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BOJAAS

RESEARCH ARTICLE

Can *Axonopus compressus* (Beauv.), *Cynodon dactylon* (L.) Pers. or *Eleusine indica* (L.) Gaertn. be used as green solution to soils heavily polluted with crude oil?

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EDV; Conceived idea, designed study, Edited of manuscript. OOO; Collected data, data analysis, draft manuscript, KOI; Collected data, data analysis, laboratory & statistical analysis

ABSTRACT

The objective of this study is to assess the survival of three grass species, *Axonopus compressus* (Beauv.), *Cynodon dactylon* (L.) Pers, and *Eleusine indica* (L.) Gaertn. in crude oil polluted soil and propose an explanation for their responses. Top Ultisol soils (0-15 cm depth) collected from demonstration farm were pooled to make composite sample, air-dried and sieved (<2mm). Experimental pots were prepared from the composite sample and individually treated with 0%, 10% or 20% (w/w) crude oil by gradual manual mixing. Ten equally sized stumps of a grass species were sown in a pot. Each species had 9 pots representing three replicates per oil treatment, arranged in a completely randomized design. Parameters assessed were plant height, number of leaves, stem circumference, leaf length, root length and biomass. Soil pH was monitored every 2 weeks for 10 weeks. Total petroleum hydrocarbon (TPH) content and GC-FID analyses of soil samples before and after plant growth were done. Bacterial species associated with the soil samples after plant harvest were determined. Growth of the test species were suppressed by oil when compared to plants grown in unpolluted soil. Mean height of *A*. *compressus* plants recorded 10 weeks after planting (WAP) were 24.37, 12.27 and 9.78 cm for 0, 10 and 20% oil polluted soil respectively. *C*. *dactylon* and *E*. *indica* failed to sustain growth in 20% soil. TPH was reduced from 6.704 to 2.965 mg/kg and 6.176 to 2.032 mg/kg for 10 and 20% soil respectively by *A*. *compressus*, 6.704 to 2.503 mg/kg and 6.176 to 1.819 mg/kg for 10% and 20% soil respectively by *C*. *dactylon*, and 6.704 to 2.282 mg/kg and 6.176 to 1.522 mg/kg for 10 and 20% soil respectively by *E*. *indica*. Soil pH increased from 5.8 – 8.3 and 4.5 - 7.8 in 10 and 20% soil respectively where *A. compressus* plants were grown. Soil pH where *C*. *dactylon* and *E*. *indica* plants were grown did not exhibit this. The study suggests that *A. compressus* is able to sustain growth in 20% crude oil polluted soil by modifying soil pH from acidic to neutral conditions.

Keywords Crude oil, *Cynodon dactylon*, *Eleusine indica*, *Axonopus compressus*, soil pollution, survival

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INTRODUCTION

Crude oil exploration, exploitation, transportation, and storage in Nigeria have brought damaging impacts on the environment (soil, air and water), even though the economy and prosperity of the nation are tied to these activities. Hydrocarbons are categorised as common environmental contaminants in industrialised countries (Schaefer and Juliane, 2007; Phillips *et al*., 2009; Zhang *et al*., 2009). These damaging impacts have provoked the scientific community to look-up for acceptable, less toxic, environmentally-friendly and sustainable cleanup solutions on contaminated environs. Phytoremediation and bioremediation are suggested as effective and low cost technologies for recovering polluted soils (Fernandez *et al*., 2011). The support gained by phytoremediation and bioremediation is based on the effective reduction of total petroleum hydrocarbon (TPH) content of polluted soil. Furthermore, phytoremediation does not produce toxic intermediates and avoid undesired environmental and ecological effects (Geissen *et al*. 2008).

Hydrocarbon spills in the environments may be accidental or due to sabotage. Whatever the case, soil pollution by crude oil usually changes soil properties and conditions negatively, affecting plant communities (Nwilo, 1998; Nwilo and Badejo, 2005). Oil spills destroy farmlands and have significant effects on plant growth and yield (Anoliefo *et al*, 2003). Though crude oil pollution is a worldwide issue, it is widespread in many communities and local Government areas in the nine out of thirty-six states in Nigeria, where crude oil wells are located. The presence of hydrocarbon in soil makes the soil impermeable to water, plants suffer dehydration, loss of leaves and root viability, and essential nutrients (phosphorus and nitrogen) for plants are unavailable (Nicolloti and Eglis, 1998; Anoliefo and Vwioko, 2001). Crude oil at very low concentrations in soil stimulates plant growth and this is attributed to the ease of degradation by natural soil populations, increase in organic matter of soil, and improvement in soil fertility. Oil at both moderate and high concentrations is detrimental as it leads to growth retardation and death in higher plants (El-Bakatoushi, 2011). Crude oil spills affect plants by retarding seed germination, decreasing plant height, stem circumference, plant cover, photosynthetic rate and causing mortality (Adams and Duncan, 2002; Vwioko and Osazuwa, 2012). Agbogidi (2009) showed that hydrocarbons significantly reduced the availability of plant nutrients in soil. Vwioko and Fashemi (2006) stated that oil contamination of soil

creates an unfavourable condition which interferes with nutrient uptake resulting in suppressed growth of plants.

Eweis *et al*. (1998) stated that directly and/or indirectly, plants are involved in degradation of petroleum hydrocarbons into products (e.g., alcohols, acids, carbon dioxide and water) that are less toxic and less persistent in the environment than the parent compounds. One suggestion about the indirect role of plants in the degradation process of petroleum hydrocarbons is connected to the release of enzymes from plant roots which are capable of transforming contaminants by catalysing chemical reactions in the soil (El-Bakatoushi, 2011). Phytoremediation of hydrocarbon will be successful if the species used exhibits tolerance or resistance to oil pollution. The presence of some plant species (e.g., grasses) in contaminated environments produces greater disappearance of TPH than with other species. El-Bakatoushi (2011) stated that the precise mechanisms of plants response to toxicity of crude oil are not clear. It varies according to degree of habitat disturbance and between different plants and among populations of same species inhabiting different habitats. Researchers should explore how to gain relevant and necessary understanding of mechanisms employed by plant species growing in oil polluted soil. There are plant species identified as potential phytoremediant of hydrocarbon contaminated soil (Anoliefo and Vwioko, 2001; Anoliefo *et al*., 2003). However, the mechanisms used by these plants to survive and grow continuously are not clear. The objective of this study was to assess the survival of three grass species, *Cynodon dactylon* (L.) Pers., *Eleusine indica* (L.) Gaertn. and *Axonopus compressus* Beauv. in crude oil polluted soil as potential phytoremediation agents as well as propose a mechanistic explanation underlying their responses.

MATERIALS AND METHODS

This study was carried out in the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

Plant materials

Equal-sized stumps of three grass species, *Cynodon dactylon* (L.) Pers., *Eleusine indica* (L.) Gaertn. and *Axonopus compressus* Beauv., were identified and used in this study. They were obtained from an open field within the premises of Senior Staff quarters, University of Benin.

Collection of composite soil sample

Top soil (0-15 cm depth) was collected from the Faculty of Agriculture demonstration farm. The type of soil is Ultisol. The soil was air-dried and sieved to exclude gravels, stone and other debris. The composite sample was used to prepare 27 experimental pots, each with 3kg of soil.

Collection of crude oil

The crude oil was obtained from Nigerian Petroleum Development Company (NPDC), Ologbo flow station, along Benin-Sapele expressway, Edo State, Nigeria.

