PHYTOREMEDIATION OF HYDROCARBON CONTAMINATED SOILS USING CARPET AND ELEPHANT GRASSES

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ABSTRACT
This study is geared towards mitigating hydrocarbon contamination of soils through a nature friendly approach known as phytoremediation. By implication, phytoremediation refers to the use of plants to selectively remove pollutants from the soils. Two plants were selected for the study and they are Pennisetum Purpureum (elephant grass) and Axonopus (carpet grass).The trial research tried setout to compare the two plants under laboratory condition by evaluating their potentials to degrade crude oil in contaminated soils. The plants were transplanted into plastic bows filled with loamy soils and provided with adequate water, sunlight, and organic manure. Crude oil was spilled and the plants were observed for weeks. After 6 to 12 weeks, total hydrocarbon content, soil physicochemical properties and bacterial count were measured for. An average hydrocarbon loss of 75% and 81.6% for Pennisetum Purpureum and Axonopus respectively after 6weeks and 91.0% and 93.5% after 12weeks of remediation. These values differed greatly from those obtained in the control. The percentage hydrocarbon loss recorded in this study indicates that over a wide range of field condition, both plant are good hydrocarbon digesters and carpet grass can join the list of phytoremediation plants in addition to its cost effective and environmental friendliness.

Key words: pennisetum purpureum, elephant grass, axonopus, carpet grass, hydrocarbon, phytoremediation, soils, contamination

1. INTRODUCTION

The increased rate of oil spill incidents in Nigeria cannot be overemphasized as it is becoming daily occurrence. It also required time and money to clean up. It is to attain total clean-up of the menace caused by oil spill on land and water that brought about Biotechnology which is a treatment technology for remediation of contaminated land and water. It involves biodegradation of contaminants by stimulating the indigenous microbial population. This is done by oxygenation, nutrients addition or exotic species of microorganism are introduced to argument. The various biological techniques involved in bioremediation include fungi, compost, and microbial remediation (Alexander et al., 1990). Phytoremediation takes advantage of the fact that a given plant acts as a solar driven pump, which can extract and concentrate certain heavy metals from the environment (Raskin et al., 1994). This remediation technique maintains the biological properties and physical structure of the soils. Oil spill causes environmental pollution to the ecosystem especially the oil producing region of the world. Two different grass species were employed and their remediation rates compared (i.e. the rate of remediation of crude oil pollution on agricultural soils using Elephant grass and Carpet grass). The objective is to check if phytoremediation using carpet grass could facilitate the quick metabolism of crude oil and to ascertain the effect of crude oil contamination on the elephant and carpet grasses after remediation. Owing to the menace caused by crude oil spill on land, the study will be investigating the potential difference between elephant and carpet grasses in crude oil remediation of contaminated soils. This served to enlist carpet grass among viable options for phytoremediation. In phytoremediation, the plants remove the contaminants from site and break

them down for easy absorption, they are also contained and stabilized as the plants act as a filter or trap. The advantage of this technique is that the site can be cleaned up without excavating the affected soil and workers are safe since they do not come in contact with the pollutant. Elephant grass is a tall grass of tropical grassland with very high productivity both as a forage grass for livestock and as a bio fuel. Elephant grasses are drought and fire tolerant. It thrives on poorly drained soils as well as dry soils. Carpet grass on the other hand is known to be tough, native to tropical and subtropical climate. It is not fast growing but is adapted to all soil types. Oil spill can occur through human error, equipment failure, blowouts, sabotage, accident and natural causes. Oil spill can occur beneath the soil surface as a result of pipeline rupture. Aquatic oil spill is when it is discharged into water bodies (pond, lake, river, etc.). Gaseous oil spill refers a situation where oil escape into the atmosphere. Surface oil spill tend to occur on the surface of the ground sometimes partly or completely submerging vegetation. Contamination of soils with hydrocarbon is one of the major environmental problems associated with industrialization and dependence on petroleum and its product. Techniques for treatment of crude oil contaminated soil include incineration, thermal desorption, solvent extraction, soil washing, fungi remediation, compost remediation, bioremediation and phytoremediation.

