Scientia Africana, Vol. 18 (No. 3), December, 2019. Pp 75-96
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EVALUATION OF PLANT GROWTH PERFORMANCE IN BIOREMEDIATED PETROLEUM CONTAMINATED SOIL IN RIVERS STATE, NIGERIA.

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Received: 12-11-19
Accepted: 13-11-19

ABSTRACT

This study was carried out using complete randomized design to evaluate growth performance of Telfairia occidentalis (pumpkin) in certified bioremediated petroleum contaminated soils obtained from Ogoniland in Rivers State, Nigeria. Four soil samples- a pristine soil and three bioremediated soils of different ages (6 months, 12 months, and 18 months after bioremediation) were collected and transferred into plastic buckets for the study. The physico-chemical characteristics of the soil samples were determined before planting 9 seeds of Telfairia occidentalis in the various soil samples contained in the buckets. Thereafter, seedling emergence vigour and final seedling emergence were determined for each set up. Plant growth indices such as plant height, root length, stem girth, leaf area, and chlorophyll content were also monitored bi-weekly for a period of 12 weeks (3 months). Experiments were conducted in both dry and wet seasons. Results indicated the presence of residual total petroleum hydrocarbons (TPH) in three bioremediated soil samples albeit at varied concentrations. However, TPH concentrations in soils were lower than the Department of Petroleum Resources (DPR) intervention limit. Seedling emergence occurred 11 days after planting. Telfairia occidentalis grown on pristine soil had the highest final percent seedling emergence in the dry season whereas, seeds sown in 18m-AB soil showed the highest final percent seedling emergence during the wet season. There were significant differences in the growth performances of Telfairia occidentalis grown on pristine soil and bioremediated soils ($p<0.05$) as plant growth was considerably hindered in bioremediated soils in both seasons. Best Telfairia occidentalis growth performance was obtained on pristine soil followed by bioremediated soil that had been left fallow for 18 months after intervention. Data from this study suggest that bioremediated petroleum contaminated sites may not be suitable for crop production even months after intervention due to residual hydrocarbon contaminants in soil.

Keywords: bioremediated soil, petroleum, pumpkin, plant performance

INTRODUCTION

Environmental pollution due to petroleum spills goes beyond what can be sensually perceived as there are dire effects that threaten biodiversity, ecosystem and environmental balance owing to leaching, extension and bioaccumulation of contaminants in soil by plants with possible effects on living organisms (Ortínez *et al*., 2003). Bioremediation, which is the treatment of contaminated environments by utilizing biological (especially microbial) mechanisms, is one of the several protocols that have been developed and applied for restoring such oil-polluted sites to their pristine nature. However, *ex-poste* studies suggest that such bioremediated sites still possessed some levels of residual substances that are toxic to surrounding flora and fauna (Malik *et al*., 2001; Khan *et al*., 2008). This is because; a decrease in the level of total petroleum hydrocarbons (TPH) does not imply decrease in toxicity. Phillips *et al*. (2000) stated that partial degradation and emergence of intermediary metabolites may give rise to elevated toxicity in soil. Conventional chemical analyses only predict decrease in TPH and presence of residual contaminants and persistent heavy metals whereas, the generation of intermediary degradation metabolites which are not often captured could raise food safety concerns and exacerbate ecotoxicity in bioremediated soils (Phillips *et al*., 2000). In a previous study, soil toxicity was shown to worsen with the presence of intermediary metabolites, residual hydrocarbons and persistent heavy metals in bioremediated sites (Phillips *et al*., 2000). Hence, there is need for bioassays in the form of ecotoxicity tests to understand the overall effect of the residual contaminants and metabolic intermediates on the ecosystem (Plaza *et al*., 2005). These bioassays will integrate the potentially harmful effects of all pollutants and metabolites present in the sample, not only the target compounds usually monitored by chemical methods.

Recently, the high demand for food has made food security a topical issue. The Niger Delta region has quite a number of farmers that rely on growing food crops for their livelihood. The issue of land pollution has for a long period of time deprived these people of viable land for their farming activities. Petts *et al.* (2000) stated that the effect of industrial activities from history can have deleterious effect on humans and the environment, depreciate the value of land, and also limit the safe re-use of lands. As a result, the natives of the Niger Delta region tend to rush into food crop cultivation as soon as a contaminated site has been remediated.

Since plants rely on soil for their survival, any change in their growth and development may be an indication of the presence of deleterious substances in the soil. There are several ecotoxicity tests (e.g. use of plants, animals, and microorganisms) of which the use of plants promises to be more dependable for assessing efficiency of bioremediation. The use of plants as bio indicators gives quick and first-hand information about the level of toxicity during the bioremediation and post-bioremediation process. Hence, the efficiency of a remediation process can be assessed with plant bioassay (Molina-Barahona *et al*., 2005). It has been established that contaminants can cause several alterations in plant growth and development (Khan *et al*., 2008). Thus, after a certified bioremediation process, evaluating the effect of these compounds (metabolic intermediates and residual hydrocarbons) may have on plant tissues irrespective of their minute concentrations in soil is vital. The combination of data from remediation potential, ecotoxicity and chemical analysis is required to correctly

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evaluate the ecological risk present in bioremediated contaminated soils. A successful bioremediation strategy ensures safe and healthy food crop production as well as environmental sustainability (Alburquerque *et al*., 2011). However, there is dearth of information on the performance of plants grown on certified bioremediated soils in Nigeria.

Hence, this study used plant bioassay to determine the effects of residual contaminants and intermediary metabolites in bioremediated petroleum contaminated soil and their implications for crop production and food security.

MATERIALS AND METHODS

Collection of Soil Samples

Four (4) soil samples from Ogoniland, Rivers State, Nigeria were collected for the study. Three of the soil samples were collected from bioremediated petroleum contaminated sites while the fourth soil sample was collected from a pristine site and served as the control. The bioremediated sites were selected based on their ages (6 months, 12 months, and 18 months after intervention) following remediation certification. Surface soil (0–30 cm depth) was collected from each selected bioremediated site and control site using a Dutch soil auger and composited into a clean, sterile polythene bag. Thereafter, the soil samples were transferred into plastic containers that were used to hold the soil before transport. Composite sampling was carried out at each of the sites to have a good representation of each sampling site. Table 1 shows the location and intervention age of bioremediated petroleum contaminated sites where samples used in this study were obtained.

Table 1: Location and intervention age of bioremediated petroleum contaminated sites where samples used in this study were obtained

Sampling Site	Location	Age of Bioremediation (months)
Control	Bera	Pristine
6m-AB	K-Dere	6
12m-AB	Bodo	12
18m-AB	Biara	18

Physicochemical Analyses of Soil Sample

Particle size (PS) analysis of soil samples was determined using the ASTM 6913 method and the PS distribution was described by the percentage of clay (<0.002 mm), silt (0.002–0.05 mm), fine sand (0.05–0.25 mm), coarse sand (0.25–1.0 mm) and gravel (1.0–2.0 mm). A pH meter (Mettler Delta) was used to determine the soil pH while the total organic matter (TOM) of soil was assessed using the ASTM D2579 method. Nitrogen and phosphorus contents of soil were determined colorimetrically using UV 1800PC spectrophotometer however, the former was in accordance with EPA 352.1 and APHA 4500-NO_3^- B while the latter was in accordance with APHA 4500-P-D and Stewart (1989). The cation exchange capacity (CEC) was determined using the ASTM 7503 method and consisted of potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg). In accordance with ASTM D5765, EPA 1625 and USEPA 8270B, the Varian CP 3800 gas chromatograph (GC) was used to determine the test soils' content of total petroleum hydrocarbon (TPH). The concentration of polycyclic aromatic hydrocarbons (PAH) in each soil sample was ascertained using Agilent 6890 Gas Chromatograph-Mass Spectrometer in accordance to ASTM D7363. The methods used for determination of heavy metal content of soil were: ASTM D8064 (nickel and copper); ASTM D3559 (lead) and ASTM D3557 (chromium) using a flame atomic absorption spectrophotometer.

