POTENTIALS OF SOME PLANT SPECIES FOR PHYTOREMEDIATION OF CRUDE OIL POLLUTED SOIL DERIVED FROM COASTAL PLAIN SAND

BY

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DECLARATION

I Ijah, Christiana James with Registration Number ASS/Ph.D/12/003, hereby declare that this work titled "Potentials of some Plant Species for Phytoremediation of Crude Oil Polluted Soil Derived from Coastal Plain Sand" is a product of my own research effort under the supervision of Prof. Anthony Egrinya Eneji and Dr. Otobong Benjamin Iren and has not been presented elsewhere for the award of a degree or certificate.

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CERTIFICATION

This is to certify that the thesis titled "Potentials of some Plant Species for Phytoremediation of Crude Oil Polluted Soil derived from Coastal Plain Sand" was carried out by Ijah, Christiana James with Reg. No.: ASS/Ph.D/12/003 and has been examined and found worthy of the award of the degree of Doctor of Philosophy (Ph.D) Degree in Soil Science (Soil Chemistry).

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ABSTRACT

Soil and water pollution due to oil exploration activities has become a key environmental challenge in Nigeria's Niger Delta region. Phytoremediation is an environmentally-friendly approach to managing soil pollution. Greenhouse and field studies were conducted to evaluate the potentials and the associated mechanisms of some native plant species for the phytoremediation of crude oil polluted soils. Twelve native plant species (*Axonopus compressus*, *Pennisetum purpureum*, *Eleusine indica*, *Panicum maximum*, *Leuceana leucocephala*, *Gliricidia sepium*, *Talinum fructicosum*, *Chromoleana odorota*, *Cyperus rotundus*, *Calapogonium mucunoides*, *Jatropha curcas*,*Centrosema pubescens*) were studied under four levels of crude oil pollution [0, 2.5, 5.0 and 7.5 % (w/w)]using a Completely Randomized Design (CRD) in a greenhouse experiment . Two species (*P. purpureum* and *L. leucocephala*) with considerable phytoremediation potentials from the green house experiment were studied in the field at four levels of crude oil, with or without organo-mineral fertilizer (5 t/ha) and Brassinolide (250 ml per plant). The field treatments were laid out in a Randomized Complete Block design (RCBD) with three replications. After 4 months in the greenhouse, soil pollution significantly ($P < 0.05$) decreased plant biomass 42 %, soil pH by 35%, available P by 44%, exchangeable Ca by 45%, exchangeable Mg by 40%, exchangeable K by 50%, Na by 61 %, and ECEC by 43%. Conversely, it increased soil organic carbon by 18%, total nitrogen by 19% and base saturation by 33%. Total heterotrophic bacteria ($2.34x10^5$)and fungi ($2.29x10^3$) count decreased significantly in the polluted soil but hydrocarbon utilizing bacteria ($5.22x10^5$) and fungi ($2.47x10^3$)increased significantly ($P<0.05$). After 6 months in the field, soil pollution significantly increased soil pH by 27%, avail. P by 41%, Ca by 22%, Mg and K by 20% and base saturation by 23% while organic carbon decreased by 24%, total nitrogen by 35%, exchange acidity by 36% and ECEC by 25%. . Plant biomass,

total heterotrophic bacteria ($8.6x10^5$), heterotrophic fungi ($3.8x10^3$) count, hydrocarbon utilizing bacteria ($9.0x10^5$) and Fungi ($4.1x10^{3)}$ were significantly increased. Under polluted soils, the plants partitioned more heavy metals (Pb, Ni, Cd) to the roots and stem than the leaf. Whether in the greenhouse or in the field, there was a significant ($p<0.05$) reduction in total petroleum hydrocarbon (TPH) content of the soil and an increase in its absorption by the plants. *Pennisetum purpureum* had the highest absorption efficiency of 79.8 %, followed by *Leaceana leucocephala* (61.0 %). The possible mechanisms for the high uptake of TPH by these two plants were due to rhizodegradation (the interaction effect between plant and soil microorganisms), phytoextraction (metal accumulation in plants) and phytovolatilization (transfer of contaminants to gaseous state). Plant root exudates also act as a nutrient source for hydrocarbon degrading microbes which help in the absorption of the pollutants from the soil. The release of root- associated enzymes capable of transforming organic pollutants helps in catalyzing chemical reactions in the soil environment. Therefore, *P. purpureum and L. leucocephala* have good potentials to bi-accumulate contaminants from crude oil polluted soils and are recommended for phytoremediation.

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CHAPTER ONE

INTRODUCTION

The progress of civilization since independence has been phenomenal, but rapid industrialization also brought with it the danger of soil pollution. Today, everything around us like the air we breathe and the water we drink even the soil we grow our crops on is severely polluted. Pollution of agricultural soils is one of the most prevalent problems associated with the exploration and processing of petroleum hydrocarbon (Ayotamuno *et al.,* 2006).

Crude oil otherwise known as black gold is a major source of revenue and support for Nigeria economy (Odeyemi and Ogunseita, 1985). Increased population and the high demand for petroleum products has exacerbated oil spills in the environment. This oil is mainly discharged into the environment through leakages from pipe-line or flow-line, hose failure, sabotage and during accidents (Odu, 2000). In the Niger Delta region of Nigeria, it has been estimated that about 0.7-1.7 million tons per year of crude oil is spilled into agricultural soils, oceans and rivers (Odeyemi and Ogunseitan, 1985).

Crude oil comprises of both hydrocarbon and non-hydrocarbon compounds, including metallic elements such as copper, uranium, nickel, iron, vanadium, lead, cadmium and aluminium (Bremmer and Tabalabai, 1973). These pollutants have been found to affect and alter the chemical and biological properties of soils. Pollution occurs when a change in the environment adversely affects the quality of human life, including soils and plants. Eneje and Ebomotei (2011) noted that soil pollution with crude oil increased the soil organic carbon, total nitrogen, bulk density and reduced the soil water holding capacity and phosphorus. Onyeike *et al.*, 2002; Akubugwo *et al.*, 2009 and Ezeigbo *et al.,* 2013 reported an increase in exchangeable cations in crude oil polluted soil. Ijah and Antai, (2003a) observed a decrease in pH of crude oil polluted soil. Oil

contamination inhibits photosynthesis, transpiration and respiration which in turn affect the yield of crops (Chindah and Braide, 2000).

The survival of many life forms depends on the quality of the soil. Chaney *et al,* (2005) observed that subsistence farmers feeding on rice grown on polluted soils, especially hydrocarbon polluted soil are at risk from dietary exposure to cadmium. Soil supports the terrestrial life through detoxification of pollutants, biomass production, restoration and resilience of ecosystems and cycling of some nutrients like carbon, boron, phosphorus, sulphur and water (Lal, 2001). Soil quality is depleted as the soil is contaminated through individual or combined processes such as crude petroleum oil pollution. When a soil is polluted, its capacity to produce is reduced.

Many techniques have been adopted and used to clean up polluted soils. These include, soil excavation, soil washing/flushing, chemical immobilization, stabilization, electrokinetics, covering the polluted soil with clean soils and the dilution method. These techniques are rather costly, labour intensive and further degrade the soil (GOC, 2003; Lundstedt, 2003). Thus, biological method such as phytoremediation is most suitable for the remediation of hydrocarbon contaminated soils.

Phytoremediation also known as botano-remediation, green remediation or vegetative remediation is a technique that uses plants and their associated micro-organisms to degrade, extract, contain or render harmful substances harmless in the soil (EPA, 2000; Helmisaari *et al*., 2007). Phytoremediation has been singled out as the best and available technologies for the remediation of hydrocarbon contaminated soils. This technology is believed to be less disruptive to the soil as well as cost-effective (Eredei *et al*., 2005). Besides being easy to implement, it is more eco-friendly and aesthetically pleasing than the traditional methods (Henry, 2000). Phytoremediation also prevents the excavation and transportation of pollutants from one place to another, thus, reducing the risk of spreading the contaminants. It also has the great potential to treat a wide range of

toxic substances such as organic and inorganic contaminants (Meagher, 2000). However, the choice of plant species is a key consideration for successful phytoremediation.

Searching for the most effective plant species to clean up hydrocarbon polluted soils is a critical step in phytoremediation trials. Mathematical modeling has been used to evaluate the appropriate plant species (Thomas *et al.*, 2003) but in general, the selection of plant species for phytoremedition for specific sites is empirical and based on preliminary results obtained from pot experiment. (Kirkpatrick *et al.*, 2006; Liste and Felgentreu, 2006; Euliss *et al.*, 2007). According to Kamath *et al.*, (2004), plant species selected for phytoremediation are required to be fast growing, hardy and easy to plant and maintain. It must also possess high biomass, be adaptable to local climate, compactible to soil properties like pH, water content and structure and must have the ability to degrade the contaminant concerned. Although phytoremediation is considered cheap and environmentally friendly, field application of this method of soil remediation has only been practiced in developed countries. It is not commercially adopted in most countries like Nigeria. Even though over 400 plant species for phytoremediation of crude oil polluted soils have been recognized, most of them are exotic with low biomass production. Thus, there is need to source for alternative local plants for phytoremediation.

1.1 Justification of the study

Oil pollution is of great concern the world over. Even at the micro-level, contamination of the environment by crude oil is a global problem in that it leads to loss of vegetation, biodiversity and food insecurity. Considering the detrimental effects of crude oil pollution on soil and plants and its attendant implications for food security and environmental integrity, it has become necessary to source for a more cost effective, affordable, adaptable and environmentally- friendly method to restore polluted soils back to their original state.

