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Phytoremediation potential of sorghum (*Sorghum bicolor* (L) Moench) and African yam bean (*Sphenostylis sternocarpa*) Hoechest ex A. Rich) harms on crude oil polluted soil

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ABSTRACT: A study on Phytoremediation potential of Sorghum (*Sorghum bicolor*) and African yam bean (*Sphenostylis sternocarpa*) on crude oil polluted soil was conducted in the botanic garden of university of Port Harcourt. 30kg of soil was weighed and put into planting bags. 100ml. 200ml, 400ml and 800ml of crude oil were measured using measuring cylinder and mixed with the soil and the untreated soil sample (control). These served as treatments and were replicated 5 times. After two weeks of pollution, viable seeds of *Sorghum bicolor* and *Sphenostylis sternocarpa* were planted and morphological and physico-chemical parameters were taken and the data collected were subjected to Analysis of Variance. The result shows that the biomass of the untreated soil sample (control) was found to be the highest, while the 800ml treatment recorded the lowest, The morphological parameters are Plant Height, Number of Leaves and Leaf Area. At week 12 for sorghum, treatments(1,2,3 and 4) concentration of crude oil had mean Plant height of 65.28cm,65.56cm,63.5cm and 59.9cm respectively while control had 66.77cm.For Number of leaves, treatments(1,2,3,and 4) had 74cm,75cm,76cm,and 69cm respectively while control had 78cm.For Leaf Area treatments.(1,2,3,and 4) had 320cm^2, 311cm^2, 281cm^2 and 269cm^2 respectively while control had 341cm$^{2.}$.That for African yam bean, treatments(1,2,3,and 4) had mean Plant height of 66.78cm,67.62cm,64.04cm,and 28.72cm respectively while control had 72.82cm.For Number of leaves treatments {1,2,3,and 4} had 123cm,123cm,121cm,and92cm respectively while control had 127cm.For Leaf Area treatments(1,2,3,and 4) had 103cm^2, ,99cm^2, ,91cm^2 ,and 77cm^2 while control had 116cm^2. The heavy metals analyzed in the soil (control) before planting are Zinc = 0.342g/ml, lead = 0.188g/ml and Nickel = 3.49g/ml while the soil polluted with crude oil: Zinc = 1.719g/ml lead = 0.256g/ml and Nickel = 4.245g/ml. The heavy metal content of sorghum analysed for after 12 weeks are Zinc = 0.13g/ml, lead = 0.14g/ml and Nickel = 0.175g/ml while that of African yam bean analyzed for are Zinc = 0.14g/ml, Lead = 0.16g/ml and Nickel = 0.179g/ml. Lastly heavy metals found in the soil at the end of 12 weeks was Zinc = 1.449g/ml, lead = 0.44g/ml and Nickel = 3.891g/ml. Result from the experiment shows that sorghum is a better phytoremediating plant than African Yam bean.

Key words: Phytoremediation, African Yam bean, Sorghum and Crude oil

Introduction

Sorghum bicolor (L) Moench is a monocotyledonous plant belonging to the family poaceae (Sub-family panicoideae,) class liliopsida, sub-class commelinidae (order cyperales), and genus *Sorghum bicolor* Andropogoneae. The species are native to tropical and sub-tropical regions of all continents in addition to the South-West pacific.

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It is a cane like grass, that grows up to the height of 6m tall with large branched clusters of grains. Sorghum are non-rhizomatous, culms nodes are either glabrous or shortly tomentose. It also include all cultivated Sorghums as well as a group of semi wild plants often regarded as weeds Sorghum contains hydrocyanic acid and the alkaloid hordenine. Sometimes plants accumulate toxic levels of nitrate (Moton, 1981). *Sorghum bicolor* is an African Crop, which is widely distributed throughout the world. Different cultivars are found in different regions depending on the climate. It is adapted to a wider range of ecological conditions. It is mostly a plant of hot dry regions still survive in a cool weather as well as water logged habitats. It is self and cross pollinated by wind. Cultivation is by seed normally sown in rows 75-100cm part at rate of 3-9.kg/ha; higher seed rates used more humid areas. The major diseases reported on Sorghum include the following; cercospora sorghi, colletotrichum graminicola (Anthracnose of leaves and stems), helminthosporium turcilum (leaf blight).