2. LITERATURE REVIEW

Crude oil is a dark liquid hydrocarbon with a mixture of aliphatic and aromatic hydrocarbon and some non-hydrocarbon compounds. Crude oil occur naturally in geologic time from the conversion of organic matter biomass, it is derived from dead organism (Xie and Lui, 2009). It can be grouped into Paraffin, Naphthalene Aromatic, and unsaturated hydrocarbons. Paraffin are characterized by the saturated non-cyclic chain structure (e.g. Methane). Naphthalene belong to the saturated hydrocarbons (e.g., cycloalkane such as cyclo-pentane and hexane). Aromatic - They are the unsaturated hydrocarbons like benzene. Crude oil may be light or heavy, they both have their various characteristics and uniqueness. Light crude are none volatile, spread rapidly when spilled, while heavy crude which have high viscosity and low volatility, do not spread rapidly when spilled. The products derived from refined crude have chemical and physical characteristics which depend on the nature of the crude. Gasoline and petroleum contain similar hydrocarbon compounds with a narrow distillation temperature band (IMO, 1998). Oil spillage is the accidental and unintended discharge of liquid hydrocarbons into the environment during the cause of processing, transferring oil or while in storage (Onoyere, 2003). Oil spill due to exploration and exploitation activities in the environment affect land and aquatic habitats, the arable and pastural land are constantly diminished and about 25% of cultivated land are affected by soil degradation due man's activities (Inyang, 1993). The liquid hydrocarbon is made up of various constituents and prominent among the constituent are the heavy and light liquid which evaporate easily into the atmosphere called natural gas (Jessen, 1996).

According to Shell Petroleum Development Company (SPDC) classification of oil spill, oil spill is classified into: minor spill which is less than 25 barrel, medium spills which is greater than 250 - 2500 barrels and major spills which is greater than 2500 or 2500 – 25000 barrels of oil discharge into water or land, coastal ground or offshore (Onoyere, 2003). The various biological techniques involved in bioremediation include fungi, compost, and microbial remediation (Alexander et al., 1990). Phytoremediation takes advantage of the fact that a given plant acts as a solar driven pump, which can extract and concentrate certain heavy metals from the environment (Raskin et al., 1994). This remediation technique maintains the biological properties and physical structure of the soils. Indian mustard can extract heavy metals (lead and chromium) (Delille and Bassères, 1998) and sunflower can concentrate uranium in their roots (Raskin et al., 1994). Phenanthrene may be trapped within soil structure or pore diameter usually for soils with pore diameter less than 100mm and soil texture tend to influence the

availability of the contaminant (Alexander et al., 1997). Soil organic binds lipophilic compounds thereby reducing their bioavailability (Cunningham and Berti, 1993).

3. SOIL PROPERTIES AND OIL SPILL

When oil spill on land it will spread over the surface and will sink into the permeable soil or rock. The degree of remediation depend on the types, nature of material and volumes of oil (Bostert and Bartia, 1984). Less volatile oil rapidly penetrate into the soil, wet or clay type of soil will resist penetration of the oil. But none viscous oil will continue to spread from an oily mass especially if the ambient temperature is favorable. The spreading continues until the carrying capacity of the soil is reached, subsequent movement of oil is as a result of its displacement by water and is typically in the direction of the groundwater flow. Thus oil reappears in spring and such case may result in contamination of surface water (Bostert and Bartia, 1984). All form of oil spillage must be checked if our environment must remain a healthy one. This can only be achieved when the right equipment and technology are applied. Soil pH is a measure of the acidity or basicity of soils. The optimum pH range for most plant is between 6 and 7.5, many plants have adapted to thrive at pH values outside this range. Total organic carbon (TOC) is the amount of carbon bound in an organic compound and is often used as a zone specific indicator of soil quality. It is also the carbon store in soil through the decomposition of plant and animal, it plays key role in nutrient recycling and can help improve soil structure. Factors influencing organic matter in soil are climate condition such as rain fall, temperature, soil aeration, pH level, microbial population of the soil, increased use of fertilizer and irrigation. Organic matter in the soil has beneficial effects on agriculture, soil quality and soil structure. It enhance aeration and water penetration. Soil moisture is the amount of moisture present in the soil. Bacteria count is soils reflects soil health, the less the count, the less healthy the soil is. When the contaminants get into water bodies, they interfere with the water quality and these could trigger health and environmental effects (Lichtenthaler and Haag, 1989).