Plant Seed Germination and Growth Study

The complete randomized design (CRD) was used in this experiment. The methods employed for seed germination and plant growth experiments were adopted from the Organization for Economic Co-operation and Development, OECD Guideline for Testing of Chemicals (OECD, 1984). The effects of the various bioremediated soil samples were determined using seed germination and growth of fluted pumpkin seedlings during dry and wet seasons as toxicity indices. The viability and content of the seeds were first assessed. Thereafter, the pristine soil as well as the bioremediated soils were transferred into plastic buckets before the seeds were planted. The pumpkin seeds were planted after spreading them out to dry for 2 days following extraction from the pod. The number of seeds (9) planted per soil sample was recorded. The time it took each of the seeds to germinate and emerge from the soil was also recorded. Plant growth indices such as stem girth, plant height, leaf area, root length, and total chlorophyll content were measured at 2 weeks intervals for a period of 3 months. Fully expanded new leaves were selected for measurement and the measurement of stem girth was with a Vernier caliper at a point 5cm above the soil (Achakzai *et al.*, 2012). The growth characteristics data were subsequently presented in graphs for each growth parameter. All experiments were carried out in triplicates and results presented as mean value of each analyzed parameter.

Determination of Total Chlorophyll Content

Each leaf sample was weighed (0.1g) and placed in a vial containing 10 ml of acetone. This was kept under room temperature (25±2°C) for 2 days and the total chlorophyll color in the extract was read at 660 nm and 643 nm using UV spectrophotometer. Total chlorophyll content (mg/kg) was calculated using the formula: Abs at 660 + 16.5 × Abs at 643 (Allen *et al.*, 1974). All experiments were carried out in triplicates and results presented as mean total chlorophyll content.

Data Analysis

Results obtained on growth attributes of pumpkin from the respective experiments were pooled, their means determined and subsequently subjected to statistical analysis (ANOVA) using the SAS software to determine if there are significant differences in the means obtained. One-way ANOVA, *t*-test and the least significant difference (LSD) were used to test the means for significance of variance. Spatial variance equality in the means of plant growth attributes at P values < 0.01 and $P < 0.05$ were calculated (Oyegun, 2003).

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RESULTS AND DISCUSSION

The physico-chemical characteristics of the uncontaminated and bioremediated petroleum contaminated soils used in this study are as presented in Table 2. The four soil samples (pristine soil and three bioremediated petroleum contaminated soils) used had similar sand, silt and clay composition and pH range of 6.35 to 7.48. The ranges of each soil type in samples were; fine sand (65.6 -68.4 %), silt (19.5-22.6 %) and clay (10.7-14.5 %) and the soil texture was sandy loam. The relatively higher concentrations of sodium, magnesium and calcium (Table 2) in pristine soil may be the reason its pH was slightly higher (7.48) when compared to the three bioremediated soil samples.

Some of the constituents of oily waste are potentially toxic heavy metals which penetrate the soil and being naturally persistent end up staying over a long period of time, eventually affect soil ecosystems (Sarma and Prasad, 2016). In this study, the metal (Pb, Cu, Cd and Ni) contents in all four soil samples were below EGASPIN guideline for heavy metals. This may seem contradictory especially for the bioremediated soil as these heavy metals have been previously reported to be associated with petroleum (Bacosa *et al*., 2012) and are non-degradable, being elements. However, our investigation revealed otherwise as stated above. Therefore, it may seem that the heavy metal concentrations in the soil sample were decimated by vertical (leaching) or horizontal migration as a result of intense precipitation associated with the study area, biotreatment strategies or water run-off/floods. The potassium and phosphorus levels were low in both the pristine and bioremediated soils while the nitrogen content was maximum in 18m-AB soil followed by 6m-AB soil, pristine soil and 12m-AB soil. Total petroleum hydrocarbons (TPH) was not detected in pristine soil but was present in certified bioremediated soil samples albeit, at varied concentrations lower than the Department of Petroleum Resources, DPR (2002) intervention limit of 5000 mg/Kg but above the target value of 50 mg/kg: 161.25 mg/Kg (6m-AB soil); 51.72 (12m-AB soil) and 91.5 mg/Kg (18m-AB soil). In the case of PAH, it was not detected in pristine as well as in bioremediated soils. PAH, just like heavy metals, co-exist with crude oil and when introduced into the environment via accidental spills or wet deposition can result in soil contamination which threatens plants, animals and all living organisms (Bacosa *et al*., 2012) as they are actively carcinogenic, teratogenic and mutagenic. Their non-detection in soil samples analyzed in this study may be attributed to a variety of mechanisms by which PAHs are degraded in the environment, including chemo-oxidation, photo-oxidation, and microbial degradation (Juhasz and Naidu, 2000). Leaching into sub surface compartments in soil may also be responsible for their non detection.

Table 2: Physicochemical characteristics of pristine and bioremediated petroleum contaminated soils used in this study

Physico-chemical Parameters	Pristine soil-(control)	Bioremediated soil- 6m-AB	Bioremediated soil- 12m-AB	Bioremediated soil- 18m-AB
Sand (%)	65.6	68.4	65.8	66.8
Silt (%)	22.6	20.6	19.7	22.5
Clay (%)	11.8	11	14.5	10.7
pH	7.48	7.40	6.35	6.50
TOM (%)	1.232	1.095	1.232	0.958
N (mg/kg)	38.007	46.94	35.34	73.49
P (mg/kg)	0.571	0.48	0.283	0.218
CEC- K (mg/kg)	ND	ND	ND	ND
Na (mg/Kg)	0.4613	0.3468	0.3646	0.3056
Ca (mg/Kg)	2.864	0.102	0.041	0.10
Mg (mg/Kg)	0.3831	0.1663	0.1438	0.1948
TPH (mg/kg)	ND	161.25	51.72	91.5
PAH (mg/kg)	ND	ND	ND	ND
Lead (mg/kg)	0.04	0.11	0.07	0.05
Cadmium (mg/kg)	ND	ND	ND	ND
Copper (mg/kg)	0.035	0.016	ND	0.005
Nickel (mg/kg)	0.067	ND	ND	0.054

Nine pumpkin seeds were planted in each of the different soils during dry and wet seasons and the seedling emergence time and total emergence percentage obtained are as presented in Figs. 1a – 2b.

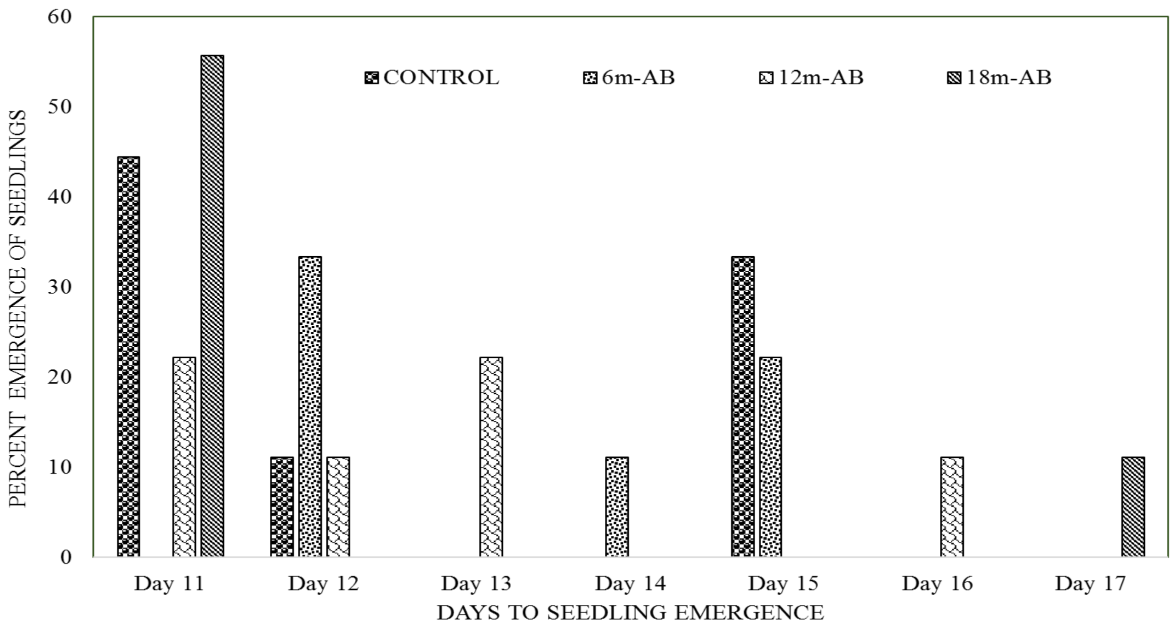

Fig. 1a: Seedling Emergence Vigour of Pumpkin (*Telfairia occidentalis*) Planted During the Dry Season in Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

