



Mitigation of the Effect of Crude Oil Polluted Soil on the Growth and Development of Cassava-*Manihot esculenta* Crantz with Organic and Inorganic Amendments

P. O. Eremrena^{1*} and L. A. Akonye¹

¹Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author POE designed the study, wrote the protocol and the first draft of the manuscript. Author LAA managed the literature searches, analyses of the study performed the spectroscopy analysis. Author POE managed the experimental process and author LAA identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

NPK and compost of *Centrosema pubescens* were studied at the University of Port Harcourt Botanic garden for their effect on the growth components of *Manihot esculenta*, Crantz (NR 8082) in a crude oil polluted soil. The soil samples were polluted at four different levels (0%, 2%, 4% and 6%) with crude oil and amended with organic supplement (decomposed *Centrosema pubescens*) and NPK fertilizer at the rate of 0.25 Kg per 5 Kg of soil to the various levels of crude oil contaminated soil, alongside a control. A total of 48 plastic buckets were used, each treatment was replicated three times. Mean values of Physico- chemical properties of experimental soils were expressed. Quantitative observations showed that amelioration treatments recorded significant ($P=0.05$) increase in plant

*Corresponding author: E-mail: peter.ermreana@uniport.edu.ng;

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 at $P=0.05$ significantly increased percentage total organic carbon, total organic matter and total hydrocarbon content (THC) while pH, percentage total nitrogen, phosphorus and cation nutrients (Ca, K and Mg) were significantly decreased at two weeks after pollution. The results also showed that the amendment treatments at $P=0.05$ significantly decreased crude oil toxicity at different degrees by improving the nutrients content and decreasing the total hydrocarbon content of the soil.

Therefore, *Centrosema pubescens* and NPK fertilizer in single and combined treatments is effective in remediation of crude oil polluted soil for cassava cultivation.

Keywords: Mitigation; growth; *Manihot esculenta*; crude oil; pollution; amelioration; compost.

1. INTRODUCTION

Crude oil contamination constitutes one of the most prevalent sources of environmental degradation in the industrialized world. Human technological and scientific advances have caused environmental changes that are impossible to evaluate and fully comprehend. Our ability to change the environment has increased faster than the ability to predict the effect of that change. Pollution of the environment is one of the major effects of human technological advancement. This has raised considerable concern on the subject of crude oil pollution especially on arable agricultural land.

Pollution is thus defined as the introduction of deleterious substance into the environment that endangers human health and other natural resources [1]. It results when a change in the environment harmfully affects the quality of human life including effects on animals, microorganism and plants [2,3,4,5]. 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) in quantities that are poisonous or capable of causing immediate physical, chemical and biological damage to the affected ecosystem [6,7,8,9,10,11]. One of the environmental challenges posed by this oil pollution is the alteration in the physical, chemical and biological nature of the soil which subsequently affects the growth of plants [12,13,14,15,16]. The discharge of crude oil on land also affects the physicochemical properties of the soil, thus causing deleterious effect on seed germination and growth [17,18,19,20,21].

Petroleum (crude oil) pollution has been found to affect the cultivation and production of economic crops including Cassava especially in Niger Delta.

Consequently, the presence of these hydrocarbons in soils has resulted to further deposition of heavy metals and other various components of hydrocarbon thereby, decreasing the fertility of the soils. This change in the physical, chemical or biological characteristics of the air, water or soil have harmfully affected the health, survival or activities of humans or their organisms [11,22,23]. Therefore, there is need to remediate pollution site to improve plant performance. The study attempts to investigate the growth performance of Cassava

(NR 8082) in crude oil contaminated soil amended with *Centrosema pubescens* and NPK. 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 effect 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.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

Soil samples obtained from University of Port Harcourt Botanical garden was used for the study. The crude oil was obtained from Nigerian National Petroleum Corporation, Eleme, Rivers State. Cassava (*Manihot esculenta* Crantz) NR 8082 variety cuttings and NPK 15:15:15 were sourced from Agricultural Development Programme (ADP) in Rivers State. Leaves of *Centrosema pubescens* were harvested from farms at Alakahia community opposite University of Port Harcourt Teaching Hospital (UPTH) and Abuja campus.

