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GROWTH PERFORMANCE OF CASSAVA –*Manihot esculenta crantz* IN CRUDE OIL CONTAMINATED SOIL AMENDED WITH COMPOST OF *centrosema pubescens* BENTH AND INORGANIC FERTILIZER

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ABSTRACT
Studies on the performance of Manihot esculenta, Crantz (TMS 30572) in a crude oil polluted soil was investigated in the Botanic Garden of University of Port Harcourt. The soil samples were polluted at four different levels (0%, 2%, 4% and 6%) with crude oil and amended with organic supplement (decomposed Centrosem apubescens) and NPK fertilizer at the rate of 0.25Kg per 5Kg of soil to the various levels of crude oil contaminated soil, alongside a control. Quantitative observations showed that amelioration treatments (C. pubescensand NPK) recorded significant (P=0.05) increase in plant height, petiole length, leaf number, leaf area, fresh weight, dry weight and moisture content, than those of the control. Results of edaphic physico-chemical parameters showed that crude oil pollution significantly increased percentage total organic carbon, total organic matter and total hydrocarbon content (THC) at P=0.05 while pH, percentage total nitrogen, phosphorus and exchangeable bases (Ca, K and Mg) were significantly decreased at two weeks after pollution. The results also showed that the amendment treatments significantly decreased crude oil toxicity at different degrees at (P=0.05) by improving the nutrient content and decreasing the total hydrocarbon content of the soil.

Key words: Growth, Manihot esculenta, crude oil, pollution, Amendments, Contamination

INTRODUCTION
The contamination of the environment (mainly terrestrial and aquatic) by crude oil is referred to as oil pollution, and it is estimated that 80% of oil pollution is as a result of spillage (Odu, 1977).
Spill of crude oil into the environment particularly into the soil causes toxicity in the soils, which is due to the presence of heavy metals that hampers soil productivity, thus affecting the growth of the Plant (Audrey, 2001). Pollution is the introduction of contaminants into a natural environment that causes instability, disorder, harm or discomfort to the ecosystem. Petroleum oil, regardless of its complexity constitutes a major threat to plant growth and development (Carls, 2001).
One of the environmental challenges posed by oil pollution is the alteration in the physical and chemical nature of the soil which subsequently affects the growth of plants (Tanee and Akonye, 2009). Petroleum hydrocarbon contamination may

affect plant by retarding seed germination and reducing plant height, stem density, photosynthetic rate and biomass or resulting in complete mortality (Pezeshki, *et al*., 2000).

In Nigeria, most of the terrestrial ecosystem and shore lines in oil producing communities are important agricultural land under continuous cultivation. Any contact with crude oil usually results in damage to the soil, microorganism and plants (Adedokun and Ataga , 2007). Oil pollution prevents normal oxygen exchange between soil and the atmosphere, due to hydrophobic properties of oil (Onuoha*etal.*, 2003). The most serious result of pollution is its harmful biological effects on human health and on the food chain, birds and marine life. Pollution can destroy vegetation that provides food and shelter and it can seriously disrupt the balance of nature and in extreme cases can cause the death of humans (Amakiri and Onofeghara, 1983).Crude oil spills lead to insufficient aeration, a reduction in the level of available plant nutrients and a rise in toxic levels of certain elements such as cadmium, copper, manganese and iron (Nwaichi, and Wegwu, 2010).

In view of the devastating effect of oil pollution to the soil and plants, the application of remediation techniques like amendments, bioaugumentation etc becomes necessary. A soil amendment is any material added to a soil to improve its physical properties, such as water retention, permeability, water infiltration, drainage alteration and structure (Davis and Wilson, 2005). Petroleum has been found to affect the cultivation and production of economic crops including cassava, especially in the Niger Delta. Cassava has been reported to be vulnerable to crude oil pollution as it affects the physical and biochemical characteristics of the plant (Anyanwu and Tanee, 2008). There is therefore the need to remediate such polluted site to improve plant performances. Bioremediation as been proven to be a cost- effective treatment tool, if used properly, in cleaning certain oil-contaminated environments.