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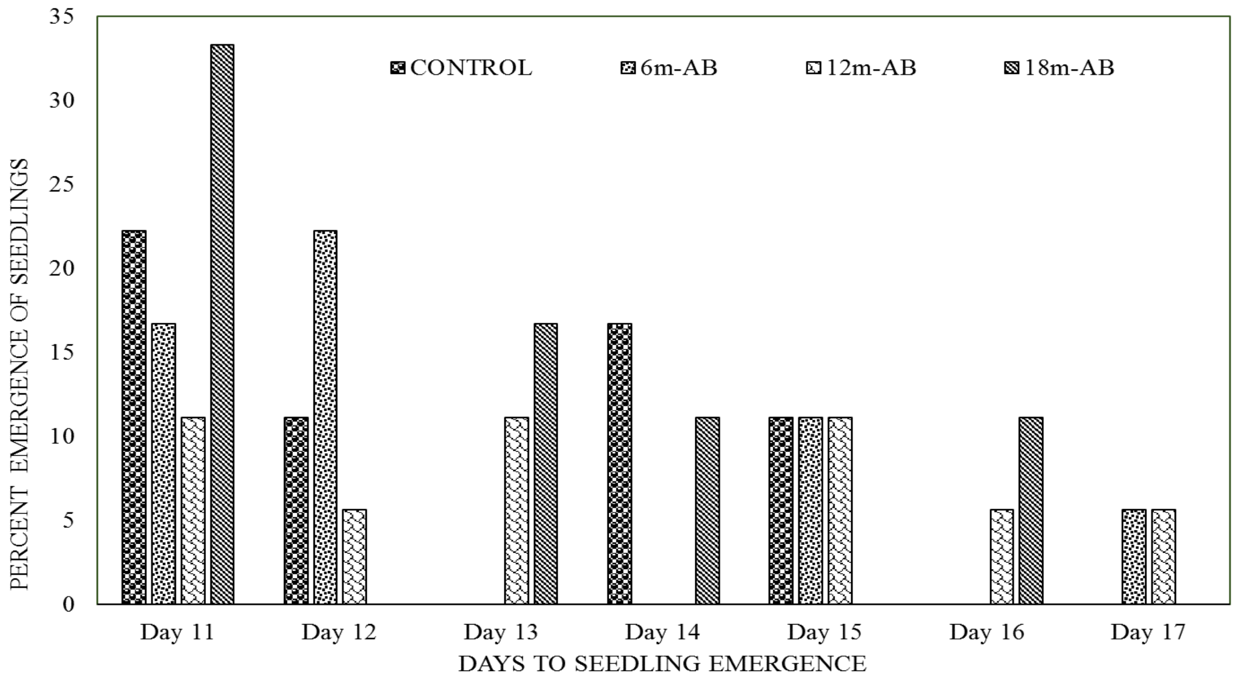

Fig. 1b: Seedling Emergence Vigour of Pumpkin (*Telfairia occidentalis*) Planted During the Wet Season in Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

Tolerance of plants to residual contaminants and metabolites in bioremediated soils and the ability to germinate in these test soils varied greatly between test soil samples. Generally, there was a delay in germination of some seeds sown in bioremediated soils. The pumpkin seeds were largely affected by the residual contaminants in the bioremediated soil which resulted in non-emergence, delay in germination or facilitated emergence. For instance, seedling emergence was delayed in 6m-AB and 12m-AB soils whereas, germination was stimulated in 18m-AB soil.

In both seasons, seed emergence from soil commenced 11 days after the seeds were planted in various soils and continued thereafter over a 6-day period. Seed emergence vigour was lower in bioremediated soils except in 18m-AB soil which stimulated seed emergence vigour during both seasons (Fig. 1a and b). Residual pollutants and intermediary metabolites in the bioremediated soils may have been responsible for the delayed emergence of pumpkin seeds that were planted in bioremediated soils (6m-AB and 12m-AB). Adam and Duncan (1999) had reported that relatively low levels of diesel oil contamination in soil resulted in delayed seed emergence and reduced germination rates in a variety of plant species they studied. In the both seasons, seedling emergence was highest in 18m-AB at the onset (11^{th}) of emergence (Figs. 1a and b). For instance, in the wet season, pumpkin seeds sown in 18-AB soil had the highest seed emergence of 33.3 % on the 11^{th} day (Fig. 1b). The reason for this trend in 18m-AB soil might not be unconnected to stimulation of germination by some degradation metabolites in 18m-AB soil. Moreover, since 18m-AB soil had been fallowing for 18 months after bioremediation, the assumption would be that sustained natural attenuation had taken place thus, ameliorating the effects of the residual contaminants. Despite this however, 12m-AB soil which had been left fallow for only 12 months had a lower TPH content (51.75 mg/Kg) than 18m-AB soil (91.5 mg/Kg). This was rather unexpected and alluded to the fact that the bioremediation strategy deplored was more effective in reducing the TPH content in 12m-AB soil than in 18m-AB soil but less effective in reducing the ecotoxicity potentials of 12m-AB soil when compared to 18m-AB soil. A similar observation had been made by Shen *et al.* (2016) who reported increased phytotoxicity and *Photobacterium phosphoreum* ecotoxicity despite reduction of TPH concentration by 64.4% after forty days of bioremediation. In that report, they attributed the increased ecotoxicity of the soil to the formation of intermediate metabolites which are characterized by high toxicity potentials and suggested that the use ecotoxicity values are a more valid and reliable index for evaluating the efficacy of bioremediation techniques compared to only employing TPH concentrations. Notwithstanding, the highest final percent emergence was obtained in pristine soil in the dry season with a value of 88.8 % (Fig. 2a). In the wet season, the highest final percent emergence (72.2%) was obtained for seeds sown in 18-AB soil at the end of the emergence period (Fig. 2b).

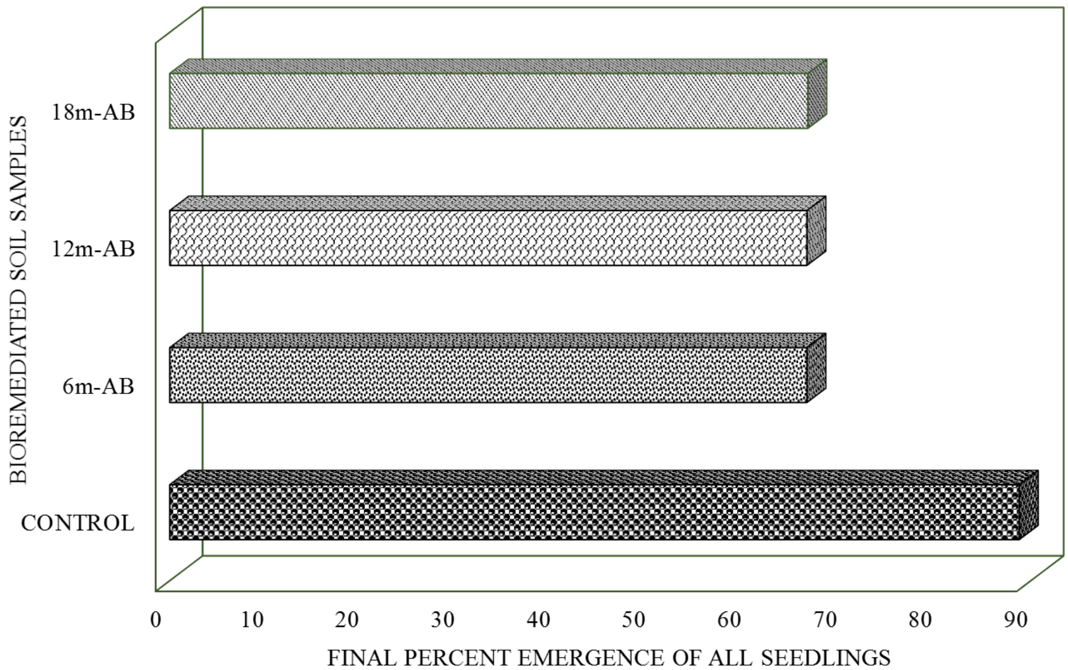

Fig. 2a: Final Percent Emergence of Pumpkin (*Telfairia occidentalis*) Seedlings Planted During the Dry Season in Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

