Effects of simulated crude oil pollution on the growth of *Manihot esculenta* Crantz

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ABSTRACT

The susceptibility of *Manihot esculenta* to Nigeria’s Forcados blend crude oil was investigated for 8 weeks using soil supplemented with 0-5% v/w oil/soil. Parameters considered include, plant height, leaf area, dry weight, relative growth rate, total chlorophyll and nitrate reductase activity of the plant. A significant (p=0.05) reduction in these parameters were observed in plants exposed to oil treatments. Comparatively, the control plants had a mean height and chlorophyll level of 43.27±3.08 cm and 3.2468±0.0038 mg g⁻¹ f.wt respectively after 8 weeks, while plants grown in soil containing 5% oil correspondingly had 23.70±0.00 cm and 1.9374±0.0052 g⁻¹ f.wt. The oil treatment also had a significant inhibitory effect on the nitrogen metabolism of the plant as indicated by nitrate reductase activity. The results of this study showed the toxic effects of Nigeria’s Forcados blend crude oil on *Manihot esculenta*.

Keywords: Crude oil, toxicity, inhibition, environment, safety

1. INTRODUCTION

Modern industrial civilisation depends heavily on the use of fossil fuels as a source of energy and raw material for many products. Crude oil is a naturally occurring, bituminous liquid composed of various organic chemicals (Anonymous, 2004). Pollution from crude oil can occur as spillage from onshore or offshore operations of the petroleum industries. Major causes of crude oil pollution include leaking storage tanks and pipelines, land disposal of petroleum waste and accidental or intentional spills (White et al., 2006). The Niger Delta region in Nigeria is richly endowed with oil and gas. It is the region that accounts for the total crude oil produced in Nigeria and is the most hit by the pollution problems associated with exploration of crude oil. The drive by the federal government to diversify Nigeria’s economy led to the presidential initiative on cassava production and export in 2002. The goal was to promote cassava as a foreign exchange earner as well as to satisfy national demand. To meet the volume of demand, the Government will have to invest extensively in the production and processing of cassava. Comparing the output of various crops in Nigeria, cassava production ranks first, followed by yam, sorghum, millet and rice. The estimated annual production of cassava is approximately 34 million tonnes (IITA, 2004).

Cassava, *Manihot esculenta* Crantz is the principal food for poor people in the tropics. Typically it is grown in areas where the mean annual temperature is greater than 18 °C. All the species of the genus *Manihot* are native to Brazil (Nassar, 1986). In Africa, cassava is used principally for human consumption. The main value is from its starchy roots, either fresh, boiled or in numerous processed forms. Cassava roots are composed of peel which is about 10-20% of the tuberous roots; the cork layer is 0.5-2% of the total tuber weight. The edible fleshy portion makes up 80-90% of the tuber. It is composed of 60-65% water, 30-35% carbohydrate, 1-2% protein, 0.2-0% fat, 1-2% fibre and 1-1.5% mineral matter of the tuber flesh (Nassar and Costa, 1976). It is used for animal feed and as a raw material for producing starch, starch based products and starch derivatives. Cassava flour, made from cassava roots is now being used in many industries and its possibility to replace wheat flour is being exploited in Nigeria.

1.1. Experimental plant

*Manihot esculenta* belongs to Euphorbiaceae family and it is a short-lived perennial up to 1 to 5 meters tall. It has tuberous adventitious roots and is usually propagated by stem cuttings. It is cultivated for its tubers which are either eaten as staple food after been processed or for industrial purposes that utilized the high quality starch. The study reported here aimed to investigate the effects of Forcados blend crude oil on the growth, nitrate reductase activity and chlorophyll content of *Manihot esculenta* Crantz. *Manihot esculenta* was chosen for the study because it is a popular crop plant widely cultivated in the Niger Delta where crude oil pollution is a common and a major problem.

2. MATERIALS AND METHODS

Stem cuttings of *Manihot esculenta* used for the study were collected from a farm in Lagos, Nigeria. Cuttings of relatively equal weight and length were used for the study. Forcados blend crude was collected in a single batch from Shell Petroleum Company, Port Harcourt, Nigeria. The study was carried out at the Biological garden of University of Lagos. Sandy loam soil was treated with crude oil to obtain 1, 2, 3, 4, 5 % v/v oil/soil and placed in nursery bags. Treatment of the soil with crude oil was carried out manually by mixing a known weight of soil with a known volume of crude oil. This was carefully mixed in a plastic bowl to ensure thorough mixing. Soil devoid of crude oil served as the control. One stem cutting was put in a slanting position in each bag representing each treatment and this was replicated six times. All treatments were watered regularly to keep soils moist. The experimental period was 8 weeks and all parameters were measured in triplicates.