The study attempts to investigate the growth performance of cassava (var. TMS 30572) in crude oil contaminated soil amended with leaves of *Centrosema pubescens*. The choice of cassava for this study is necessitated by the fact that it is the most common crop cultivated in the Niger Delta where crude oil pollution is inevitable. It is expected that result obtained from this study will widen our knowledge on the effects of oil pollution on the growth of plants and how *Centrosema pubescens* and NPK fertilizer can be used to improve such conditions for better performance of crops.

MATERIALS AND METHODS

The top soil(loamy soils) used for the study were collected from the University of Port Harcourt Botanic Garden. The crude oil was obtained from Nigerian National Petroleum Corporation, Eleme, Rivers State. Cassava(*Manihot esculenta* Crantz) TMS 30572 variety cuttings and NPK 15:15:15 were obtained from Agricultural Development Programme (ADP) in Rivers State. Leaves of *Centrosema pubescens* were obtained from farms at Alakahia community opposite University of Port Harcourt Teaching Hospital(UPTH) and Abuja campus. Physico-chemical properties of the experimental soils (uncontaminated loamy soil, and contaminated loamy soil) were analyzed using standard procedure (Allison, 1965; A.O.A.C., 1975; Udo and Ogunwale,1986).

A 3x4 factorial arrangement fitted into completely randomized design were used, each treatment was replicated three times.

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Top soil(loamy soil) weighing 5kgfor each bucket were used. The soil was mixed thoroughly with different levels of crude oil thus, 0%,2.0%, 4% and 6% and placed in a plastic buckets based on each treatment. A total of 72 plastic buckets were used for the experiment. The buckets were perforated at the bases and sides to allow for aeration and drainage. These were allowed to stand for one week for the oil to acclimatize to the soil before emediation.

Leaves of *Centrosema pubescens* and NPK 15:15:15were used to ameliorate the crude oil contaminated soil. The reasons for the choice of these two materials are that they are easily available and have high nitrogen content which is always a limiting factor in a Crude oil polluted soil. Each ameliorating material was weighed 0.25kg for each 5kg soil per bucket. There mediation/treatments were in this order,
Soil with 0%, 2%, 4% and 6% crude oil contamination respectively,
Soil with 0%, 2%, 4%,6% crude oil contamination and NPK,
Soil with 0%,2%, 4% and 6% of crude oil and *C. pubescens*,
Soil with 0%,2% ,4%and 6% of crude oil mixed with *C. Pubescens* and fertilizer
After treatment was carried out, a period of two weeks was allowed for the NPK and leaves of *Centrosema pubescens* to decompose in the soil before planting. Three cassava cuttings, ranging from4-5cm long were planted thereafter.

The following growth and biochemical parameters were analysed from plant and soil before and after harvest: plant height, petiole length, leaf number, fresh weight, dry weight, total organic carbon (TOC), total organic matter (TOM), pH, available phosphorus, Total Nitrogen Manganese, Zinc, Copper, Lead, Iron, Cadmium and Chromium. The shoot length (plant height) was measured with a metre tape in centimetres from the soil surface to the plant apex. The plants were uprooted from each bucket and weighed immediately on a weighing balance, model PN 163 to avoid moisture loss. This was done to obtain the fresh weights. To get the dry weights, the plants were taken to the laboratory, oven-dried at80^{o}C for 24 hours to get rid of all moisture and ensure a constant weight. It was then weighed on a PN 163 model weighing balance. Leaf chlorophyll content was extracted from 1.0g of leaf sample. The sample was homogenized by adding small amount of 85%acetone. 25ml aliquot of extract was added to 50mldiethyl ether in a separating funnel. The optical density at 660nm and 643nm in 1cm cell was measured using ether as a reference. Leaf carbohydrate content was analysed by extracting 1.0gof dry leaf sample and digested with Perchloric acid and the sugar was determined colorimetrically by the An throne method. The nitrogen content was determined by the Kjedahl method (Stewart, *etal*.,1974) in which 1.0g of leaf sample was heated on an electro thermal hot plate, until digest turned to sky-blue, then diluted with 100mls of diluted water.30mls of 40% NaOH added and the sample was heated to release ammonia. The distillate was titrated with 0.01M hydrochloric acid.
All data collected were subjected to statistical analysis such as Analysis of variance (ANOVA) and standard error means. New Duncan Multiple range test (NDMRT) was employed to separate means at 5% level of significance.