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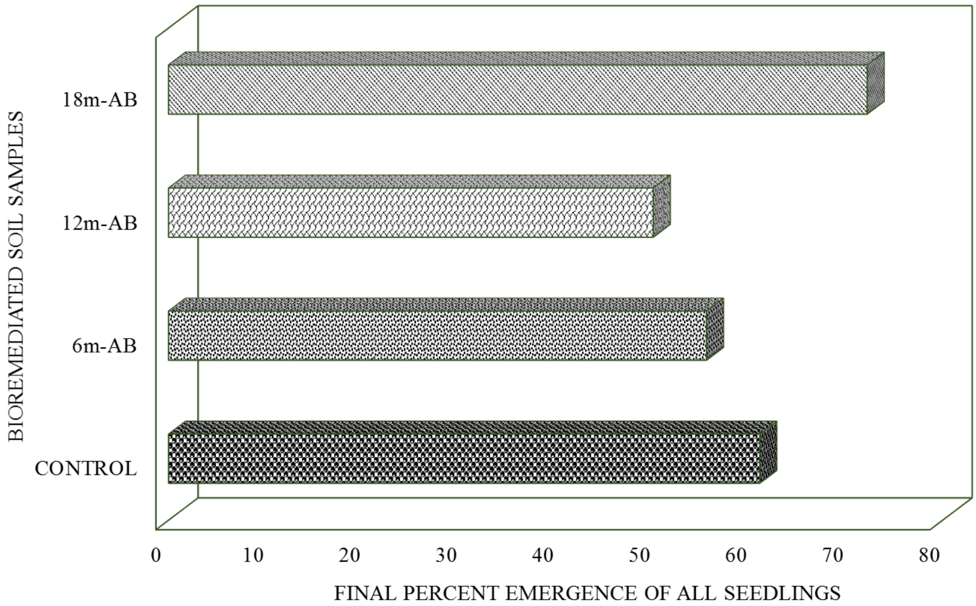

Fig. 2b: Final Percent Emergence of Pumpkin (*Telfairia occidentalis*) Seedlings Planted During the Wet Season in Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

There were significant differences in data obtained on percent seedling emergence of pumpkin during the dry and wet seasons and this may be attributed to decreased precipitation and increased temperature regimes in soil during the dry season which may have stimulated seed germination.

After emergence, pumpkin seedlings were further studied to determine the effect of the residual contaminants in bioremediated soil on the later stages of plant development. Increase in stem girth of the pumpkin seedlings with time in all test soils during the wet and dry seasons was observed (Fig. 3a and b). In the dry season, mean stem girth was highest in pumpkin plants grown on pristine soil (control) with a value of 14.2 mm and least in plants grown on 18m-AB soil with a value of 7.22 mm. Similar trends were obtained in the wet season with plants grown on pristine soil showing the highest mean stem girth. Gradual stem width depression/collapse was observed with time (after 3 weeks) in plants grown on bioremediated soil set ups during the dry season (Fig. 3a). However, in the wet season, there was a steady increase in mean stem girth throughout the study. A similar study carried out to determine effect of petroleum impacted clay soils on germination or growth of *Telfairia occidentalis* indicated ecotoxicity potentials as the contaminated soil manifested a general reduction in germination and growth performance of the vegetable, including stem girth (Adekunle *et al*., 2015).

Generally, increase in mean stem girth was more rapid and consistent in plants grown during the wet season in various test soils than in those grown during the dry season. For instance, plants grown on pristine soil (control) had the highest final mean stem girth of 17.23 mm during the wet season whereas, those grown during the dry season on pristine soil had a final mean stem girth of 14.2 mm (Fig. 3b).

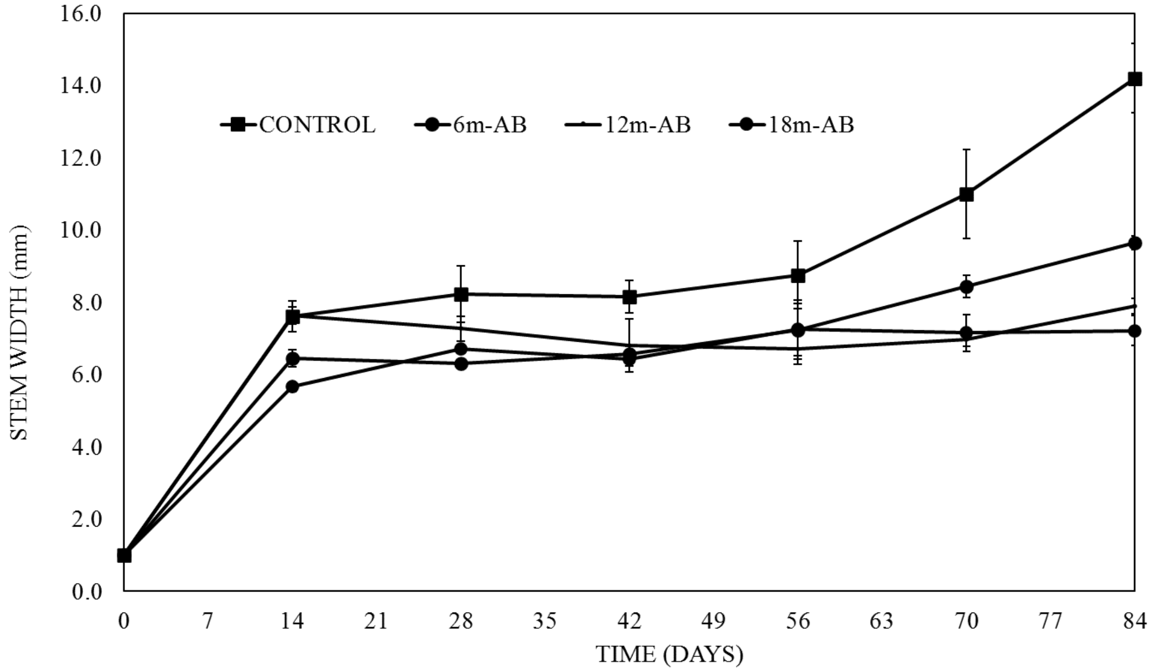

Fig. 3a: Changes in Stem Width (mm) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Dry Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

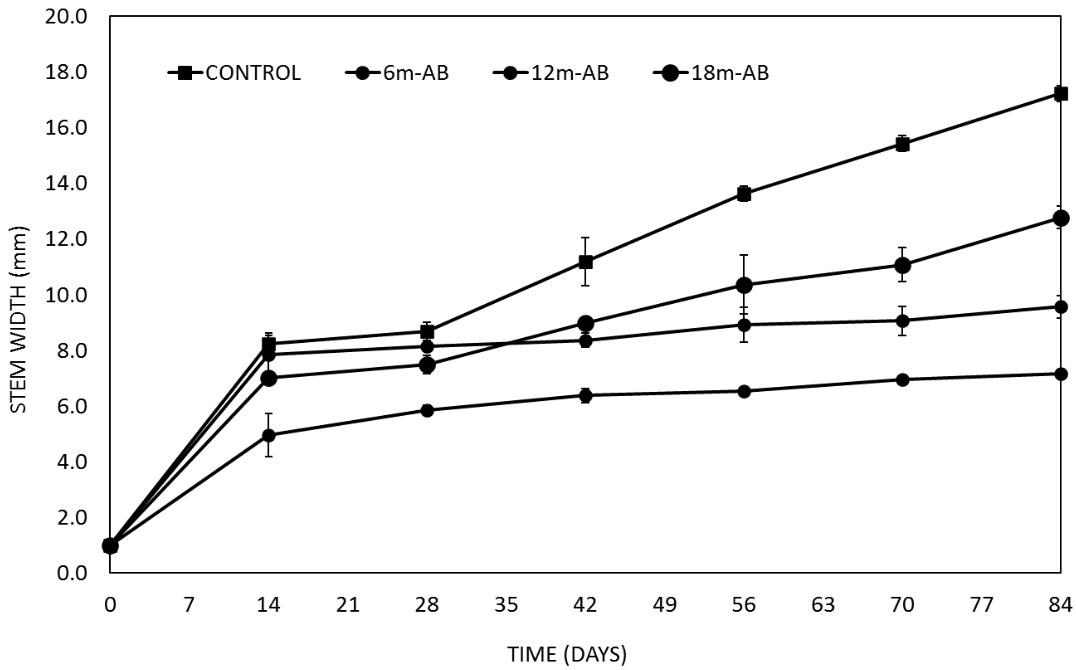

Fig. 3b: Changes in Stem Width (mm) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Wet Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

