.40±1.24 respectively.

There was an enhanced nitrogen content in treated/ amended soils such that the C/N ratio of 50:1. The concentration of PO₄²⁻ increased from 206.00±2.20mgkg⁻¹ in control soil to 6300.50±1.58 mgkg⁻¹ and 4300.62±1.43 mgkg⁻¹ for soil treated with DPM, OPBA and OPBA + DPM respectively. Basic cations including Ca, Mg, K and Na recorded higher concentrations in treated soils when compared with the control soil. Microbial uptake of nutrients known as immobilization from agronomic viewpoint reduces availability of nutrients for plant growth (Subba Rao, 2009). The rate of immobilization of nutrient such as nitrogen depends among other factors on the C/N ratio

below 25 seems to be ideal for maximum decomposition since there will be no release of mineral nitrogen from residues over and above the threshold value. In the present study, the C/N ratio of amended ranged between 8-10:1 as against the 50:1 C/N ratio of for the control. In other words, a favourable soil environment was created on application of the amendment such that critical balance of C/N ratio less than 25 was established resulting in improved soil quality. The acid nature of control soil may be responsible for the reduced values of basic cations whereas organic amendment caused increase in the pH from 5.62±1.50 in the control soil to a range of 6.97±1.23 to 9.24±1.89 in soils treated with organic amendments. This enhanced pH creates a geo-

chemical environment that promotes absorption by electrostatic or coulombic attraction of cations (Ca²⁺, Mg²⁺, K⁺, Na⁺) to soil properties. This explains why the concentrations of these basic cations as provided by the amendment were relatively high. When sorped forms of cations are in equilibrium with soil particles, they serve as long-term source of essential nutrients that is slowly released into soil as soil solution concentration of the cation decrease with time (Udofia, 2018). The general deductions made from results of this study is that crude oil contaminated state of soil could be reverse, returning it to optimum level for fertile soil under amendment with oil palm bunch ash and dried poultry manure.

Table 1: Chemical Composition of Crude Oil Contaminated Soils under Organic Amendment and Control at the end of Experimental Periods.

Parameters	Control soil (No amendment)	Amended Soils		
		DPM	OPBA	OPBA+DPM
pH	5.62±1.50	6.97±1.23	9.24±1.89	7.40±1.24
EC(dSm ⁻¹)	3.26±1.96	4.28±0.96	6.01±1.25	5.44±1.44
OC(gkg ⁻¹)	402.20±3.45	304.30±2.05	145.00±1.20	201.70±260
ON(gkg ⁻¹)	7.95±2.8	35.79±1.50	149.69±3.20	22.52±2.18
C/N ratio	50:1	8.5:1	9.8:1	10:1
PO ₄ ²⁻ (mgkg ⁻¹)	206.00±2.20	6,300.50±1.58	3,500.60±1.84	4,300.62±1.43
Ca(mgkg ⁻¹)	1,200.45±2.25	8,540.45±1.55	7,440.56±2.15	8,223.43±1.65
Mg(mgkg ⁻¹)	800.50±1.23	677.37±1.25	1,089.35±1.55	4,435.52±1.35
K(mgkg ⁻¹)	620.50±1.8	1,946.47±1.60	3,883.65±1.45	2,657.28±1.75
Na(mgkg ⁻¹)	106.50±2.1	1,456.95±2.15	1,965.25±2.45	1,891.19±2.50

Values are means of three replicates ± SD determinations.

Table 2: Mean SPAD Readings of Chlorophyll Concentration Index, Chlorophyll-a, Chlorophyll-b, Carotenoids and Nitrogen Status in Leaves of Cassava from Crude Oil Contaminated Soil as Influenced by Variety and Organic Amendments

