Growth and Biochemical Performances of Two Cassava (Manihot esculenta, Crantz) Varieties to Crude oil Polluted biostimulated Soil.

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Abstract: Growth and biochemical performances of two varieties (NR 8082 and TMS 30572) of Manihot esculenta; Crantz (cassava) in a crude oil polluted soil biostimulated with both organic and inorganic fertilizers were carried out. 5kg soil polluted with 250ml of crude oil were remediated with 20g NPK 15:15:15, 50kg poultry droppings and 10g NPK15:15:15 + 30g poultry droppings (combined) alongside a control (polluted soil but no remediation). After two months post-biostimulation, two stem-cuttings of each of the two cassava varieties were planted in each remediation treatment with 12 replicates each and allowed for 20 weeks. Results showed that addition of these biostimulation materials (NPK and poultry droppings) to the crude oil polluted soil improved the growth and biochemical performances of the two varieties. Highest significant (p=0.05) yields, especially the shoot length, fresh weight and dry weight yields were recorded in the NPK 15:15:15 treatment option while the control recorded the least. Result also showed that the treatment in combined form had no advantage over the single treatments in terms of growth and biochemical performances. Therefore, NPK 15:15:15 and poultry droppings are good biostimulation materials for the remediation of crude oil polluted soil for cassava cultivation, and NPK 15:15:15 (inorganic fertilizer) appeared to be a better treatment option than poultry droppings (organic fertilizer.)

Key words: pollution, poultry droppings, yield, NPK 15:15:15, biostimulation.

INTRODUCTION

The greatest threat to the Niger-Delta ecoregion of Nigeria is environmental pollution caused by crude oil spillage. Crude oil pollution is defined as the introduction of crude oil or its derivatives with its associated gases into the environment (air, water and land) in quantities that can be harmful or capable of causing immediate physical, chemical and biological alterations to the affected ecosystem[17]. One of the environmental challenges posed by this oil pollution is the alteration in the physical and chemical nature of the soil which subsequently affects the growth of plants[4]. 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[7,14]. Petroleum (crude oil) pollution 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 affect the physical and biochemical characteristics of the plant[17,3,18].

There is therefore, need to remediate such polluted site to improve plant performances. Biostimulation which is the promotion of growth of indigenous oil degraders by the addition of nutrient or other growth limited substrates; has been proven to be an affective strategy to enhance oil biodegradation. Lee et al.[5] reported that both organic and inorganic fertilizers are capable of stimulating crude oil degradation. Odokuma and Ibor[19], Venosa et al.[20], Akonye and Onwudije[41]; and Tanee and Kinako[19] have proved the effectiveness of biostimulation in crude oil polluted soil for plant growth. Tanee and Akonye[48] reported an improved biomass yield of cassava in a crude oil polluted phytoremediated soil.

The study attempts to investigate the growth and biochemical responses of cassava (var. Tms 30572 and NR 8082) to crude oil polluted remediated soil using inorganic and organic fertilizers as biostimulating materials. 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 and biochemical characteristics of plants and how local biostimulating materials such as NPK fertilizer and poultry droppings can be used to improve such conditions for better performances of crops.

MATERIAL AND METHODS

Sources of Materials: The top loamy soil used for the study were collected from the University of Port Harcourt Botanic Garden. The crude oil was obtained from Nigeria National Petroleum Corporation, Eleme, Rivers State; the poultry droppings were collected from a poultry farm in Bori, Rivers state; while the cellophane bags and NPK 15:15:15 were obtained from Agricultural Development Programme, Rumuodomaya, Port Harcourt.

Description of the Experimental Site: The study was carried out at the Botanic Garden University of Port Harcourt in the Niger Delta region of Nigeria under natural climatic and environmental conditions of total rainfall of 2551.3mm, mean minimum temperature range of 23.08°C and mean maximum temperature range 37.5°C. The Niger Delta region is situated in the Gulf of Guinea lying between Longitude 5°E to 8°E and Latitude 4°N to 6°N[12]. The area experienced two distinct seasons; a dry season and rainy season. It is a warm-wet climate. The soil of the area ranged from well-drained to moderately drained except in some areas where the soils were poorly drained[13]. The soil is always low in nutrient content due to the leaching of the nutrient down the earth profile by rainfall which is always very heavy in the area[14].

