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Effectiveness of *Vigna Unguiculata* as a Phytoremediation Plant in the remediation of Crude Oil polluted soil for Cassava (*Manihot Esculenta*; Crantz) Cultivation

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ABSTRACT: The effectiveness of *Vigna unguiculata* as a phytoremediation plant in the remediation of crude oil polluted soil for cassava cultivation was carried out. Soil polluted with 50ml/kg of crude oil was phytoremediated with *Vigna unguiculata* alongside a control and double control. After 2 months of remediation, two cassava (*Manihot esculenta*) varieties (NR 8082 and TMS 30572) were planted. The level of remediation as well as the crop performance (growth and yield) and leaf chlorophyll content were determined. Results showed that Total Hydrocarbon Content in the phytoremediation soil reduced significantly to 600 mg/kg as against 1300 mg/kg in the control. The crop performance (growth and yield) also improved as well as the leaf chlorophyll content of the two varieties ($p=0.05$). The result also showed that TMS 30572 performed better in shoot length (37.3±0.38), below-ground fresh (51.24±0.77) and total dry weight (28.37±0.20) than NR 8082 of 29.9±0.83, 24.97±0.33, and 19.7± 0.19 respectively in the phytoremediated soil. Therefore, phytoremediation using *Vigna unguiculata* is thus recommended as a good phytoremediation measure for crude oil polluted soil for cassava cultivation especially TMS 30572. @ JASEM

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 (Chronopoulos *et al*., 1997). Petroleum hydrocarbon contamination may affect plants by retarding seed germination and reducing height, stem density, photosynthetic rate and biomass or resulting in complete mortality (Mendelssohn *et al*., 1990; Lin and Mendelssohn, 1996; Pezeshki *et al*., 2000). Cassava is one of the economic crops cultivated in the Niger- Delta where crude oil pollution is common. This crop has been found to be vulnerable to crude oil pollution (Tanee and Anyanwu, 2007). Anyanwu and Tanee (2008) also reported that cassava (var. TMS 30572) cannot tolerate post-planting crude oil pollution at concentration as low as 500 ml/m^2. This may be due to the reduction in plant nutrients in the soil (Xu and Johnson, 1997; Akonye and Onwudiwe, 2004) or phytotoxicity of the oil (Siciallano and Germida, 1998). It therefore, means that for effective cultivation of this cassava to meet man's demand, it is imperative to get rid of these pollutants by applying some remedial measures.

Phytoremediation has emerged as a viable option for the remediation of petroleum hydrocarbon polluted sites (Frick *et al*., 1999; Tanee and Kinako, 2008). The use of phytoremediation as a clean up option may not only degrade contaminants but could enhance habitat recovery through the stimulation of vigorous vegetative plant growth (Lee *et al*., 2001). In majority of studies, grasses and legumes such as bush beans (*Phaseolus vulgaris* L.), Switch grass (*Panicum virgatum*), alfalfa (*Medicago sativa* L.) and sorghum (*Sorghum bicolor*) have been singled out for their potentials to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbons (Reilly *et al*., 1996; Qiu *et al*., 1997).

Literature on the use of *Vigna unguiculata* in phytoremediation work is lacking. Cowpea (*Vigna unguiculata* L.) is a leguminous plant belongs to the family *Fabaceace*. The seeds are edible as it contain proteins. It is mostly grown in crop rotation to replenish lost fertility especially nitrogen in the soil. *Vigna unguiculata* is chosen for this study because of its ability to fix nitrogen in the soil which is always a limiting factor in crude oil polluted soil This study attempts to examine the effectiveness of *Vigna unguiculata* as a phytoremediation plant in the remediation of crude oil polluted soil for cassava cultivation. This will go a long way in ameliorating some of the problems posed by crude oil in the cultivation of agricultural crops such as cassava.

MATERIALS AND METHODS.

The study was carried out at the University of Port Harcourt, Rivers state of Nigeria. The study site is located about 26 km North-west of the city of Port Harcourt along the East-west road in the tropical rainforest belt of Southern Nigeria.

Surface soil from the University of Port Harcourt Botanic Garden was collected and mixed thoroughly. It was then filled into 60 black cellophane bags of radius 12.5 cm and height 17 cm leaving a space of 6 cm from the top end of the bags to make allowance for crude oil and water addition. 5000g of soil were placed in each bag. The bags were perforated at the sides and bases to avoid water logging and also to increase the soil aeration. The bags were arranged in three (3) rows designated as A, B and C of 20 replicates each.

Crude oil obtained from Nigeria National Petroleum

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Corporation (NNPC) Eleme, Rivers state was applied as the pollutant. 250 ml of crude oil representing 5% by volume of pollution was added and thoroughly mixed with the soil, given a concentration level of 50 ml/kg of soil. Rows A and B received crude oil while row C was not polluted.