Crude oil application to experimental soil samples

The crude oil treatments (pollution) of 0%, 10% and 20% of the weight of soil samples were measured and applied. For each respective experimental soil sample, the application of crude oil was done gradually by thoroughly mixing with hand, thereafter watered and left to stand in the field overnight. The 0% crude oil treatment was used as control. Each crude oil treatment was replicated three times.

Planting of grasses into experimental pots

Ninety (90) stumps for each grass species were planted. The stumps were of equal size and separated into individuals; and individually transplanted into moist soil. Ten individual stumps were planted into each experimental pot. A total of 270 individual stumps were used in the study.

Plant Data Measurements

Heights of plants were measured as the length of the stem from soil level to the apex of the plants. Measurement was taken using a metre rule from one plant selected in each experimental pot every two-weeks. Stem circumference was done by wrapping a thin thread around the stem below the first node and placing the thread on a metre rule to obtain the length. The third and fourth leaves from the stem apex of a plant were marked and used to record measurements of leaf length of plants. Number of leaves *per* plant was carried out by counting every two-weeks. The number of plants found alive and survived in each experimental pot was used to calculate the percentage survival of plants. This was carried out every two weeks. Average root length of plants was taken after plant harvest. Due to the fibrous root system of the plants, mean length of ten roots *per* plant was recorded. Fresh weights of whole plants were taken after plant harvest. Dry weights of plants were recorded after oven drying to obtain constant weights at 55°C for 48 hours.

Experimental soil analysis

Physicochemical analysis of composite soil sample was carried out using standard procedures. For example, particle size analysis by hydrometer method, organic carbon was by Walkley and Black method (Bremner and Jenkinson, 1960), nitrogen was by Kjedahl method (Bremner, 1960), phosphorus was by Bray and Kurtz (1945) method, sodium and potassium by flame photometric method (Orhue and Osaigbovo, 2003), and exchangeable bases and acidity by titration (Orhue and Osaigbovo, 2003).

Total petroleum hydrocarbon (TPH) contents of crude oil polluted soil before and after plant growth were carried out according to Salanitro *et al*. (1997). Samples of soil were collected from each experimental pot using stainless steel scoop and labelled. A mixture of acetone and dichloromethane (1:1) was used as extraction solvent, and fractionation was done using n-hexane solvent in silica gel columns. GC-FID analysis of extracts for composition of aliphatic and aromatic hydrocarbons

present in the different experimental soil samples was done also (Vwioko and Omamogho, 2012).

Monitoring pH of experimental soils during plant growth was carried out in each experimental pot every two-weeks for ten weeks. This was done by taken the pH of soil to water (1:3) mixture using hand held pH meter.

Soil-borne bacterial analysis was carried out. Ten weeks after plant growth in different experimental pots, the soil samples were collected and used for analysis of bacterial species. Serial dilutions and the pour plate methods were used for the inoculation on sterilsed nutrient agar (NA) medium, incorporated with antifungal agent (0.5 ml of griseofulvin per plate prepared by dissolving 300 mg of griseofulvin in 12.5 ml sterile distilled water). The plates were incubated at 37°C for 24-48 hours to develop colonies. Viable colonies were recorded as respective of the microbial in colony forming units per gramme (cfu/g). The isolation and identification of the bacterial isolates were carried out according to the methods of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Statistics

The study was carried out as a completely randomised design. Mean and standard deviation were calculated for the data collected in the study. Data were analysed as one - factor ANOVA, which tested the effects of crude oil concentration on plant species every 2- week interval. Interaction effects were not statistically tested. Comparison of means was done using Duncan multiple range (DMR) test. All analyses of data were done using GENSTAT (8th edition, 2005).

RESULTS

The results obtained in this study are shown in Tables 1-8 and Figures 1-6. Table 1 shows the physicochemical properties of the soil used for the experiment before cultivation.

Table 1: Physicochemical properties of soil used for the experiment

Properties	Unit	Amount
Sand	gkg^{-1}	840
Silt	gkg^{-1}	80
Clay	gkg^{-1}	80
pH		6.70
Conductivity	$uScm^{-1}$	1280
Organic carbon	gkg^{-1}	18.16
Total nitrogen	gkg^{-1}	0.89
Available phosphorus	$mgkg^{-1}$	122.85
Exchangeable calcium	$mgkg^{-1}$	2600
Exchangeable magnesium	$mgkg^{-1}$	463.2
Exchangeable potassium	$mgkg^{-1}$	50.7
Exchangeable sodium	$mgkg^{-1}$	41.4
Exchangeable acidity	$mgkg^{-1}$	0.00
ECEC	$mgkg^{-1}$	3155.3

Sand content was 840 gkg^{-1}; while silt and clay was 80 gkg^{-1} each. The pH was had a value of 6.70, while organic carbon was 18.16 gkg^{-1}. Calcium, magnesium, potassium, and sodium contents are also shown in Table 1.

Table 2 shows the height of surviving plants grown in crude oil contaminated soils. The values obtained indicate that within the first four weeks, plants in control soil exhibited extension growth. Plants grown in 10% and 20% crude oil polluted soils exhibited marginal increase in growth. Six WAP, the values recorded for *C. dactylon* and *E. indica* in 20% polluted soils show the degeneration and death of all mature plants and were left with minute stubs. The stubs eventually degenerated as well.

Table 3 shows stem circumference of *A. compressus, C. dactylon* and *E. indica* plants grown in crude oil contaminated soil samples (0%, 10% and 20%). The values obtained showed the effect of the oil contaminant on the growth of the plant species. The values of stem circumference obtained for plants grown in contaminated soil were reducing as the study progressed because of gradual death of mature plants. Values are based on those plants that were observed at that particular week. Two species, *C. dactylon* and *E. indica* completely lost all mature plants after 6 weeks of growth in 20% oil contaminated soils.

Table 4 shows leaf length of *A. compressus, C. dactylon* and *E. indica* growing on crude oil contaminated soil samples (0%, 10% and 20%). After ten weeks of growth, plants grown in control soil (0%) had the highest leaf length values for the three species. This was followed by plants grown in 10% contaminated soil. *A. compressus* plants grown in 20% contaminated soil gave some values. *C. dactylon* and *E. indica* had no plants in 20% contaminated soils after ten weeks of growth

Figure 1 shows root length of *A. compressus, C. dactylon* and *E. indica* grown on crude oil contaminated soil samples (0%, 10%, and 20%) after plant harvest. Root lengths of the individual species were statistically significant except for *E*. *indica*. This shows that root length values obtained for a species were affected by oil concentration in soil. *A*. *compressus* cultivated in polluted soil with 0%, 10% and 20% had root length values of 3.60 ± 0.26^{a} cm. 5.33 ± 0.55^{b} cm and 7.33 ± 0.52^{c} cm respectively. *C. dactylon* cultivated in polluted soil with 0%, 10% and 20% had root length values of 14.73 ± 1.32^{a} cm, 14.07 ± 1.79^{a} cm and 23.33 ± 3.50^{b} cm respectively. *E. indica* cultivated in polluted soil with 0%, 10% and 20% had root length values of 3.73 ± 0.33^{a} cm, 4.43 ± 0.67^{a} cm and 4.67 ± 0.88^{a} cm respectively

Figure 2 shows percent survival of the 3 grass species in crude oil polluted soil. *Axonopus compressus* had a constant survival rate of 100% (Figure 2A); *C. dactylon* and *E. indica* had a drop in their percent survival with increasing concentration of crude oil (Figure 2B and 2C). Survival rate curves of the test plants revealed that *A. compressus* survived better than the other two plant species

Figure 3 shows the results obtained for monitoring soil pH of the different soil media where *A. compressus (A), C. dactylon (B*) and *E. indica* (C) plants were grown. In Figure 3A, the pH of soil media polluted with crude oil (10% and 20%) gradually increased from acidic conditions to above neutral (i.e. pH 7) following the growth of *A. compressus* plants. In Figure 3B, the pH of soil media polluted with crude oil (10% and 20%) did not change but remained acidic with growth of *C. dactylon* plants. In Figure 3C, the pH of crude oil polluted soil media where *E. indica* plants were grown showed increased with time. But only in 10% polluted soils that the pH values had risen to neutral conditions by ten weeks after planting.