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There was steady increase in height of emerged pumpkin plants with time in the four test soils however, the overall mean heights of plants grown in bioremediated soil were stunted when compared to the ones grown on pristine soil (control) which had the highest mean plant height obtained (118.67 cm and 141.17 cm) in dry and wet seasons respectively. Delayed seed emergence cannot be blamed for this effect since some seeds sown in bioremediated soils germinated at the same time as those in pristine soil, yet their growth was impaired probably due to the presence of the residual pollutants and metabolic intermediates. For example, the pumpkin seeds sown in 18m-AB soil germinated well in the midst of the residual contaminants (Fig. 1a and b) however, in same soil, plant height was markedly retarded to over 36.3 % and 28.9 % of the control's plant performance for dry and wet seasons respectively. The same pattern was observed for pumpkin grown on other bioremediated soils. The least mean plant height of 60.9 cm was obtained in pumpkin grown on 12m-AB soil during the dry season (Fig. 4a) and for pumpkin grown on 6m-AB soil (76.97 cm) during the wet season (Fig. 4b) thus, suggesting that the bioremediated soils elicited phytotoxic activity and retarded the growth of the pumpkin plants during the study. Salam *et al*. (2016) had reported that the addition of soil contaminated with TPHs in a test soil decreased mean relative height growth by about 25 % in *Salix schwerinii* E. L. Wolf. Cunha *et al*. (2012) also posited that the mean height growth of *Salix rubens* and *Salix triandra* reduced by about 20 – 25 % in soil polluted with petroleum TPHs when compared to uncontaminated soil.

Increased mobility of residual contaminants and metabolites facilitated by increased precipitation may have exacerbated the growth retardation effect observed in plants grown on bioremediated soil in the wet season. This mechanism is also expected to give rise to occasional release of considerable loads of these residual pollutants into the underground water in the vicinity of the bioremediated sites. Snousy *et al.* (2016) had stated that due to shallow ground-water levels and infiltrating water, hydrophobic organic contaminants present in a contaminated soil can be remobilized from the soil. This observation is also in sync with the findings of Halimah *et al.* (2011), who reported that chlorpyrifos easily leached into lower layer of a peat soil profile (0–45 cm depth) due to heavy precipitation. Ismail *et al.* (2002) also noted that simulated rainfall enhanced the mobility of pesticides in a soil column.

Mean plant height was highest in plants grown on uncontaminated pristine soil (141.17 cm) in the wet season. Generally, in terms of plant height, pumpkin performed better in the wet season than in the dry season in all four test soils.

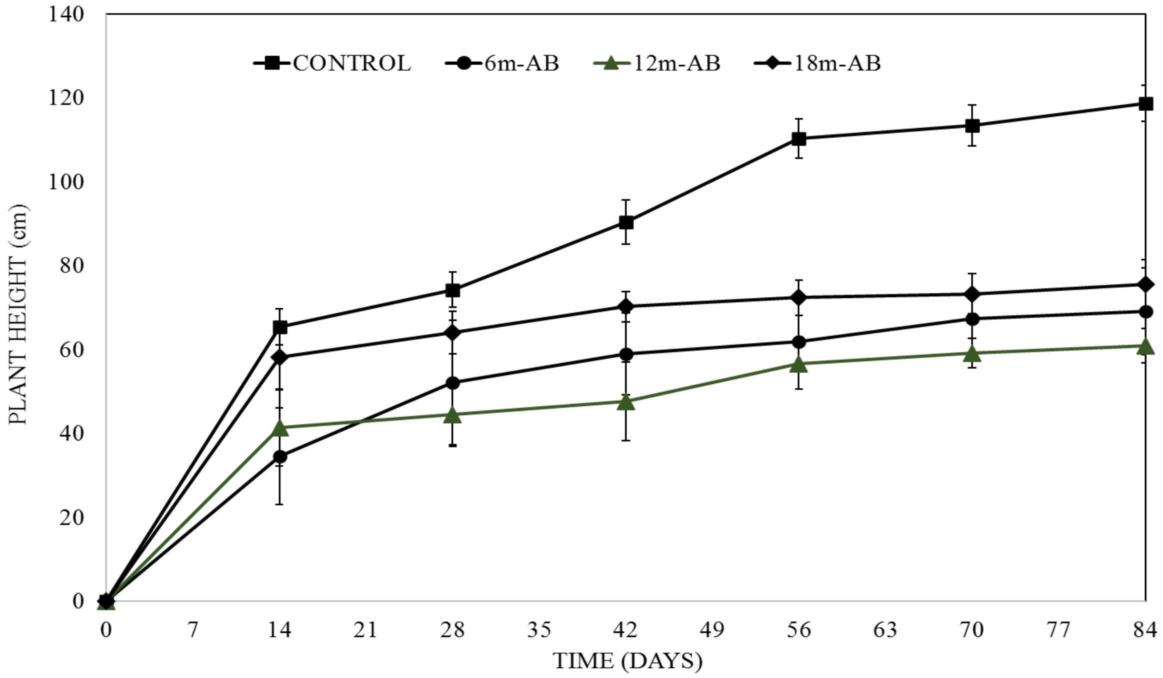

Fig. 4a: Changes in Plant Height (cm) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Dry Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

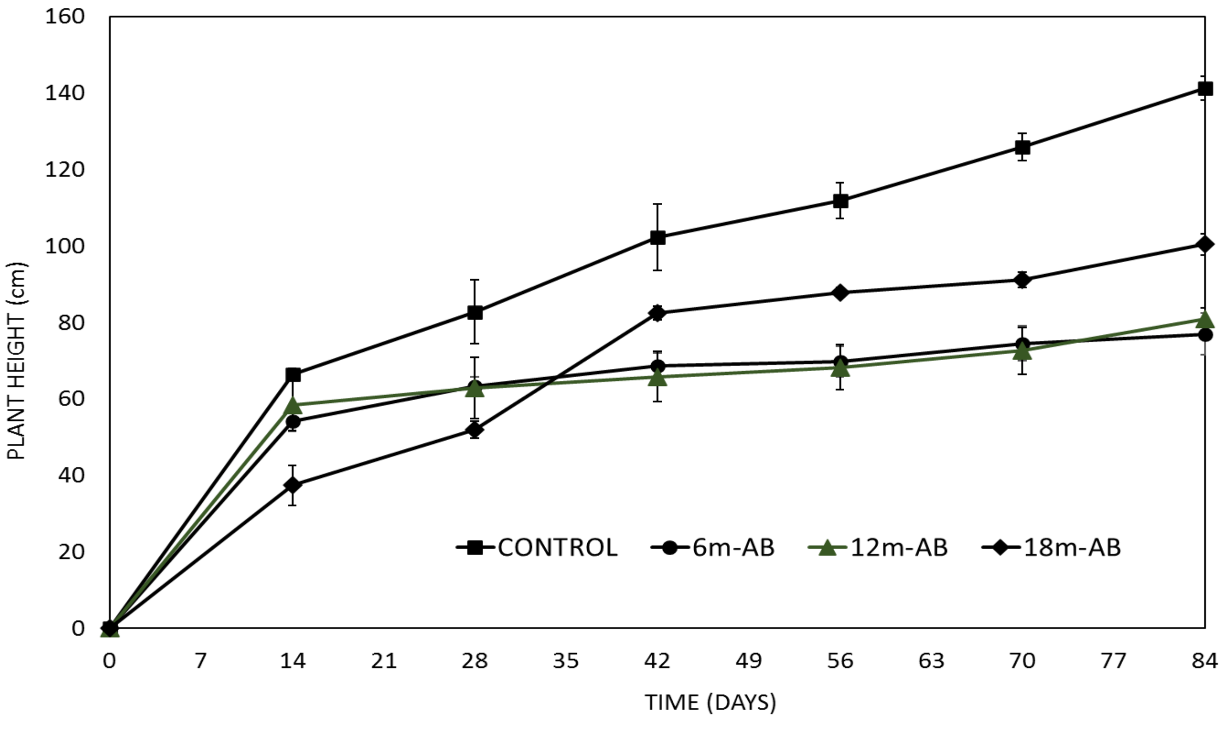

Fig. 4b: Changes in Plant Height (cm) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Wet Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

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Increase in leaf area with time was obtained in pumpkin grown on all test soils in the dry season with those grown on pristine soil (control) showing the widest mean leaf area of 105.78 cm^2. The smallest mean leaf area (51.67 cm^2) was observed in plants grown on 6m-AB soil (Fig. 5a). In the wet season, widest mean leaf area of 143.65 cm^2 was observed in pumpkin grown on pristine soil while, the least mean leaf area (67.33 cm^2) was observed in plants grown on 6m-AB soil (Fig. 5b). Generally, increase in leaf area was more prominent during the wet season. Brown necrotic spots were observed in plants growing in bioremediated soils (6m-AB and 12m-AB). These spots were probably not due to infection, but attributable to pollutant induced phytotoxicity. Similar observation had been made by Tripathi *et al*. (2004) who reported the presence of necrotic spots on *Cassia siamea* Lamk as a result of heavy metal-induced phytotoxicity.