Cassava Variety	Soil Treatment	CCI (µgcm ²)			Chl. a (µgcm ⁻²)			Chl. b (µgcm ⁻²)			Carotenoids µgcm ⁻²			N ₂		
		12	24	36	12	24	36	12	24	36	12	24	36	12	24	36
Local Variety	A	WAS	W	W	W	WA	W	W	W	WA	W	WAS	W	WA	WA	
		AS	AS	AS	AS	S	AS	AS	AS	S	AS	AS	S	S	S	
		AS	AS	AS	AS	S	AS	AS	AS	S	AS	AS	S	S	S	
	B	50.01	49.	49.	28.	29.	30.5	20.	18.	18.	4.3	7.8	8.1	0.2	0.28	0.7
		48.54	46.	47.	29.	29.	29.4	19.	19.	19.	4.5	6.4	7.6	0.2	0.26	0.3
		49.25	47.	49.	27.	28.	29.5	20.	19.	20.	6.5	9.5	9.9	0.3	0.32	0.3
	C	31.45	30.	30.	18.	19.	19.1	14.	13.	14.	2.5	3.5	4.5	0.1	0.15	0.2
		59.5	58	61.	34.	34.	36.5	22.	21.	20.	5.94	9.7	9.61	0.5	0.28	0.3
		54.2	55.	55.	32.	33.	34.3	23.	24.	24.	5.42	6.2	6.30	0.2	0.25	0.26
	D	57.3	58.	58.	33.	35.	34.6	22.	23.	23.	5.35	8.7	9.15	0.2	0.29	0.29
		33.7	33.	32.	20.	19.	19.	15.	14.	14.	2.65	3.5	4.3	0.1	0.10	0.15
		55.70	60.	60.	54.	57.	57.	24.	23.	24.	5.89	9.3	9.68	0.2	0.25	0.28
NR 8082	54.60	56.	58.	50.	52.	54.	22.	22.	23.	5.65	8.7	8.45	0.2	0.25	0.27	
	64.25	63.	60.	59.	58.	56.0	27.	25.	26.	6.22	10.	9.80	0.3	0.32	0.31	
	32.50	31.	33.	19.	19.	21.0	15.	14.	14.	2.88	3.6	4.25	0.1	0.15	0.16	
TMS 30572	D															

A = DPM – Dried poultry manure; B = OPBA – oil palm bunch ash; C = OPBA+DPM; D = Control; TMS – Tropical Manihot Species, NR – New Released, LV – Local variety, CV – Cassava Variety, Chl.a – Chlorophyll - a and Chl.b – Chlorophyll - b.

Mean SPAD readings of plant pigments (chlorophyll concentration index – CCI, chlorophyll-a, chlorophyll-b, carotenoids and leaf nitrogen status) of cassava grown on crude oil contaminated soil as affected by cassava variety and organic amendments are presented in Table 2. Results revealed that local variety cassava grown in crude oil contaminated soil but amended with Dried Poultry Manure (DPM) significantly (P ≤ 0.05) affected plant pigmentation with similar significance recorded for NR 8082 variety. However, TMS 30572

grown in OPBA + DPM treated crude oil contaminated soil significantly (p ≤ 0.05) enhances CCI (60.61 µgcm⁻²), Chl-a (56.05 µgcm⁻²), Chl-b (26.20 µgcm⁻²), Carotenoids (9.80 µgcm⁻²) and N₂ status 0.39 after 36 weeks of planting. When effect of cassava variety and organic amendments on crop performance and productivity was considered, results of chlorophyll concentration index-ccl, chlorophyll-a, chlorophyll-b, carotenoids and leaf nitrogen status based on SPAD measurements revealed no statistically (p ≤ 0.05)

difference in readings obtained for both cassava crop varieties. However, the interactive effect of both cassava variety and type of amendment was significant. It is apparent that in crude oil contaminated soil environment, the cultivar, TMS 30572 can be brought to high productivity when such soils are treated with combined OPBA+DPM amendments. Results of the optical properties of the cassava cultivars (Table 2), further revealed that chlorophyll-a, was generally higher in concentration than chlorophyll-b and the carotenoid contents. This corroborates findings by Math (2015), who reported that chlorophyll-a is the chief pigment associated with photosynthesis while chlorophyll-b and carotenes are accessories that absorb light and transfer the energy to chlorophyll-a. The SPAD meter reading can also be applied in the evaluation of health status of plants. There exist high correlations between values of chlorophyll-a/chlorophyll-b ratios obtained and plant morphometric property such as leaf blade Thickness. Accurate measurement of leaf thickness is difficult and time consuming because leaf blades are subject to changes under different pressures and temperature. Therefore, leaf chlorophyll contents which can be rapidly estimated *in situ* by SPAD was used to estimate chlorophyll – a/ chlorophyll – b ratio and the value used to ascertain observed leaf blade thickness and hence the health status of the plant.