2.2 Analysis of Soil Samples

Physico-chemical properties of the experimental soils (loamy soil, and contaminated soil) were analyzed using standard procedure [24,25,26,27].

A total of 48 plastic buckets were used, each treatment was replicated three times.

2.3 Pollution Treatment

Top loamy soil weighing 5kg for 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. 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 remediation.

2.4 Remediation Materials/ Treatment

Leaves of *Centrosema pubescens* and NPK 15:15:15 were used to ameliorate the crude oil contaminated soil. The reasons for the choice of these two materials are that they are cheap, 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. The remediation/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 three weeks was allowed for the NPK and leaves of *Centrosema pubescens* to decompose in the soil before planting. Three cassava cuttings, ranging from 4-5cm were planted thereafter.

2.5 Growth and Biochemical Parameters

The following growth and biochemical parameters were analysed: Plant height, petiole length, leaf number, fresh weight, dry weight, total organic carbon (TOC), total organic matter (TOM), pH, available phosphorus, Total Nitrogen and heavy metals. The shoot length (plant

height) was measured with a metre tape in centimetres from the soil surface to the plant apex. The plant were uprooted from each bucket and weighed immediately 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 at 80°C for 24 hours to get rid of all moisture and ensure a constant weight. It was then weighed. Leaf chlorophyll content was extracted from 1.0 g of leaf sample. The sample was homogenized by adding small amount of 85% acetone. 25 cm³ aliquot of extract was added to 50 cm³ diethyl ether in a separating funnel. The optical density at 660 nm and 643 nm in 1 cm cell was measured using ether as a reference. Leaf carbohydrate content was analysed by extracting 1.0 g of dry leaf sample and digested with Perchloric acid and the sugar was determined colorimetrically by the Anthrone method. The nitrogen content was determined by the Kjeldahl method (20) in which 1.0 g of leaf sample was heated on an electro thermal hot plate, until digest turned to sky-blue, then diluted with 100cm³ of diluted water. 30 cm³ of 40% NaOH added and the sample was heated to release ammonia. The distillate was titrated with 0.01 M hydrochloric acid.

2.6 Statistical Data Evaluation

All data generated were subjected to statistical analysis such as analysis of variance (ANOVA) and standard error at P=0.05. T-test and New Duncan multiple range test were employed to separate means according to the procedure of Statistical Package for Social Sciences for Windows version 17.0 (SPSS Inc., Chicago, IL).

3. RESULTS

Result showed that addition of remediation treatments to the polluted soil affected the shoot length of NR 8082 cassava variety at different degrees. The treatments improved the shoot length of NR 8082 cassava varieties at 90 days after planting (Fig. 1). There was statistical significant differences in average plant height among the contaminated unamended plants; Contaminated, Compost amended plants; Contaminated NPK amended plants and contaminated Compost + NPK amended plants at P=0.05. That is, the shoot length of the cassava variety increased significantly with time. The result obtained shows that significant differences (P=0.05) exist between the various concentration of crude oil used for the experiment during the data study period.

Treatment option Compost + NPK recorded the highest shoot length throughout the growth period while the control (natural attenuation) recorded the least.

The petiole length of NR 8082 cassava variety was enhanced considerably in all the amended soil samples contaminated with crude oil than in the soil treated with crude oil alone (Fig. 2).

However the petiole length decreased as the concentration of the crude oil increased. The

result showed that high crude oil contamination decreased the petiole length of the plant. Significant differences between treatment means ($P=0.05$) was also obtained.