Puccinis purpurea (rust), *Ramalispora sorghi* (sooty strip) sclerosphora sorghi (downy mildew); plants are also severely attacked by various species of striga (*S. lutea, S. hermontheca*) nematodes isolated from sorghum include the following species: *Hoplololaimus pararobustos* and *Meloidogyne javanica*. *Sorghum bicolor* is an important crop providing food and fodder in the semi-arid tropics of the world. It is a staple food for more than 500 million people in more than 30 countries, although maize has to some extent replaced it's used in Southern Africa. *Sorghum* is used for food (as grain and in sorghum syrup or "Sorghum Molasses") as fodder in the production of alcoholic beverages as well as biofuels.

The whole plant is used for building; fencing, weaving, broom making and firewood. Industrially it can be used for vegetable oil, waxes and dyes. Also the seed is used as food, in brewing "Kiffir beer" the kiffir corn malt and corn meal is fermented to make leting (a sour mash), the pith is eaten and the sweet culm chewed/watt and Breyer-Brandwijk, (1962). The seeds are used as coffee substitutes or adulterant (Morton, 1981). In folk medicine, Sorghum is reported to anti-abortive, cyanogenetic demulcent, diuretic, emollient, intoxicant and poison. Sorghum is a folk remedy for cancer, epilepsy, flux and stomach ache (Duke and Wain, 1991). The root is used for malaria in Southern Rhodesia; the seed has been used for breast disease and diarrhea, the stem for tubercular swellings. In India, the plant is considered anthelminthic and insecticidal and in South Africa, in combination with *Erigeron canadense* L. it is used for eczema (watt and Breyer-Brand Wijk, 1962). *Sphenostylis sternocarpa* (Hoechest ex A. Rich/Harms is well known as the African yam bean. It belongs to the family fabaceae (leguminosae). This is this third largest family of the flowering plants (after compositae and orchidaceae) with 650 genera and 18,000 species (Gill 1988). It belongs to the sub-family papillonoideae, tribe phaseolene, sub-tribe phaseolinae, and genus sphenostylis. The crop has twining vigorous vines which could be green or pigmented red. The vines twine clockwise around the stakes or climb other supports to a height of about 3 meters or more. The leaves are compound trifoliate. Pods are usually linear housing about 20 seeds. These vary in size, shape, colour pattern etc.

African yam bean *sphenostylis sternocarpa*) is the most economically important among the seven species of *sphenostylis* and it is one of the most important tuberous legumes. The domestication, cultivation, and distribution of the crop are very evident in the tropics of Africa.

The genus consists of several species but only three are found in West Africa (Hutchinson and Dalziel, 1958) these include:

(a). *Sphenostylis holoserica* (welw ex. Baker) Harms. A twining herb with tuberous root stock reported from Senegal, Ghana and Tanzania.

(b). *Sphenostylis schweinfurthii* Harms. An erect under shrub found in the open savannah in Ghana, Central African and Nigeria.

(c). *Sphenostylis sternocarpa* (Hoechst ex. A Rich) Harms. Because tropical Botany has been relatively neglected, there are thousands of premising species that await research and study.

Ecology

The origin of African yam bean as indicated by GRIN includes the following countries within the tropical regions of Africa: Nigeria is very significant for this crop production (potter 1992) where extensive cultivation had been reported in the Eastern, Western and Southern Areas of Nigeria. It is mostly inter-planted with yam and cassava and was cultivated mainly by children and women.

There are different varieties of the African yam bean, these are the Grey, Brown, Dark brown, Brown speckled, Black, Red and milky varieties.