Soil Collection and Pollution Treatment: Top loamy soil collected were thoroughly mixed to have a uniform mixture. 5kg of soil were put in each black cellophane bag of volume 8348cm³ leaving a space of 3cm from the top to make allowance for the addition of pollutant, water and remediation materials. A total of 96 bags were used for the experiment. The bags were perforated at the bases and sides to prevent waterlogging of the soil. 250ml of crude oil was added to each bag and thoroughly mixed with the soil making a concentration of 50ml/kg soil. These were allowed to stand for one week for the oil to be acclimatized to the soil before remediation.

Remediation Treatment: Two biostimulation nutrients (NPK 15:15:15 and poultry droppings) were used. 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. The bags-containing soil were separated into 4 rows of 24 bags each. Each row was treated with a particular remediation material. 20g of NPK 15:15:15 was applied to each bag in the first row; 50g of poultry droppings applied to each bag in the second row; 10g of NPK 15:15:15 and 30g poultry dropping (combined) applied to each bag in the third row while the fourth row received no remediation treatment (control). The set up was monitored for two months for remediation to take place before cassava planting.

Cassava Planting: Two varieties (TMS 30572 and NR 8082) of *Manihot esculenta*; Crantz (cassava) were used for the study. Stems of these two varieties were cut to a length of 25cm each. Each row remediated with a particular material and the control were separated into two (2) sets. Two stem-cuttings of variety NR 8082 were planted in set one while variety TMS 30572 in set two. A 4x2 factorial arrangement fitted into a randomized complete block design (RCBD) with 12 replicates each was used for the planting. The stem cuttings were planted at a slanting position at an angle of 60°C with at least 8 stem buds above the soil surface.

Growth and Biochemical Analyses: The growth and biochemical responses of the two varieties were analysed at 5 week interval for a period of 20 weeks. The following growth and biochemical parameters were analysed: shoot length (plant height), below-ground fresh weight yield, total fresh weight yield, above-ground dry weight yield, total dry weight yield, chlorophyll content, carbohydrate content and nitrogen content. 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 bag and weighed immediately on a weighing balance, model PN163 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 on a PN163 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 50ml diethyl ether in a separating funnel. The optical density at 660nm and 643 in 1 cm cell was measured using ether as a reference. Leaf carbohydrate content was analysed by extracting 1.0g 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 Kjedahl method[16] in which 1.0g of leaf sample was heated on an electro-
thermal heated hot plate; until digest turned to skyblue; then diluted with 100 mls of diluted water. 30 mls 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 (SEM). New Duncan Multiple Range Test (NDMRT) was employed to separate means.

**Results:** Result showed that addition of biostimulation materials to the crude oil polluted soil significantly (p=0.05) increased the shoot length (plant height) of the two cassava varieties (Table 1). Highest significant plant height was recorded in the NPK 15:15:15 treatment option than the other treatment options for the both varieties. Although, there was no significant difference (p=0.05) between the poultry dropping and NPK + poultry dropping (combined) treatment options for var. TMS 30572.

In Table 2; the below – ground fresh weight yields of the two cassava varieties were found to improve with time both within and between treatment options when compared with the control. NPK 15:15:15 treatment was found to be most effective in improving the below-ground fresh weight yield in the two varieties than the other treatment options while the control produced the least below-ground fresh weight yield. Similar results were recorded for the total fresh weight yield of both varieties in the different treatment options as shown in Table 3, except in TMS 30572 where the total fresh weight yield was significantly (p=0.05) higher in the NPK + poultry dropping (combined) treatment than the poultry droppings treatment.

The above – ground dry weight yield (Table 4) and the total dry weight yield (Table 5) of the two varieties showed similar responses to the different treatment options in the crude oil polluted soil. Significant (p=0.05) highest yield (above-ground dry weight and total dry weight yields) were recorded in the NPK 15:15:15 treatment while the control recorded the least. There was no significant difference (p=0.05) in these yield parameters between the poultry droppings treatment and the NPK + poultry droppings (combined) treatment.