RESULTS

Result showed that addition of amendment materials to the crude oil polluted soil significantly (P=0.05)increased the plant height of cassava (fig.1). Highest significant plant height was recorded in the compost and NPK combined treatment. There were significant (P=0.05) reductions in petiole length and leaf number with increasing concentration of crude oil (figs. 2 and3). In fig. 4 the fresh shoot weight of cassava were found to improve with time when compared with the control. Compost + NPK 15:15:15 was found to be most effective in improving fresh shoot weight than the other treatment options while the control produced the least. Similar results were recorded for the dry shoot weight(fig. 5).Addition of ameliorating materials (*Centrosemapubescen s*and NPK) to crude oil polluted soil also improved the biochemical properties of cassava. Tables 1,2, 3 and 4 showed a significant (P=0.05) improvement in the leaf chlorophyll, leaf carbohydrate (CHO) and leaf nitrogen (N) in the different remediation treatment as compared to the control (no remediation). Compost + NPK 15:15:15 treatment recorded the highest (P=0.05) leaf chlorophyll content followed by compost. In leaf nitrogen content Compost + NPK 15:15:15 recorded the highest followed by Compost with no significant difference between them.

Table 1: Physico Chemical Properties of Experimental Soil before Harvest

	Concentration (%)			
Parameters	0	2	4	6
pH	5.7±0.10	5.6 ±0.20	5.3±0.13	5.0 ±0.12
Total N(%)	0.42±0.02	0.25 ±0.04	0.17 ±0.09	0.05 ±0.01
Available P (mg/kg)	105 ±0.52	52.7 ±0.23	39.2 ±0.21	30.7 ±0.19
Mn (mg/kg)	19.9 ±0.17	21.2 ±0.21	29.7 ±0.23	31.6 ±0.30
Zn (mg/kg)	13.7 ±0.32	12.6 ±0.29	10.7 ±0.20	10.2±0.18
Cu (mg/kg)	21.6 ±0.32	13.7 ±0.22	12.4 ±0.24	11.6 ±0.19
Pb (mg/kg)	27.0 ±0.30	19.2 ±0.21	17.6 ±0.20	15.2 ±0.18
Fe (mg/kg)	22.6 ±0.21	29.4 ±0.27	30.4 ±0.30	34.6 ±0.40
Cd (mg/kg)	13.7 ±0.12	16.2±0.18	17.5 ±0.19	17.9 ±0.20
Cr(mg/kg)	11.6 ±0.13	12.7 ±0.16	15.6 ±0.21	16.2 ±0.25
K(mg/kg)	106.4 ±0.52	73.6 ±0.45	40.4 ±0.30	32.1±0.29
Na (mg/kg)	410.5 ±0.67	423.1 ±0.56	428.7 ±0.46	457.2 ±0.69
Mg (mg/kg)	52.0 ±0.42	57.7 ±0.45	59.8 ±0.52	61.3 ±0.30
Ca (mg/kg)	55.9 ±0.24	56.2 ±0.30	59.7 ±0.28	60.2 ±0.35
Total organic C (%)	1.20±0.18	3.52 ±0.26	4.06 ±0.35	5.29 ±0.28
Total organic matter (%)	2.74 ±0.10	2.17 ±0.20	1.06 ±0.19	0.45 ±0.05
THC (mg/kg)	557.50 ±2.11	6798.59 ±7.08	7714.29 ±5.13	8709.67 ±8.05
Sand (%)	78.02 ±0.82	76.21 ±0.76	74.30 ±0.57	73.20 ±0.59
Silt (%)	16.50 ±0.34	12.20 ±0.26	14.10 ±0.30	14.24 ±0.26
Clay (%)	5.20 ±0.23	10.27 ±0.45	11.02 ±0.56	12.36 ±0.58
Moisture content (%)	62.42 ±0.82	57.61 ±0.50	48.06 ±0.48	42.16 ±0.38

Mean ± Standard Error

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Table 2: Physico Chemical Properties of Polluted Soil at 90 Days after Planting (Tms 30572)