The economic potentials of African yam bean are immense. Apart from, the production of two major food substances, the value of the protein in both tubers and seed is comparatively higher than what could be obtained from most tuberous and leguminous crops. African yam bean is more than twice the protein in sweet potato (Ipomea batatas) or Irish Potato (Solanum tuberosom) and higher than those in yam and cassava.

Insecticidal and Medical Usefulness

African yam bean (*Sphenostylis Stenocarpa)* as a crop is less susceptible to pests and diseases compared with most legumes; an unidentified yellow mosaic virus that attacks the plant has been observed at the international constitute of tropical Agriculture Nigeria. Maduewesi (as cited by Okigbo, 1973) stated that the pests and diseases which are found in African yam beans grown under mixed cropped with yams and cassava are powdery mildew, leaf spot, stem rust and viruses. No major insects pest have been identified but several grasshopper and crickets are known to damage young seedlings and some lepidopterous larvae, leaf rollers and thrips may pose sporadic problems on the crop; also the crops are less susceptible to nematode attack (Ezuch, 1984).

Importance of African Yam Bean

The dry beans are usually eaten, or with yams, maize or in soups. The seeds are soaked for several hours before being cooked to make a meal, which is said to cause giddiness if taken in excess.

African yam beans take 4-6 hours to cook but are claimed to be very filling, giving a lot of staying power and causing those who have eaten it to drink a lot of water. Apart from serving as food for man, livestock feed has been derived from the seeds and used to feed our poultry animals especially in countries where they are processed to improve the nutritive value. The African yam bean is grown primarily for its dry seeds, which are a nutritious pulse.

With the acknowledgement of the nutritional potentials of African yam bean, the crop many well contribute to solving food security problems in Africa if its diversity is saved for future genetic improvement. Crude oil can be said to be a mineral oil or hydrocarbon composed primarily of hydrogen and carbon only. This is justified by the qualitative analysis in which hydrocarbons gave carbon (iv) oxide and water on combustion. Hydrocarbons are broadly classified as saturated (single covalent bonds) or unsaturated (double or triple covalent bond). Crude oil is obtained over about 2000-3000m beneath the earth's surface. It is now common knowledge that crude oil pollution on soils makes it unsatisfactory for plant growth, metabolic functions such as transpiration, translocation. This result from insufficient aeration of the soil because of displacement of air from space between the soil particles by crude oil (Rowell, 1977). The effects of crude oil deposits on soles mostly interferes with the chlorophyll content, lending to the impairment of plant's photosynthetic capacity. Other possible morphological changes brought about by oil in soils where plants were cultivated include: (1) Stunted growth (2) leaf burning (3) leaf chlorosis (4) leaf fold or curl (5) seizure in leaf growth (6) leaf abscission etc. Certain species of plants are usually more susceptible to oil pollution than others. Plants with rhizomes or underground storage organ tend to be more resistant than annuals and other swallow noted plants.

Baker (1983) suggested that oil pollution can kill plants and inhibit growth of plant by various mechanisms, which include:

(a) By altering soil properties.
(b) By acting as a physical barrier and preventing gaseous exchange.
(c) By being toxic, directly on the plants.
(d) Direct toxic effect leading to the disruption of metabolic functions. More still, oil spills cases over the years have also resulted in entire habitat destruction. For example, oil may be retained in sediments for several years exerting long terms effect on species composition and abundance.

The effects of oil pollution

Oil pollution incidents leave the environment with some negative impacts which usually comprise the following:

1. **Agriculture**: Oil spill deposits could render agricultural lands unproductive. Oil deposits on plants causes an interference with normal physiological functions mainly by reducing transportation and photosynthesis. It also causes discoloration, wrinkling, wilting and shedding of their foliage parts.
2. **Health:** Communities without alternative source of water supply are often exposed to health hazards when oil spills occurs-which usually contaminates well, springs, stream, rivers-seas and other open water bodies. On

humans the effect of taking in crude oil contaminated foods, water and marine organisms ranges from epidemic diseases to reduced productivity, social and mental imbalance or even death.