Table 2: Height (cm) of surviving plants of *Axonopus compressus*, *Eleusine indica* and *Cynodon dactylon* grown in different concentration of crude oil polluted soil

Plant species	Crude oil conc in soil	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
Axonopus compressus	0%	14.33 ± 1.74^{a}	15.77 ± 1.21^{b}	17.67 ± 0.50^{b}	22.10 ± 2.31^{b}	24.37 ± 2.58^{b}
	10%	13.03 ± 1.21^{a}	12.67 ± 0.95^{b}	11.23 ± 0.87^{a}	12.17 ± 0.79^{a}	12.27 ± 0.87^{a}
	20%	11.73 ± 2.61^{a}	9.33 ± 2.09^{a}	9.67 ± 3.18^{a}	9.74 ± 3.12^{a}	9.78 ± 1.09^{a}
Cynodon dactylon	0%	10.50 ± 0.57^{a}	11.40 ± 0.89^{a}	13.90 ± 1.96^{a}	15.80 ± 2.87^{b}	18.27 ± 2.43^{c}
	10%	10.60 ± 0.49^{a}	10.27 ± 1.86^{a}	10.00 ± 1.37^{ab}	13.53± 0.38^{b}	13.57 ± 0.55^{b}
	20%	10.50 ± 1.53^{a}	8.8 ± 2.66^{a}	4.00 ± 1.82^{b}	2.83 ± 2.03^{a}	1.63 ± 1.33^{a}
Eleusine indica	0%	11.37 ± 0.81^{a}	12.77 ± 0.73^{b}	15.60 ± 0.59^{b}	19.43 ± 0.39^{c}	28.83 ± 3.76^{c}
	10%	11.50 ± 1.25^{a}	12.03 ± 1.86^{b}	9.06 ± 2.94^{ab}	9.63± 2.09^{b}	11.20 ± 1.95^{b}
	20%	8.20 ± 1.10^{b}	10.67 ± 0.84^{a}	1.17 ± 1.07^{a}	1.17 ± 1.07^{a}	1.06 ± 1.00^{a}
F-prob		<0.01	<0.01	<0.01	<0.01	<0.01

Figures are presented as mean ± S.D (standard deviation). Means with similar alphabets as superscript in one column for each species are not significantly different at 0.05 level of significance using Duncan multiple range test. WAP- weeks after planting

Table 3: Stem circumference of surviving plants of *Axonopus compressus*, *Cynodon dactylon* and *Eleusine indica* grown in different concentration of crude oil polluted soil

Species	Crude oil conc in soil	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
Axonopus compressus	0%	0.80 ± 0.17^{a}	0.97 ± 0.17^{b}	1.33 ± 0.09^{b}	1.23 ± 0.29^{b}	1.40 ± 0.32^{b}
	10%	0.63 ± 0.13^{a}	0.57 ± 0.12^{a}	0.53 ± 0.03^{a}	0.53 ± 0.09^{a}	0.53 ± 0.09^{a}
	20%	0.50 ± 0.11^{a}	0.47 ± 0.03^{a}	0.40± 0.10^{a}	0.40± 0.10^{a}	0.40 ± 0.10^{a}
Cynodon dactylon	0%	0.43 ± 0.07^{a}	0.87 ± 0.12^{a}	1.23 ± 0.18^{b}	1.50 ± 0.17^{b}	1.23 ± 0.18^{b}
	10%	1.17 ± 0.24^{b}	1.10 ±0.42^{a}	0.47 ± 0.17^{a}	0.42 ± 0.11^{a}	0.42 ± 0.11^{a}
	20%	0.47 ± 0.09^{a}	0.33 ± 0.03^{a}	0.20 ± 0.06^{a}	0.00 ± 0.00	0.00 ± 0.00
Eleusine indica	0%	0.67 ± 0.88^{ab}	0.93 ± 0.09^{b}	1.13 ± 0.67^{c}	1.17 ± 0.13^{b}	1.73 ± 0.12^{b}
	10%	0.97 ± 0.12^{b}	0.70 ± 0.12^{b}	0.47 ± 0.09^{b}	0.30 ± 0.06^{a}	0.30 ± 0.06^{a}
	20%	0.33 ± 0.08^{a}	0.23 ± 0.09^{a}	0.10 ± 0.10^{a}	0.00 ± 0.00	0.00 ± 0.00
F-prob		<0.03	<0.03	<0.03	<0.03	<0.03

Figures are presented as mean ± S.D (standard deviation). Means with similar alphabets as superscript in one column for each species are not significantly different at 0.05 level of significance using Duncan multiple range test. WAP- weeks after planting

Table 4: Leaf length of surviving plants of *Axonopus compressus*, *Cynodon dactylon* and *Eleusine indica* in grown in different concentrations of crude oil polluted soils

Species	Crude oil conc in soil	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
Axonopus compressus	0%	5.20 ± 0.85^{b}	6.53 ± 1.39^{b}	7.10 ± 1.22^{b}	8.20 ± 0.95^{b}	8.73 ± 0.79^{b}
	10%	3.30 ± 0.42^{a}	2.80 ± 0.26^{a}	2.37 ± 0.69^{a}	2.17 ± 0.64^{a}	1.50 ± 0.17^{a}
	20%	1.50 ± 0.23^{a}	1.20 ± 0.58^{a}	0.83 ± 0.03^{a}	0.77 ± 0.15^{a}	0.63 ± 0.12^{a}
Cynodon dactylon	0%	1.83 ± 0.20^{a}	3.00 ± 0.28^{b}	3.57 ± 0.18^{c}	4.57 ± 0.33^{b}	5.23 ± 2.96^{b}
	10%	1.57± 0.74^{a}	1.43 ± 0.58^{b}	1.37 ± 0.52^{b}	2.17 ± 0.64^{a}	1.50 ± 0.73^{a}
	20%	0.83 ± 0.18^{a}	0.50 ± 0.12^{a}	0.33 ± 0.09^{a}	0.00 ± 0.00	0.00 ± 0.00
Eleusine indica	0%	6. 23 ± 0.23^{b}	8.07 ± 0.43^{c}	9.73 ± 1.13^{b}	10.53 ± 1.05^{b}	10.53 ± 1.05^{b}
	10%	0.83 ± 0.13^{a}	1.50 ± 0.23^{b}	0.83 ± 0.03^{a}	0.67 ± 0.09^{a}	0.67 ± 0.09^{a}
	20%	0.70 ± 0.15^{a}	0.33 ± 0.12^{a}	0.17 ± 0.17^{a}	0.00 ± 0.00	0.00 ± 0.00
F-prob		<0.02	<0.02	<0.02	<0.02	<0.02