4. EFFECTS OF OIL SPILL ON THE ENVIRONMENT

Despite all the regulations, groundwater contamination due to petroleum activities remains largely unattended. This is evident in the groundwater samples taken from Luiwi in Ogoni land (where oil exploration and exploitation activities have been on till production stopped in 1993) were analyzed in the United State. It was observed that the sample contained 18ppm of hydrocarbons. This amounts to 360 times the level allowed in drinking water in European Union (EU).Another sample from Ikwere in Rivers state, Nigeria contained 34ppm and about 680 times the EU standard for drinking water. The groundwater in Warri and Abraka and the effects of gas flaring on rain and surface water in Okpai and Beneku respectively in Delta State were examined and the result established that while groundwater the was relatively safe in both cases the rain and surface waters needed treatment before it could be consumed (Nwankwo and Ogagarue, 2011). The formation strata and groundwater potential in Sapele metropolis (Amukali, 2012). That of Jesse and Oghara was determined by correlating results of seismic refraction survey and those of electrical resistivity studies (Okolie et al., 2007). The submission was that the numbers of layers delineated differ, but the viable aquifer at Sapele and Jesse is generally within 25m below the surface although false and contaminated aquifer may be intercepted at 10 -15 m. On the contrary, Oghara is more of sandy formation with aquifer above 35 m thick (Inyang, 1993). Groundwater is most vulnerable to hydrocarbon contamination due to spills when they are shallow and in unconfined aquifers as observed in the cases cited above. Generally, when petroleum comes in water a very fast partitioning between the water, air and sediments(Knap, 1982). The insoluble fraction forms a layer of 0.01 to 3.0 mm thickness on the water layer (Lichtenthaler and Haag, 1989). During the first few hours some parts evaporate and

other parts are absorbed in the sediment. When the hydrocarbons are concentrated enough non-aqueous phase liquids (NAPLs) can be formed. The remaining hydrocarbons are present in the aqueous layer or as a film on the water surface. The lighter fractions are removed within twenty-four hours by evaporation (Nwilo and Badejo, 2008). However, the evaporation of alkanes is possible until an 18 carbon chain (Dushenkov et al., 1995). The mass loss due to evaporation can range from 0.1% for heavier oils to 17.3% for lighter oils(Delille and Bassères, 1998).
Total Hydrocarbon Content is used to describe the quantity of the measured hydrocarbon impurities present. It is usually measured in methane equivalent. Source of hydrocarbon contaminant - flow stations, distribution depots, oil production refineries, automobile industry, haulage yard. Effect of hydrocarbon content on plant and animals - Causes tumor on farm animals, causes cancer on human, affect skin, body fluid, reduce immunes system (ATSDR, 2012). A plant suitable for phytoremediation should possess the following characteristics: ability to accumulate metals, to selectively extract metal from soils, tolerate hydrocarbon accumulation, fast growing, highly effective biomass and easily harvestable (Alexander et al., 1997). Plants which do not translocate metal could be useful for phyto- stabilization and land scape recreation. Plant play a direct role in the degradation of organic contaminant. Plant provides soil and root associated microbes with soluble exudates that increase microbial number and activity. Plant can remediate through co-metabolism which a compound that cannot support microbial growth on its own can be degraded when another growth support substrate is present (Cunningham and Berti, 1993). Environmental factors influencing plants use in phytoremediation of contaminated soil include soil structure, organic matter contents, oxygen availability, temperature, nutrients, solar radiation, toxicity level or contaminant level.

5. METHODOLOGY

Parameters considered are total hydrocarbon content, total bacteria count, pH, total organic nitrogen, electrical conductivity, moisture content and total dissolved solids. The crude oil was taken to the laboratory for chronology to find out the amount and species of hydrocarbon present in the crude oil. The experimental design involved collection of soil samples, pouring the soils into 11bowls of 5 liters each, adapting elephant and carpet grasses into 5 bowls each, and later crude oil was spilled into each of the specimen. The grasses were placed under good planting condition for proper adaptation and allowed to grow for 6weeks. The grasses were presumed grown and ready for the role it is to play. Then about 0.5 liters of bunny light was spilled on the soils in each of the 11bowls using from a perforated can. The said treatment was monitored on daily basis and later soil samples were collected and sent for Laboratory Analysis at 6 and 12 weeks respectively. The THC was estimated using ASTM 3921. 10g of air dried homogenous samples were measured in beakers using Mettler pm 2000 weighing balance to the nearest 0.01g. 30ml of n-hexane was added and stirred for 1minute in a magnetic stirrer. The supernatant were decanted into another beaker. Another 30ml of n-hexane was added again to the left over in the beaker and extraction continued. The extraction process was repeated one more time to ensure complete extraction. In each step, supernatant were decanted and collected in the same beaker. 2g of activated silica gel was added to the extract to remove the biogenic, stirred and filtered using glass wool. The silica gel step was repeated until all the biogenic were removed. Extracts were collected using 100ml volumetric flask and make up to mark with n-hexane. Absorbance of extracts were measured against n-hexane blank at 420mm using Hatch UV Spectrophotometer Model 2000. THC was deduced as follows:

$$\text{THC in mg/kg} = \frac{A*B}{KC}$$

where A is measured absorbance; B = final volume gram extracts in ml, K= spectrophotometer calibration constant and C = weight of sample used.