2.1. Plant height

The heights of the plants were measured from the stem cuttings to the terminal bud using a metre rule. This was done at a regular interval of 7 days.

2.2. Leaf area determination

This was determined by comparing the weight of a cut out traced area of the leaves with standard paper of known weight to area ratio (Eze, 1965).

2.3. Relative Growth Rate (RGR)
The experimental plants were carefully uprooted and the root parts rinsed in running tap water. They were then put in marked paper bags and oven-dried at 70 °C for 2 days. The RGR of the plants were determined according to the method described by Causton, (1994) using the relationship:

$$\text{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \text{ g g}^{-1} \text{week}^{-1}$$

Where $W_1$ and $W_2$ are plant dry weights at $t_1$ and $t_2$ ($4^{th}$ and $8^{th}$ week) respectively.

2.4. Mineral elements composition
One gram of the sample was ashed in a muffle furnace at a temperature of about 550 °C. The sample was then cooled and moistened with 1ml concentrated trioxonitrate (v) acid (KNO$_3$). It was then evaporated to dryness and returned to the muffle furnace for 1 hour, after which it was cooled and 5ml of 5M HCl was added. It was then heated in a water bath and filtered through a hardened filter paper into a 100ml volumetric flask. 3ml of diluted hydrochloric acid (0.01M) was added to it, warmed and passed through filter paper. This last process was repeated 4 times and it was then filtered with hot water. The volume was then made up to the 100ml mark by adding distilled water. The mineral elements (Ca, P, Mg and K) were then determined spectrophotometrically.

2.5. Estimation of total chlorophyll content
Extraction and estimation of chlorophyll was done using the method of Maclachlam and Zalik (1963) as described by Singh and Rao, (1981). The total chlorophyll content was estimated spectrophotometrically at 645nm and 663nm corresponding to the limits of minimum and maximum absorption of chlorophyll. This was done in triplicates and total chlorophyll content calculated.

$$C_a = (12.3D_{663} - 0.86D_{645}) \frac{v}{d \times 1000 \times w}$$

$$C_b = (19.3D_{645} - 3.6D_{663}) \frac{v}{d \times 1000 \times w}$$

Where $D_{645}$ and $D_{663}$ = Optical Density at 645nm and 663nm respectively
$v = $ volume of acetone = 10ml
$w = $ weight of plant sample = 1g

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**Figure 1** Effects of different concentrations of crude oil on the height of *Manihot esculenta*. Different letters indicate significant difference at p=0.05 according to Tukey’s pairwise comparison.

**Figure 2** Effects of different concentrations of crude oil on the leaf area of *Manihot esculenta*. Different letters indicate significant difference at p=0.05 according to Tukey’s pairwise comparison.

**Figure 3** The relative growth rate of *Manihot esculenta* exposed to different concentrations of crude oil. Different letters indicate significant difference at p=0.05 according to Tukey’s pairwise comparison.

**Figure 4** Effect of crude oil treatment on the total chlorophyll content of *Manihot esculenta*. Different letters indicate significant difference at p=0.05 according to Tukey’s pairwise comparison.

Odjega et al.
2.6. Nitrate reductase activity
The extraction and assay of nitrate reductase was estimated by a modified method of Fan et al. (2002). Fresh leaves (0.2g) was ground and extracted in 2ml of distilled water, centrifuged (10000g) for 5 min at 4 °C. 1 ml of the crude extract was incubated at 25 °C in the dark for 1 hour in 3 ml of the substrate assay solution in a test tube. The substrate assay solution contained 1 ml each of 0.1M KNO₃, 15ml H₂O and 0.1M potassium phosphate buffer (pH 7.5). 1ml of the solution was transferred using a pipette after the incubation period into a clean test tube. 1ml each of 1% sulphanilic acid and 0.02% naphthylenediamine (NED) was added, the mixture was thoroughly shaken and left to stand for 1 hour to allow full colour development. A blank was prepared by mixing 1ml of substrate assay solution, 1ml of 1% sulphanilic acid and 1ml of 0.02 % naphthylenediamine in a test tube. The mixture was allowed to stand for 1 hour for colour development. Development due to nitrite was then measured spectrophotometrically at 540nm. The values were compared to a standard curve generated using solutions of NaNO₂.

2.7. Statistical analysis
All measurements in triplicates were analysed using one way analysis of variance and differences in means were determined by Tukey’s family comparisons.