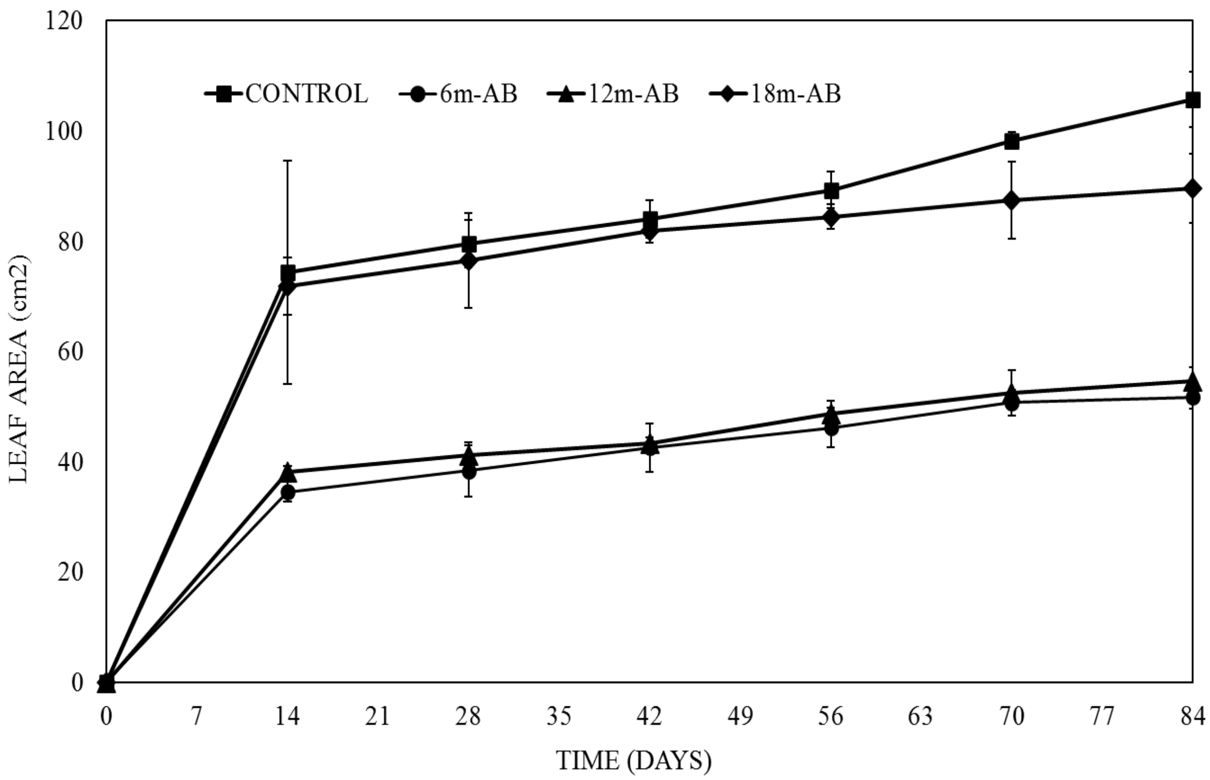

Fig. 5a: Changes in Leaf Area (cm^2) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Dry Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

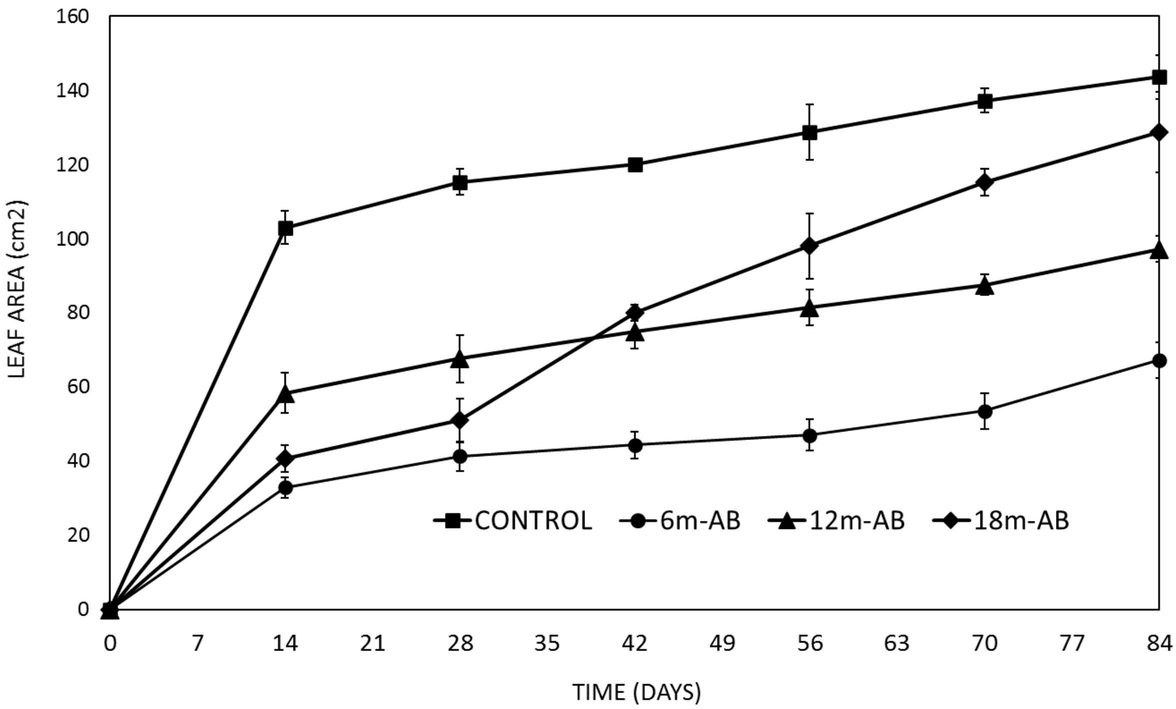

Fig. 5b: Changes in Leaf Area (cm^2) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Wet Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

The extent of pumpkin root extension in various test soils was also determined and results suggest that elongation of pumpkin roots was facilitated by stress imposed by the presence of residual contaminants and metabolites in bioremediated soils especially during the dry season. Support for this inference on facilitated root elongation on exposure to pollutants had been adduced by Tripathi *et al*. (2004) who stated that when plants are exposed to various stresses like heat, oxidative, pathogenesis, pollutants and heavy metals, they respond at cellular level via rapid and transient acceleration in the rate of expression of specific functional genes which may accelerate root elongation. Visioli *et al*. (2016) had also stated in a previous report that *Lepidium sativum* L exhibited root stimulation despite wood-based biochar contamination in soil albeit, the response was attributed to its higher metal tolerance. On the other hand, it may also seem that rapid root elongation obtained in bioremediated soils was a negative chemotactic movement initiated by the plants in a bid to access non-polluted segments of soil and evade the stress elicited by the pollutant concentrations in its ambience. For example, plants grown on 18m-AB soil had the longest mean root length of 30.77 cm in the dry season (Fig. 6a). Adam and Duncan (1999) had posited, in an earlier research to determine the pattern of root development of a selected plant species in a model soil system contaminated with diesel oil, that plant roots avoid diesel oil contaminated areas of the soil completely if they have access to uncontaminated soil to grow into. However,

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roots will grow through contaminated soil segments, if uncontaminated soil is unavailable, until they find an area of uncontaminated soil. At lower contamination levels (up to 10g diesel/kg soil), roots entered the contaminated segment after a period of acclimation. However, the roots began to move into contaminated soil once majority of the surrounding uncontaminated soil had been utilized. To further buttress this phenomenon, it has been stated that root length is an important attribute involved in nutrient uptake (Casper and Jackson, 1997) hence, it is expected that larger roots would exist in plants of high biomass (Ji *et al*., 2011) since roots serve as conduits to transport nutrients and water to above-ground plant tissues (Hodge, 2004). On the contrary, in this study, plants with relatively longer roots did not exhibit enhanced growth performance due to phytotoxic effects of the residual contaminants and metabolic intermediates in soil. For instance, the accelerated root elongation in plants grown on 18m-AB soil was reflected by decreased stem girth, plant height, leaf length and leaf area when compared to plants grown on uncontaminated soil thus, suggesting that stunted growth of these plants was due to undesirable chemical properties of the bioremediated soil.