	Concentration (%)			
Parameters	0	2	4	6
pH	5.6±0.14	5.4±0.28	5.3±0.68	5.0 ±0.20
Total N(%)	0.36±0.09	0.14±0.08	0.08 ±0.06	0.04 ±0.03
Available P (mg/kg)	72.1±0.50	13.0 ±0.22	11.0 ±0.30	9.2 ±0.54
Mn (mg/kg)	10.7±0.20	10.7 ±0.30	11.3±0.35	12.1±0.40
Zn (mg/kg)	9.6 ±0.36	6.1±0.48	4.7 ±0.30	4.2±0.62
Cu (mg/kg)	14.1 ±0.25	7.2±0.26	6.1±0.23	5.7±0.19
Pb (mg/kg)	9.2 ±0.54	6.1±0.30	4.2 ±0.82	3.6 ±0.40
Fe (mg/kg)	16.1 ±0.27	17.2 ±0.42	18.2 ±0.39	18.9±0.56
Cd (mg/kg)	9.3 ±0.28	9.4±0.38	11.6 ±0.26	11.9 ±0.66
Cr(mg/kg)	10.2 ±0.17	10.7 ±0.29	11.2 ±0.56	11.5 ±0.32
K(mg/kg)	82.1 ±0.63	20.6 ±0.59	19.6 ±0.52	17.0±0.24
Na (mg/kg)	297.2 ±0.90	310.7 ±0.95	325.2 ±0.82	360.7 ±0.86
Mg (mg/kg)	34.2 ±0.55	35.1±0.68	36.7 ±0.55	37.0±0.48
Ca (mg/kg)	22.1 ±0.38	23.4 ±0.48	24.2±0.84	24.8 ±0.49
Total organic C (%)	0.09±0.24	2.97 ±0.39	3.12±0.46	3.22 ±0.65
Total organic matter (%)	1.07 ±0.28	0.92 ±0.28	0.74 ±0.25	0.66±0.08
THC (mg/kg)	539.17 ± 1.41	193.14 ± 1.73	267.18± 1.45	384.93±2.43
Sand (%)	78.04±0.94	77.20 ±0.78	75.21 ±0.85	74.06 ±0.90
Silt (%)	15.32 ±0.68	11.14 ±0.58	13.07 ±0.48	13.30±0.50
Clay (%)	5.30 ±0.26	10.20 ±0.75	11.02 ±0.44	12.24±0.88
Moisture content (%)	60.21 ±0.82	57.36 ±0.96	47.18±0.89	42.52±0.45

Mean ± Standard Error

Table 3: Nutrient Contents In *Manihotesculenta* (TMS 30572)At 90 Days After Planting (Control + Pollution Treatment)

	Concentration (%)			
Parameters	0	2	4	6
CHO (%)	56.72±0.58	44.30±0.48	38.06±0.21	33.61±0.25
Protein (%)	49.30±0.54	34.13±0.28	30.24±0.65	24.07±0.25
T.N(%)	6.21±0.15	5.02±0.28	4.21±0.14	3.62±0.19
T.Chlorophyll(mg/l)	641.07±0.98	482.30±0.91	426.46±0.82	334.20±0.58
Ca (mg/kg)	138.3±0.45	92.6±0.74	84.7±0.30	82.1±0.45
Mg (mg/kg)	113.6±0.16	90.7±0.46	80.2±0.53	64.2±0.85
K (mg/kg)	61.6±0.45	62.6±0.28	67.4±0.24	70.3±0.18
Na (mg/kg)	240.6±0.61	221.7±0.35	210.6±0.45	204.4±0.56
P (mg/kg)	150.2±0.14	120.7±0.24	114.2±0.24	90.6±0.43
Mn(mg/kg)	36.7±0.27	37.2±0.35	38.5±0.45	39.2±0.42
Zn(mg/kg)	4.0.0±0.07	23.2±0.43	32.1±0.52	32.7±0.23
Pb (mg/kg)	13.7±0.64	24.2±0.42	30.2±0.53	32.9±0.45
Fe (mg/kg)	14.0±0.22	15.2±0.45	18.7±0.54	20.7±0.28
Cu (mg/kg)	13.2±0.28	18.6±0.75	30.2±0.16	32.6±0.48

Mean ± Standard Error

Table 4: (Amelioration treatment) Nutrient contents in *Manihotesculenta* (TMS 30572) at 90 Days after Planting