This study employed the use of commonly found plant species amongst the grasses, legumes, arable crops and shrubs for the remediation of soils contaminated with crude oil. Besides restoring biodiversity to areas that have been disturbed, these species will enhance and conserve wildlife habitats and save money over alternative clean up methods. Unlike many introduced plants species, once established, they do not require pesticides and water. Also the use of these plant species in site restoration of crude oil polluted soils may serve to restore the habitats and thus create native parks, sanctuaries and other green areas for general human benefits. Finally, it is believe that, the findings from this study will give new insight into the reclamation of crude oil polluted soil and reduced land abandonement and frustration of rural farmers while greatly supporting decision makers, soil scientist, agronomist, researchers and oil companies in developing effective clean up strategies and curbing civil uprising.

1.2 Objectives of the study

The main objective of this study was to evaluate the potentials of some plant species for phytoremediation of crude oil polluted soil derived from coastal plain sand

The specific objectives were to;

1. Screen and identify suitable plant species for the phytoremediation of crude oil polluted soil,
2. Determine the effect of oil pollution on soil physico-chemical properties,
3. Determine the effects of oil pollution and phytoremediation on soil microbial population and species,
4. Explore the possible mechanism of phytoremediation by the plant species, and
5. Determine the effects of integrated use of plant species, organomineral fertilizers and phytohormonal treatment (Brassinolide) in the remediation of crude oil polluted soil.

CHAPTER TWO

LITERATURE REVIEW

2.1 Soil contamination

Contamination of soils with petroleum hydrocarbon and their subsequent degradation has become a major concern because of the critical role of soil resources in promoting sustainable environment and economic development. Both inorganic and organic compounds in soils may not only adversely affect their production potentials but may also compromise the quality of the food chain and the underlying ground water. However, hydrocarbon in whatever form are generally the most common contaminant that requires remediation due to their negative impact on the environment. In organic chemistry, a hydrocarbon is defined as any compound consisting entirely of carbon and hydrogen. The majority of hydrocarbons found naturally occur in crude oil, where decomposed organic matter provides an abundance of carbon and hydrogen which when bonded can catenate to form seemingly limitless chains (Clayden *et al.*, 2001). Hydrocarbons act as a source of fuels and lubricants as well as raw materials for the production of plastics, fibres, rubbers, solvents, explosives and industrial chemicals (Francis, 2008).

Many hydrocarbons occur in nature; besides making up fossil fuels, they are also present in trees and plants. The structures and the chemistry of individual hydrocarbons depend in large part on the types of chemical bonds that link together the atoms of their constituent molecules.

2.2 Types of hydrocarbons

a) Saturated hydrocarbons (alkanes)

Saturated hydrocarbons (alkanes) are the simplest of the hydrocarbon species that contain only one bond between carbon atoms and the carbon atoms are saturated with

hydrogen. The general formula for the saturated hydrocarbons is $C_nH_2n+_2$. Saturated hydrocarbons are the basis of petroleum fuels and are either found as linear or branched species. Hydrocarbons with the same molecular formulae but different structural formulae are called structural isomers (Silberberg, 2004).

b) Unsaturated hydrocarbons

Unsaturated hydrocarbons are hydrocarbons that have double or triple bonds between carbon atoms.Those with double bond are called alkenes with a formula C_nH_2n (Silberberg, 2004). Those containing triple bond are called alkynes with general molecular formula $C_nH_2n-_2$.

c) Aromatic hydrocarbons

Aromatic hydrocarbons otherwise known as arenes are hydrocarbons that have at least one aromatic ring. They have a relatively low solubility in water, but are highly lophilic (Halsall *et al.*, 1994).

d) Cycloalkenes

Cycloalkenes are hydrocarbons consisting of one or more carbon rings to which hydrogen atoms are attached. They are derived from the straight chain analogue by folding and joining the ends of the chains to form rings or circles of carbon atom. The carbon rings are called cyclic hydrocarbons and if they are saturated, the molecules are called cycloalkanes. They contain two hydrogen atoms fewer than the corresponding open-chain alkanes.

The smallest possible ring contains only three carbon atoms and is known as cyclopropane. Larger rings with over thirty carbon atoms are possible but the five-membered rings (cyclopentanes) and the six-membered rings (cyclohexane) are the most common and most widely studied. A saturated hydrocarbon containing one ring has a general molecular formula C_nH_2n (Silberberg, 2004).

2.3 Composition of hydrocarbons

The composition of hydrocarbons varies between sources and in source itself. Therefore it is not possible to give an exact composition of oil in general. However, hydrocarbon contains at least the following groups of chemicals: alkenes, cycloalkanes, aromatics and polyaromatic (Peterson, 1994). It further contains some additional nitrogen and sulphur containing compounds.

2.4 Petroleum hydrocarbons and toxicity in the environment

The toxicity of petroleum hydrocarbons depend on the solubility and the bioavailability of the hydrocarbons. It is assumed that the water soluble fraction is the most environmental harmful fraction because it is direct available for uptake by microbes. The partitioning of a hydrocarbon depends on the hydrophobicity of the compound which is expressed by Kow, the partitioning of the organic compound between an octanol and water phase. The height the biological available concentration in microbes can attain depends on the Kow, and the time in contact with the hydrocarbon (Peterson, 1994). Hydrophobic hydrocarbons are toxic for both plants and soil microbes by the accumulation in the membrane, which causes the loss of the membrane integrity (Sikkema and De Bont, 1995). Another uptake route for hydrocarbons is through sediment. The most hydrophobic hydrocarbons will absorb in the sediment and are toxic to the animals living within the sediment.

2.5 Fate of petroleum hydrocarbons in the environment.

Weathering processes of hydrocarbons include adsorption to soil particles, volatilization of hydrocarbons, and dissolution of hydrocarbons in water (Barakat *et al.*, 2001). When petroleum come in contact with water, a very fast partitioning between the water, air and the sediment part of the environment take place (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 liquid (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. Studies have showed that the amount of hydrocarbon that evaporates strongly depends on the nature of the oil. The evaporation of alkanes is possible up to 18 carbon chain (Knap, 1982). The mass loss due to evaporation can range from 0.1 % for heavier oils and 17.3 % for lighter oils (wang and Fingas, 1998). Evaporation of lighter fractions stimulates biodegradation, because the lighter fractions are more toxic to degrading bacteria (Delille and Basseres, 1998). After the partitioning the degradation starts in the different compartments.

a) Hydrocarbons dissolved in water

When hydrocarbon comes in contact with water, an emulsion is formed in the aquatic environment due to the increased viscosity of the oil after evaporation of volatile compounds. This makes degradation less favourable (Nicodem and Fernandes, 1997). In fact bacteria are only able to degrade hydrocarbons dissolved in water. This explains the persistence of larger PAHs (Wodzinski and Bertolini, 1972). Only some fractions are dissolved in water after oil spill in the environment, and this can be as low as only 2 % (Nicodem and Fernandes, 1972). Other parts are absorbed in the sediment or soil. Lighter 3 or 4 ring aromatic molecules are soluble in water (31.7 mg/L), but the PAHs consisting of 5 or more aromatic rings are not soluble in water (0.003 mg/L) and will become associated with the sediment (Shor and Kosson, 2004). This makes them more persistent. Research carried out by Ke and Bao, 2009 revealed that the presence of humic acid play a vital role for solvability of PAHs and are insoluble in the absence of humic acids.

b) Sorption of hydrocarbons to sediments and soil

Sediment absorption is important for degradation of hydrocarbons because it makes the hydrocarbon in general less available for degradation. Uptake of hydrocarbons

by microbes was shown to be much slower from the sediment than when the hydrocarbons are in solved state (Pignatello and xing, 1994).The sorption of organic compounds depends on a lot of factors. First the composition of the sediment is an important factor. Secondly, the presence of other organic substances in the soil can have an influence. Also the environmental conditions like PH, salinity and water temperature play a key role in the process of absorption. In an experiment to assess the factors affecting the association of fatty acids with mineral particles in sea water (Meyers and Quinn, 1973) reported that, PH was found to have a minor influence, with a 6-9 % decrease in absorption with an increase of one PH unit in the sediment.

c) Adsorption

The process of adhesion of hydrocarbon compounds to the layer of molecules of solutes, liquids or gases covering the surface of solids or liquids is known as adsorption. The majority of polycyclic aromatic hydrocarbons (PAHS) are generally found attached to solids and a very small amount (11%) is found dissolved in water (Karlsson & Viklander, 2008). This implies that polycyclic aromatic hydrocarbons are mostly adsorbed in soil particles. When hydrocabons are absorbed to solids suspended in aquatic environment, they can undergo sedimentation. This is a key factor in their remediation.

Hydrocarbons are predominately adsorbed to suspended particulate matter; they can however also be present in the vapour phase and are transferred to soil and water through wet or dry deposition (Grimalt *et al.,* 2004). In the soil, PAHs can be adsorbed to particulate matter and transported by surface runoff.

d) Volatilization

The process of volatilization involves the transfer of hydrocarbons to the vapour phase. The molecular weight of compounds, the movement of water and weather conditions all affect the rate of volatilization. Some PAHs volatilize from the water column than others. For example HMW-PAHs with five benzene rings or above do not

tend to volatilize due to their high melting points. However, the volatilization half-life of a high molecular weight PAH such as PYR ranged from 115 hours to 3.2 years (conditions not stated) (CCME, 2008).

2.6 Hydrocarbons degradation

There are a lot of mechanisms known for hydrocarbon degradation. The most studied ones are without doubt the bacterial pathways which are able to degrade several hydrocarbons compounds. Light is also able to degrade a lot of hydrocarbons. Some volatile parts of the oil will simple desorbs or evaporate immediately after the pollution occurs, but this is not always advantageous because this slows the degradation of the remaining part of the oil. An overview of the possible degradation mechanism is shown in figure 1. Nicodem and Fernandes (1997) investigated the photochemical processes and the environmental impact of petroleum spills and found that, a major cause of petroleum degradation is light, most notably in tropical regions. First the presence of light has a positive influence on degradation of some hydrocarbons in the presence of algae (Munoz and Guieysse, 2003), but it also has the possibility to degrade petroleum components in a direct photochemical way. The photochemical reactions caused by light are mainly able to effects the physical properties of some of the oil fractions.They are able to alter the emulsion formation and the solubility of the petroleum fractions. This is done by inducing reaction between oil components and other molecules, which makes the molecules more polar and water soluble, so creating new compounds with other physical and toxicological properties (Nicodem and Fernandes, 1997). The change in the original hydrocarbon concentration has also effect on toxicity.