It is important to act fast and clean-up an oil spill and prevent the oil spill from spreading to a larger area. Oil spillage can happen in an open water body, close to store or on lands, where it can also contaminate ground water.

Objective of the Study

This study was designed to determine; the extent of impact caused by crude oil pollution on the growth and development of *Sorghum bicolor* and *Sphenostylis sternocarpa*. This is as regards to the disruption of normal functioning of the plants activities that hinders growth potentials. The physical growth parameters to be considered include: leaf area, leaf height, number of leaves and yield of plant.

Materials and Methods

The various materials used during the experiment includes; 50 Sac-planting-bags, crude oil, electronic weighing balance, top loam soil, Petri dishes, seeds of *Sorghum bicolor*, and *sphenostylis stenocarpa*, blotter paper, distillated water, beakers photo colorimeter, magnetic stirrer, cylinder, conical flask, murfle furnance, P^H meter (Jenway 3015 pH meter) foil paper, methyl orange indicator (H_2S0_4), Nutrient Agar for total Heterotrophic bacteria count, LAB M Product. Magnetic stirring, rod, spatula, conductivity meter (Jenway 4010 conductivity meter) shovel.

Study Area/Experiment Site

The study site of the project was carried out in the botanic garden of the University of Port Harcourt, Rivers State Nigeria. The research project was conducted in an open space with little shade.

Experiment Design

The type of experimental design used is the completely Randomized Design (CRD). In this type of design, the bags (treatments) are arranged completely at random to the experimental unit. The Randomization gives all treatments an equal opportunity, this reduces experimental error and avoid bias and presumption.

Filling and arrangement of bags

The top loam soil from the University of Port Harcourt Botanic Garden was collected behind the screen house and filled with shovel into each of these sac planting bags after removing the debris from the soil. The 50 sac bags were filled to the tune of 30kg and were arranged 20cm apart in rows and column.25 bags were used for each plant making them a total of 50 bags, and the bags are arranged in 5 different coloured batches of 5 replicate each designated as Green (100ml), Red (200ml), Orange (400l), Black (800ml) and Blue (control).

Viability test and planting of seeds

The sorghum and African yam bean seeds were bought from Choba Market located at east-west road Obio/Akpor, Rivers State. A germination test was carried out on the seeds. Ten grains of each plants were put into five petri-dishes containing a blotter paper soaked with water, the place was covered and allowed to stay for 48 hours. 86% germination was recorded for sorghum while 73% was for African yam bean.

Plate 1: Sorghum

Plate 2: African yam bean

Pollution with crude oil

For the purpose of this research project work, crude oil was obtained from Agbada in Igwuruta flow station of Rivers State. Different treatments of the crude oil were weighed in the laboratory as follows:

T1-	(100ml)
T2	(200ml)
T3	(400ml)
T4	(800ml)
T5	(control)

There were five treatments which were replicated five times giving a total of 25 for sorghum and 25 for African yam bean.

Soil Analysis

Soil sample were collected during each sampling for physico-chemical analysis. Soil sampling stated two weeks after soil contaminations i.e. after soil pollution have been carried out. The following physico-chemical properties were taken after soil pollution with crude oil. Parameters calculated for are soil P^H, conductivity, alkalinity, total bacteria count, total fungi count, total heterotrophic bacterial count and total heterotrophic fungi count and heavy metals such as (zinc, lead, nickel).

Parameters Assessed

In the course of this experiment, two major groups of parameters were assessed, morphological parameters such as the (plant height, plant area, leaf area, yield of plants), and the physico-chemical parameters which include, P^H, alkalinity, conductivity, Heterotrophic fungi count, hydrocarbon utilizing bacteria, total heterotrophic bacteria, and hydrocarbon utilizing fungi, including heavy metals. The latter was carried out in the plant physiology laboratory of the department of Plant Science and Biotechnology of the Faculty of Science in University of Port Harcourt.