Figures are presented as mean ± S.D (standard deviation). Means with similar alphabets as superscript in one column for each species are not significantly different at 0.05 level of significance using Duncan multiple range test. WAP- weeks after planting

Table 5: Number of leaves per plant recorded for *Axonopus compressus*, *Cynodon dactylon* and *Eleusine indica* in grown in different concentrations of crude oil polluted soils

Species	Crude oil conc in soil	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
Axonopus compressus	0%	14.33 ± 1.74^{a}	15.77 ± 1.21^{b}	17.67 ± 0.50^{b}	22.10 ± 2.31^{b}	24.37 ± 2.58^{b}
	10%	13.03 ± 1.21^{a}	12.67 ± 0.95^{b}	11.23 ± 0.87^{a}	11.17 ± 0.79^{a}	10.07 ± 0.87^{a}
	20%	11.73 ± 2.61^{a}	9.33 ± 2.09^{a}	8.67 ± 3.18^{a}	7.44 ± 3.12^{a}	6.13 ± 1.09^{a}
Cynodon dactylon	0%	10.50 ± 0.57^{a}	11.40 ± 0.89^{a}	13.90 ± 1.96^{a}	15.80 ± 2.87^{a}	18.27 ± 2.43^{b}
	10%	10.60 ± 0.49^{a}	10.27 ± 1.86^{a}	10.00 ± 1.37^{ab}	13.53± 0.38^{a}	12.17 ± 0.55^{a}
	20%	10.50 ± 1.53^{a}	8.8 ± 2.66^{a}	7.00 ± 1.82^{b}	0.00 ± 0.00	0.00 ± 0.00
Eleusine indica	0%	11.37 ± 0.81^{a}	12.77 ± 0.73^{a}	15.60 ± 0.59^{b}	19.43 ± 0.39^{b}	28.83 ± 3.76^{b}
	10%	11.50 ± 1.25^{a}	12.03 ± 1.86^{a}	9.06 ± 2.94^{ab}	9.63 ± 2.09^{a}	11.20 ± 1.95^{a}
	20%	8.20 ± 1.10^{a}	10.67 ± 0.84^{a}	2.17 ± 2.17^{a}	0.00 ± 0.00	0.00 ± 0.00
F-prob		<0.01	<0.01	<0.01	<0.01	<0.01

Figures are presented as mean±S.D (standard deviation). Means with similar alphabets as superscript in one column for each species are not significantly different at 0.05 level of significance using Duncan multiple range test. WAP-weeks after planting

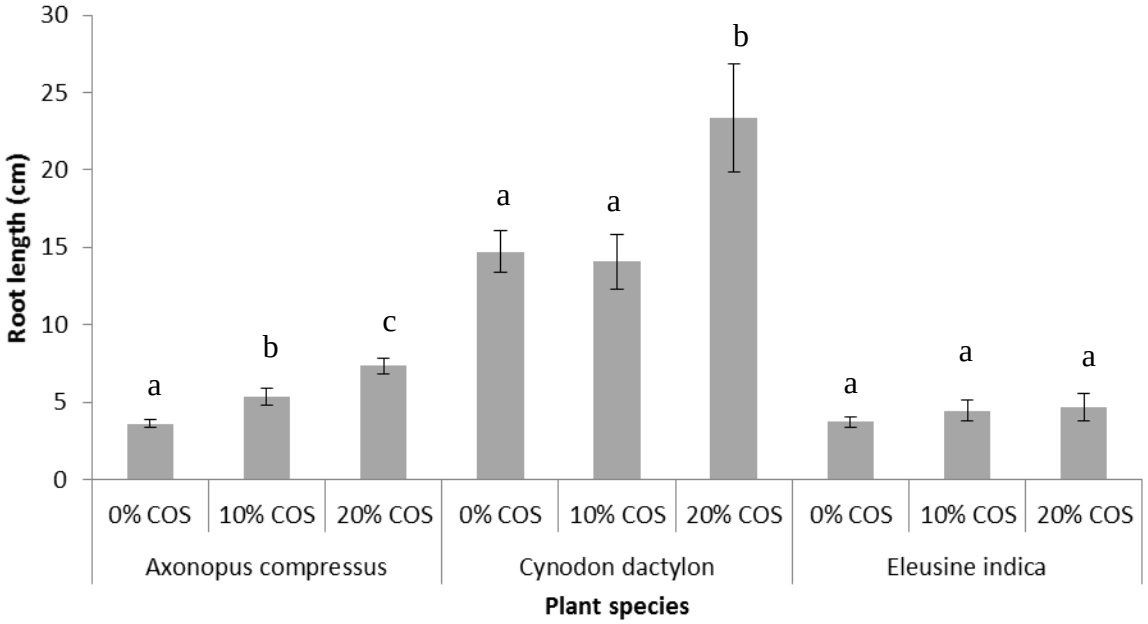

Figure 1: Root length (cm) of *Axonopus compressus*, *Cynodon dactylon* and *Eleucine indica* after 10 weeks of plant growth in crude oil polluted soil (COS).

Table 5 shows the number of leaves of *Axonopus compressus, Cynodon dactylon* and *Eleusine indica* plants grown in crude oil contaminated soil at concentration 0%, 10% and 20%. The formation of leaves was observed with plants grown in uncontaminated soil. The number of leaves produced was increasing as the weeks after planting increased. Plants grown in contaminated soil exhibited reduction in the production of leaves. Table 6 shows plant biomass for *Axonopus compressus, Cynodon dactylon* and *Eleusine indica* after harvest. The biomass decreased as crude oil treatments increased for all the three species. Fresh weight and dry weight values showed similar pattern of decrease as oil concentration in soil increased. Table 7 shows the soil-borne bacteria associated with the different test plants / crude oil polluted soils after ten weeks of plant growth. Bacteria species isolated and identified include: *Pseudomonas spp, Klebsiella spp, E. coli, Staphylococcus sp, Enterobacter spp, Salmonella spp*, and *Serratia sp*p. It was observed that two species of bacteria- *Klebsiella* and *Serratia* were not recorded for soil samples where *Cynodon dactylon* and *Eleusine indica* were grown. Figure 4 shows the chromatograms of different aliphatic and aromatic hydrocarbons detected in 0% crude oil in soil before planting. Figure 4A indicates the aliphatic hydrocarbons, C_8 – C_{21} present. These are saturated hydrocarbons. Figure 4B shows aromatic hydrocarbons, usually cyclic and unsaturated.