The leaf number increased with time for the cassava variety NR 8082. Highest number of leaves was recorded at 90 days after planting. The leaf number of the plant (Fig. 3) in the amended treatment Compost + NPK at 0% contamination had the highest leaf number. The

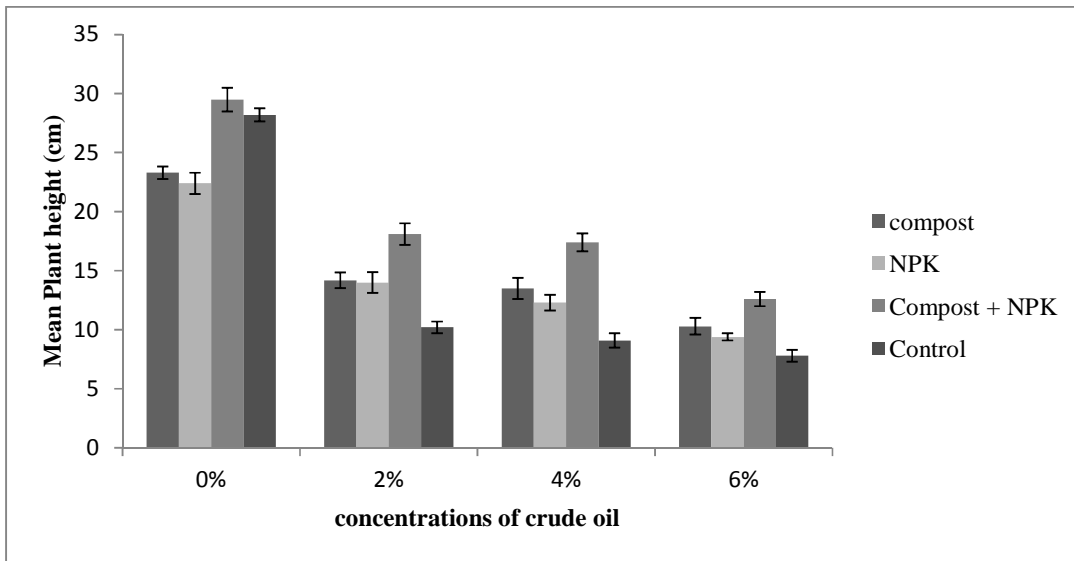


Fig. 1. Mean plant height (cm) at 90 days after planting control and amendment (NR 8082)

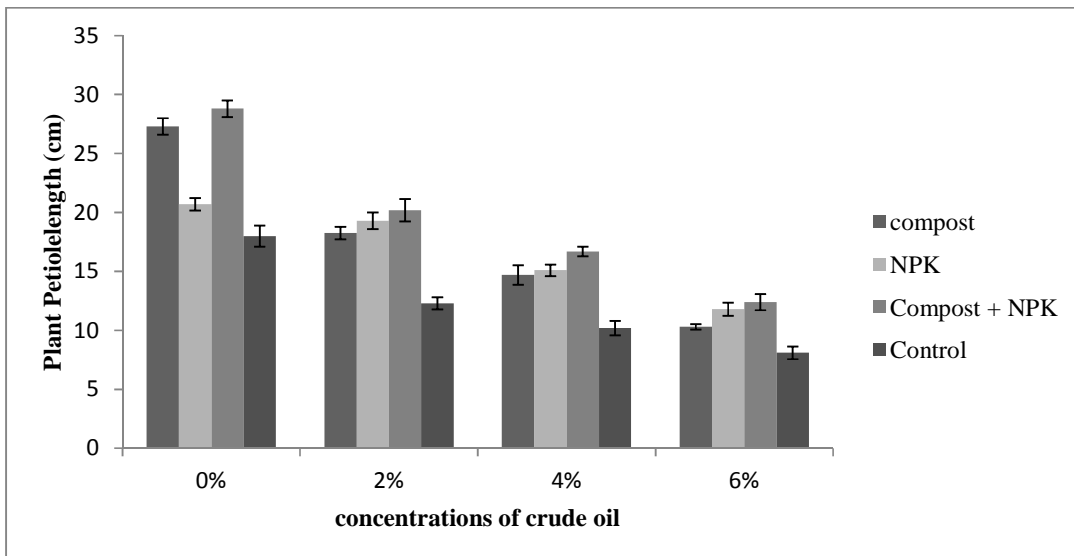


Fig. 2. Plant petiole length (cm) at 90 days after planting control and amendment (NR8082)