2.6.1 Mechanisms of hydrocarbon degradation by light

There are three major mechanisms involved in photo-degradation of hydrocarbons (Nicodem and Fernandes, 1997). These mechanisms can be classified as either direct or indirect photolysis (Plata and Sharpless, 2008).

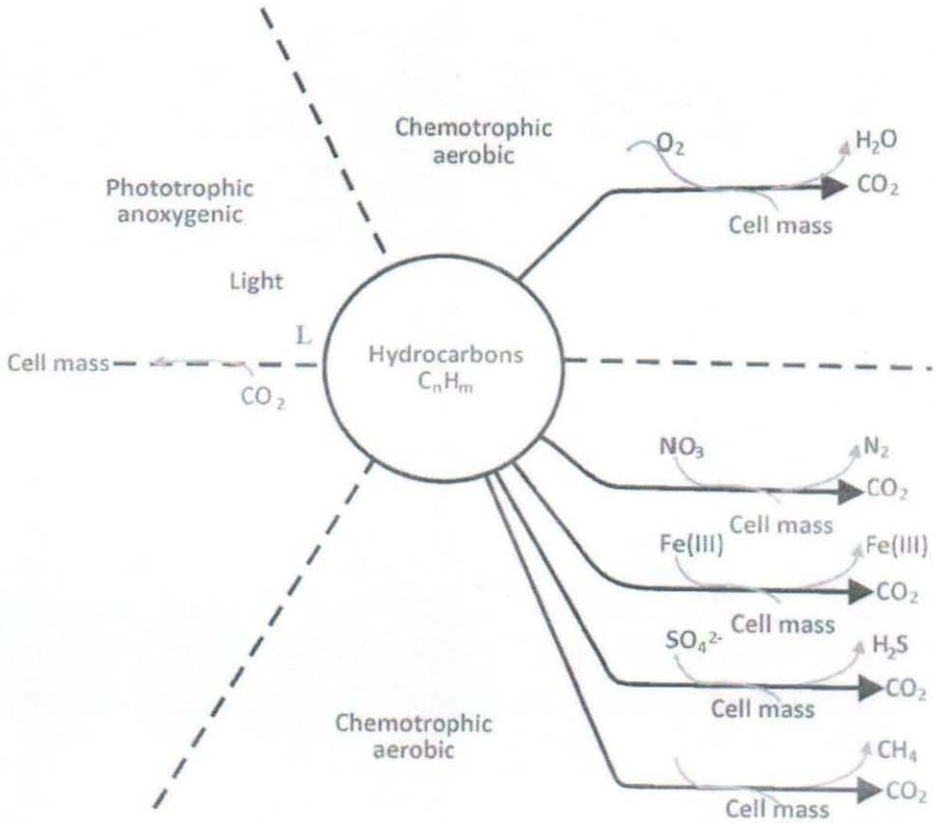


Figure 1: Possible degradation mechanisms for hydrocarbons

Source: Widdel and Rabus (2001)

Direct photolysis take place when the molecule of interest absorbs energy from light and further degrades. Degradation is called indirect photolysis when another molecule absorbs the energy from light and reacts to degrade the molecule of interest. Indirect photolysis can be divided into single oxygenation and radical oxidation (Lichtenthaler and Haag, 1989). An overview of the reactions is given in Figure 2 (Fasnacht and Blough, 2003). In this figure P is the PAH molecule.

a) Direct mechanism, direct photolysis

The first mechanism starts with excited hydrocarbons (aromatics) or other polar molecule which react with an oxygen molecule (Fasnacht and Blough, 2003). The aromatics are for this reaction exited to their triplet state (reaction 9+15). The absorbed energy is then transferred to an oxygen molecule (reaction 17) in a complex formed between the PAH and oxygen (reaction 16). This result in the formation (18) of a single oxygen molecule and the original PAH molecule (Lichtenthaler and Haag, 1989). The single oxygen molecule is able to react with aromatic and sulphur containing cyclic molecules.

b) Indirect photolysis, radical oxidation and electron transfer

The second mechanism that is thought of influencing the degradation of hydrocarbon compounds is radical formation. Photo ionization causes the removal of an electron from the PAH (reaction 1) which results in a PAH radical. This PAH radical can react further with water or hydroxide ions to form secondary radicals (reaction 2), also called radical oxidation. This radicals can react further to form products (reaction 3 and 4), (Fasnacht and Blough, 2003).This mechanism is mostly caused by a photochemical reaction which involves both single oxygen formation and free radicals (Nicodem and Fernandes, 1997).

c) Oxygenation by electron transfer

The third mechanism involves oxygenation by electron transfer. In this reaction

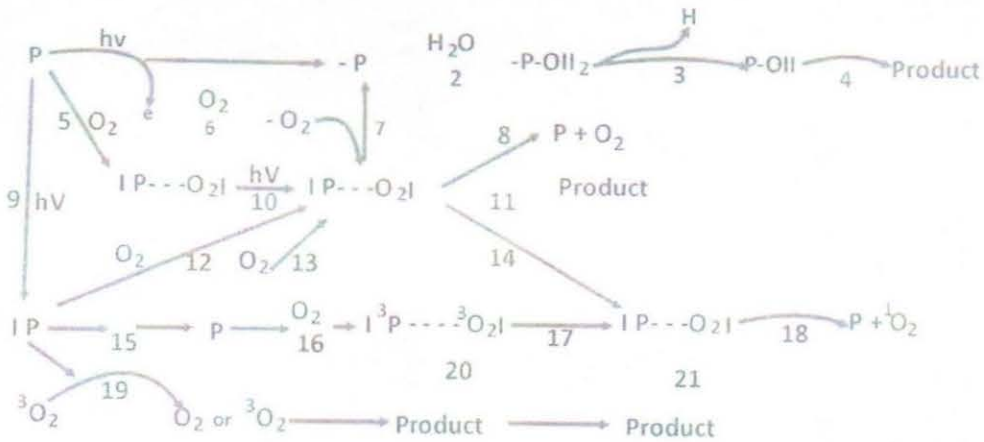


Figure 2: Mechanisms for photodegration of polycyclic hydrocarbons

Source: Fasnacht and Blough (2003)

light causes the transfer of an electron from the PAH molecule to oxygen (reaction 5and10). This electron transfer is thought to take place at the air-oil surface and creates anion and scation radicals which undergo solvent separation (Fasnacht and Blough, 2003). This can lead through reaction 7 to the same situation as during photo oxygenation (Nicodem and Fernandes, 1997; Fasnacht and Blough, 2003). One of the experiments in which this mechanism was tested was the formation of hexadecanoic acid from an oil solution as reported by (Nicodem and Fernandes, 1997).

2.7 Biodegradation of Hydrocarbons

Biodegradation is a major weathering process of hydrocarbons and an important natural attenuation process. Rates of biodegradation vary with different microbial populations, hydrocarbons, and geochemical ahnd hydrological conditions present in the subsurface. Nearly all soils and sediments have populations of bacteria and other organisms capable of degrading petroleum hydrocarbons (Kennedy *et al.,* 2000). Hydrocarbon-degrading bacteria can be present in low numbers in unpolluted environments; however, microbial populations can adapt and reach high densities after coming into contact with released petroleum compounds (Wisconsin Department of Natural Resources, 1994). Generally, petroleum hydrocarbons and other organic molecules with abundant carbon-hydrogen bonds are good food sources (electron donors) because they contain high-energy electrons. Soil and ground-water bacteria use a variety of natural electron acceptors in the degradation process.

Bacteria responsible for biodegradation commonly are categorized by their terminal electron acceptor processes (TEAP). These include aerobic bacteria, which use dissolved oxygen as their TEAP, nitrate-reducing bacteria, iron- and manganese-reducing bacteria, sulfur-reducing bacteria, and methanogenic bacteria. Pseudomonas bacteria are free--swimming aerobic bacteria known to degrade BTEX (Chapelle, 2000).

Biodegradation rates of the various types of petroleum hydrocarbons depend on the presence of TEAP. The sequence of preferential electron acceptor processes has been shown to cause zones of different electron-accepting processes dominating in different redox zones in contaminant plumes (Godsy *et al.,* 1999). Geochemical and microbiological data can be used to delineate the zones and to obtain information on possible degradation rates. Biodegradation rates of low to moderate weight aliphatic, alicyclic, and aromatic hydrocarbons can be high if ideal conditions are present. Resistance to biodegradation typically increases as the molecular weight of the hydrocarbon increases (Wiedemeier *et al.,* 1995).

A number of limiting factors have been recognized to affect the biodegradation of hydrocarbons. The composition and inherent biodegradability of the hydrocarbon pollutant is the first and foremost important consideration. Among physical factors, temperature plays an important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as the physiology and diversity of the microbial flora. Temperature also affects the solubility of hydrocarbons (Foght *et al.,* 1996). Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with decreasing temperatures. Nutrients are also very important ingredients for successful biodegradation of hydrocarbon pollutants, especially nitrogen, phosphorus, and in some cases iron. Some of these nutrients could become limiting factors, thus affecting the biodegradation processes. Biodegradation is a major attenuation process for hydrocarbons released into the environment. The underlying mechanisms that ultimately drive the mineralization of hydrocarbons have been proven to be enzymes actions.