One week after pollution, each bag in Row A was planted with 10 seeds of *Vigna unguiculata* obtained from mile III market, Port Harcourt. After germination the seedlings were thinned to 5 seedlings per bag to avoid overcrowding. Row B was the control with pollution but no phytoremediation treatment while Row C was the double control with no pollution and no phytoremediation treatment. At the end of the 2 months, the concentration of Total Hydrocarbon Content of the soil was measured using the spectophometer method to ascertain the level of remediation. After 2 months of remediation, the *Vigna unguiculata* in Row A were uprooted and chopped with knife and mixed with the soil before the cassava planting. Each Row was then separated into 2 sub-groups of equal number of 10 replicates.

Stem-cuttings of 25 cm each of two cassava varieties (NR 8082 and TMS 30572) collected from Agricultural Development Programme (ADP), Port Harcourt were planted in one sub-group of Rows A, B and C while TMS 30572 planted in the other sub-groups of the Rows. Two stem-cuttings of each variety were planted in each bag in the slanting position with half of the length of the stem-cutting in the soil.

A 2x3 factorial fitted into a randomized complete block design was used. The experiment was done under natural climatic (environmental) conditions with a total rainfall of 2551.3 mm and mean minimum and maximum temperatures of 23.08^{o}C and 37.5^{o}C respectively (Source: Federal Ministry of Aviation, Port Harcourt).

The following parameters were measured: shoot length (cm), above-ground fresh weight (g), below-ground fresh weight (g), total dry weight (g), and leaf chlorophyll content (%)

The shoot length was measured with a metre tape from the base of the stem to the apex (above-ground) of the plant. The fresh weights were obtained by uprooting the plant from each bag and weighed on a weighing balance (model PN 163) immediately after harvest to avoid water loss. The dry weights were obtained by oven-drying the plant at 70^{o}C to get rid of all moisture and ensure a constant weight. Then weighed on a weighing balance (model PN 163). The leaf chlorophyll content was extracted from 1.0g of leaf sample with aqueous acetone. 25 ml extract was transferred to ether and the optical density at 660 nm and 643 nm in 1cm cells was measured using ether as a reference. All data collected were subjected to statistical Analysis of Variance (ANOVA), T-test and standard error mean (SEM).

RESULTS AND DISCUSSION

Phytoremediation using *Vigna unguiculata* remediated the polluted soil by reducing the Total Hydrocarbon Content (THC) in the soil to 600 mg/kg as against 1300 mg/kg in the control (no phytoremediation). This invariably affected the growth and yield as well as the chlorophyll content of the two cassava varieties.

Results showed that there was significant difference in the shoot length of the two cassava varieties in the different treatments (phytoremediation, control, and double control) with a significant improvements in the phytoremediation and double control soil with time (p=0.05) but the reverse was the case in the control soil (Fig. 1). At the 20th week there was no significant difference between the phytoremediation and double control plots in shoot length of var. TMS 30572.

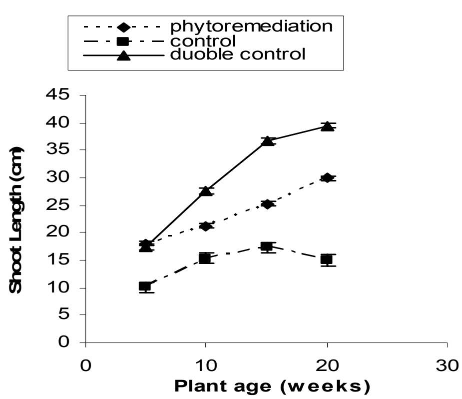

Fig.1a. Shoot Length (cm) of var. NR 8082 ± SEM.

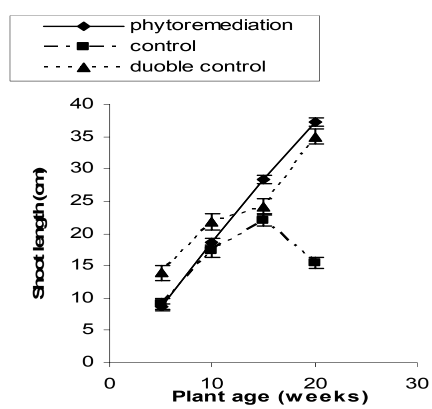

Fig.1b. Shoot Length (cm) of Var. TMS 30572 ± SEM.

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The fresh weight yield of the two varieties showed a similar result as the shoot length in which phytoremediation caused an increased fresh weight (below and above-ground) yield as against the control soil (Figs. 2 and 3). The above-ground fresh weight and below-ground fresh weight of TMS 30572 in the phytoremediation soil significantly rise above their respective double control.