	COMPOST				**NPK**				**NPK+ COMPOST**			
Parameters	**0%**	**2%**	**4%**	**6%**	**0%**	**2%**	**4%**	**6%**	**0%**	**2%**	**4%**	**6%**
CHO (%)	56.81±0.68	53.10±0.56	50.13±0.46	45.21±0.38	56.34±0.69	50.35±0.56	47.32±0.50	44.06±0.45	57.02±0.42	56.01±0.72	54.36±0.44	53.17±0.42
Protein (%)	49.37±0.45	44.17±0.30	43.86±0.56	41.19±0.35	49.21±0.44	42.07±0.58	38.16±0.36	35.12±0.48	49.37±0.28	48.32±0.48	47.26±0.68	45.31±0.64
T.N (%)	6.28±0.28	6.33±0.84	6.12±0.58	5.73±0.28	6.24±0.28	5.84±0.38	5.20±0.45	4.97±0.48	6.21±0.56	6.32±0.48	6.28±0.56	6.02±0.46
T.Chlorophyll (mg/l)	643.0±0.80	584.2±0.46	482.1±0.38	477.0±0.43	644.0±0.86	570.1±0.80	467.2±0.82	436.1±0.86	649.2±0.85	602.1±0.82	584.7±0.85	542.7±0.65
Ca (mg/kg)	137.2±0.28	112.6±0.85	102.0±0.68	100.2±0.38	136.1±0.65	98.7±0.45	95.0±0.75	92.4±0.38	137.0±0.87	132.1±0.65	127.0±0.38	118.6±0.65
Mg (mg/kg)	114.1±0.54	96.1±0.56	84.0±0.45	78.4±0.38	114.3±0.65	89.5±0.28	80.7±0.36	74.6±0.48	114.6±0.33	103.1±0.64	98.2±0.54	90.6±0.48
K(mg/kg)	61.4±0.65	68.2±0.28	75.3±0.40	80.9±0.54	61.6±0.43	70.2±0.45	76.2±0.54	82.1±0.42	61.5±0.38	69.4±0.42	75.7±0.52	84.6±0.45
Na (mg/kg)	240.7±0.80	302.1±0.20	261.9±0.76	230.4±0.20	240.5±0.36	260.2±0.36	252.1±0.30	243.2±0.80	240.6±0.65	293.2±0.30	272.0±0.56	252.6±0.56
P (mg/kg)	151.2±0.42	146.7±0.25	145.2±0.26	139.6±0.50	151.2±0.28	145.2±0.62	138.7±0.58	130.2±0.24	151.3±0.68	143.6±0.56	147.2±0.72	145.6±0.62
Mn (mg/kg)	34.9±0.44	50.2±0.20	53.8±0.44	59.2±0.48	34.8±0.58	42.4±0.32	46.7±0.34	49.0±0.75	34.7±0.46	52.1±0.31	58.2±0.30	60.4±0.26
Zn (mg/kg)	4.2±0.62	36.0±0.28	47.0±0.42	50.2±0.36	4.0±0.14	32.7±0.34	35.7±0.34	42.1±0.29	4.1±0.32	48.2±0.44	54.2±0.44	59.6±0.52
Pb (mg/kg)	13.2±0.04	28.3±0.54	39.6±0.30	48.0±0.18	13.2±0.12	26.0±0.60	30.7±0.38	33.4±0.38	13.3±0.24	30.2±0.72	42.6±0.65	48.4±0.84
Fe (mg/kg)	13.7±0.28	23.0±0.42	24.7±0.45	25.0±0.53	13.9±0.45	19.3±0.72	20.4±0.65	21.2±0.68	13.7±0.74	24.0±0.54	26.6±0.12	30.1±0.38
Cu (mg/kg)	13.6±0.15	23.6±0.64	31.6±0.50	35.6±0.34	13.5±0.12	21.7±0.36	30.2±0.46	30.7±0.55	13.6±0.13	26.7±0.68	38.3±0.74	39.6±0.65