Total Bacteria Count- total heterotrophic bacteria was determined using APHA 92 15B/9600B (pour plate0 method. Serial dilution of the sample was carried using sterile water, aliquot of the 10-fold dilution were placed on nutrient agar for the enumeration of heterotrophic bacteria. Plates were incubated at 35ºC for 24-48hours. Total microbial colonies were deduced as follows:

$$\text{Plate count (cfu/g)} = \frac{\text{Number of Colonies in Plate x Dilution factor}}{\text{Volume inoculated into plates}}$$

The pH and conductivity were determined using laboratory meter (Hanna Hi 3220).

Total organic carbon was determined using Blacky and Walky wet oxidation method. In this case, 0.5g of dried and homogenized sample was weighed into Erlenmeyer flask. 10ml of IN $K_2Cr_20_7$ was added using10ml pipette. 20ml concentrated H_2SO_4 was added and swirl, then allowed to stand in asbestos mat for 30minutes. 10ml of distilled was added and shaken and titrated with 0.5N of ferrous ammonium sulphate that was previously standardized, 2drops of ferroin indicator was used to mark the end points. % TOC was calculated thus:

$$\frac{(M_{eq}K_2Cr_2O_7 - M_{eq}\ FAS)\ 1.33 \text{ x } 100 \text{ x } 0.003}{\text{Weight of sample (g)}}$$

where $M_{eq}K_2Cr_2O_7$ = mili-equivalent of $K_2Cr_20_7$ (volume x normality)Meq FAS) = mili – equivalent of ferrous ammonium sulphate(volume x normality) and 1.33 = constant for soils and sediments; 100 is conversion for percentage, 0.003 is mili- equivalent weight of carbon.

Total Nitrogen was determined by modified Kjeldahl Method. 1g of sample was mixed with 4ml salicyclic/sulfuric acid indigestion flask and allowed to stand overnight. Then 0.5g of sodiumthiosulphste was added and mixture heated cautiously until frothing ceased. Mixture were cooled and added 1.1g of Titanium dioxide catalyst and heated again to clear solution. Mixture was boiled for 2hours and allowed to cool. 2ml of water added while shaking and content added into distillation apparatus. 5ml of boric acid was added to 100ml conical flask. The flask was placed under the condenser f the distillation apparatus. 20ml of sodium hydroxide was run slowly into the distillation chamber through funnel. About 40ml of the condensate was distilled and titrated with sulphuric acid to a violet end point using 3drops of mixed indicator at pH 5. The deductions are as follows;

$$WN = \frac{(V_1 \text{ x } V_0) \text{ x } C\ (H^+) \text{ x } MN \quad \text{x } 100 \ + \ WH_2O}{100M}$$

Where W_N = total nitrogen (g/kg); V_1 = volume sulphuric acid for sample (ml titration), V_0 = volume sulphuric acid for Blank (ml) titration, $C(H^+)$ = concentration of H^+ (mols/liter); M_N = molar mass of nitrogen in g/ml. M = mass of air dried sample (g), WH_2O = water content of soil sample based on oven dried soil (%by mass).

Moisture content determined using the oven dried technique. 20g of wet soil was wrapped in aluminum foil and kept in an oven at 150^0C. The soil samples were weighed again after oven drying. The mass of the dried soil sample from that of the wet soil sample was determined thus; the mass of the water in the sample was deduced from the difference between the weights of wet and dried soil samples. The moisture content or gravimetric water content was calculated using the expression following:

$$\text{Gravimetric water content} = \frac{\text{mass of water x100}}{\text{mass of oven dry soil}} 1$$

6. RESULT AND DISCUSSION

The result of the analysis is summarized in the table and figures following. The tables1 and 2 following shows the summaries of the values obtained for the relevant physical, chemical properties of soil samples, the bacteria count, total hydrocarbon content of the soils analyzed which were used as indices of pollution and remediation