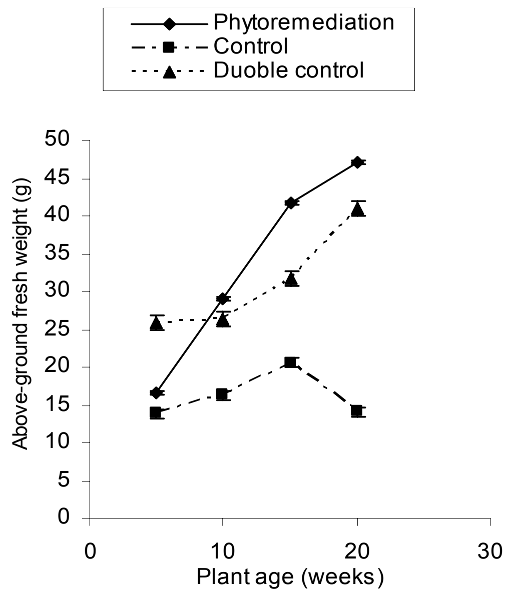

Fig. 2b. Above-ground fresh weight yield (g) of var. TMS 30572 ± SEM

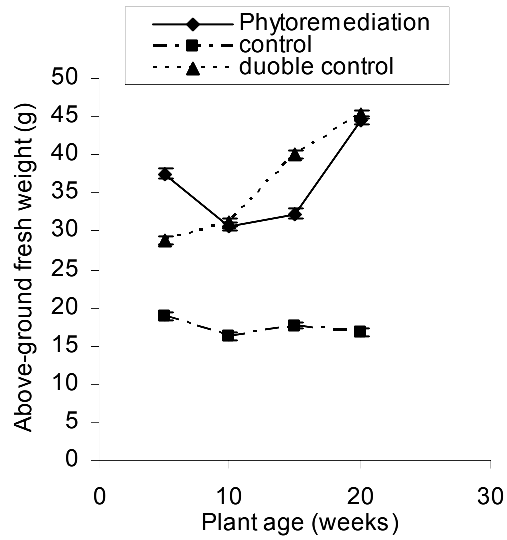

Fig. 2a: Above-ground fresh weight yield (g) of var.NR 8082 ± SEM

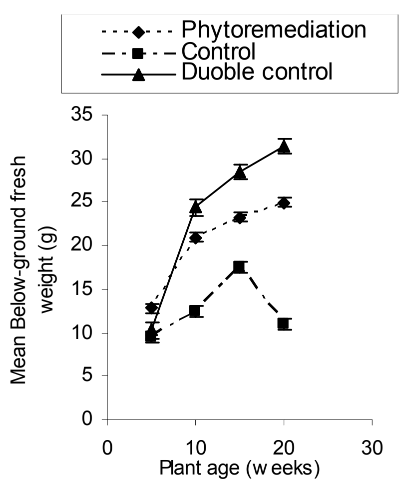

Fig.3a. Below-Ground Fresh weight Yield (g) of NR 8082 ± SEM.

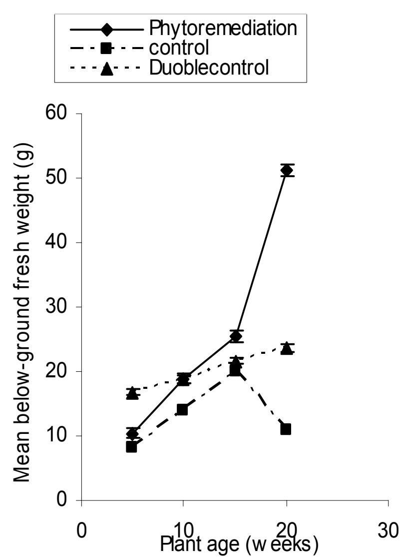

Fig 3b: Below-Ground fresh Weight Yield (g) of TMS 30572 ± SEM.

There was also an improvement in the total dry weight of the cassava varieties grown in phytoremediation soil (Fig. 4). Significant improvement occurred in var TMS 30572 with 28.36g as against 19.89g in the double control and 7.59g in the control at the 20th week.

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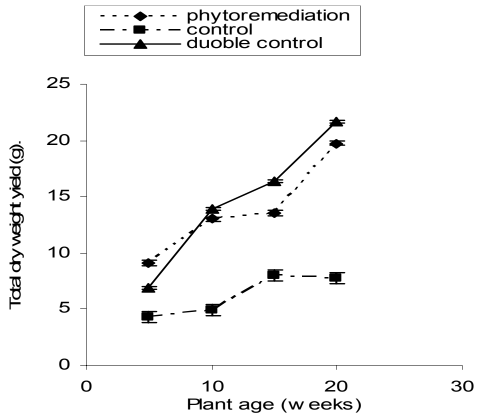

Fig 4a: Total dry weight yield (g) of NR 8082 ± SEM.