Mean ± standard error

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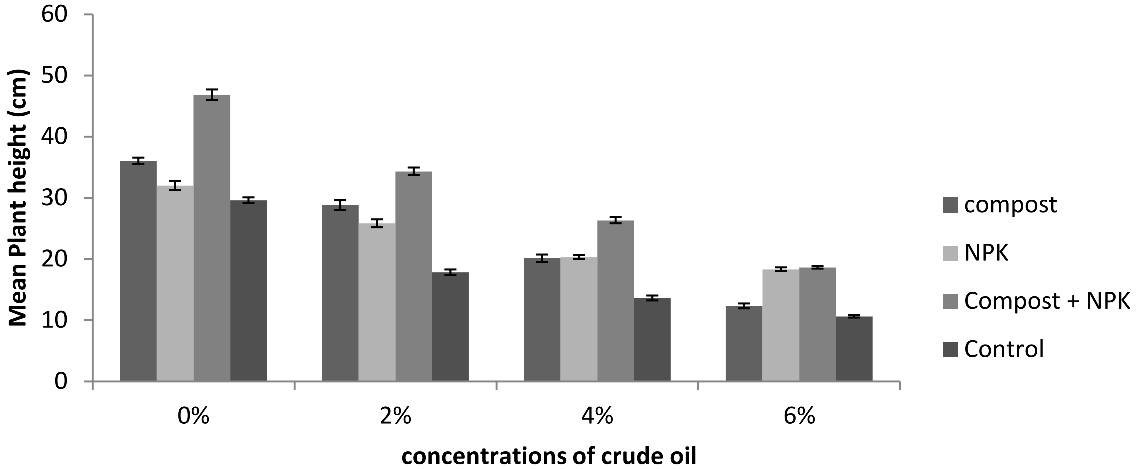

Fig. 1. Mean Plant height (cm) at 90 days after planting (control and amendment) (TMS 30572)

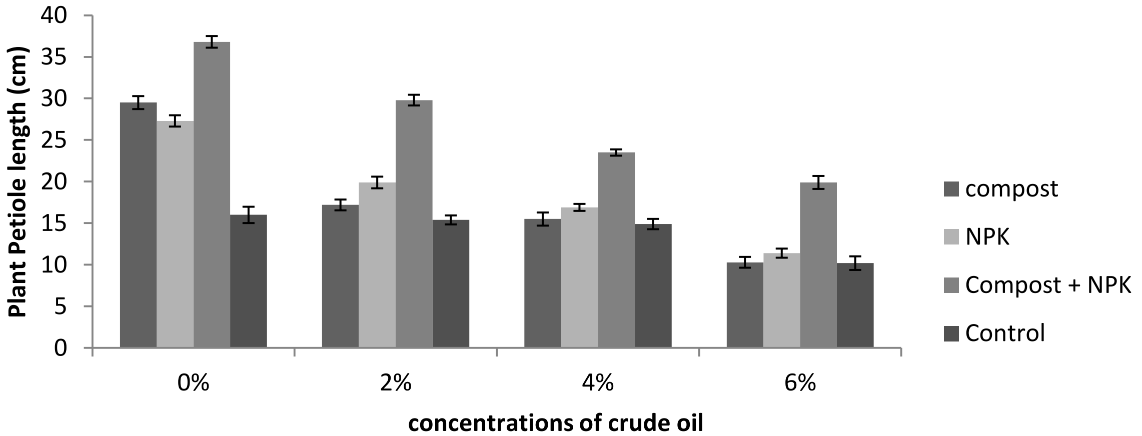

Fig. 2: Plant Petiole length (cm) at 90 days after planting (control and amendment) (TMS 30572)