Addition of ameliorating materials to crude oil polluted soil also improved the biochemical properties of the two cassava varieties. Tables 6, 7 and 8 showed a significant (p=0.05) improvement in the leaf chlorophyll, leaf carbohydrate and leaf nitrogen contents respectively in both cassava varieties in the different remediation treatment as compared to the control (no remediation). NPK 15:15:15 treatment recorded the highest (p=0.05) leaf chlorophyll content for NR 8082 in NPK 15:15:15 treatment followed by NPK + poultry dropping (combined) treatment while in TMS 30572, highest yields were recorded in NPK 15:15:15 and NPK + poultry droppings (combined) of values 39.37 ± 0.00 and 39.16 ± 0.55 respectively with no significant difference between them. In the Leaf carbohydrate content (%), NR 8082 showed the highest significant yield in the NPK + poultry droppings (combined) treatment than the other treatment options while in TMS 30572, highest leaf carbohydrate was recorded in NPK 15:15:15 and poultry droppings treatments options (Table 7). In leaf nitrogen content (%), NR 8082 showed the highest yield in the poultry droppings and NPK + poultry droppings (combined) treatments with no significant difference between them, followed by NPK 15:15:15 treatment option while the least was recorded in the control. In TMS 30572, highest leaf Nitrogen (%) was recorded in NPK 15:15:15 treatment followed by poultry droppings and NPK + poultry dropping (combined) with no significant difference between them.

**Discussion:** Growth is an important outcome of the physiology of plants. Any distortion in the physiology of the plant due to environmental factors like water, pollution, temperature, etc. will be physically manifested in their growth form. For instance, Odjegba and Sadiq[11] suggested that any condition that disrupts the normal plant – water relationship of the roots within the soil will negatively affect the normal growth of the plant. Crude oil has been reported to affect growth and overall performance of plants[14,17,3].

Result showed that addition of biostimulation materials improved the growth (shoot length, fresh weight and dry weight yield); and the biochemical characteristics (leaf chlorophyll, leaf carbohydrate and leaf Nitrogen contents) of the two varieties. This is an indication that these materials (NPK 15:15:15 and poultry droppings) have potentials to ameliorate crude oil polluted soil for crop cultivation. These materials (NPK and pouting dropping) have Nitrogen as their constituent in which added to the soil removed nutrient limitation for microbial activity of biodegradation[5,2,10]; thereby reducing the hydrocarbon content of the soil[19]. Low hydrocarbon content have been reported to improve the soil structure through the increase in soil-water infiltration and soil aeration[16], which invariably can stimulate plant growth[6]. Increase in soil nitrogen causes an increase in vegetative growth of cassava[15].

Highest shoot length, fresh weight and dry weight yields were recorded in the NPK 15:15:15 treatment option. The cause might be traceable to the ameliorating materials used in which NPK (inorganic
### Table 1: Mean Shoot Length (cm) of the two cassava varieties in the different remediation treatment options.

<table>
<thead>
<tr>
<th>Wks/trtment</th>
<th>Var. NR 8082</th>
<th>NPK 15:15:15</th>
<th>Poultry droppings</th>
<th>NPK + Poultry droppings</th>
<th>Control (no remediation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>17.7±0.70d</td>
<td>16.5±0.32c</td>
<td>17.5±0.29g</td>
<td>10.1±0.18d</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>14.5±0.77b</td>
<td>10.0±1.00a</td>
<td>9.0±1.56c</td>
<td>9.1±1.28c</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>28.9±0.59c</td>
<td>19.1±0.70d</td>
<td>23.3±2.32b</td>
<td>15.4±1.00f</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>21.1±0.85b</td>
<td>29.6±0.76c</td>
<td>17.3±0.66d</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>38.4±0.10d</td>
<td>27.3±0.43b</td>
<td>30.6±0.09a</td>
<td>15.0±2.11f</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>42.9±0.96d</td>
<td>27.3±0.43b</td>
<td>30.6±0.09a</td>
<td></td>
</tr>
</tbody>
</table>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

(b) Var. TMS 30572:

<table>
<thead>
<tr>
<th>Wks/trtment</th>
<th>Var. NR 8082</th>
<th>NPK 15:15:15</th>
<th>Poultry droppings</th>
<th>NPK + Poultry droppings</th>
<th>Control (no remediation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>14.5±0.77b</td>
<td>10.0±1.00a</td>
<td>9.0±1.56c</td>
<td>9.1±1.28c</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>19.9±0.37d</td>
<td>29.2±1.20d</td>
<td>17.6±0.15g</td>
<td>17.3±0.96f</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>24.2±0.32d</td>
<td>44.0±2.69c</td>
<td>24.9±2.22e</td>
<td>22.0±1.03f</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>37.2±1.42c</td>
<td>34.0±1.15d</td>
<td>30.7±0.18d</td>
<td>15.5±0.04d</td>
</tr>
</tbody>
</table>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

### Table 2: Mean Below-ground fresh weight yield (g) of the two cassava varieties in the different remediation treatment options.