Heavy Metals

The test for metals (zinc, lead and nickel) were carried out on two samples (pure soil and soil +crude oil samples). The extraction method was used in this test. The steps for this method are as follows:

- 1g of each sample was weighed into a digestion flask (pyrux conical flask). 5ml of concentrated NO_3 (nitric acid and 3ml of 62% purchloric acid were added.
- The flasks containing the different samples were heated to digest the content within 15 minutes.
- The cleared looking digest was diluted with 50ml of distillated water and filtered into a plastic vial through a whatman filter paper No. 541.
- The filtrate was used for the analysis of zinc (zn), lead (pb), and nickel (ni) contained in the sample by an atomic absorption spectrophometer.

Plant Weight

a) **Plant fresh weight:** The weight of the fresh plant was recorded after harvesting.
b) **Root dry weight:** The root of each plant was wrapped with a foil paper and dried at 80^{0c} for 48 hours and weight was recorded.
c) **Shoot dry weight:** The weight of the shoot was dried and recorded; the drying process was the same as with root dry weight.
d) **Root /shoot Ratio:** This was done by dividing the weight of the root with the weight of the shoot. Average root or shoot weight was measured by dividing the total root or shoot weight by the number of plants.

Data Analysis

The data collected were statistically analyzed using SAS (2007, version 9.1) statistical package for all treatment tested, the mean were separated using least significant different (LSD) at 5% level of probability.

Results

Results of physicochemical tests carried out

Table 1: Different tests carried out on samples collected

Sample	Pure Soil	Soil +crude oil
pH value	4.98	5.06
Conductivity	3	3
Alkalinity (mg/l)	4.0	4.8
Zinc (mg/l)	0.342	1.719
Lead (mg/l)	0.188	0.256
Nickel (mg/l)	3.49	4.245

Table 2: Different tests carried out on the physicochemical parameters

	THBC	HUB	TF	HUF
CRUDE	$32+26={}^{58}/_{2}=2.9_{X}10^{5}$	$7.7_{X}\,10^{5}$	$8.7_{X}\cdot 10^{4}$	$8.6_{X}\cdot 10^{3}$
PURE SOIL	74+74=148/2 $7.4_{X}10^{5}$	$3.7_{X}10^{5}$	$3.5_{X}10^{4}$	$2.0_{X}\cdot 10^{4}$

Germination Percentage

The result obtained at the early stage of the experiment, both plants were able to sprout on the polluted soil, sorghum recorded 96% germinations for all treatment which was higher than that of African yam for the treatments with higher concentration of crude oil. African yam bean recorded 89% germination for treatments 1, 2 and 5 (100ml, 200ml and 0ml of crude oil respectively) while treatment 3 and 4 (400ml and 800ml of crude oil respectively) had 62% and 54% germinations respectively.

Below are the analysis of plants heights, leaf area and number of leaves taken in week two and week 12.