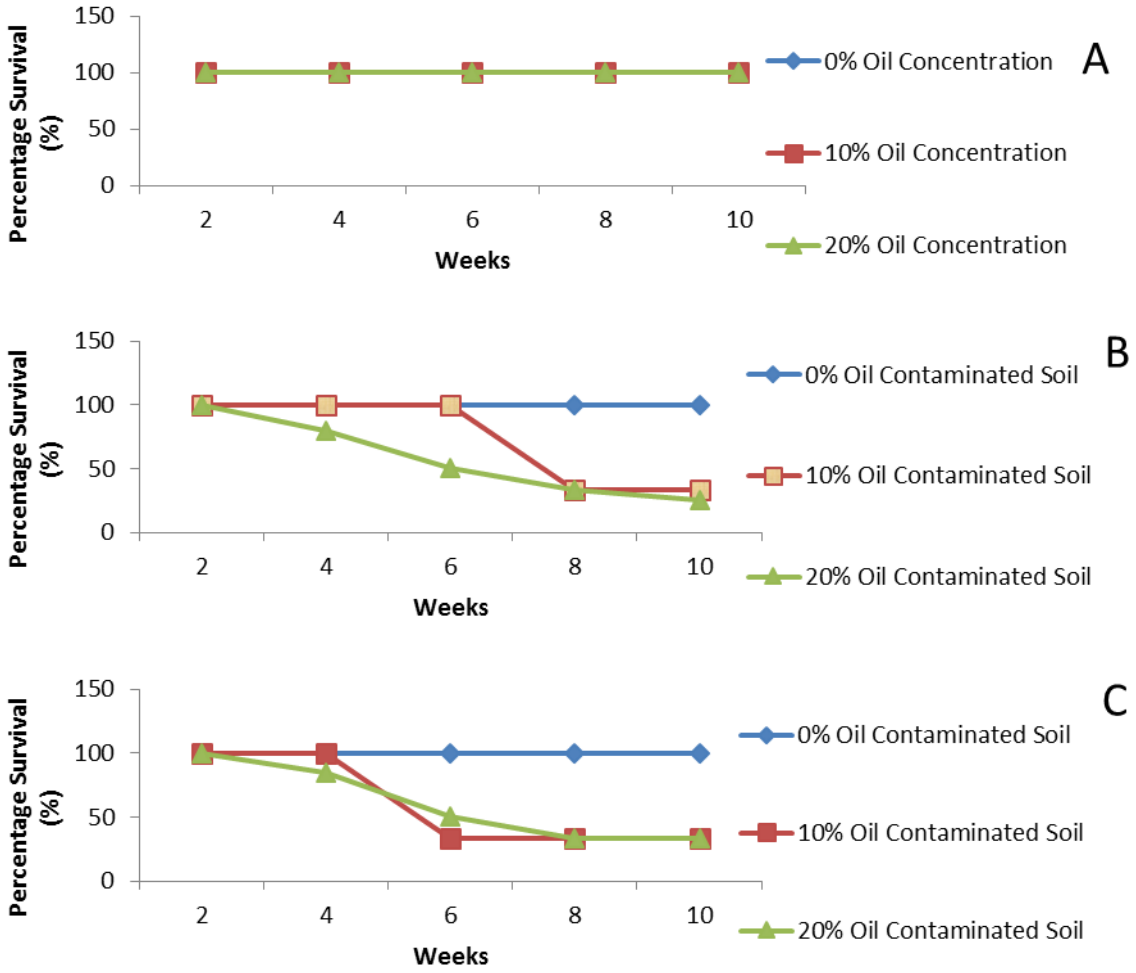

Figure 2: Percentage survival of *Axonopus compressus* (A), *Cynodon dactylon* (B) and *Eleusine indica* (C) in polluted soil after 10 weeks of plant growth

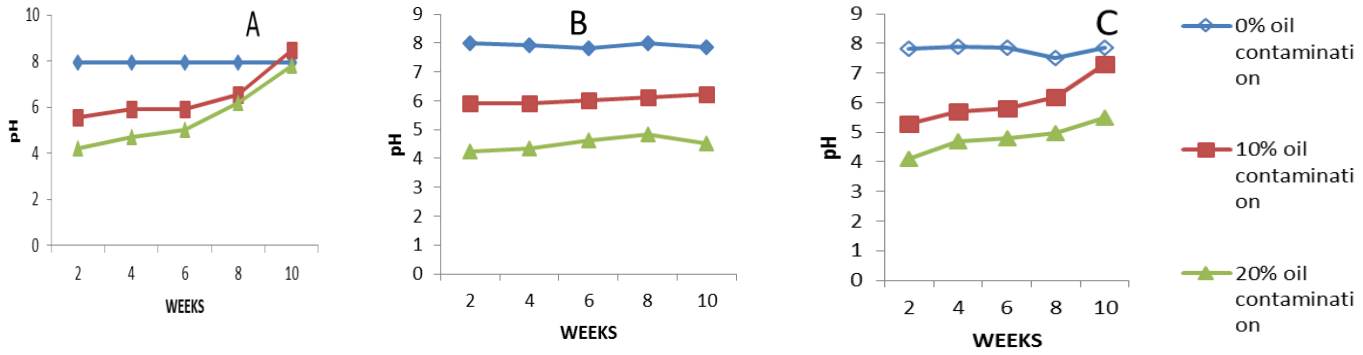

Figure 3: pH values recorded for crude oil contaminated soil where *Axonopus compressus* (A), *Cynodon dactylon* (B) and *Eleusine indica* (C) plants were grown after 10 weeks of plant growth

Table 8 shows the total petroleum hydrocarbon (TPH) content of the different experimental soil samples before and after plant harvest. Degradation of hydrocarbons was recorded in all soil media where the plants were grown. The TPH values for 0%, 10%, and 20% crude oil treated soils after plant harvest were less than 50% of the values recorded before plant growth.

Figure 5 shows the chromatograms obtained from GC-FID analysis for aliphatic hydrocarbons present in 20% crude oil polluted experimental soils. The peaks recorded in the chromatogram for aliphatic hydrocarbons detected in 20% oil polluted soil before planting (5A) were conspicuous. The heights of the peaks were lower for soil with *A. compressus* growth (5B). Aliphatic hydrocarbons labelled range from C_8 – C_{33}. Peaks for the aliphatic hydrocarbons content detected when *C. dactylon* was grown in 20% oil polluted soil (5C) showed degradation as well. Labelled aliphatic compounds were from C_8 to C_{27}. Similarly, the growth of *E. indica* in 20% oil polluted soil (5D) showed reduction in heights of peaks of labelled aliphatic hydrocarbons. Labelled aliphatic hydrocarbons were C_8 to C_{32}.

Table 5: Number of leaves per plant recorded for *Axonopus compressus*, *Cynodon dactylon* and *Eleusine indica* in grown in different concentrations of crude oil polluted soils

Species	Crude oil conc in soil	2 WAP	4 WAP	6 WAP	8 WAP	10 WAP
Axonopus compressus	0%	14.33 ± 1.74^{a}	15.77 ± 1.21^{b}	17.67 ± 0.50^{b}	22.10 ± 2.31^{b}	24.37 ± 2.58^{b}
	10%	13.03 ± 1.21^{a}	12.67 ± 0.95^{b}	11.23 ± 0.87^{a}	11.17 ± 0.79^{a}	10.07 ± 0.87^{a}
	20%	11.73 ± 2.61^{a}	9.33 ± 2.09^{a}	8.67 ± 3.18^{a}	7.44 ± 3.12^{a}	6.13 ± 1.09^{a}
Cynodon dactylon	0%	10.50 ± 0.57^{a}	11.40 ± 0.89^{a}	13.90 ± 1.96^{a}	15.80 ± 2.87^{a}	18.27 ± 2.43^{b}
	10%	10.60 ± 0.49^{a}	10.27 ± 1.86^{a}	10.00 ± 1.37^{ab}	13.53± 0.38^{a}	12.17 ± 0.55^{a}
	20%	10.50 ± 1.53^{a}	8.8 ± 2.66^{a}	7.00 ± 1.82^{b}	0.00 ± 0.00	0.00 ± 0.00
Eleusine indica	0%	11.37 ± 0.81^{a}	12.77 ± 0.73^{a}	15.60 ± 0.59^{b}	19.43 ± 0.39^{b}	28.83 ± 3.76^{b}
	10%	11.50 ± 1.25^{a}	12.03 ± 1.86^{a}	9.06 ± 2.94^{ab}	9.63 ± 2.09^{a}	11.20 ± 1.95^{a}
	20%	8.20 ± 1.10^{a}	10.67 ± 0.84^{a}	2.17 ± 2.17^{a}	0.00 ± 0.00	0.00 ± 0.00
F-prob		<0.01	<0.01	<0.01	<0.01	<0.01