<table>
<thead>
<tr>
<th>Wks/trtment</th>
<th>Var. NR 8082</th>
<th>NPK 15:15:15</th>
<th>Poultry droppings</th>
<th>NPK + Poultry droppings</th>
<th>Control (no remediation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>11.7±0.65b</td>
<td>12.8±0.03d</td>
<td>12.57±1.10b</td>
<td>9.50±0.27b</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>24.47±0.28c</td>
<td>24.44±2.38c</td>
<td>19.36±0.32b</td>
<td>12.40±1.19b</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>27.94±0.12d</td>
<td>27.42±0.41c</td>
<td>21.95±3.78b</td>
<td>17.55±0.52b</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>34.52±0.86d</td>
<td>25.61±1.44d</td>
<td>25.50±2.51c</td>
<td>10.97±0.61c</td>
</tr>
</tbody>
</table>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

### Table 3: Mean Total fresh weight yield (g) of the two cassava varieties in the different remediation treatment options.

<table>
<thead>
<tr>
<th>Wks/trtment</th>
<th>Var. NR 8082</th>
<th>NPK 15:15:15</th>
<th>Poultry droppings</th>
<th>NPK + Poultry droppings</th>
<th>Control (no remediation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td></td>
<td>56.42±0.84c</td>
<td>27.80±1.52c</td>
<td>37.89±3.85c</td>
<td>27.98±1.49c</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>66.75±1.65c</td>
<td>49.52±3.43c</td>
<td>40.05±0.51b</td>
<td>28.55±1.22c</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>73.42±0.36d</td>
<td>52.85±0.56d</td>
<td>44.18±3.72b</td>
<td>35.20±0.78b</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>87.72±2.66c</td>
<td>57.08±1.32d</td>
<td>54.63±0.26c</td>
<td>27.71±0.96c</td>
</tr>
</tbody>
</table>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05
### Table 4: Mean Above-ground dry weight yield (g) of the two cassava varieties in the different remediation treatment options.

<table>
<thead>
<tr>
<th>Wks/treatment</th>
<th>Var. NR 8082</th>
<th>Wks/treatment</th>
<th>Var. TMS 30572</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NPK 15:15:15</td>
<td>Poultry droppings</td>
<td>NPK + Poultry droppings</td>
</tr>
<tr>
<td>5</td>
<td>10.51±0.39*</td>
<td>3.03±0.14*</td>
<td>5.43±0.63*</td>
</tr>
<tr>
<td>10</td>
<td>9.98±0.55*</td>
<td>4.87±0.70*</td>
<td>4.23±0.20*</td>
</tr>
<tr>
<td>15</td>
<td>10.99±0.02*</td>
<td>5.61±0.24*</td>
<td>4.47±0.32*</td>
</tr>
<tr>
<td>20</td>
<td>11.96±0.23*</td>
<td>6.43±0.21*</td>
<td>6.03±0.09*</td>
</tr>
<tr>
<td></td>
<td>NPK 15:15:15</td>
<td>Poultry droppings</td>
<td>NPK + Poultry droppings</td>
</tr>
<tr>
<td>5</td>
<td>10.01±0.12*</td>
<td>4.04±0.03*</td>
<td>3.42±0.30*</td>
</tr>
<tr>
<td>10</td>
<td>8.54±0.58*</td>
<td>5.88±0.07*</td>
<td>4.03±1.06*</td>
</tr>
<tr>
<td>15</td>
<td>7.67±0.31*</td>
<td>7.52±0.19*</td>
<td>5.13±0.13*</td>
</tr>
<tr>
<td>20</td>
<td>10.07±0.04*</td>
<td>8.31±0.75*</td>
<td>7.71±0.15*</td>
</tr>
</tbody>
</table>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

### Table 5: Mean Total dry weight yield (g) of the two cassava varieties in the different remediation treatment options.