In the wet season, the longest mean root length of 41.5 cm was observed in plants grown on pristine soil during the wet season while the shortest mean root length of 26.9 cm was observed in plants grown on 6m-AB soil (Fig. 6b). Retarded root elongation in bioremediated soil may be due to soil compaction as a result of its TPH content (which probably acted as a physical barrier to root elongation). Moreover, the residual pollutants in the bioremediated soil may also have affected cell division in root tips. Vaazquez *et al*. (1999) and Gunsse *et al.* (2000) had reported that high amount of aluminum induced the inhibition of root growth by affecting the root elongation due to reduced cell division.

Generally, root elongation of the pumpkin plants was more rapid in the wet season than in the dry season. Increased precipitation in the wet season and the inundation of the soil with water may have diluted out toxicant concentrations in soil pores resulting in decreased bioavailability of the pollutants, metabolites and thus amelioration of the stress (toxic) effect on the plant roots. The relatively poor root development obtained in pristine soil during the dry season may be as a result of the drought conditions prevalent in test soil during that period. Moreover, plants grown in all four test soils exhibited formation of roots with no noticeable differences in root structure derived from different test soils.

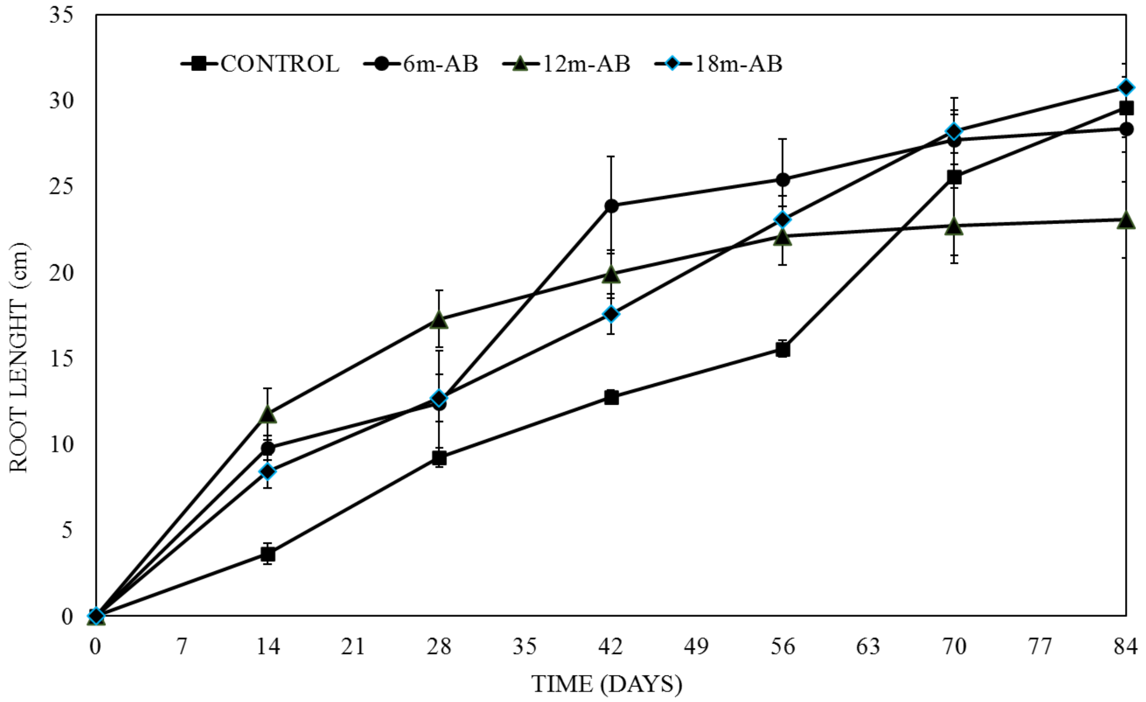

Fig.6: Changes in Root Length (cm) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Dry Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

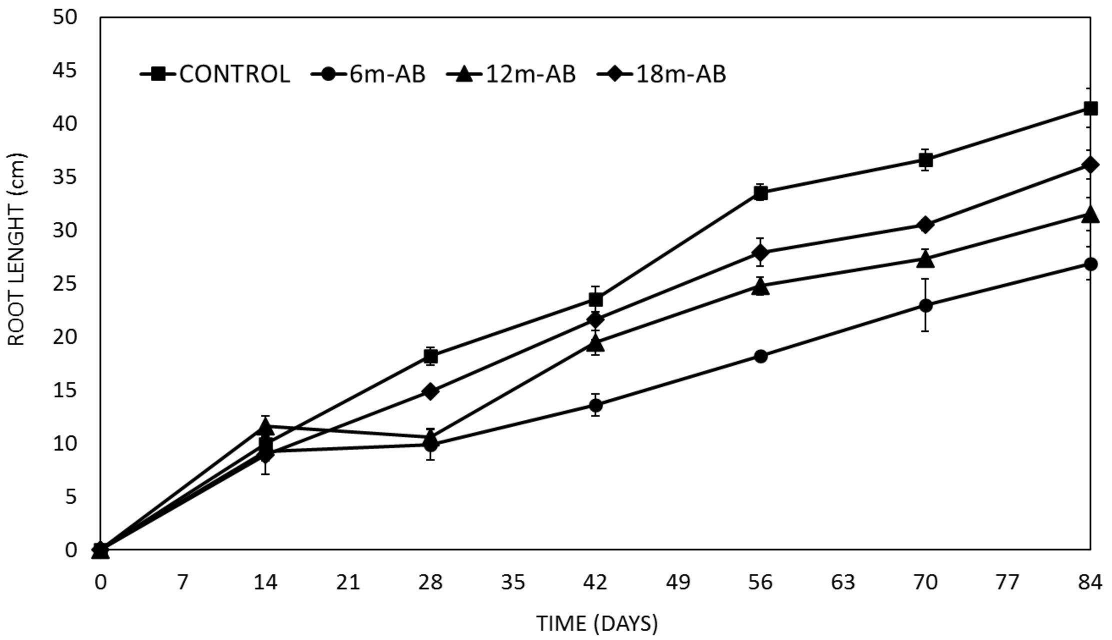

Fig. 6b: Changes in Rooth Length (cm) of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Wet Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

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The mean total chlorophyll content of leaves differed significantly (p<0:05) amongst pumpkin grown on the various bioremediated soils (Fig. 7a and b). Plants grown on bioremediated soils had their mean total chlorophyll content significantly reduced (p<0:05) in both seasons when compared to that obtained for plants grown on pristine soil. The toxic effect of the contaminants on chlorophyll content in soil was more pronounced when plants were grown on 6m-AB soil followed by 18m-Ab soil in both seasons.

In all pumpkin plants grown on various test soils, chlorophyll content of the leaves declined gradually with time till the end of study with more rapidity in decline obtained in leaves of plants grown on bioremediated soils. Chlorophyll content of pumpkin leaves was also lower during the dry season when compared to the wet season. In the dry season, plants grown on pristine soil had the highest mean total chlorophyll content of 561.73 mg/kg whereas, plants grown on 6m-AB soil had the least mean total chlorophyll content of 360.7 mg/kg (Fig. 7a). A similar trend was obtained with regards to chlorophyll content of leaves during the wet season. A previous study had also reported reduced chlorophyll content in leaf samples of plant families found in petroleum polluted sites when compared to pristine forest and secondary forest sites that were non-polluted (Arellano *et al.*, 2017). It was concluded in that report that the natural photosynthetic gradient of a forest canopy can be used to differentiate plant stress caused by petroleum pollution; a suggestion that was made based on the results obtained together with the analysis at various vertical canopy levels.