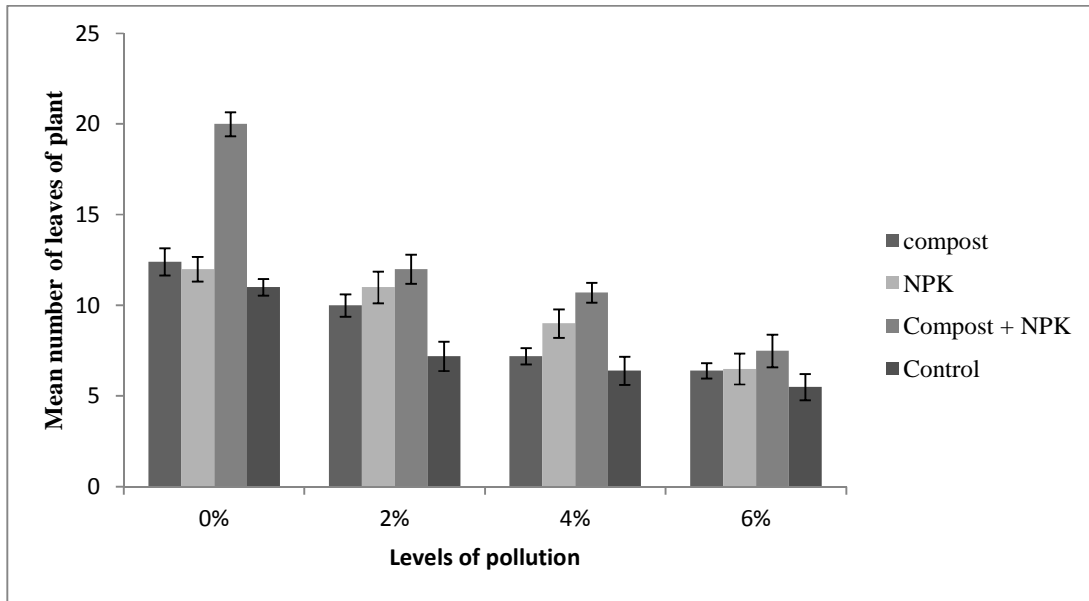


Fig. 3. Mean number of leaves of plant 90 days after planting control and amendment (NR 8082)

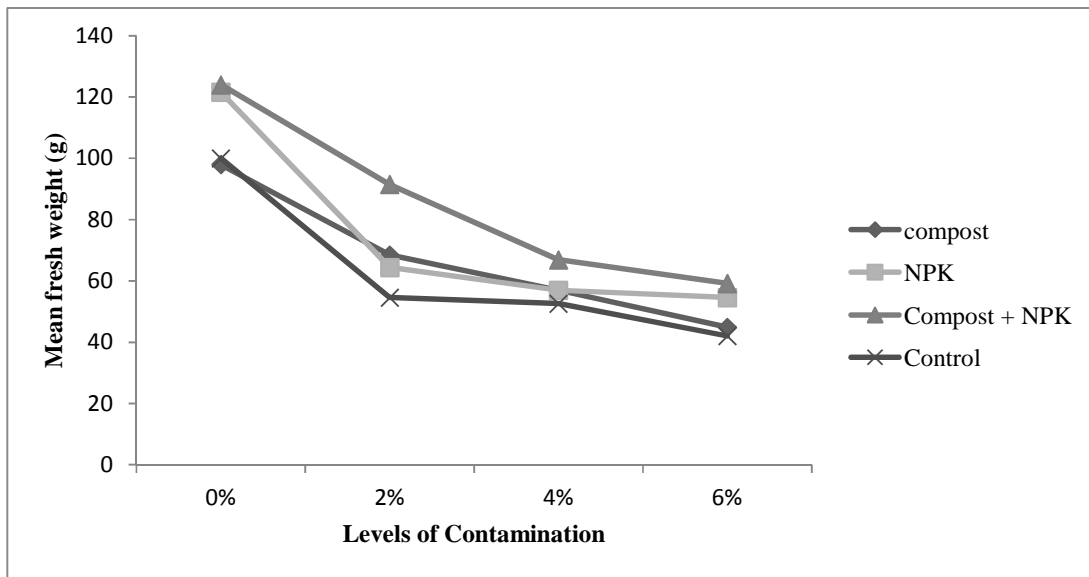


Fig. 4. Mean fresh weight of variety NR 8082

leaf number of the plant decreased as the percentage concentration of the crude oil increased. However the soil without amendment had lower leaf number as the concentration of crude oil increased. The 2% crude oil contamination recorded high leaf number as a result of low toxicity. There was significant difference between and within treatments at $P=0.05$. Growth in fresh weight decreased with