Multiple lines of evidence generally are needed to demonstrate biodegradation processes at contaminated sites (Wiedemeiser *et al,* 1995). The lines of evidence used to examine biodegradation of petroleum hydrocarbons include (1) chemical data that

indicate decreasing concentrations of petroleum hydrocarbons, (2) geochemical data that indicate depletion of electron acceptors, and (3) laboratory or field microbiological data that indicate the bacteria present at a site can degrade petroleum hydrocarbons (U.S. Environmental Protection Agency, 1994).

2.8 Effects of biodegradation

The effect of biodegradation is a change in petroleum composition. Some parts are readily degraded by bacteria, and other compounds are degraded only slowly due to leak of degrading enzymes/ mechanisms by bacteria or because the hydrocarbons were toxic to the bacteria. This causes a difference in degradation for different oil, and thereby creates a different in toxicity between oil (Wang and Fingas, 1998). The degradation of aliphatic hydrocarbons is faster than degradation of other compounds. A degradation of 50 to 65 % was observed for hexadecane (Rhykerd and weaver, 1995) similarly, Hund and Traunspurger (1994) found that the PAH with less rings are degraded faster and the bigger PAH molecules are only slowly degraded.

Another property of oil that can be changed by biodegradation is the solubility of oil. Some bacteria are able to make so called biosurfactants. These biosurfantant are able to decrease the surface tension of the solution more than two times, and are therefore more effective than synthetic detergents. Lai and Huang (2009) reported that, the effect of biosurfactants also increases with concentration of petroleum pollution. In a related view, Miller and Bartha (1989) observed that enhanced solubility can also have an effect on further biodegradation, because it has been shown that solubility enhances uptake of hydrocarbons by bacteria. This is because the oxygenases are membrane bound and are only available by soluble organic molecules.

2.9 Effects of crude oil pollution on soil properties

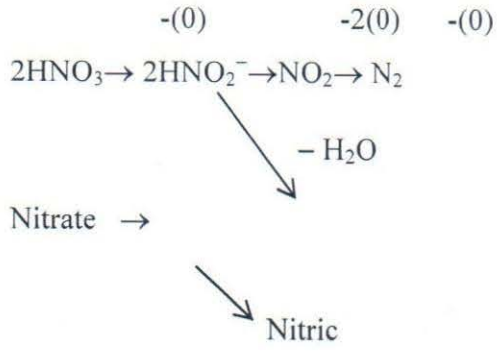
The environmental consequences of crude oil pollution on soil properties are enormous. Abosede (2013) investigated the effects of crude oil pollution on some soil

physical properties and found that crude oil contamination had no significant influence on the textural class of the soil. Similarly, Marinescu *et al.*, (2001) also reported no significant effect of crude oil pollution on granulometric fraction of the soil. In assessing the effect of crude oil pollution on soil physicochemical properties and germination of *Amarantus hybridus*, Eneje and Abomotei (2011) observed a significant decrease in the pH of polluted soils with corresponding increase in organic carbon content. In a related study (Ogboghodo *et al.*, 2004a; Udo, 2008) it was reported that increases in organic carbon occurred with increases in the concentration of crude oil.

Nudelman *et al.,* (2002) reported that, the adverse effect of crude oil pollution on the soil depends basically on a number of factors, such as the permeability of the soil, adsorption properties of the soil and the partition coefficient. Similar studies carried out by Mashalah *et al.*, (2006) using sandy loam soil with crude oil confirmed significant changes on the physicochemical and the microstructure of the polluted soils. The contaminated soil was found to reduce the cation exchange capacity and the double layer of the soil. Also the heavy metal contents of the soil increased with increases in crude oil. Benka-Cooker and Ekundayo (1995) reported a significant build-up of lead, iron and zinc in crude oil polluted soils of the Niger-Delta. Similarly, Kulakow *et al.*, (2002) observed a decrease in fresh biomass of ryegrass after 30 days of growth in soil polluted with 25 g of petroleum hydrocarbon while Tesar *et al.*, (2002) reported 82 % reduction in plant biomass in soil contaminated with 5 g of diesel oil. Udo (2008) and Asia *et al.* (2007) showed that zinc distribution is more in oil polluted soil than non-polluted soils and attributed its distribution to depend on certain soil properties such as clay, organic matter and soil pH.

Crude oil pollution has also been reported by Townsend *et al.* (2003), Ijah, *et al.* (2003a) and Sulton *et al.* (2013) to cause anaerobic condition in soil by smoothing soil particles and blocking air diffusion in the soil pores and affect microbial communities.

Ibia (2012) stated that, under anaerobic conditions, most soil nitrates (NO_3) are denitrified and lost to the atmosphere. $2NO_3 + 12H + 10^{e-} \quad N_2 + 6H_2O$ under such conditions, the nitrifying bacteria are inhibited and other anaerobic organisms (Bacillus, Pseudomonas e.t.c) become active, threrby reducing the nitrates to nitrogenous gases which become lost to the atmosphere. Also leaching losses of NH_4^+ occur rapidly under anaerobic conditions. A generalized trend in nitrate reduction (Denitrification) is as shown below:



Nitrate reduction in anaerobic soils

In a study by Kayode *et al.* (2009) to investigate the impact of spent lubricating oil on physicochemical properties of soil, it was observed that oil increased soil porosity, and reduces soil capillarity, soil aeration, water holding capacity and phosphorus contents. The authors also reported that, oil destroys soil structure by increasing the bulk density which in turn can lead to reduction in root penetrations of crops and subsequently impedes nutrient up-take. An increase in the bulk density of soil especially above 1.49 cm^{-3} may lead to reduction in crop yield (Janssen and Vander-weert, 1977). Similarly, Vwioko *et al.* (2006) and Hinojosa *et al.* (2004) stated that, oil pollution of soil lead to build up of heavy metals, especially copper, nickel, cadmium, zinc, chromium and lead in the soil.

Plant root absorb heavy metals in the soil most especially where there is contamination (Jordao *et al.,* 2006). When these heavy metals are taken up by plant roots, it result in chlorosis, weak plant growth, yield reduction, reduced nutrient uptake,

disorder in plant metabolism and reduced ability to fix molecular nitrogen in leguminous plants (Guala *et al.*, 2010). The uptake of these heavy metals by plants and accumulation in the food chain is a serious threat to both animal and human health (Spryskyy *et al.*, 2007).

The presence of heavy metals in human body is toxic and they accumulate in the soft tissues. High level ingestion of toxic metals has undesirable effects on humans which become obvious only after several years of exposure (Khan *et al.*, 2008).Although some heavy metals at low concentration are considered essential micronutrients for plants, at high concentrations they may cause metabolic disorder and become growth inhibitor to most plants species (Fernades and Henriquez, 1991).

In an assessment of the environmental impact of oil spill at Ikot Ada Udo in Akwa Ibom State, Udo (2008) observed an increase in nitrogen content and attributed it to the nitrogen compounds present in the spilled oil. Similar findings were reported in Eneje and Ebomotei (2011) and Odu (1972). Onyeike *et al.* 2002; Akubugwo *et al.* 2009 and Ezeigbo *et al.* 2013 observed an increase in exchangeable cations in crude oil polluted soil.

Isirimah *et al.* (1989) and Okolo *et al.* (2005) reported that crude oil pollution increases base saturation while Udo (2008) showed that crude oil pollution increases exchangeable acidity in the soil.

2.10 Effects of oil pollution on plants

Pollution of the soil with crude oil had been reported to affect the growth parameters of plants. In a study by Udo and Fayemi (1975), they observed that oil pollution led to reduction in chlorophyll content in the leaf, nutritional composition and higher level of heavy metal uptake in the fruit. Sharma (1984) stated that the adverse effect of crude oil pollution on plants ranges from morphological aberration, reduction in biomass to stomata abnormalities. Osuagwu *et al.* (2013) reported a delay sprouting,

reduction in leaf length, leaf area, growth and yield of air potato (*Discorea bulbifora*). They attributed the adverse effect on air potato to the disruption in water and nutrient uptake owing to the effects of crude oil in the soil and the depletion of essential nutrients like nitrogen, phosphorus and potassium content in the soil (Baran *et al.*, 2002). Bossert and Bartha,(1984) reported that toxic compounds of petroleum hydrocarbon could also inhibit plant growth. Similar findings were reported in Vwioko *et al.* (2005) on the effect of higher concentration of crude oil on germination of *Ricinus communus*.

The effects of crude oil contaminated soil on the growth parameter of seashore (*Paspalum vaginatum*) seedlings were investigated by Bamidele and Igiri (2011). The result showed that crude oil imposed physiological stress on the seedlings and there was also a significant difference in plant growth with respect to time of crude oil application. In another study, Nwazue (2011) reported that oil pollution affected the growth rate, vitamin C content, nutrient content and the chemical composition of plants and the physicochemical parameters of the soil.

The susceptibility of *Manihot esculenta* to Nigeria's Forcados blend crude oil was studied by Odjegba and Atebe (2007) for a period of 8 weeks using soil polluted with 0-5 % w/w oil/soil. The authors observed a significant reduction in plant parameters such as plant height, leaf area, dry weight, growth rate, chlorophyll and nitrate reductase activity of plant exposed to oil treatments. Similarly, Oyedeji *et al.* (2012) reported reduced growth rate, germination, plant height and stem girth on *Abelmoschus esculentus* L. exposed to soil polluted with crude oil.