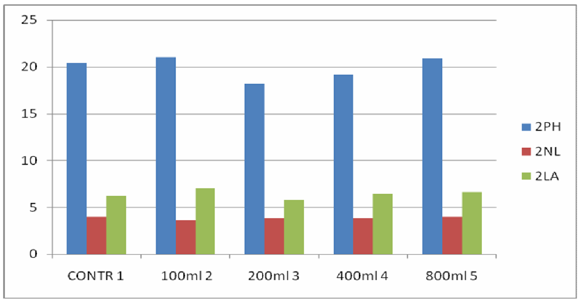

Fig. 1: Sorghum at 2 weeks

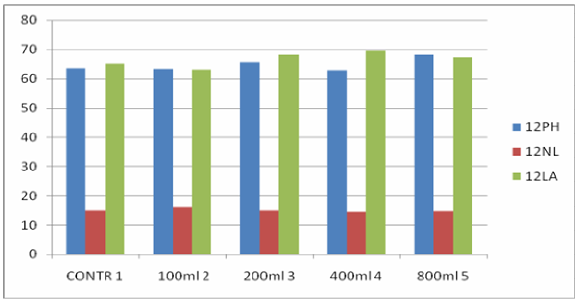

Fig: Sorghum at 12weeks

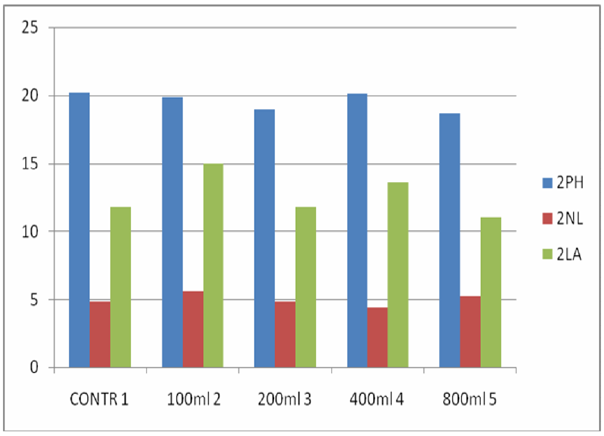

Fig. 3:African yam bean week 2

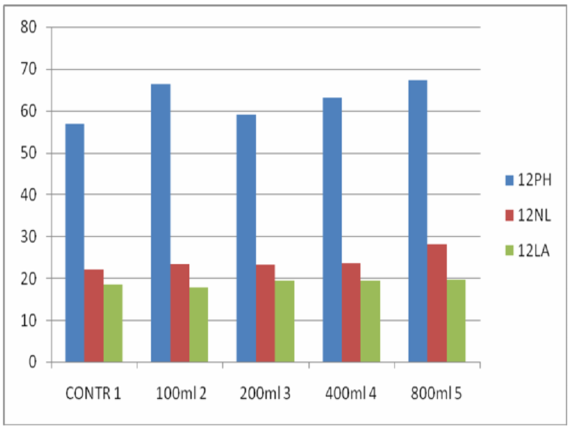

Fig. 4:African yam bean week 12

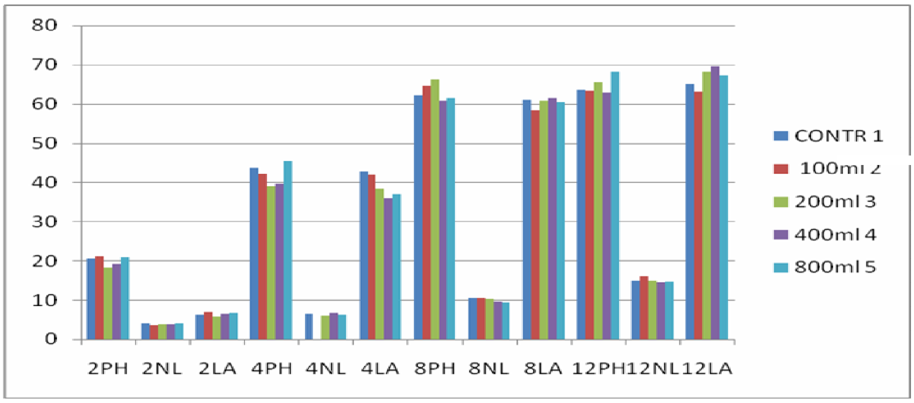

Fig. 5: Complete analysis of Sorghum

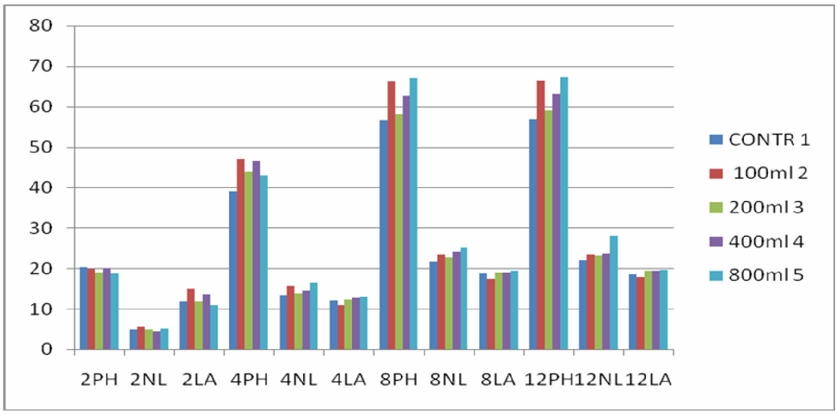

Fig. 6: complete analysis of African yam bean

Discussion

From the results in the above chapter, it was observed that sorghum bicolor did better in crude oil polluted soils than *Sphenostylis sternocarpa* in the aspect of growth. At first (two weeks after planting), both plants showed impressive rate of growth in all treatments. However, as the experiment continued, the plants (sorghum bicolor and *Sphenostylis sternocarpa*) in the higher concentrations (T3 and T4) started to grow poorly having thin stems, poor leaf production, and chlorosis. At the end of the experiment i.e. at week 12, African yam bean plants in soils of treatment (3 and 4) having higher concentrations (400ml and 800ml) of crude oil, had mean plant heights of 63.04cm and 28.72cm respectively, but when compared to those of lower concentrations (100ml and 200ml) and the control (0g)of crude oil, the mean plant heights were 66.78cm (for 100ml),67.62cm (for 200ml) and the control treatment had mean of 72.82cm, this shows that African yam bean had better growth in control treatment than in treatment (1 and 2). The above comparison shows that heavy amount of crude oil (400ml and 800) in the soil greatly affected the growth and development of African yam bean. Also, comparing the dry weights of the plants, treatments 3 (400ml of crude oils) and 4 (800ml of crude oil) and had lower treatment with lower weights of (6.6g and 6.23g respectively) when compared to that of treatment 1 and 2 respectively.