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Table 1. Physico-chemical properties and the bacteria count in soil samples

Sp	pH		THC		TOC		COND		TN		BC		MC	
	W6	W 12	W6	W 12	W6	W 12	W6	W 12	W 6	W 12	W 6	W 12	W 6	W 12
C_1	6.00	6.50	300	150	0.64	0.30	52	30	0.07	0.04	$1.6X10^5$	$1.0X10^5$	30	53
C_2	6.20	6.90	250	120	ND	0.15	55	20	0.05	0.02	$1.63X10^5$	$0.5X10^5$	42	50
E_1	5.58	7.00	350	100	0.96	0.43	284	150	0.09	0.06	$1.2X10^5$	$0.1X10^5$	45	49
E_2	6.00	7.10	400	95	0.96	0.26	230	110	0.10	0.04	$1.22X10^5$	$0.06X10^5$	30	43
C_t	10.0	10.0	1500	500	8.00	8.00	500	500	5.00	5.00	$5.2X10^5$	$5.2X10^5$	75	75

KEY: w – week; TN- total nitrogen, Bacteria Count - Moisture Content- Total Hydrocarbon Content, cond- conductivity,

C_{1-2} carpet grass specimen 1-2, E_{1-2} elephant grass, SP- sample, C_t - control

Table 2. Cumulative changes in the physical and chemical properties of soil samples

Sp	pH		THC		TOC		COND		TN		BC		MC	
	W6	W 12	W6	W 12	W6	W 12	W6	W 12	W 6	W 12	W 6	W 12	W 6	W 12
SC	6.1	6.7	275	135	0.67	0.23	53.5	25	0.06	0.03	161500	75000	41	51.5
SE	5.8	7.1	375	98	0.96	0.33	257	130	0.10	0.04	121000	8000	37.5	46
SC_t	10	10	1500	500	1500	500	5.0	5.0	8.0	8.0	$5.2X10^5$	$5.2X10^5$	75	75

Key: SC – Specimen with carpet grass; SE – Specimen with Elephant grass; SC_t – Control Specimen

7. DISCUSSION OF FINDINGS

There is no significant difference in the rate of remediation between the elephant and carpet grasses at both 6^{th} and 12^{th} week of the adaptation for pH, THC, TN, TOC, BC and MC but variation was observed for conductivity values. The bacteria count indicates microbial activities which enhances phytoremediation. This trend was different for the control site because no plant was adapted therein. pH of soil samples in the specimen became alkaline at the beginning of adaptation. This did impeded plants adaptation but the pH reduced from 10 to 6 after the 6^{th} week. This may not be unconnected to the production of acid radicals by nitrifying bacteria. Similarly, total nitrogen decreased throughout the adaptation period as shown in tables 1 and 2. This may be accounted for by the high bacterial activity in the soils and possible leaching of ammonia, denitrification in wet soils and plant uptake of nitrogen as also submitted in (FitzPactrick, 1986). Also nitrogen reduction during biodegradation can be attributed to conversion of nitrous compounds to nitrogen (due to biochemical reactions induced by

pseudomonas bacilli) which escapes to the atmosphere even as Banks et al (1997) submitted. Phytovolatalization tend to occur during phytoremediation which is observed when leaf burn (chlorosis) occurs, and this was observed within the 6th week. This is attributable to the uptake by plant roots of volatile organic compounds and their consequent translocation within the plants and transpired through the stem and leaves. This aligns with the submission of Lee and Banks (1993). The percentage reduction in the THC for both treatment grasses was significant and progressive with the remediation period. The result of the remediation exercise across the specimen are correlated in the plots of the observed values in figures 1 to 14 following .