The effect of crude oil pollution on germination and growth of *Glycine max* (soy beans) was investigated by Ekpo *et al.* (2012), who found that crude oil pollution significantly ($P < 0.05$) affected the growth of *Glycine max* at higher than at lower pollution level. Similarly, Shukry *et al.* (2013) noted changes in composition of mineral elements in jojoba plants due to crude oil pollution. Omosun *et al.* (2008) examined the

response of the growth and anatomy of *Amaranthus hybridus* in crude oil polluted soils and found that plant height, number of leaves, leaf area, plant weight were higher in the control plot than in oil impacted soils. Ali *et al.* (2009) reported that crude oil pollution caused an adverse effect on olive (*Olea europaea* Linn.). Contamination of the soil by crude oil led to leaf chlorosis, dryness and death of seedlings. Plant heights and total dry weights were significantly ($P < 0.05$) reduced as a result of pollution.

Lopes *et al.* (2009) investigated the survival and morpho-anatomical modifications of the free floating water hyacinth (*Eichhornia crassipes)* and the semi-aquatic grass *Echinochloa polystachya* under different concentrations of crude oil. They observed that higher concentrations led to mortality in both species; however, lethal (LD_{50}) values showed that *E. polystachya* was more sensitive, than *E. crassipes*. Reagan (2014) in a study to investigate the effect of Bonny Light crude oil on stem sprouting of *Talinum fructicosum* observed that the different concentrations of crude oil significantly ($P < 0.05$) affected the number of leaves.

Agbogidi (2011) stated that, contamination of soil with crude oil significantly reduced biomass accumulation in *Jatropha curcas* seedlings compared with seedlings grown in uncontaminated plots. He also observed a negative interaction between soil crude oil level and weight gain in the plants. In an earlier study, Agbogidi (2010) reported that spent engine oil affected germination in six cultivars of cowpea. Similarly, Okonokhua *et al.* (2007) observed that, plant height, root number, root length and grain yield of maize were adversely affected by crude oil pollution. Kekere *et al.* (2011) reported that crude oil pollution negatively affected total leaf area, stem girth, total biomass as well as crop yield in *Vigna unquiculuta.*

Oil pollution affects plants by creating condition which makes essential plant nutrient like nitrogen, oxygen, phosphorus and potassium needed for plant growth unavailable (Adam and Duncan, 2002). Petroleum hydrocarbon may form a film on the

seed of plant, preventing the entry of oxygen and water (Adam and Duncan, 2002) and toxic hydrocarbon molecules can inhibit the activities of enzymes such as amylase and starch phosphorolase and thereby affecting the assimilation of starch (Achuba, 2006). Henner *et al.* (1999) reported that petroleum hydrocarbon consisting of small molecules and those that are water soluble are more phytotoxic for the germination of seed. Anoliefo *et al.* (2006) found that oiled shoots of crops like pepper and tomatoes may wilt and die off due to blockage of the stomata thereby inhibiting photosynthesis transpiration and respiration.

2.11 Effects of crude oil pollution on soil microbes

Soil microbes refer to the group of microorganisms for which the soil is their natural habitats (Tamames, 2010). They are made up of both prokaryotes (*Bacteria actinomycetes*, blue-green algae) and the eukaryotes (Fungi, *Microscopic algae* and the Protozoans). The diversity and activity of soil microorganisms play a key role in recycling of plant nutrients, break down of organic matter, maintenance of soil structure, fixing of nitrogen, promote plant growth and detoxification of noxious chemicals. Crude oil pollution has been reported as one of the factors that affect soil microbe activities in the soil.

Onuoah *et al.* (2003) and Franco *et al.* (2004) observed that, pollution of the soil with crude oil upset the microbial biomass thereby reducing and/or damaging it. Similarly, Olukunle and Boboye (2013) investigated the effect of crude oil pollution on soil microbes. The result indicated an alteration in the microbial community after pollution; Bacillus *spp.*, *Clostridium sporogens* and *Micrococcus luteus* were no longer present after two weeks of pollution with crude oil. Odu (1981) reported a reduction in the activities of soil microbes in crude oil polluted soil and attributed it to reduced air availability. This arises from selective destruction of aerobic bacteria and fungi thus leaving the resistant and adaptive microbial strains to proliferate.

Also, Saadoun (2002) observed a significant reduction in bacterial, streptomycetes and fungi counts in soils polluted with crude oil. Leahy and Colwell (1990), found that fresh spills and or high levels of pollution often kill or inhibit large sectors of soil microbial population, whereas soils with lower concentration of crude oil shows greater numbers and diversity of microorganisms. In addition, Saadoun (2002) reported that old-contaminated soils showed greater numbers of microorganisms, while fresh contaminated soils showed lower numbers. According to Odu (1981), the ability of soil microbes to degrade pollutants in crude oil polluted soils depends on a number of factors such as temperature, viscosity of the oil, coarseness of the soil and the oil in the environment. In tropical soils, crude oil disappears rapidly in well-drain soils but degradation is slowed by poor aeration (Odu, 1981). Roscoe *et al.* (1989) reported an increase in anaerobic microorganisms in crude oil polluted soils.

The rate of petroleum hydrocarbon biodegradation in nature is determined by the populations of indigenous hydrocarbon-degrading microorganisms, the physiological capacities of these populations plus other various abiotic factors that may influence the growth of the hydrocarbon degraders (Atlas, 1981). Leahy and Colwell (1990) confirmed that hydrocarbon biodegradation depends on the composition of the microbial community and its adaptive response to the presence of hydrocarbons.

2.12 Techniques used for remediating crude oil polluted soils

Several techniques for rehabilitating hydrocarbon polluted soils have been developed and adopted in recent times, but most of them are technically difficult, labour intensive and further degrade the valuable component of the soils. Besides being costly, most of them are only applicable to the temperate zone. Remediation methods includes physical (mechanical) and biological methods (phytoremediation). Physical methods include soil washing/leaching, excavation and landfilling, incineration and thermal desorption and vacuum extraction; biological methods are infiltration gallaries, biopiles

and land farming. Generally, biological methods are one half to one third the cost of physical methods (Toma, 1994). Physical and biological methods are outlined here reference to their particular strength and weaknesses.

a) Soil washing

Soil washing is an in-situ process employing chemical and physical extraction and separation techniques to remove a broad range of organic, inorganic, and radioactive contaminants from soils (U.S. EPA, 1989, 1990b; Everson, 1989; Anderson, 1994a). The process entails excavation of contaminated soil, mechanical screening to remove various oversized materials, separating coarse-and fine grained fractions, treatment of these fractions, and management of the generated residuals. It is a separation and volume reduction process that is typically used in conjunction with other technologies. Concentrating the contaminants in a smaller volume for further treatment enables a more overall cost-effective treatment (Anderson, 1994b).

Surface-associated contaminants are removed through abrasive scouring and scrubbing using water that sometimes is augmented by surfactants or extractions. The soil is then separated from the spent washing fluid, which carries with it some of the contaminants. The recovered soils consist of a clean coarse fraction (sand and gravel textured soils, generally > 50 um), a contaminated fine fraction (materials that floats on the washing solution). For the process to be effective, essentially all of the chemical contaminants must be associated with the fine grain fraction. The fine grain material generally requires further treatment, such as stabilization-solidification (Anderson, 1994b, 1994a; Lynch and Henes, 1989; Sims, 1990; Kim *et al.*, 1991; U.S.EPA, 1994).

The major problem using this method is that abrasive additives can cause a major harm to the natural flora and further disrupt the environment (loss of mineral cycling capacities) (Atlas and Bartha, 1993). Additional steps to remove soil additives after

clean-up, non-specificity of cleaning agents, high labour requirements and low treatment volumes may also serve to reduce efficiency and increase costs of soil washing.

b) Excavation and landfilling

This option involves excavating hydrocarbon contaminated soil with heavy equipment and placing it in a regulated landfill. When on-site landfilling is not feasible, soil must be containerized and shipped to a licensed waste manager. These factors and the need for ongoing monitoring to control fugitive leachate emissions make excavating and landfilling costly and logistically difficult to implement (U.S. EPA, 1994).

c) Incineration and thermal desorption

Thermal desorption and incineration use heat to volatilize and destroy hydrocarbon contaminants. Incineration uses a closed-vessel combustion unit to completely destroy hydrocarbon components at high temperature, whereas thermal desorption can be carried out *in* or ex-*situ* and uses lower temperature ranges to volatilize hydrocarbon components from the soil. Volatilized components are then captured and or treated. Influent/effluent streams for both processes face varying regulatory restrictions and monitoring requirements (Kostecki and Calbrese, 1990). These factors combined with low treatment volumes reduce efficiency and increase costs for large-scale treatment, making incineration and/or thermal desorption inappropriate.

d) Vacuum extraction

In vacuum extraction, a pump draws air through wells constructed above the water table within the contaminated soil. Contaminants volatilize into the vapour phase where they are then captured, treated or exhausted. This in- situ treatment method removes the need for excavation and ex-situ remediation. It is not possible, however for treatment of soils with tight formations (clay) (Kostecki and Calbrese, 1990).

e) Biopiles and landfarming

Biopiles are similar to landfarms in that they are both above-ground, engineered systems that use oxygen, generally from air, to stimulate the growth and reproduction of aerobic bacteria which, in turn, degrade the petroleum constituents adsorbed to soil. While landfarms are aerated by tilling or plowing, biopiles are aerated most often by forcing air to move by injection or extraction through slotted or perforated piping placed throughout the pile (U.S. EPA, 2007).

They can be coupled with biostimulation (addition of nutrients) and or bioaugmentation (inoculation with microbes). Biopiles involve placing soil in mounds or windrows to promote higher temperatures. For landfarming, soil is excavated, spread thinly (15-30 cm) over a large area to ensure adequate aeration and periodically tilled. The amount of equipment required depends on the degree of process control required.

Regulatory guidelines for volatile organic carbon (VOC) emissions may require that off-gases from the treatment cells be captured and treated. Biopiles and/or landfarms can be used for all soil types and can treat large volumes of soil efficiently and economically. These methods are rather costly besides, they do more damage to the environment. However, the need arises to develop more cost effective and environmentally friendly methods that will not only clean-up the environment, but will also restore the soils to its original status before the pollution. Phytoremediation which involves the use of various plant species and its associated microorganisms appears to be more promising in this regard.