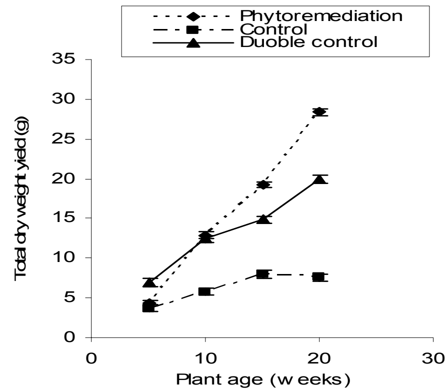

Fig.4b: Total dry weight yield (g) of TMS 30572 ± SEM.

Improvement in growth and yield parameters especially in the phytoremediated soil may be as a result of increase in nitrogen content and reduction in the phytotoxicity of the soil (Tanee and Kinako, 2008). Increase in soil nitrogen causes an increase in the vegetative growth of cassava (Purseglove, 1985).The increase in nitrogen is justifiable since *Vigna unguiculata* (a legume) is capable of fixing nitrogen in the soil by symbiotic association with *Rhizobium* (a bacterium). Thus the plant (*Vigna* sp) supplies the available nitrogen at contaminated sites (Gudin and Syrath, 1975). Hence removed the nutrient limitation for microbial activity of biodegradation (Lee *et al*.,1993; Odokuma and Ibor, 2002). The reduced concentration level of Total Hydrocarbon Content in the phytoremediated plot can stimulate plant growth (Li *et al*, 1990).

The reduction in the growth/ yield parameters in the control could be related to water and nutrient stress due to oil. Odjegba and Sadiq (2002) 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.

There was an improvement in leaf chlorophyll content of the two cassava varieties with time in the phytoremediated soil as against the control as shown in Fig.5.

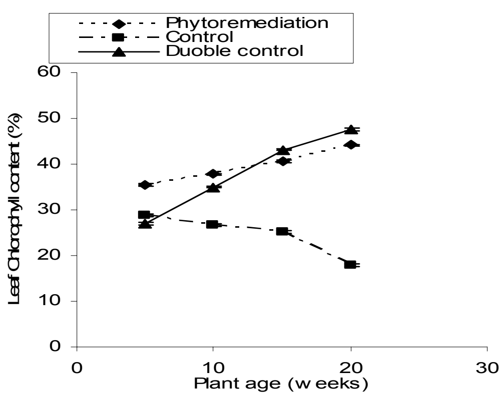

Fig 5a: Leaf Chlorophyll content (%) of NR 8082 ± SEM.

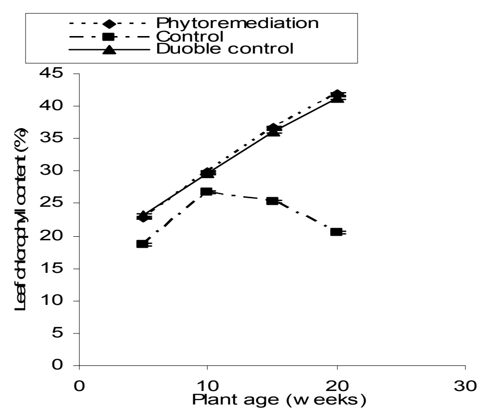

Fig 5b: Leaf Chlorophyll content (%) of TMS 30572 ± SEM .

*Note SEM means standard error mean.

Nitrogen and magnesium are necessary for the formation of chlorophyll. Thus any condition that obstruct the normal uptake and metabolism of these elements in plants will invariably affect chlorophyll synthesis. It might be suggested that phytoremediation provides these basic elements needed for chlorophyll synthesis. Chlorophyll content determines the photosynthetic rate, hence increase in photosynthetic products. This might also contribute to the increase in biomass of the two cassava varieties in the phytoremediated soil.

T-test analyses revealed that there was significant differences in the growth and yield parameters between the two cassava varieties in the phytoremediated soil with TMS 30572 performing better in shoot length, below-ground fresh weight and total dry weight than NR 8082 at the 20th week. The reason might be attributed to their physiological, biochemical, morphological and genetic constitution in which TMS 30572 was able to utilized certain

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factors for better growth and yield than NR 8082. It can be concluded that the use of *Vigna unguiculata* as a phytoremediated plant is a good remedial option in a crude oil polluted soil for cassava cultivation.

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