increase in levels of pollution (Fig. 4). There was significant difference between and within treatments at $P=0.05$. Application of amelioration agents to pollution levels showed improvement in fresh weight of *M. esculenta*. However, the increase in fresh weight was considerably higher at 2% pollution treatments than 6% pollution. The control gave the highest significant increase than other treatments.

The result of the Dry weight (g) at 90 days after planting is presented in Fig. 5. Differences in treatment options also resulted in differences in Dry weight. The highest mean dry weight was obtained from plants grown in the treatment Compost + NPK. The least mean dry weight was obtained from plants grown in the treatment cassava + 6% crude oil polluted soil.

The moisture content was not affected by the impact of crude oil (Fig. 6). There was no significant difference between moisture content between and within treatments for the Cassava variety NR 8082 at P=0.05. Addition of

ameliorating materials 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.

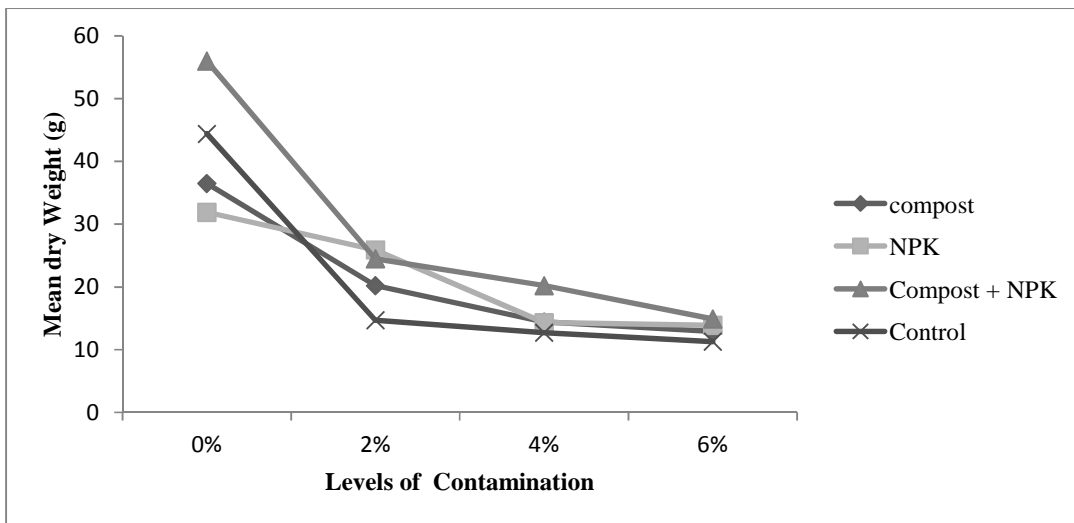


Fig. 5. Mean dry weight (g) of variety NR 8082

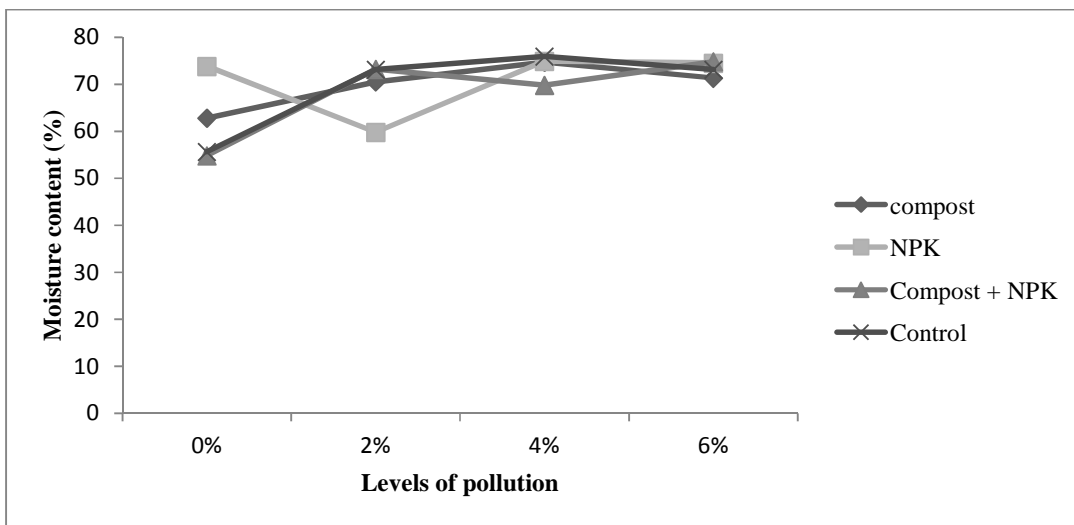


Fig. 6. Moisture content (%) of variety NR 8082

Table 1. Physicochemical properties of experimental soil (5)

Parameters	Concentration (%)			
	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***Table 2. Physico chemical properties of polluted soil after harvest (NR 8082)**

Parameters	Concentration (%)			
	0	2	4	6