2.13 Phytoremediation overview

Phytoremediation is a technology that refers to the use of green plants and its associated soil microbes to extract remove or detoxify pollutants from the soil, sediment, groundwater, surface water and waste water. It utilizes a variety of plant biological processes and the physical characteristics of plants to aid in the clean-up of contaminated

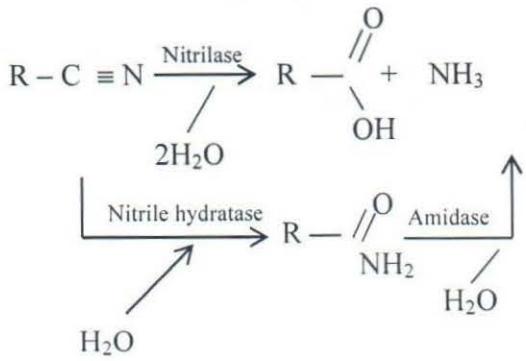
site. Phytoremediation involves series of processes within the different processes occurring at differing degrees for different conditions, media, contaminants and plants. This method of soil clean-up is potentially applicable to a variety of contaminants sujch as petroleum hydrocarbons, chlorinated solvents, metals, radionuclides, nutrients, pentachlorophenol (PCP) and polycyclic aromatic hydrocarbons (PAHs) (U.S. EPA, 2001).

Phytoremediation technology takes advantage of the natural processes of plant species. These processes include, water and chemical uptake as shown in the reaction: $2C_2OH42 + 61O_2 \rightarrow 42H_2O + 40CO_2$ metabolism within the plant, exudate release into the soil that leads to contaminant loss, the release of root associated enzymes capable of transforming organic pollutants and the physical and biochemical impacts of plant roots. The uptake and translocation of organic compounds such as petroleum hydrocarbon in plants (Briggs *et al.*, 1982; Bell, 1992 and Schnoor, 1997) are dependent on their hydrophobicity, solubility, polarity and molecular weight. Briggs *et al.* (1982) reported that the translocation of non ionized compounds to shoots of plant was optimum for intermediate polarity compounds that were moderately hydrophobic with less translocation for more polar compounds. More hydrophobic compounds have been reported to be more strongly bound to root hence resulting in less translocation within the plant (Briggs *et al.*, 1982; Schnoor *et al.*, 1995 and Cunningham *et al.*, 1996). Very soluble organic compounds will not be sorbed onto roots as well as lower solubility compound or translocated within the plant (Schnoor *et al.*, 1995). In contrast to the very soluble organic compounds, soluble inorganic compounds such as macro and micro nutrients can be readily taken up by plants. Uptake of the inorganic compounds is mediated by active or passive uptake mechanisms within the plant (Brady, 1974) whereas uptake of organic compounds is generally controlled by hydrophobicity and polarity.

Plant uptake of organic compounds has been reported to depend on the type of plant, age of the contaminant and other soil properties. Paterson *et al.* (1990) found that more than 70 organic chemicals which represented many classes of compounds were taken up and accumulated by 88 species of plants and trees. Similarly, Qiu *et al.* (1997) reported that when Pentachlorophenol (PCP) was introduced into the soil, 21% was absorbed in root system and 15 % in shoots after 155 days in the presence of grass.

Plant roots and other plant materials have been reported to have some biochemical impacts in the soil. Exudates such as simple phenolics and other organic acids can be released from living cells or the entire cell contents during root decay. These exudates can change the form of the metal and the uptake of metal ions and simultaneous release of protons, which acidified the soil and promote metal transport and bioavailability (Ernst, 1996). In some cases, the changed metals specification can lead to increased precipitation of the metals. The presence of organic compounds in the root exudates can also stimulate microbial growth in the rhizophere which in turn influence the chemical conditions within the soil ((U.S. EPA, 2001)). Contaminant loss may also be enhanced or increased as root decay due to the release of substrates and the creation of air spaces in the soil. AATDF (1998) reported that a high increase total petroleum hydrocarbon loss occurred as white clover was dying and the roots were degrading in a field study.

Another role played by plants in the degradation of petroleum hydrocarbons involved the release of enzymes from the roots. The enzymes are capable of transforming organic contaminants by catalizing chemical reactions in soil. Rao et al., (2010) reported that niger nitrilase was able to transformed a large range of different substrates at high rate as compared with bacterial nitrilase. Enzymatic pathway for hydrolysis of nitriles capable of transforming different substrates with bacterial nitrilase is shown below: Schnoor *et al.* (1995) identified plant enzymes as a causative agent in the transformation



Enzymatic pathways for hydrolysis of nitriles

of contaminants mixed with sediment and soil. Isolated enzymes systems include dehalogenes, nitroreductase, peroxidase, laccase and nitrilase. These findings suggest that enzymes may have significant spatial effects extending beyond the plant itself and temporal effects continuing after the plant has died (Cunningham *et al.*, 1996).

2.14 Mechanisms of phytoremediation

There are basically seven processes by which plants and soil microbes clean up hydrocarbon polluted soil and ground water. These mechanisms include:

- Rhizodegradation
- Phytoextraction
- Phytovolatilization
- Phytostabilization
- Rhizofiltration
- Phytodesalination
- Biological hydraulic containment

a) Rhizodegradation

Rhizodegradation also known as phytostimulation refers to a process by which plant roots in conjunction with the rhizopheric microoganisms are used to remediate soils

contaminated with organic compounds (Walton and Anderson, 1994a; Anderson and Ingram, 1993; McCutcheon and Schnoor, 2003).

Although plants and microoganisms, especially bacteria can degrade petroleum hydrocarbons independently, Atlas and Bartha (1998) reported that, it is the interaction between plants and microorganisms (rhizophere effect) that is the major mechanism responsible for the degradation of petroleum hydrocarbon in phytoremediation trials. Plant provide root exudates of carbon, energy, nutrients, enzymes, hormones like auxins, cytokinins, gibberellins (Escalante-Espinosa *et al.*,2005) and sometime oxygen to microbial populations in the rhizophere (Cunningham *et al.*,1996). Root exudates of sugars, alcohols and acids can amount to 10-20% of plant photosynthesis annually (Schnoor *et al.*, 1995) and provide sufficient carbon and energy to support a large population of soil microbes (e.g. approximately 10-100 vegetative microbes per gram of soil in the rhizophere (Erickson *et al.*, 1995). Due to these exudates provided by plants, the microbial population, especially bacteria activities are 5-100 times higher in the rhizophere than the bulk soil (Paul and Clark, 1996; Atlas and Bartha, 1998; McCutcheon and Schnoor, 2003; Joner *et al.*, 2006). This plant induced enhancement of the microbial population is believed to be responsible for the degradation of organic contaminants in the rhizophere.

Several studies serve as example of the rhizophere effect in the phytoremediation of petroleum hydrocarbons. Lu *et al.* (2010) found higher microbial numbers and activity coupled with increased degradation in hydrocarbon- contaminated soil planted with Goose grass (*Eleusine indica*) compared with the unplanted soil. The authors reported that plant roots stimulated the soil microbes, which in turn enhanced the degradation of the hydrocarbon. Similarly, Aprill and Sims (1990) observed higher degradation of petroleum hydrocarbon and higher microbial population in soils planted with *Axonopus compresus* compared to the unplanted soil. Adesina *et al.* (2014) reported higher

population of hydrocarbon utilizing bacteria and hydrocarbon utilizing fungi in crude oil polluted soils compared to the unpolluted soils. Lee and Banks (1993) observed that the plant root zone (rhizophere) has higher population of microorganisms than soils without plants growing in them; this appears to facilitate the biodegradation of organic compounds. Siciliano *et al.* (2003) reported that the mechanism responsible for phytoremediation of crude oil polluted soils is an increase in microbial activity.

A wide variety of soil microbes are reportedly involved in the oxidation or degradation of petroleum hydrocarbon. The bacteria group includes Pseudomonas spp, Arthrobacter, *Mycobacterium, Sphingomonas, Rhodococcus, Pseudomonas florescens, Pseudomonas aeroginosa, Bacillus subtilis, Bacillus anthrax, Alcaligenes spp, Acinetobacter iwoffi, Flavobacterium spp, Micrococcus species, Corynebacterium spp, Achromobacter,Nordia, Burkholderia* and *Sphingomonas* which are reported as the most active bacteria spp in the degradation of hydrocarbon polluted soils (Bossert and Bartha, 1984; Adebusoye *et al.*, 2007; Jones *et al.*, 1983). *Pseudomonas, Arthrobacter* and *Achromobacter* often occur in greater numbers with the *rhizopheric* soil than the bulk soil (Walton *and Anderson,* 1992). The fungi group consists of: *Penicillium, Fusarium, Trichoderma, Aspergillus ochraceus, Cunninghamella elegans, Phanerochaete chrysosporium, Saccharomyces corevisiae and Syncephalastrum racemosum* (Suntherland, 1992) that can degrade various polyaromatic hydrocarbon (PAHs) namely: (*anthracene, benz (a) anthracene, benzo (a) pyrene, fluoranthene, fluorine, naphthalene, phenanthrene, pyrene)* as well as methyl,-nitro-,and fluoro-substituted polyaromatic hydrocarbon (PAHs).