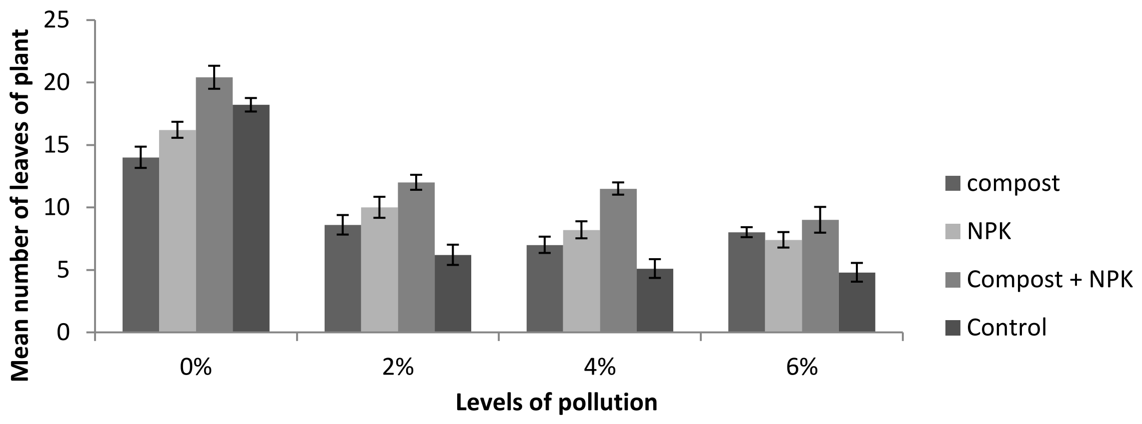

Fig.3. Mean number of leaves of plant 90 days after planting (control and amendment) (TMS 30572)