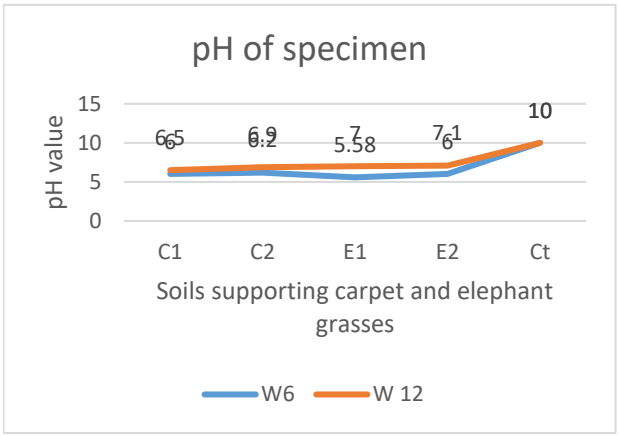

Fig. 1. pH across soil specimens

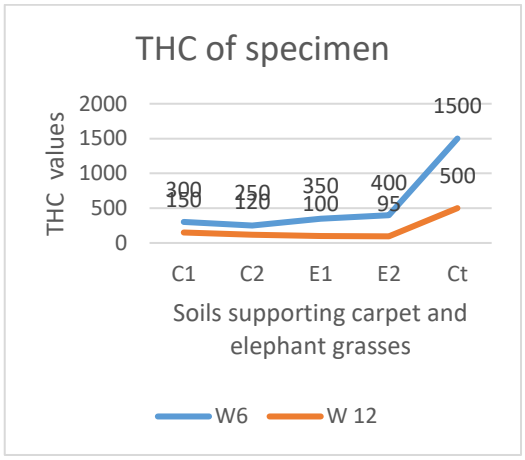

Fig. 2. THC across soil specimens

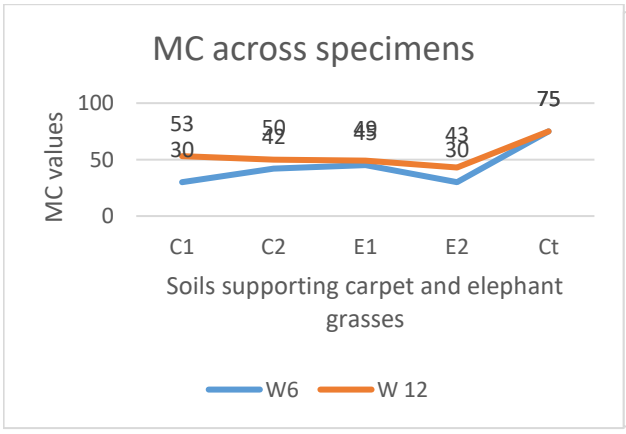

Fig. 5. MC across soil specimens

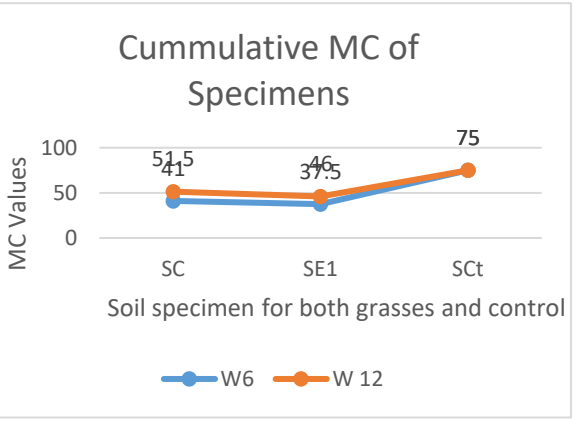

Fig. 6. Cummulative MC across soil specimens

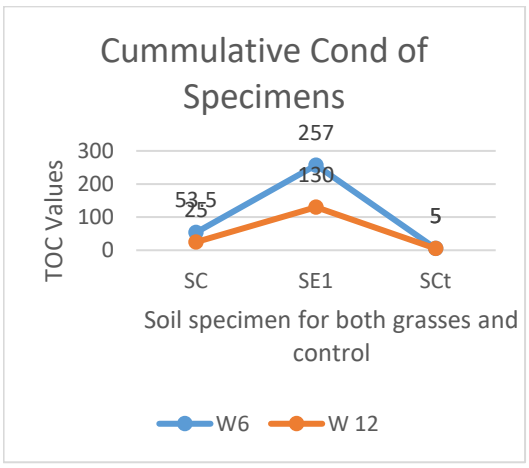

Fig. 7. Cummulative Cond across soil specimens

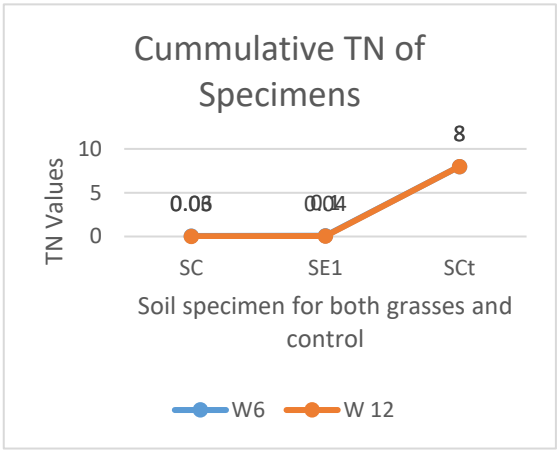

Fig. 8. Cummulative TN across soil specimens

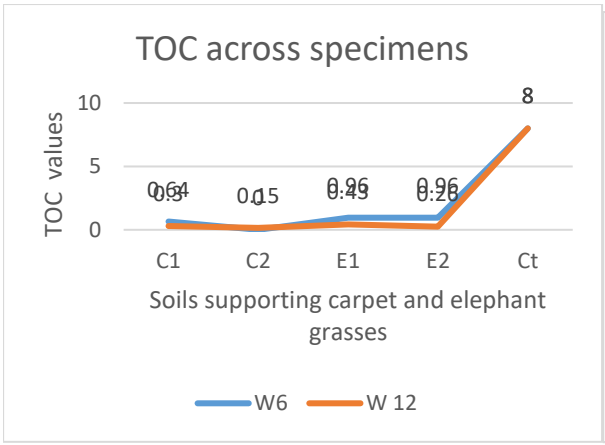

Fig. 3. TOC across soil specimens

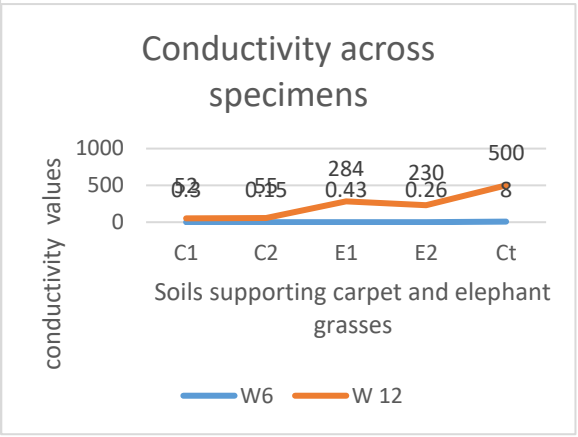

Fig. 4. Conductivity across soil specimens

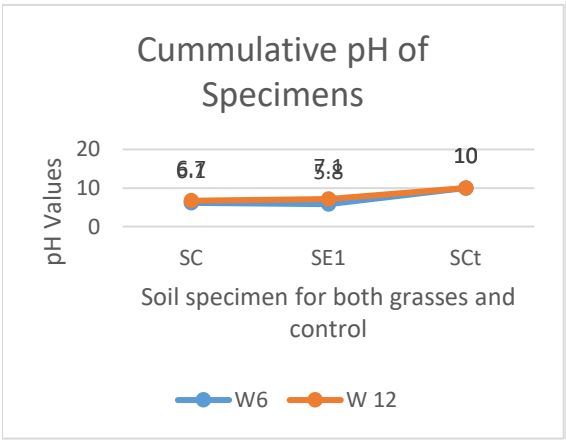

Fig. 9. Cummulative pH across soilspecimens

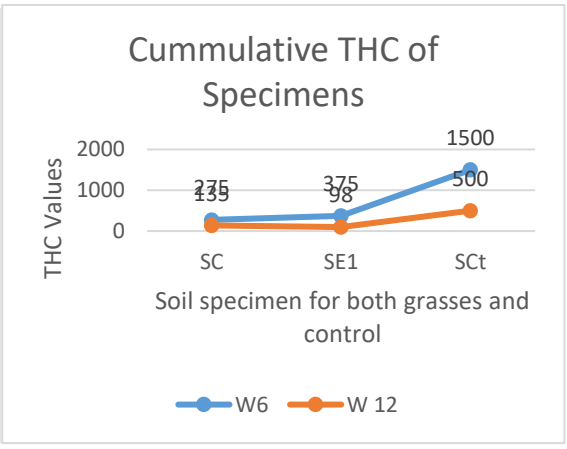

Fig. 10. Cummulative THC across soil specimens

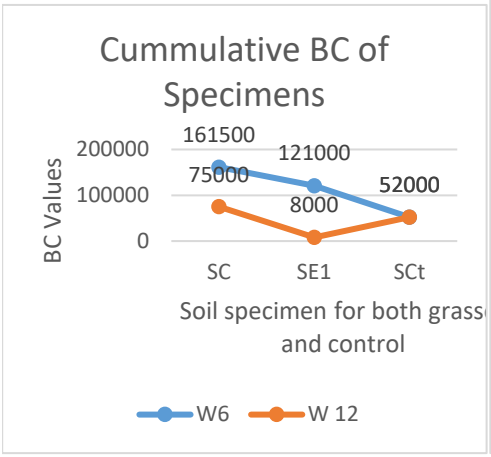

Fig. 11. Cummulative pH across soil specimens

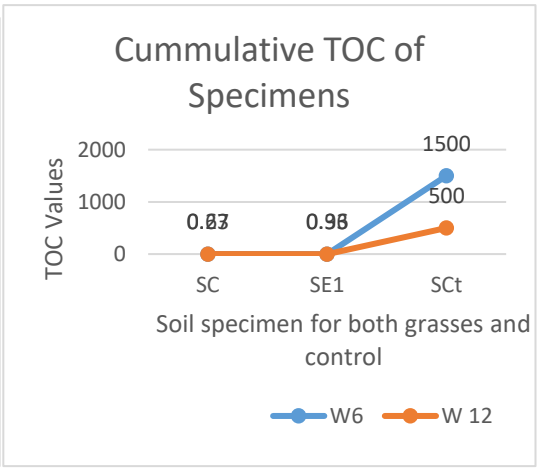

Fig. 12. Cummulative THC across soil specimens