pH	5.6±0.11	5.4±0.21	5.2±0.14	4.9 ±0.14
Total N(%)	0.34±0.04	0.12 ±0.05	0.09 ±0.08	0.02 ±0.05
Available P (mg/kg)	70.2±0.56	12.4 ±0.24	10.7 ±0.22	8.2 ±0.20
Mn (mg/kg)	10.2 ±0.18	10.4 ±0.25	11.2±0.26	11.6 ±0.40
Zn (mg/kg)	9.3 ±0.42	5.2±0.30	4.3 ±0.24	3.8±0.20
Cu (mg/kg)	13.0 ±0.34	6.6 ±0.22	5.8 ±0.25	5.4±0.17
Pb (mg/kg)	8.6 ±0.32	4.9±0.25	3.7 ±0.22	3.0 ±0.19
Fe (mg/kg)	15.4 ±0.24	16.5 ±0.30	17.0 ±0.32	17.8 ±0.50
Cd (mg/kg)	8.3 ±0.14	9.7±0.28	10.4 ±0.23	11.0 ±0.25
Cr(mg/kg)	9.7 ±0.15	10.7 ±0.18	11.0 ±0.28	11.4 ±0.28
K(mg/kg)	78.5 ±0.55	20.8 ±0.55	18.2 ±0.34	16.0±0.25
Na (mg/kg)	296.2 ±0.70	300.6 ±0.58	314.0 ±0.48	341.7 ±0.70
Mg (mg/kg)	30.2 ±0.45	30.7 ±0.34	31.2 ±0.55	32.2±0.35
Ca (mg/kg)	20.2 ±0.28	20.9 ±0.38	21.6 ±0.29	22.0 ±0.37
Total organic C (%)	0.09±0.20	2.92 ±0.27	3.07±0.40	3.16 ±0.30
Total organic matter (%)	1.02 ±0.17	0.84 ±0.25	0.76 ±0.24	0.64±0.08
THC (mg/kg)	540±1.07	4881±2.43	5420±2.07	6373±3.82
Sand (%)	78.03 ±0.86	76.22 ±0.78	74.28 ±0.59	73.17 ±0.60
Silt (%)	15.38 ±0.45	12.17 ±0.28	14.09 ±0.36	14.22±0.29
Clay (%)	5.40 ±0.25	10.24 ±0.44	11.04 ±0.52	12.39 ±0.48
Moisture content (%)	60.32 ±0.84	55.41 ±0.54	45.16 ±0.50	40.07 ±0.39

Mean ± Standard Error

4. DISCUSSION

Growth is an important outcome of the physiology of plants. Any distortions in the physiology of the plant due to environmental factors like water, pollution, temperature, etc will be physically manifested in their growth form. Plant height, Petiole length, number of leaves, moisture content, leaf area, fresh and dry weight are some growth parameters in plants.