Sing (2006) reported a group of terrestrial fungi, namely: *Aspergillus, Cephalosporium* and *Penicillium* that have the potential to degrade crude oil hydrocarbons. Das and Chandran (2010) listed some bacterial - *Acinetobacter, Gordonia, Brevibacterium, Aeromicrobium, Dietzia, Burkholdena* and *Mycobacterium* isolated

from petroleum contaminated soil with proven to be the potential organisms for hydrocarbon degradation. Also, the degradation of polyaromatic hydrocarbons by *Sphingomonas* was reported in Daugulis and McCraken (2003). Kasai *et al.* (2002) isolated *Flavobacterium* spp. from crude oil polluted marine environment which is capable of degrading aromatic hydrocarbon in crude oil. Edlund and Jansson (2006) found that members of the class *Gramma proteobacteria* (*Pseudomonas spp.*) and *Flavobacterium species* were the most dominant bacteria in a highly polychlorinated biphenyl-polluted sediments before and after dredging. Said *et al.* (2008) isolated *Bacillus spp., Staphylococcus spp., Pseudomonas spp.* and *Acinetobacter spp.* as potential microbes capable of degrading Polyaromatic Hydrocarbons (PAH's) from a polluted sediments. Also, reported that *Acinetobacter* played major role during petroleum hydrocarbon degradation (Margesin *et al.*, 2003; Quatrini *et al.*, 2008).

b) Phytoextraction

Phytoextraction or phytoaccumulation refers to a process in which metal accumulating plants (hyper-accumulator) are used to transport and concentrate metals from the soil into the harvestable parts of roots and aboveground shoots (Brooks, 1997; Morikawa anud Erkin, 2003). According to Sinha *et al.* (2004) some plants act as both "accumulators and excluders". Accumulators survive despite the high concentration of pollutants in their aerial tissues. They have the capacity to biodegrade or biotransform the pollutants into inert forms in their tissues whereas the excluders restrict pollutant or contaminant uptake into their biomass.

Plants have evolved highly efficient mechanisms to obtain nutrients from the soil even when present at low levels. Plant roots, aided by plant-produced chelating agents and plant induced pH changes and oxidation – reduction reactions are able to solubilize and take up nutrients, especially micronutrients from very low levels in the soil, even from nearly insoluble precipitates. Another approach that has been taken to enhance

phytoextraction is the use of soil amendments to solubilize metals and bring them into the soil solution (Blaylock *et al.*, 1995). Several studies have shown that plants take up contaminants through the root system and accumulate them in the roots and shoots.

Olatunji *et al.* (2014) investigated the uptake of lead, chromium and cadmium via root, shoot and foliage of *Panicum maximum* exposed to different levels of lead, chromium and cadmium and found a general increase in their uptake by the different tissues of *P. maximum*. Accumulation of heavy metals in *P. maximum* ranged from 13 to 45% lead, 13 to 65% chromium and 11 to 52% cadmium with tissue abundance decreasing in the order chromium > lead > cadmium. Generally the concentration of heavy metals in *Panicum maximum* tissue decreased in the order root > stem > foliage.

Cho-Ruk *et al.* (2006) noted that some species of plants have been successful in phytoaccumulating contaminants such as cadmium, lead, chromium, arsenic and various radionuclides from soils. Phytoextraction, one of the phytoremediation method can be used by plants to extract heavy metals from soil using its ability to uptake metals which are essential for plant growth (Fe, Mn, Zn, Cu, Mg, Mo and Ni). Some metals with unknown biological function (Cd, Cr, Pb, Co, Ag, Se, Hg) can also be accumulated. In a green house study to evaluate the efficacy of three tropical grasses, *Vertiver* (*V. zizanoides*) imperata (*L. cylindrica*) and *Pennisetum purpureum* to phytoremediate heavy metals, Ng *et al.* (2016) observed that the three grasses showed significantly high ($P < 0.05$) accumulation of heavy metals in the plant tissue.

In a field sampling to assess the phytoextraction of Pb, Cu, Cr, Cd and Zn in cement polluted soil using *Sida acuta* and *Pennisetum purpureum*, Ogunkunle *et al.* (2014) observed that *Sida acuta* and *Pennisetum purpureum* were able to phystabilize Cr, Cd and Zn while Cr and Cd were phytoextracted from the cement polluted soil. Abdel-Salam (2012) reported that elephant grass was the most efficient phytoremediator for cadmium followed by sunflower and sorghum.

Chen and Cutright (2001) documented the influence of different levels of EDTA on the phytoextraction ability of *Pennisetum pedicellatum* for cadmium and zinc and determined that Cd and Zn uptake in the root and shoot was generally high. Schnoor (2002) reported that, phytoextraction is more effective with vigorously growing plants that are easily harvested and which accumulate large concentration of contaminants in harvestable form. Njoku *et al.* (2009) stated that, the capacity of a given plant species to reduce the level of crude oil in oil contaminated soil can help to restore polluted soils back to its original state for agricultural use. Some plant species have shown the ability to absorb and hyper-accumulate metal contaminant such as lead, cadmium, chromium, arsenic and various radionuclides from soil (Tanngahu *et al.*, 2011).

c) Phytovolatilization

Phytovolatilization involves contaminants being taken up by the roots of plants, converted to a gaseous state and released into the atmosphere by transpiration. Phytovolatilization may also refer to the diffusion of contaminants from the stems or other plant parts that the contaminants travel through before reaching the leaves (McCutcheon and Schnoor, 2003). This process of phytoremediation uses a solid or liquid contaminant and transforms it to an airborne vapour. The vapour can either be the pure pollutant or the pollutant can be metabolized by the plant before it is vapourized as in the case of mercury, lead and selenium (Boyajian and Carriera, 1997; Black, 1995; Wantanabe, 1997).

Phytovolatilization is mainly applied to groundwater, but it can also be applied to soil, sediments and sludge.

d) Phytodegradation

Phytodegradation refers to the degradation of organic contaminants within the plant tissues. Plant produces enzymes such as dehalogenase and oxygenase that help catalyze degradation by changing contaminants to less toxic substances.

Phytodegradation has been observed to remediate some contaminants such as chlorinated solvents, herbicides and munitions and can also remove contaminants in soil, sediments or groundwater (U.S. EPA, 2006).

e) Rhizofiltration

Rhizofiltration is the adsorption of contaminants onto plant roots or adsorption of contaminants in the solution surrounding the rhizophere. Rhizofiltration is similar to phytoextraction, but the plants are used primarily to address contaminated groundwater rather than soil. In rhizofiltration process, the contaminants are removed from the soil when the plant is harvested.

f) Phytostabilization

Phytostabilization involves the use of certain plant species to retain contaminants in the soil and prevent further mobility of the contaminants to the ground water, and it also reduces bio-availability of metal into the food chain. In phytostabilization process, contaminants can be stabilized in the roots or precipitated within the rhizophere. Phytostabilization is used to remove metals and other inorganic contaminants in soil and sediment (U.S. EPA, 2001)

g) Phytodesalination

Phytodesalination involve a process where halophytes (plants adapted to saline soil) are used to extract salt from the soil tp improve fertility.

h) Biological hydraulic containment

Biological hydraulic containment refers to a process wher plants draw water upward through the soil into the roots and out through the plant, which decreases the movement of soluble containments downwards, deeper into the site and into the ground water.

2.15 Factors affecting phytoremediation

a) Choice of plant

In phytoremediation process, plant species are screened and those that have a high remediating potential are selected (Prasad *et al.*, 2003). Plant species are the major determinants of the uptake of contaminants (Burken and Schnoor, 1996). The success of any phytoremediation techniques depends on the identification of suitable plant species that can produce large amounts of biomass using established crop production and management practices (Rodriguez *et al.*, 2005).

b) Soil type

Soil type entails various characteristics such as soil structure, texture and organic matter content. Alexander *et al.* (1997) reported that phenanthrene (group of chemicals called polycyclic aromatic hydrocarbons) may be trapped within and sorbed to the surfaces of nanopores (nano particles) that are inaccessible to organisms.

Soil texture can also affect the mechanisms of phytoremediation by influencing the bioavailability of the contaminant Brady, (1974) observed that clay is capable of binding molecules more readily than silt and sand. Similarly, Carmicheal and Pfaender (1997) found that soils with larger particles (e.g sand) typically had greater mineralization of polycyclic aromatic hydrocarbon (PAHs) than soil with smaller particles (e.g silt and clay) possibly due to the greater bioavailability of the contaminants in the sandy soil. Edward *et al.* (1982) found that the amount 14^C anthracene taken up by soybean in soil was considerably lower than the amount taken up by plants in nutrient solution.

Cunnningham *et al.* (1996) observed that soil organic could bind lipophilic compounds thereby, reducing their bioavailability. A high organic matter content (>5 %) in soil usually leads to strong adsorption, hence, low availability while a moderate organic matter content (1-5 %) may lead to limited availability (Otten *et al.*, 1997).

c) Weather

Phytoremediation is mostly affected by the differences in climatic zone. For example, in temperate climate, phytoremediation is restricted to the growing period only whereas in the tropic, plant growth occurs all year round (Kamath *et al.*, 2004). Among the elements of climate, temperature affects the rates at which the various mechanisms of phytoremediation take place. Eweiss *et al.* (1998) and Wright *et al.* (1997) reported that the rate of microbial degradation or transformation doubles for every10°C increase in temperature.

d) Water availability

Some researchers like Kamath *et al.* (2004) have shown that irrigation of contaminated site enhanced bioremediation of certain diesel substances. For terrestrial phytoremediation application, irrigation of plants at the initial stage of the project is important as this will encourage the growth of plant and the adsorption of contaminants. Beside, water is important to the general health of plants and soil microbes (Eweiss *et al.*, 1998). Water is not only a major component of living organism; it also serves as a medium to carry nutrients to soil biota. If the moisture content of the soil is low, there will be a loss of microbial activity and dehydration of plants. Too much water results in limited gas exchange and the creation of anoxic zones where degradation is dominated by anaerobic microorganisms.

e) Oxygen requirements

Soil microbes require oxygen for efficient degradation of petroleum hydrocarbon. In phytoremediation process, a plant can act as a net positive or negative oxygen source (Lee *et al.*, 1995). Plant acts as a net positive source by relying on organs such as parenchymatous cells to transport oxygen to the rhizophere, thereby, enhancing aerobic biological degradation (Shimp *et al.*, 1993). As a net negative source, Rentz *et al.* (2003) documented the stimulation of hybrid poplar growth and increased root density of poplar

with the addition of oxygen releasing compounds when plants were grown in crude oil smear zone soils.

f) Cost of implementing phytoremediation

Several studies have shown that the cost of implementing phytoremediation is lower than that of traditional processes such as soil excavation, pump-and-treat, soil washing or extraction (U.S. EPA, 2001). Apart from the cost incurred during the initial stage of planting the vegetation, a field-scale phytoremediation project involves preliminary greenhouse experiments with soil testing, expenditure on design, site preparation, reporting, monitoring, operation and maintenance (Green and Hoffnagle, 2004).