Figures are presented as mean±S.D (standard deviation). Means with similar alphabets as superscript in one column for each species are not significantly different at 0.05 level of significance using Duncan multiple range test. WAP-weeks after planting

Table 6: Biomass of *Axonopus compressus*, *Cynodon dactylon* and *Eleucine indica* grown on soil with different crude oil concentration

Species	Treatment	Fresh weight (g)	Dry weight (g)
Axonopus compressus	0%	4.59 ± 0.51^{b}	2.41 ± 0.65^{a}
	10%	3.69 ± 0.50^{b}	2.23 ± 0.39^{a}
	20%	2.14 ± 0.56^{a}	1.75 ± 0.48^{a}
Cynodon dactylon	0%	3.28 ± 0.24^{a}	1.47 ± 0.20^{a}
	10%	1.71 ± 0.04^{b}	2.23 ± 0.39^{a}
	20%	1.56 ± 0.32^{b}	1.00 ± 0.25^{a}
Eleusine indica	0%	7.74 ± 2.20^{b}	4.60 ± 0.84^{a}
	10%	3.41 ± 0.05^{ab}	2.10 ± 0.47^{b}
	20%	2.47 ± 0.20^{a}	1.13 ± 0.18^{b}
F-prob		<0.01	<0.01

Figures=mean ± S.D. Mean with similar alphabets in one column for a particular species are not different significantly using Duncan multiple range test

Table 7: Soil-borne bacteria species associated with the different test plant/contaminated soil after 10 weeks of plant growth

Plant species grown	***Axonopus Compressus***			***Cynodon Dactylon***			***Eleusine indica***		
Oil concentration in soil **Bacteria species**	0%	10%	20%	0%	10%	20%	0%	10%	20%
Pseudomonas sp.	+	+	+	+	+	+	+	+	+
Klebsiella sp.	+	+	+	-	-	-	-	-	+
E. coli sp.	+	+	+	+	+	+	+	+	+
Staphylococcus sp.	+	+	+	+	+	+	+	+	+
Enterobacter sp.	+	+	+	+	+	+	+	+	+
Salmonella sp.	+	+	+	+	+	+	+	+	+
Serratia sp.	+	+	+	-	-	-	-	-	-

+ = Present, - = absent

Table 8: Total petroleum hydrocarbon (TPH) content of the different experimental soil samples after 120 days after planting

Species	Crude oil concentration in soil	TPH (before planting) (mg/kg)	TPH (after harvest) (mg/kg)
Axonopus compressus	0%	7.966	5.277
	10%	6.704	2.965
	20%	6.176	2.032
Cynodon dactylon	0%	7.966	5.155
	10%	6.704	2.503
	20%	6.176	1.819
Eleusine indica	0%	7.966	5.253
	10%	6.704	2.282
	20%	6.176	1.522

TPH values stated above were obtained as average of duplicate analysis

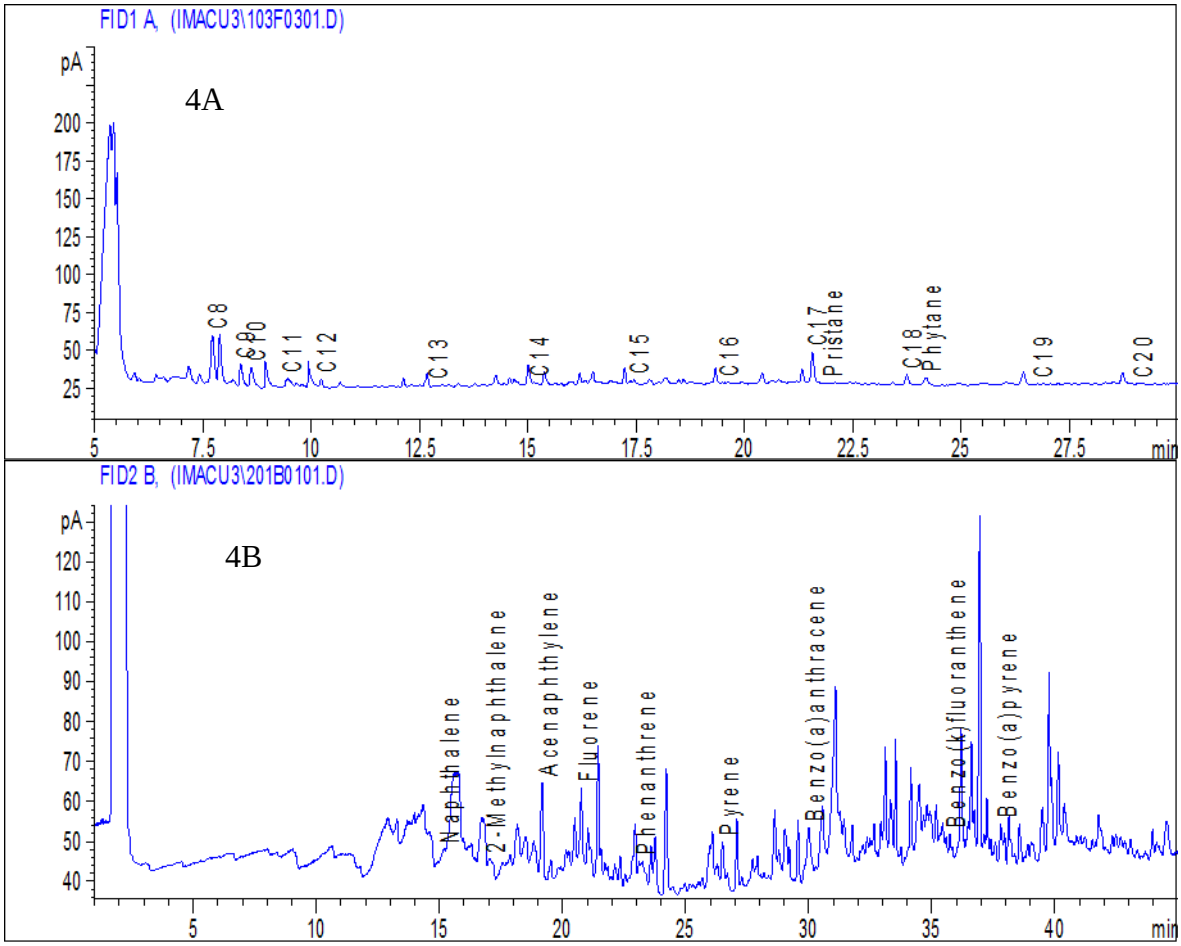

Figure 4: Chromatograms obtained from GC-FID analysis for aliphatic (A) and aromatic (B) hydrocarbon contents of 0% crude oil soil samples before planting

Figure 6 shows the chromatograms obtained from GC-FID analysis for aromatic hydrocarbons present in 20% crude oil polluted experimental soils. The peaks recorded in the chromatogram for aromatic hydrocarbons detected in 20% oil polluted soil before planting (6A) were conspicuous. Some identified polyaromatic hydrocarbons (PAH) were naphthalene, 2-methyl naphthalene, anthracene, pyrene, and benzo(a)fluoranthene. The heights of the peaks were lower for soil with *A. compressus* growth (6B). The peaks of aromatic hydrocarbons detected in 20% oil polluted soil after the growth of *C. dactylon* were low (6C). Four aromatic hydrocarbons were identified and these include naphthalene, 2-methyl naphthalene, acenaphthalene and fluorene. Other unidentified peaks are shown too. The growth of *E. indica* in 20% oil polluted soil (6D) showed reduction in heights of peaks obtained for the aromatic hydrocarbons detected. The lowest peaks were obtained in this soil sample. Five aromatic hydrocarbons were identified and these include fluorene, phenanthrene, fluoranthrene, benzo (b)fluoranthrene and benzo (a) pyrene.