<table>
<thead>
<tr>
<th>Wks/treatment</th>
<th>Var. NR 8082</th>
<th>Wks/treatment</th>
<th>Var. TMS 30572</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NPK 15:15:15</td>
<td>Poultry droppings</td>
<td>NPK + Poultry droppings</td>
</tr>
<tr>
<td>5</td>
<td>11.89±0.31*</td>
<td>4.27±0.13*</td>
<td>6.91±0.92*</td>
</tr>
<tr>
<td>10</td>
<td>17.20±0.47*</td>
<td>12.36±0.97*</td>
<td>10.72±0.25*</td>
</tr>
<tr>
<td>15</td>
<td>19.40±0.02*</td>
<td>14.24±0.28*</td>
<td>11.62±0.92*</td>
</tr>
<tr>
<td>20</td>
<td>23.73±0.14*</td>
<td>15.25±0.50*</td>
<td>13.72±0.05*</td>
</tr>
<tr>
<td></td>
<td>NPK 15:15:15</td>
<td>Poultry droppings</td>
<td>NPK + Poultry droppings</td>
</tr>
<tr>
<td>5</td>
<td>11.60±0.13*</td>
<td>5.63±0.06*</td>
<td>4.19±0.05*</td>
</tr>
<tr>
<td>10</td>
<td>15.58±0.51*</td>
<td>12.02±0.08*</td>
<td>10.25±1.17*</td>
</tr>
<tr>
<td>15</td>
<td>15.46±0.50*</td>
<td>14.27±0.61*</td>
<td>12.34±0.14*</td>
</tr>
<tr>
<td>20</td>
<td>22.64±0.10*</td>
<td>18.22±0.74*</td>
<td>18.03±0.29*</td>
</tr>
</tbody>
</table>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05

### Table 6: Mean Leaf chlorophyll content (%) of the two cassava varieties in the different remediation treatment options.

<table>
<thead>
<tr>
<th>Wks/treatment</th>
<th>Var. NR 8082</th>
<th>Wks/treatment</th>
<th>Var. TMS 30572</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NPK 15:15:15</td>
<td>Poultry droppings</td>
<td>NPK + Poultry droppings</td>
</tr>
<tr>
<td>5</td>
<td>35.25±0.13*</td>
<td>31.54±0.01*</td>
<td>26.54±0.28*</td>
</tr>
<tr>
<td>10</td>
<td>41.00±0.02*</td>
<td>29.46±0.25*</td>
<td>32.41±0.00*</td>
</tr>
<tr>
<td>15</td>
<td>42.80±0.46*</td>
<td>27.17±0.04*</td>
<td>37.80±0.00*</td>
</tr>
<tr>
<td>20</td>
<td>46.90±0.03*</td>
<td>31.22±0.15*</td>
<td>41.82±0.12*</td>
</tr>
<tr>
<td></td>
<td>NPK 15:15:15</td>
<td>Poultry droppings</td>
<td>NPK + Poultry droppings</td>
</tr>
<tr>
<td>5</td>
<td>19.85±0.05*</td>
<td>17.52±0.33*</td>
<td>21.57±0.02*</td>
</tr>
<tr>
<td>10</td>
<td>26.53±0.03*</td>
<td>19.97±0.15*</td>
<td>27.97±0.01*</td>
</tr>
<tr>
<td>15</td>
<td>33.33±0.38*</td>
<td>21.39±0.58*</td>
<td>34.08±0.17*</td>
</tr>
<tr>
<td>20</td>
<td>39.37±0.06*</td>
<td>36.98±0.04*</td>
<td>39.16±0.55*</td>
</tr>
</tbody>
</table>

Note: Mean± SEM with different superscripts between column means significant difference @p=0.05
The improvement in the biochemical properties (leaf chlorophyll, leaf carbohydrate and leaf Nitrogen contents) of the two cassava varieties in the different biostimulation treatment options can be traced to the fact that these materials (NPK and poultry droppings) provided the basic elements such as nitrogen for chlorophyll synthesis. This suggestion is true since as much as 70% of the total nitrogen is in the chloroplast. If photosynthetic rate is directly related to the chlorophyll content of the plant; it means that the amount of chlorophyll will have a direct link to the rate of accumulation of carbohydrate. Therefore, high carbohydrate content is expected in high chlorophyll content.

Results also showed that higher yields (growth) were recorded in single treatment especially NPK than in the combined treatment. That is, the combined treatment option had no advantage over the single treatments. The possible reason might be that the remediation materials when is combined form have some antagonistic features which created stress for biodegradable microorganisms for efficient degradation through it selection of energy source (requirement).

Result showed differences in the responses of the two varieties to the different remediation treatment. This may be attributed to their physiological, morphological and genetic differences[18].

Therefore, the different remediation materials can enhance the growth and biochemical performances of...
cassava in a crude oil polluted soil but more better in NPK 15:15:15(inorganic fertilizer) treatment than poultry droppings (organic fertilizer).

REFERENCES