Results of influence of cassava Variety and organic amendments on chlorophyll-a and chlorophyll -b ratio and health of crops grown in crude oil contaminated soil is presented in Table 3. The SPAD meter measurements define symptoms developed during photosynthesis as a result of plant exposure to stress (crude oil pollutants). The readings accounts for deficit of macro and micro elements in minerals nutrition of plants, changes in chlorophyll content and Nitrogen status. The interactive effect of local variety cassava (Nsak idaha) and DPM treatment of contaminated soil results in a Chl.a/Chl.b ratio of 1.65. Leaves of this crop under treatment were found to have normal blades with average chlorophyll content. Similarly, NR 8082,

under DPM treatment recorded Chl.a/ Chl.b ratio of 1.77 with moderately thick leaves, moderate chlorophyll content and improve Nitrogen and health status. However, TMS 30572 cassava variety under combined OPBA and DPM treatment recorded high Chl. a / Chl. b ration (2.13). The leaf blades were relatively thicker with high chlorophyll content per unit area of leaf surface. On the other hand, the cassava crops grown in unamend but contaminated soil recorded on the average low Chl.a/Chl.b ratio of 1.46. The observed leaves were relatively thinner amounting to low chlorophyll content. Results of SPAD leaf chlorophyll measurement to determine influence of cassava variety and organic amendments on Leaf pigmentation and crop health status (Table 3) revealed that local variety and NR8082 under DPM treatment with chl.a/chl.b ratios of 1.65 and 1.77 respectively contained normal to moderate chlorophyll content in leaves, with observed normal to moderate blade thickness and subsequently adjudged moderately healthy. On the other hand, TMS 30572 under combined OPBA+DPM treatment recorded the highest chlorophyll content per unit area of leaf surface with chl.a/chl.b ratio of 2.13 and thicker leaf blade. This was found to be most healthy of the test crops under treatment. This results corroborates Boardman (1977); Khan *et al* (2000) and Terashima *et al.* (2006) findings that chlorophyll contents of leaves and chl.a/chl.b ratios defines leave blade thickness and hence health conditions of plant. On the contrary, cassava varieties under control condition exhibited low chlorophyll-a/ chlorophyll-b ratio with relatively thin leaf blade. Crops under this condition had low leaf chlorophyll content thereby manifesting poor health. This result agrees with Jinwen *et al.* (2009) who showed that leaves become thinner when chl.a/chl.b ratios decreased. Therefore, leaf chlorophyll is directly associated with the efficiency and capacity of the photosynthetic apparatus and hence chlorophyll, carotenoids and Nitrogen content in this study was found to be directly related to good health status of plants.

Table 3: Chlorophyll -a/Chlorophyll -b Ratio and Health Status of Cassava Grown in Crude Oil Contaminated Soil as Influenced by Crop Variety and Type of Organic Amendments.

Cassava Variety	Soil Amendment	Chl. a / Chl. b	Leaf Blade Size Observation	Remarks	Conclusion	Reference
Nsak Idaha	DPM	1.65	Normal	Normal chlorophyll content in leaf	Normal	Boardman, 1977
NR 8082	DPM	1.77	Moderately Thick	Moderate chlorophyll content in leaf	Moderate health status	Khan <i>et al</i> 2000.
TMS 30572	OPBA + DPM	2.13	Thicker leaf blades	High chlorophyll content per unit area of leaf surface	Most healthy plant	Terashima <i>et al.</i> , 2006
Control		1.46	Very thin leaf blade	Low chlorophyll content	Poor health	Jinwen <i>et al.</i> 2009

TMS – Tropical Manihot Species, NR – New Released, LV – Local variety, OPBA – oil palm bunch ash, DPM – Dried poultry manure, CV – Cassava Variety.

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Conclusions: SPAD-502 is a good tool for monitoring the functional activity of photosynthesis apparatus and for quantification of chlorophyll. Adverse effect of crude oil pollution on farmland can be improved by application of oil palm bunch ash and dried poultry manure as amendments. Also, improved cassava variety TMS 30572 cultivated under treatment of combined OPBA+DPM amendment exhibited highest productivity.

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