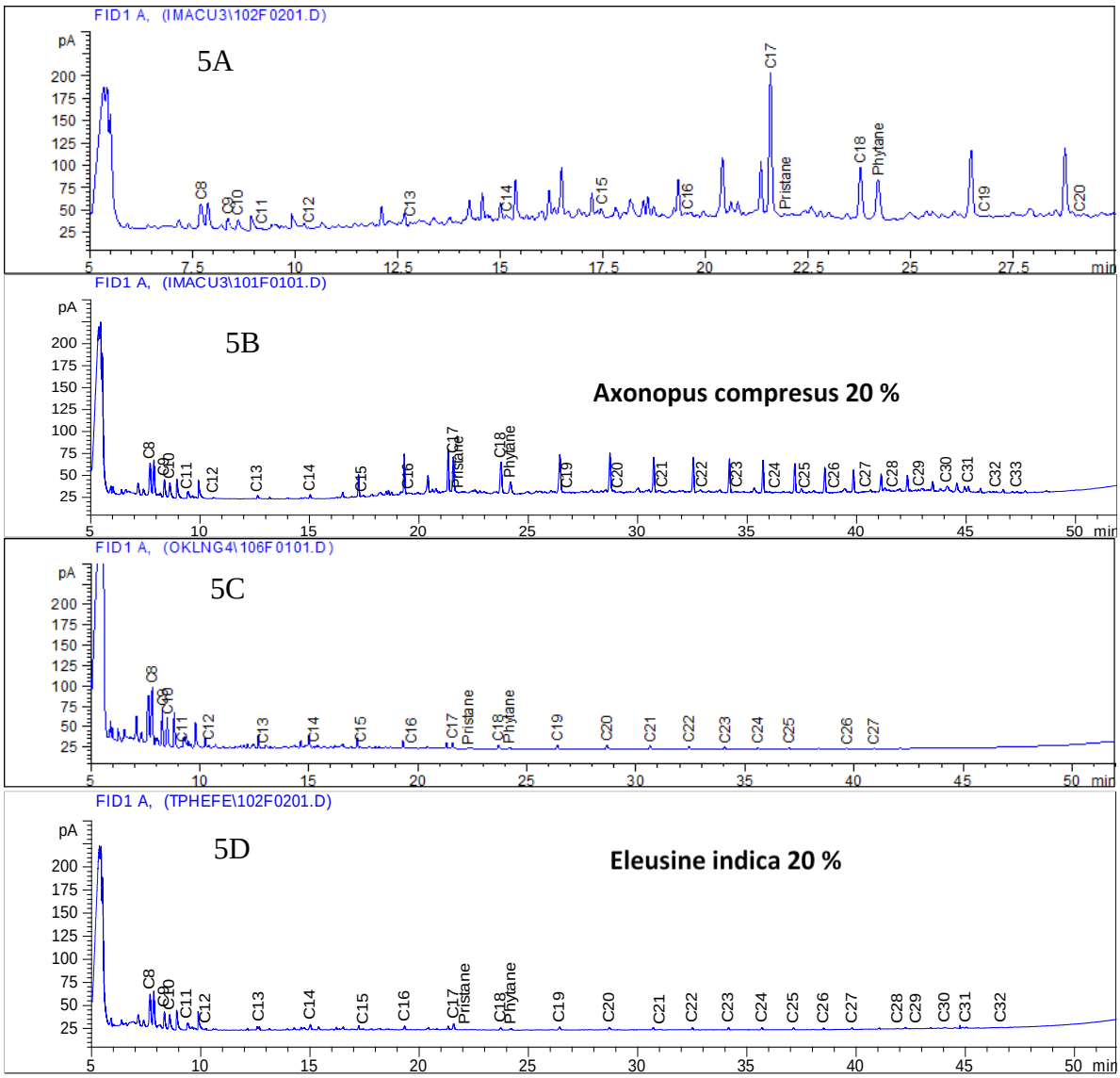

Figure 5: Chromatograms obtained from GC-FID analysis for aliphatic hydrocarbon contents of 20% crude oil soil samples before planting (5A), 20% oil polluted soils after *A. compressus* (5B), *C. dactylon* (5C) and *E. indica* (5D) growth for 120 days

DISCUSSION

Ecological devastation resulting from crude oil pollution of the environment has necessitated solutions through remediation and rehabilitation of polluted environment particularly the soil medium. Crude oil pollution interferes with plant uptake of nutrients and creates an unfavourable soil conditions (Callaham *et al*., 2002; Anoliefo *et al*., 2003; Vwioko and Osazuwa, 2012). The results obtained in the present study show that the growth of *Axonopus compressus* , *Cynodon dactylon* and *Eleusine indica* were negatively affected by the crude oil contamination. Plant heights were higher in unpolluted soil (0% concentration) than in polluted soil (10% and 20% concentration). This is in corroboration with Agbogidi (2009) who reported reduced plants' shoot growth as a result of direct reduction in water and available soil mineral. However, values of root lengths of harvested plants showed a different trend with plants in contaminated soils exhibiting higher values. *Cynodon dactylon* had the highest root length value of all the three species in this study. Many authors have suggested that nutrient availability and deficiencies were some of the challenges of plant grown in oil polluted soil (Baker 1970; Chaineau *et al*., 1997; Adam and Duncan, 2002) and thus plants invest more of their resources in root length development. This can be viewed as a strategy by the plants to extend the roots to reach unpolluted parts of the soil medium for available water and nutrients.

The fresh and dry weights of the test plants (*Axonopus compressus*, *Cynodon dactylon* and *Eleucine indica*) responded to crude oil treatment by a reduction in weight with increase in the concentration of crude oil. The observed reduction in the biomass accumulation in the plants grown in soil with higher oil levels could have resulted from nutrient immobilization as the oil created hydrophobic condition in soil and occluded soil particles, which made some vital nutrients unavailable to plants. Similar case of nutrient immobilization in soils treated with petroleum hydrocarbons have been reported by Benka-Coker and Ekundayo (1995) and Benka-Coker and Ekundayo (1997). The total petroleum hydrocarbon (TPH) recorded revealed the degradation ability of the test plants (*Axonopus compressus*, *Cynodon dactylon*, and *Eleucine indica*).