3. RESULTS AND DISCUSSION
The effects of Nigeria’s Forcados blend crude oil on growth parameters, chlorophyll and nitrate reductase activity of Manihot esculenta was investigated on soil amended with concentrations of crude oil ranging from 0-5% volume per weight of soil. After 8 weeks of growth in oil treated soil, a significant reduction was observed in all the parameters consequent upon oil treatment. The result showing the effects of crude oil on plant’s height is shown in figure 1, A significant reduction in height was observed in plants grown in oil treated soils relative to the control. A mean height of the control plants was 169.14 ± 37.1 cm, a value that was significantly (p=0.05) greater than the values observed for plants grown in oil treated soils (Figure 2). The relative growth rate (RGR) of Manihot esculenta grown in soil devoid of oil and those exposed to different concentrations of crude oil are shown in figure 3. The mineral elements detected in the control plants were higher compared to the values observed for the crude oil treated plants (Table 1).

The observed reduction in both the growth parameters and mineral element contents of plants grown in the soils amended with crude oil could be related to water and nutrient stress. This observation is in line with the findings of De Jong (1980) who reported that reduction in the dry matter yield of cereals was due to lack of intake of nutrients resulting from nutrient immobilization consequent on the adulterated nature of soil following oil application. Soil provides the direct mineral element reserves from which terrestrial plants obtain their required nutrients. These mineral elements are available to plants after the solid phase of the soil has released them into solution by solubilization and ion exchange (Epstein, 1972). Any condition that disrupts the normal plant-water relationship of the roots within the soil will negatively affect the normal growth of the plant (Sharma et al., 1980; Odjegba and Sadiq, 2002). The crude oil added to the soil affected its physicochemical properties by creating a water repelling environment around the roots thereby making the soil nutrients unavailable for absorption

Table 1 Mineral elements in the leaves of Manihot esculenta after 8 weeks of treatment with crude oil. Different letters in the same column indicate significant difference at p=0.05 according to Tukey’s pairwise comparison

<table>
<thead>
<tr>
<th>Oil Concentration (%)</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.86 ± 0.02a</td>
<td>4.91 ± 0.02a</td>
<td>7.62 ± 0.03a</td>
<td>0.07 ± 0.00a</td>
</tr>
<tr>
<td>1</td>
<td>0.71 ± 0.01b</td>
<td>3.98 ± 0.02b</td>
<td>5.99 ± 0.02b</td>
<td>0.08 ± 0.00b</td>
</tr>
<tr>
<td>2</td>
<td>0.59 ± 0.02c</td>
<td>2.76 ± 0.01c</td>
<td>5.84 ± 0.02b</td>
<td>0.05 ± 0.00c</td>
</tr>
<tr>
<td>3</td>
<td>0.53 ± 0.01d</td>
<td>2.63 ± 0.02d</td>
<td>4.48 ± 0.02c</td>
<td>0.04 ± 0.00d</td>
</tr>
<tr>
<td>4</td>
<td>0.45 ± 0.01e</td>
<td>2.08 ± 0.02e</td>
<td>2.98 ± 0.02d</td>
<td>0.04 ± 0.00d</td>
</tr>
<tr>
<td>5</td>
<td>0.32 ± 0.02f</td>
<td>1.38 ± 0.02f</td>
<td>1.84 ± 0.06e</td>
<td>0.04 ± 0.01d</td>
</tr>
</tbody>
</table>

The total chlorophyll content in the leaves of oil treated plants was significantly (p=0.05) lower than the values observed for the control (Figure 4). An estimation of the nitrate reductase activity (NRA) in the fresh leaves of both the control and oil treated plants were compared. Data showing the influence of crude oil on the NRA of Manihot esculenta are as shown in figure 5. The result showed low level of NRA in the leaves of plants grown in crude oil treated soils. Nitrogen is primarily necessary for the formation of amino acids, proteins, nucleic acids and chlorophyll. Nitrogen metabolism is mediated by an enzyme nitrate reductase located mainly in the cytoplasm of leaves of plants. It is logical to argue that conditions that impede normal nitrogen metabolism in plants would affect the synthesis of chlorophyll, the photosynthetic ability and the physiological state of the plant. The inhibitory effects of crude oil on the nitrate reductase activity of Manihot esculenta could be attributed to the toxic nature of some of its constituents on this enzyme. It has been reported that poly cyclic aromatic hydrocarbons (PAH) is a toxic and recalcitrant portion of crude oil (Wang et al., 2000). The results of this study indicating negative impact of crude oil on Man. esculenta is a great threat to food security in Nigeria because this crop is one of the most widely consumed staple foods in Nigeria and indeed West Africa. Considering the large quantity of crude oil oil going into the environment, and the fact that the plant is widely cultivated in the Niger Delta where crude oil pollution is a common and a major problem, there is an urgent need for the Government of Nigeria and the various agencies concerned with oil exploration to pay more attention to the problems of oil spillage in the country.

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