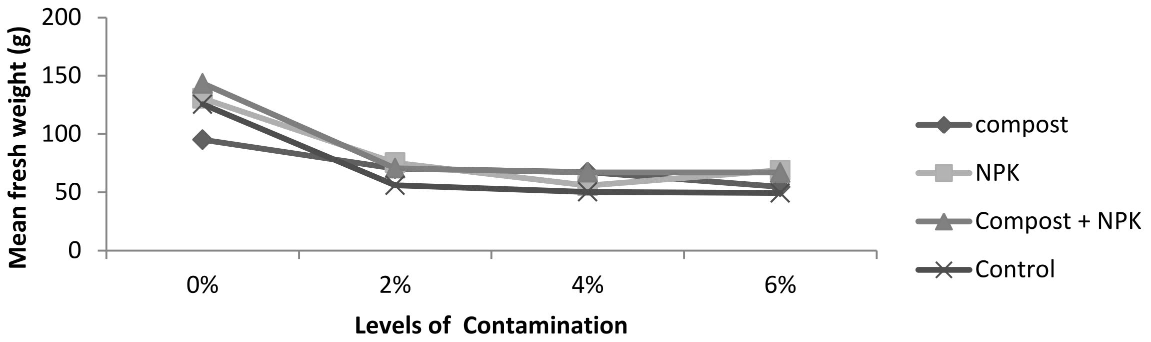

Fig. 4. Mean fresh shoot weight (g) of Variety TMS 30572

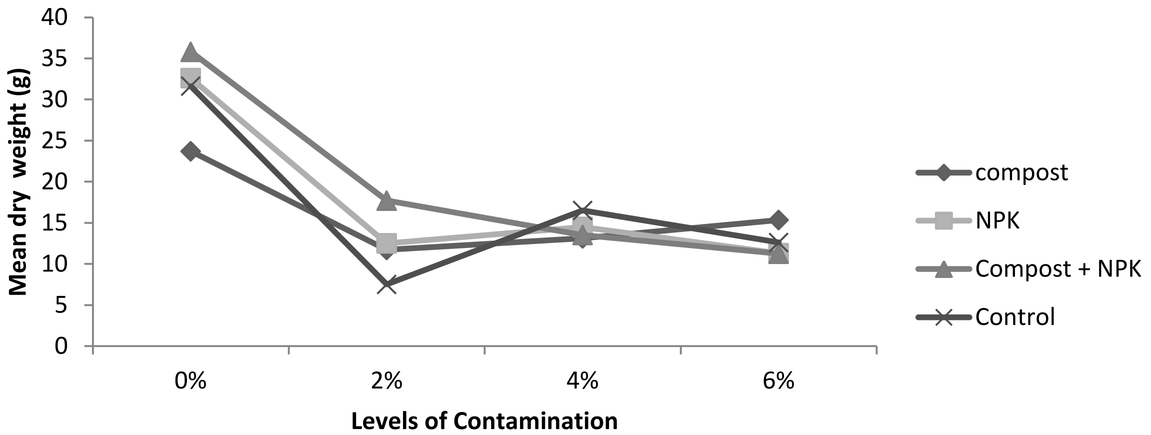

Fig.5. Mean dry shoot weight (g) of Variety TMS 30572

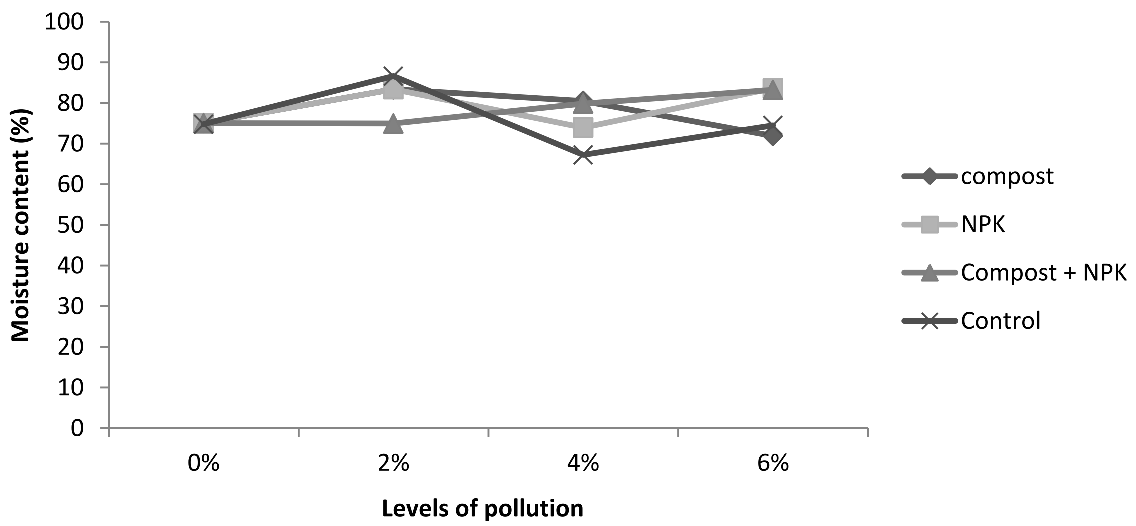

Fig. 6. Moisture content (%) of Variety TMS 30572

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DISCUSSION

The reduction in plant height noticed in polluted soil could be due to reduction in nutrient contents of the soil.Since the petroleum products are known to reduce nitrogen availability in soil (Agbogidi*et al.*, 2007).

Crude oil pollution also affected the leaf area of the crop which consequently affected the physiological activities of the cassava plant. This may be as a result of interference of the oil constituent with photosynthesis and transpiration probably by clogging the stomata (Al-Azab*et a*l., 2005). The concentration of crude oil pollution influenced the growth parameters of the crop at low and high pollution levels, respectively, compared to the control. (Offorand Akonye, 2006).Available % nitrogen and phosphorus of the soil decreased with increase in the levels of crude oil pollution,as shown in Table 1. Onuh*etal*. (2008) had also observed a decrease in nitrogen availability with increased levels of crude oil pollution. Similarly, a decrease in phosphorus availability with increased levels of crude oil pollution had been reported (Okolo*etal*., 2005; Ogboghodo*etal*., 2005; Isirimah*etal*., 1989). Exchangeable bases (Calcium, Potassium and Magnesium) were observed to have decreased with increased levels of crude oil pollution. This may be attributed to the use of these exchangeable bases by the microbes present in the experimental soil samples.

It may be suggested that the reduction in crop yield could have been due to competition for the limited nutrients between microbial population and the crop as a result of high carbon content in the soil relative to nitrogen (Inoni, 2006; Fernet, 2008), Bioremediating the contaminated soil with different remediation treatments showed an improvement in the soil status especially in the nutrients content.It has been reported that addition of nitrogen and phosphorus enhanced biodegradation of polluted soil presumably by removing the nitrogen and phosphorus limitation resulting from low natural level (Lee, *et al*., 1993). Lee, *et al*. (1995) also reported that inorganic fertilizer has a greater effect in stimulating crude oil degradation by increasing the total heterotrophic microbial growth and activity. It therefore means that NPK made available the necessary elements to assure and energize the microorganisms.

Both organic and inorganic fertilizers have proved to be very beneficial in ameliorating crude oil polluted soil by adding nutrients to the soil which creates an enabling environment for the microorganisms to thrive, thus degrading the toxic hydrocarbons in soil, making it harmless and creating an environment that supports plant growth and development. Thereforethis study shows that*Centrosemapubescens*and NPK fertilizer in single and combined treatments is effective in remediation of crude oil polluted soil for cassava cultivation.

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