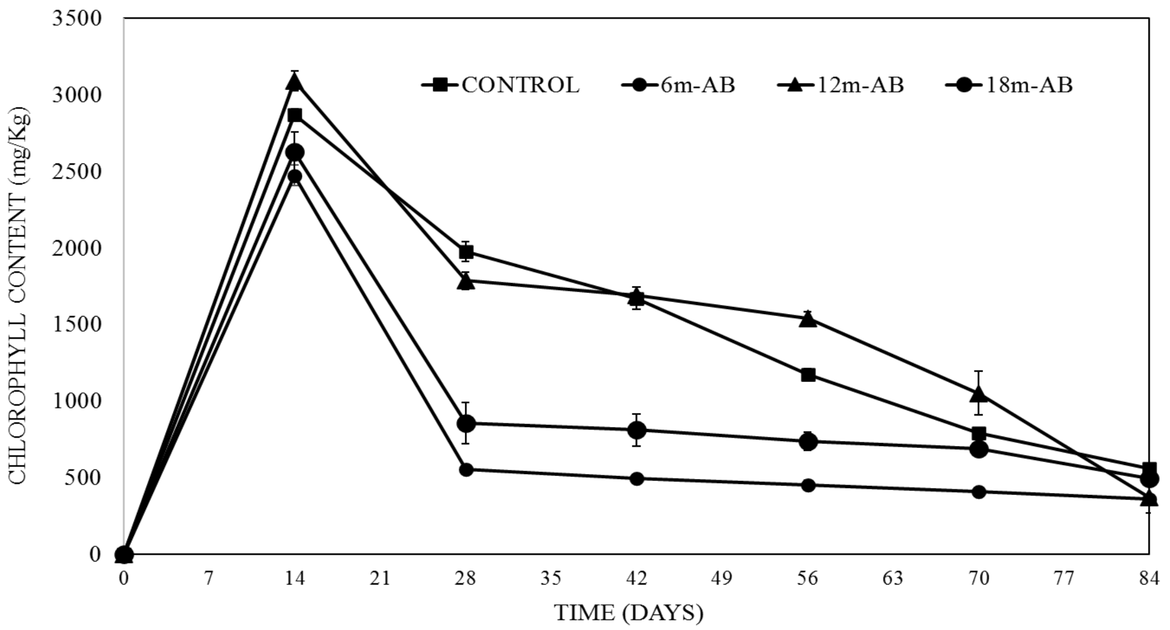

Fig. 7a: Changes in Chloropyll Content of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Dry Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

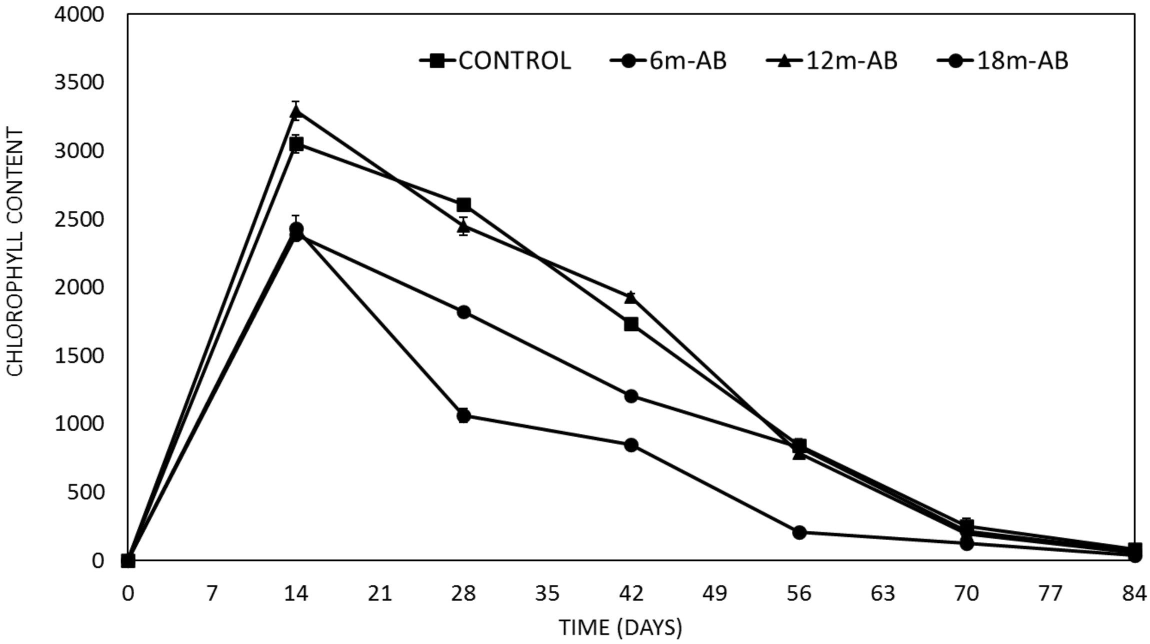

Fig. 7b: Changes in Chloropyll Content of Pumpkin (*Telfairia occidentalis*) Seedlings Grown During the Wet Season on Various Bioremediated Petroleum Hydrocarbon Contaminated Soil Samples Obtained from Ogoniland

Chlorophyll content of leaves was high at the onset of the experiment during the wet and dry seasons but declined with time and more rapidly in the wet season than in the dry season. For example, at the end of the experiment, mean total chlorophyll contents in pumpkin leaves grown on uncontaminated pristine soil were 80.37 mg/kg and 561.73 mg/kg in the wet and dry seasons respectively (Fig. 7a and b). Generally, chlorophyll content of leaves was higher in pumpkin grown on pristine soil when compared to the ones grown on bioremediated soils.

The retarded growth observed in plants grown in bioremediated soil was accompanied by decreased stem girth, plant height, leaf area, root length. This may be attributable to decreased levels of chlorophyll in pumpkin leaves coupled with undesirable chemical attributes of the residual contaminants and metabolic intermediates not to neglect the low phosphorus and potassium levels in soil (Misra and Shukla, 1986; Wong and Wong, 1989). Generally, the highest performance indices evaluated were obtained in plants grown on pristine soil probably due to a couple of factors that favoured plant development such as normal pH (7.48) and absence of TPH contaminants and degradation metabolites.

Moreover, the relatively poor performance of *Telfairia occidentalis* grown on bioremediated soils in comparison to those grown on pristine soils could also be attributed to the water logging nature (after a precipitation event) of these soils, unlike the pristine soil that had a higher water penetration capacity. This was indicative of the hydrophobicity of the soil (soil water repellency) which may have denied the

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roots of plants grown on such soils of adequate supply of pore water. A similar observation has been made by Adekunle *et al*. (2015) who opined that this phenomenon could probably due to crude oil effect.

It is well known that plant growth is impeded and their morphological and physiological aspects impacted negatively due to soil pollution from crude oil spills (Ogbo *et al*. 2009; Omosun *et al*. 2008). The presence of these pollutants makes the land unsuitable for agriculture (which ultimately reduces plant productivity and creates a threat to food security) and other economic purposes unless it is properly remediated (Sarma *et al.*, 2016). The bioremediated soil samples used in this study were deemed reclaimed but could not adequately support plant growth probably due to the presence of residual pollutants and metabolic intermediates. Hence, further intervention may be required by concerned government agencies at such sites in order to restore these affected lands to productive fields since agriculture is the primary economic mainstay of the populace in the study area.

CONCLUSION AND RECOMMENDATION

Data obtained in this study suggest that the presence of residual contaminants in soil could negatively affect seedling emergence as well as the growth performance of *Telfairia occidentalis* grown on bioremediated soil. Generally, a significant reduction in germination vigour and growth indices was observed when plants were grown in bioremediated soils of different ages after intervention. However, emergence of seedlings after *Telfairia occidentalis* seeds were sown was stimulated in 18m-AB bioremediated soil. Irrespective of the level or composition of contaminants in the soil, pumpkin had a better growth performance during the wet season compared to that of the dry season.

Amongst the bioremediated soil types, pumpkin grown on the 18m-AB soil performed better in terms of growth indices than those grown on 6m-AB and 12m-AB soil. This underscores the need for farmers to give time for post-intervention natural attenuation of bioremediated sites to take place before using such lands for crop production. We are currently evaluating the extent of pollutant and metabolite accumulation in crops grown on such bioremediated sites in our laboratory in order to determine the safety of such food product.

Acknowledgement

My sincere appreciation goes to the World Bank Africa Centre of Excellence for Oilfield Chemicals Research (*ACE-CEFOR*), University of Port Harcourt for their financial assistance during the course of this work.

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