Results indicated that there was a general improvement (increase) in the Plant height, Petiole length, number of leaves, moisture content, fresh weight and dry weight, in Cassava in all the remediated soils and the control with time. The reverse was the case in the unremediated soil (natural attenuation option), which showed a decline in growth with time. Although, there was some fluctuations in some of these growth parameters in some treatments. Variation also occur in the level of improvement of the aforementioned growth parameters between varieties and remediation treatments. This might be deduced that all the remediation treatments options remedied the polluted soil. This is understandable because there was an improvement in the nutrient status in the soil (which was lacking before remediation) with time as revealed by soil analysis.

It is a well established fact that crude oil pollution affects the physico-chemical characteristics of

soil as well as the overall performance of plants. [4,5,10,14,16,22,23]. This was also applicable to the cassava variety NR 8082.

Bioremediating the contaminated soil with different remediation treatments showed an improvement in the soil status especially in the nutrients content. Also the performance of NR 8082 cassava variety also improved with variations in the different treatments. These remediation materials amended the soil and hence the performance of cassava variety.

Result indicated that crude oil pollution reduced the Nitrogen and phosphorus content of the soil when compared with the control ($P=0.05$). This is in line with [12,14,27]; Who discovered that Nitrogen and phosphorus are always limiting factors in a crude oil polluted soil. Although, addition of different remediation materials improved the nutrient content (nitrogen and phosphorus) of the soil with time. Result revealed that there was a significant difference in the total organic carbon content between and within treatments. Crude oil increased the total organic carbon of the soil but later the TOC decreased. It can be deduced that the initial increase was as a result of the crude oil introduced. [7] reported that the reason for high carbon content in contaminated soil is that hydrocarbon have high carbon content but poor suppliers of nitrogen and phosphorus.

Table 3. Nutrient contents in *Manihot esculenta* (NR 8082) after harvest-pollution treatment + control

Parameters	Concentration (%)			
	0	2	4	6
CHO (%)	56.47 ±0.50	42.33 ±0.43	36.17 ±0.39	30.21 ±0.30
Protein (%)	48.20 ±0.56	32.06 ±0.42	28.06 ±0.36	20.15 ±0.46
T. N(%)	5.30 ±0.30	4.21 ±0.20	3.57 ±0.25	3.06 ±0.10
T. Chlorophyll(mg/l)	634.23 ±0.89	476.06 ±0.64	397.21 ±0.54	282.56 ±0.45
Ca (mg/kg)	131.6 ±0.34	80.4 ±0.20	78.8 ±0.23	79.7 ±0.30
Mg (mg/kg)	101.7 ±0.24	75.0 ±0.20	63.9±0.12	57.6 ±0.23
K (mg/kg)	53.2±0.30	54.7 ±0.35	58.7 ±0.36	60.2 ±0.41
Na (mg/kg)	220.7 ±0.30	216.6 ±0.29	197.6 ±0.17	194.0 ±0.14
P (mg/kg)	142.1 ±0.40	90.2±0.20	85.6 ±0.13	81.2 ±0.15
Mn(mg/kg)	33.2 ±0.30	35.2 ±0.26	36.1 ±0.24	37.6 ±0.35
Zn(mg/kg)	2.4 ±0.02	22.1±0.20	30.2 ±0.23	30.7 ±0.30
Pb (mg/kg)	12.1 ±0.15	23.6 ±0.22	28.7 ±0.28	31.2 ±0.17
Fe (mg/kg)	13.6 ±0.19	14.6 ±0.26	17.2 ±0.30	19.7 ±0.28
Cu (mg/kg)	12.7 ±0.42	17.2 ±0.34	29.4 ±0.23	31.9 ±0.28

Mean±Standard Error

Table 4. Amelioration treatment (NR 8082) nutrient contents in *Manihotes culenta* after harvest