Similarly, at week 12 for sorghum, treatments (3 and 4) with higher concentrations of crude oil had mean plant heights of 59.7cm and 63.5cm respectively, when compared to those of lower concentration (treatments 1 and 2) which had mean plant heights of 65.28 and 65.56cm respectively and the control treatment had mean plant heights of 66.72cm as at 12week. From the comparison above, it is observed that sorghum had the best growth in the soil without crude oil (control treatment) than those of treatments 1 and 2 (contrary to that of African yam bean). It therefore means that crude oil does not support the growth of sorghum. From the various physico-chemical analyses carried out, it was observed that the crude oil increased the $_{p}H$ of the soil (as shown in table 1). The crude oil also affected the availability of metals (Zn, Pd and Ni) in the soil and increased amount of anions (P and S) in the soil. There were also reductions in the conductivity available in the soil was slightly increased in the polluted soil, most plants do not grow well in soils with high alkalinity content. In conclusion, crude oil pollution when introduced to the soil in huge quality has gross effect on plants in the environment as observed with treatment 4 (800ml of crude oil) and treatment 3, reduction of growth, biomass and development in sorghum bicolor and *Sphenostylis sternocarpa*. Crude oil pollution should therefore be avoided especially in high concentrations to prevent the plants from low yield and its deleterious impacts. However, some lower concentration i.e. 100ml and 200ml did not show much significant effects on the rate of growth and development. Also, owing to the fact that crude oil increased soil $_{p}H$, indiscriminate dumping of disposed oil or spillages should be stopped as it will be difficult for available soil micro-organisms to decompose the waste when in great quantity. Remediation should henceforth, be carried out immediately such spillages occur either in-situ or ex-situ to avoid its possible consequences on plants.

Having carried out this experiment, the following are recommended;

- Since crude oil is capable of block pore space of soil thereby limiting the infiltration of water into the soil for plant roots, crude oil should not be disposed indiscriminately in the environment so as not to destroy our natural resources.
- The plant *Sphenostylis sternocarpa* could serve as bio-indicator (species used to detect pollution).

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