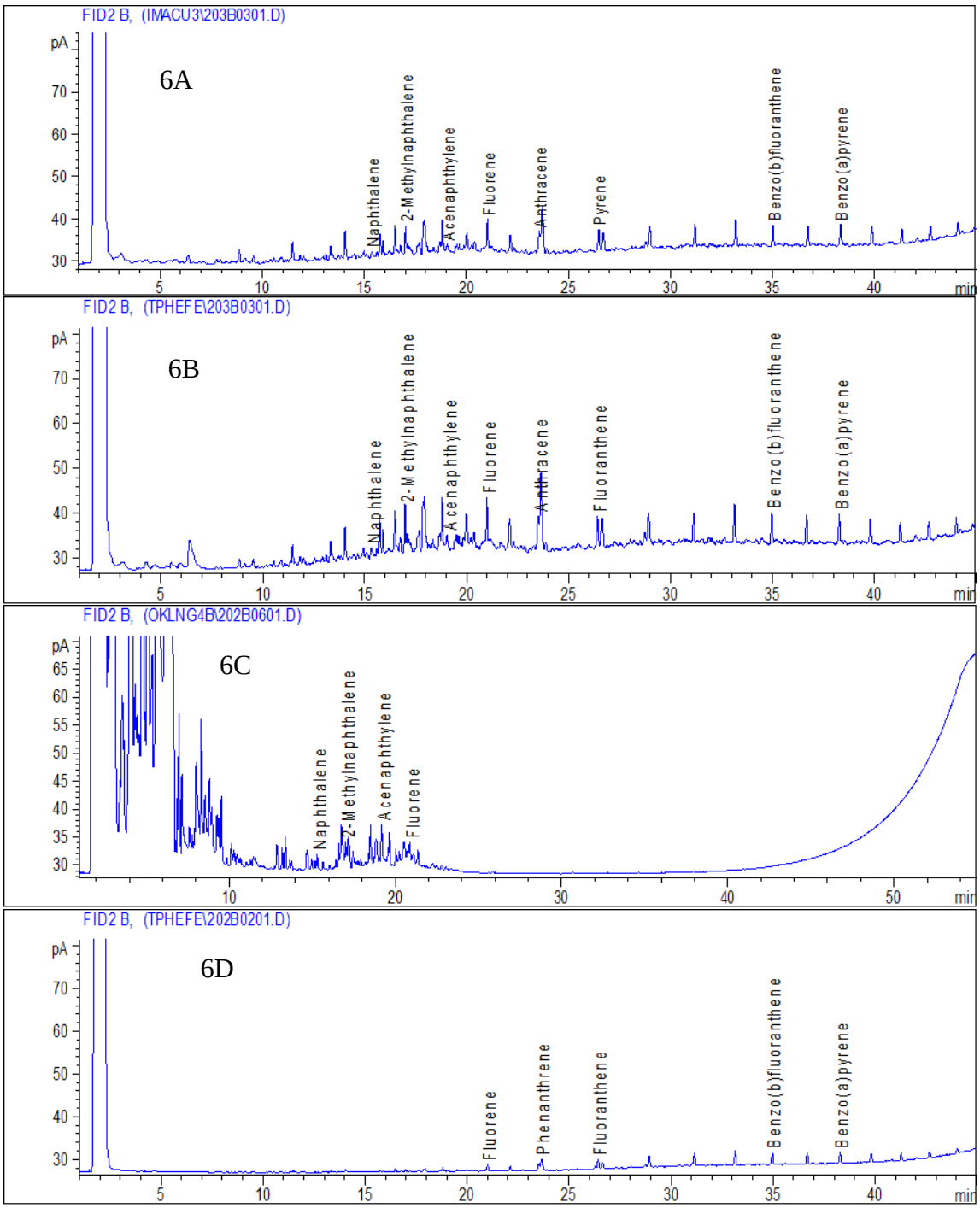

Figure 6: Chromatograms obtained from GC-FID analysis for aromatic hydrocarbon contents of 0% crude oil soil samples before planting (6A), 20% oil polluted soils after *A. compressus* (6B), *C. dactylon* (6C) and *E. indica* (6D) growth for 120 days.

The chromatograms obtained from the GC-FID analysis for the presence of aliphatic and aromatic hydrocarbons of soil samples after plant harvest showed significant degradation of crude oil in the experimental soils. In the current study these plants seem to have facilitated the metabolism of both aliphatic and aromatic hydrocarbon compounds present in crude oil (Table 5, Figures 5 and 6). The peaks of detected aliphatic and aromatic hydrocarbons were low for 20% oil polluted experimental soils where *Cynodon dactylon* and *Eleucine indica* were grown. More conspicuous peaks were observed in the chromatogram of 20% oil polluted experimental soil where *Axonopus compressus* plants were grown. The heights of peaks indicate the concentrations of the hydrocarbons detected. C_{28} – C_{33} aliphatic hydrocarbon peaks were absent in the chromatogram obtained for experimental soil where *Cynodon dactylon* was grown. Soil bacteria present in crude oil polluted soils use the contaminant as carbon source for energy metabolism. Shailubbai *et al* (1984) used *Rhodotorula* sp. to treat oil sludge and found that the susceptibility to degradation was in the following order: saturate fractions > aromatic fractions > asphaltic fractions. The reduced growth of plants in polluted soil indicated sensitive response of plants to chemical substances in the soil. Plants that are able to grow in contaminated sites take up long chains (heavy) alkenes into their roots rapidly and slowly translocate them into stem and leaves as a result of their low solubility in water

(Palmroth *et al*., 2002). Many authors have suggested that aliphatic, aromatic, naphthenic and phenolic compounds in crude oil reduce respiration, transpiration, photosynthesis and hormonal stress response in plants (Vouillamoz and Mike, 2001; Trapp *et al*, 2005). These effects however vary with individual plant species and their physiological responses to contaminants. *Axonopus compressus* survived the different concentrations of crude oil. One significant observation was the ability of *Axonopus compressus* to thrive with different degree in all forms of polluted soils used in the present study. *Axonopus compressus* had a constant survival rate of 100% at different concentration of crude oil contamination. *Cynodon dactylon* and *Eleucine indica* lost some of their plants at a higher concentration with both having a significant drop in their survival rate. The fact that *Axonopus compressus* exhibited some form of tolerance to crude oil polluted soils suggests that the plant is a potential phytoremediant.

Mechanism of Survival

Test plants in the present study were able to lower the acidity/increase the pH values with *Axonopus compressus* resulting in a significant increase in soil pH values. These findings corroborated with the work of Ighovie and Ikechukwu (2014) who reported that the growth of *Axonopus compressus* in crude oil impacted soils in Ubeji (Delta State) and Alesa Eleme (Rivers State) reduced the acidity of hydrocarbon content in soils (4.46 - 6.87 pH in Ubeji and 4.66 – 6.86 pH in Alesa Eleme) in 90 days. An interesting suggestion that should be noted also comes from the chromatograms of GC-FID analysis. The chromatograms showed that the least peaks for aliphatic and aromatic hydrocarbons detected and recorded were in the experimental soils where *Cynodon dactylon* and *Eleucine indica* were grown. This may logically show higher degradation activities and TPH reduction during growth of these plants. Irrespective of this fact, these two plant species failed to survive. It points to the fact that degradation of aliphatic and aromatic hydrocarbons alone is not an efficient mechanism as compared to combining degradation with reducing soil acidity as employed by *A. compressus*. *Axonopus compressus* was able to sustain growth at 20% crude oil conditions by modifying pH of the soil from acidic to neutral conditions. The continue increase in soil pH up to the 10th week allowed for establishment of conducive environment for growth of soil bacteria which enhances biodegradation of oil and improves the soil nutrients conditions for survival. Ighovie and Ikechukwu (2014) further added that the growth of *Axonopus compressus* enhanced the accumulation of organic matter and moisture content in oil-impacted soils of Ubeji and Alesa Eleme.

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Conflict of interest The authors declare that there is no conflict of interest with regard to this publication.

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