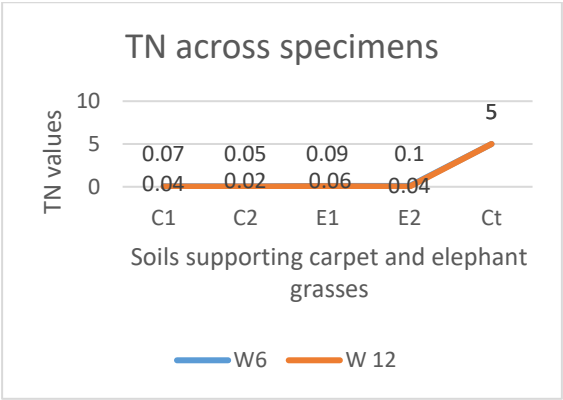

Fig.13. TN across soil specimens

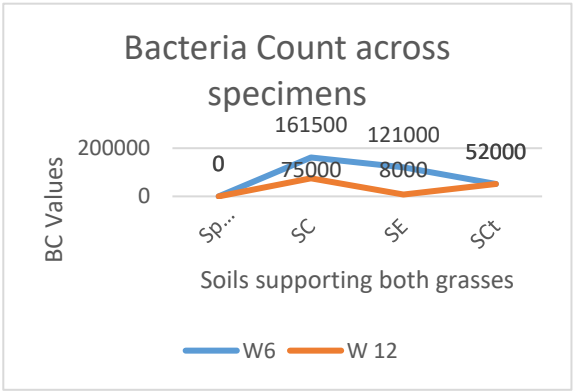

Fig. 14. BC across soil specimens

8. SUMMARY

The soil samples after 6 weeks and 12 weeks of remediation showed significant changes in their physical and chemical properties as well as bacteria count. Also, there is no significant difference in the rates of remediation of the hydrocarbon contaminated soils by the elephant and carpet grasses. It was observed that during the first 2 weeks of remediation, the site that

received carpet grass generally thrived better, the elephant grass adapted appear not to have to the contaminated soil with such ease as does the carpet grass. As the experiment progressed, leaf burn was observed more on elephant grass than in carpet grass, this indicates that carpet grass readily absorbed the contaminant, thus degrading the hydrocarbon more than the elephant grass. In the end of the adaptation period elephant grass and carpet grass presents with great potentials for phytoremediation because they are weeds. Both plants can be considered good hydrocarbon digesters. Oil spillage has become a day to day activity resulting from human activities or equipment failure which has great damage and effect on the ecosystem. There are a lot of remediation approaches both physical, chemical and biological, but phytoremediation involving the use of plants to cleanup crude oil from soil and water remains the best option. It is not only a clean technology, it is readily available and cost effective. Both plants should be used in cleaning major oil spill. Note also that if more time is allowed, more contaminants could have been mitigated and such, phytoremediation should be longtime process and larger areas of contaminated sites can be taken at once. This is because, if 12 weeks of adaptation could accomplish much using the plant specimens, more is there to be accomplished on large scale and longer time period.

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