Parameters	Compost				NPK				NPK+ Compost			
	0%	2%	4%	6%	0%	2%	4%	6%	0%	2%	4%	6%
CHO (%)	57.36±0.52	52.17±0.42	48.14 ±0.32	42.36±0.43	56.52 ±0.52	48.20 ±0.37	45.31 ±0.40	43.40±0.46	58.18 ±0.60	54.21±0.25	53.12 ±0.64	52.06±0.42
Protein (%)	49.32 ±0.42	43.63 ±0.52	43.01 ±0.60	38.12 ±0.25	48.93 ±0.30	41.35 ±0.45	36.15±0.32	33.67 ±0.23	48.72±0.57	46.43 ±0.42	45.01 ±0.52	42.07±0.36
T.N (%)	5.42±0.24	6.53±0.18	5.21±0.40	4.63±0.43	5.66±0.37	4.96±0.32	4.62±0.25	4.02±0.32	5.47±0.42	7.02±0.52	5.59±0.21	5.26±0.32
T. Chlorophyll (mg/l)	636.2±0.89	572.3±0.87	467.4±0.77	453.0±0.65	642.1±0.85	563.0±0.97	456.3±0.64	421.6±0.57	639.2±0.66	648.0±0.81	592.0±0.42	532.2±0.43
Ca (mg/kg)	132.1±0.13	100.2±0.23	92.3±0.50	90.7±0.68	133.4±0.42	91.4±0.72	86.7±0.67	87.9±0.54	134.7±0.23	124.3±0.12	112.6±0.27	105.3±0.5
Mg (mg/kg)	103.3±0.77	82.5±0.65	74.7±0.65	70.6±0.55	102.1±0.89	80.2±0.76	70.6±0.45	65.2±0.37	102.5±0.98	93.6±0.89	89.2±0.56	76.6±0.54
K (mg/kg)	54.0±0.24	67.1±0.45	72.0±0.56	79.8±0.67	54.6±0.35	69.6±0.43	75.0±0.65	80.4±0.97	58.7±0.63	68.2±0.52	75.2±0.64	89.2±0.88
Na (mg/kg)	230.1±0.12	292.4±0.10	251.7±0.24	240.6±0.22	220.9±0.19	242.1±0.32	230.7±0.21	223.1±0.34	230.7±0.18	299.0±0.36	280.6±0.35	264.2±0.28
P (mg/kg)	143.4±0.18	146.2±0.15	141.6±0.23	130.9±0.13	146.7±0.26	142.1±0.18	133.6±0.17	125.7±0.24	147.7±0.32	149.3±0.22	147.2±0.31	140.7±0.12
Mn (mg/kg)	34.1±0.12	49.3±0.26	52.6±0.34	57.2±0.35	33.9±0.28	38.7±0.32	42.5±0.28	47.2±0.34	32.7±0.21	49.6±0.29	53.7±0.39	59.4±0.18
Zn (mg/kg)	2.9±0.02	34.2±0.46	46.1±0.32	47.6±0.25	2.6±0.12	35.7±0.26	39.2±0.40	40.7±0.35	2.5±0.12	39.6±0.18	47.2±0.32	49.7±0.24
Pb (mg/kg)	12.4±0.19	27.2±0.21	39.1±0.31	44.6±0.30	12.3±0.18	25.6±0.31	30.1±0.26	32.1±0.29	12.5±0.49	28.3±0.38	40.1±0.32	47.1±0.52
Fe (mg/kg)	13.9±0.12	20.2±0.24	21.7±0.20	22.6±0.15	13.6±0.25	18.4±0.24	19.2±0.16	20.7±0.32	13.9±0.14	22.5±0.23	23.5±0.34	28.7±0.18
Cu (mg/kg)	12.8±0.25	21.7±0.32	30.7±0.21	34.2±0.28	12.6±0.19	20.3±0.12	29.7±0.36	32.0±0.23	12.8±0.31	25.2±0.42	37.6±0.24	39.1±0.25

Mean ± standard error

4. CONCLUSION

Results obtained clearly indicate that the different remediation treatment options used (either single or in combination) have the potentials to ameliorate crude oil polluted soil especially at medium concentration. It was also observed that polluted soil remediated with Compost and NPK 15:15:15 achieved the highest remediation than all the other treatment options. It is therefore, advisable that great caution should be taken for the use of any of these remediation materials especially NPK (in terms of its quantity) so as not to cause nutrient toxicity in the soil which invariably will inhibit the growth of crops. The effectiveness of any remediation materials or methods depends on the soil status, type and concentration of oil and the season of the year.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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