2.16 Techniques used to enhance phytoremediation

Several techniques especially agronomic can be used to enhance the effectiveness of phytoremediation. These techniques include the application of fertilizer and the use of phytohormonal treatment (Plant hormones).

a) Fertilizer application

Crude oil polluted soils are usually deficient in both macro and micro nutrients which are essential for healthy plant growth and stimulation of microbial contaminant degradation (Kamath *et al.*, 2004). The application of fertilizer is one of the major factors that favour phytoremediation. Ijah *et al.* (2008) and Adedokun and Ataga (2007) documented that soil amendments are needed to increase microbial activities in the soil and for effective bioremediation of contaminated soil. Similarly, Okolo *et al.* (2005) and Obasi *et al.* (2013) reported an increase in the decomposition or degradation of hydrocarbon contaminated soil augmented with poultry manure. More total petroleum hydrocarbon was lost from soils augmented with cow-dung than from the non-augmented soils (Njoku *et al.*, 2009).

Mbah *et al.* (2009) documented that amendment of spent engine oil polluted soil with organic manure improved soil physical properties and increased agronomic parameters. Wilson (2004) observed that soil amendments enhanced soil water retention, permeability, infiltration and drainage as well as aeration structure of the soil. Amadi *et al.*, (1993) reported that organic sources of nitrogen are better than inorganic sources probably due to their slow release of nitrogen which inturn help to improve soil structure and soil water relationship for plant growth.

Eneje and Uwumarongie-Ilori (2012) reported that use of poultry droppings and green manure either singly or in combination improved the chemical properties of crude oil polluted soil or in turn enhanced the solubility and removal of contaminants. Nutrient supplementation of crude oil polluted soils with poultry manure was beneficial for the growth of maize and biodegradation of oil and soil recovery (Obire and Anyanwu, 2009). Ogboghodo *et al.* (2004) also reported that adding chicken manure to soil contaminated with crude oil triggered degradation of 75 % of hydrocarbon in the soil within 2 weeks and suggested the use of chicken manure to stimulate crude oil degradation in the natural ecosystem. Millioli *et al.* (2005) noted that biological treatments were more efficient and cheaper than chemical and physical ones. However, the solubility and adsorption capacity of high molecular weight hydrocarbons limit their availability to micro-organisms. Hence addition of organic materials such as poultry and green manure singly or in combination to improve the chemical properties of the polluted soil will enhance the solubility and removal of these contaminants, improving oil biodegradation rates. Daniel-Kalio and Braide (2003) and Akonye and Onwudiwe (2004) indicated that plant sources such as sawdust and *Chromoleana odorata* L. popularly known as Siam weed have chemical and biological characteristics for amending polluted soils.

b) Phytohormonal treatment (brassinolide)

The use of hormone or phytohormones (Brassinolides) may also play an important role in phytoremediation trials. Brassinolide is a plant growth hormone, derived from Brassica, a genus of plants in the mustard family (Brassicaceae). It is one of the essential plant hormones that make the root of plant stronger and improves the ability of resistance to insects and diseases. It can strengthen the ability of plants to resist harsh environmental conditions like cold, drought, contaminated soil and it improves the uptake and translocation of micro and macro nutrients, thereby increasing plant growth and development (Sasse, 1997). Brassinolide has also been reported to regulate differentiation in tissue culture, promote cell elongation, decrease the chances of flower / fruit dropping, and is also an important element for plant growth and yield improvement.

2.17 Advantages of phytoremediation

The main advantage of phytoremediation is that it is an in situ solar driven technique. Besides being cost-effective, it is potentially less harmful to the environment.

a) The plant can be easily monitored

b) Since phytoremediation uses plants, it is aesthetically pleasing

c) The establishment of vegetation may help reduce erosion by wind and water (Wilson, 2004)

d) Phytoremediation creates habitat for animals, promote biodiversity, and can also restore ecosystems that were previously disrupted by human activity (U.S. EPA, 2006; Wilson, 2004).

2.18 Disadvantages of phytoremediation

a) Phytoremediation work best on site with low contaminants (U.S.EPA, 2006)

b) The survival of the plants is affected by the toxicity of the contaminated soil and the general condition of the soil.

c) Toxic substances may enter the food chain through grazers, birds or other animals that consumed the leaves and seeds of plant used for phytoremediation. (U.S.EPA, 2001).

d) With plant- based systems of remediation, it is not possible to completely prevent the leaching of contaminants into the groundwater without the complete removal of the contaminated ground, which in itself does not solve the problem of contamination.

e) The burning of plant leaves or branches containing harmful chemicals could contaminate the air.

f) It requires more space and time than the alternative remediation method.

g) The practice of phytoremediation techniques may be seasonal depending on location.

h) Phytoremediation is limited to the surface and depth occupied by the root.

2.19 Field application of phytoremediation using different plant species

Although phytoremediation technology is relatively new, the application of plant species to phytoremediate crude oil polluted soils has been documented by many researchers.

Various plant species have been recognized for their effectiveness in remediating soils contaminated with petroleum hydrocarbons (Table 1). In several studies, grasses and legumes have been preferred relative to others due to their potential to clean- up crude oil polluted soils (Aprill and Sims, 1990; Qiu *et al.*, 1997; Gunther *et al.*, 1996; Reilley *et al.*, 1996). However, Udom *et al.* (2004) reported that some shrubs like *Gliricidia sepium, Leuceana leucocephala and Calapogonium cerulean* combined with poultry manure significantly reduced the toxicity levels of heavy metals (Ni,Pb, Zn, Cu) found in spent engine oil contaminated soils. Plant species differ in their potentials to remediate crude oil contaminated soils (Liste and Felgentreu, 2006).

Grasses are considered superior to others probably due to their rapid growth rate, fine roots, large biomass production, strong resistance and effective stabilization of soils and therefore, usually result in excellent restoration of degraded soils, especially in the tropics and sub-tropics with high temperature and precipitation (Xia, 2004). Legumes are also preferred over non-leguminous plants because of their potential to fix nitrogen in infertile soils. They do not have to compete with soil microbes and other plants for inadequate supplies of available soil nitrogen at oil contaminated sites (Gudin and Syratt, 1975). Consequently, *Calapogonium mucunoides* and *Centrosema pubescens* would also be good phytoremediating plants since they also exhibit the ability to fix nitrogen with the help of some microorganisms found in their root nodules. Several studies on the use of plants for remediating crude oil polluted soils have been documented.

Aprill and Sims (1990) established a mixture of eight Prairie grasses in sandy loam soil to ascertain the degradation of four poly aromatic hydrocarbons was stimulated by plant growth. The extent of poly aromatic hydrocarbon (PAHs) reduction was greater in planted units compared to unplanted controls, showing that phytoremediation enhanced the removal of these compounds from contaminated soil. Apparent disappearance was greatest for benzo (a) anthracene followed by chrysene, benzo (a) pyrene and dibenzo (a, h) anthracene. This ranking correlated with the water solubility of the poly aromatic hydrocarbon (PAHs) compounds, i.e., the more water soluble the compound, the greater its disappearance from the polluted media.

Muratova *et al.* (2008) established a mixture of fifteen plants species to test their potentials for remediating a former oil-sludge pit. The extent of oil-sludge degradation was higher in planted units compared to the unplanted controls. Oil sludge as a whole was degraded successfully in soil planted with prairie grasses in the order: Perennial rye grass (46%) > crested wheatgrass (45%) > cough-grass (44%). The most effective phytoremediation was in the soil planted with rye grass (52%). Among the legumes, apparent degradation was greatest for alfalfa (41%), whereas the total petroleum

hydrocarbon reduction in the unplanted control was 34%. Gunther *et al.* (1996) found that soil planted with ryegrass lost the greatest amount of a mixture of hydrocarbons than soil that was unplanted. Parrish *et al.* (2005) observed that root maturity in ryegrass, tall fescue and yellow sweet clover contributed to the reduction in the bioavailability of poly aromatic hydrocarbons (PAHs) while Smith *et al.* (2006) reported that fescue, ryegrass, birds foot-trefoil and clover significantly reduced naphthalene and other poly aromatic hydrocarbons (PAHs) in the rhizophere.

Yateem *et al.* (2000) studied the decomposition of TPH in the rhizophere and non-rhizophere soil using three domestic plants and found that degradation was more profound with leguminous plants. Adam and Duncan (2002) found that the legume plant (*Vicia sativa*) was able to grow in soil contaminated with diesel fuel and the total number of root nodules on contaminated plants were more developed than nodules on control plants. These authors found that after four (4) months, the amount of diesel fuel left in the legume plant *Vicia sativa* was slightly less than in the rye grass planted soil.

Tanhan, (2011) listed *Chromoleana odorata* as one of the plants that has high bioaccumulation and translocation potential for heavy metals. Likewise, Atagana (2011) investigated the potential of *Chromoleana odorata* to decontaminate used engine oil impacted soil under a greenhouse condition. The residual TPH lost from the planted soil after 90 days was between 21 and 100%. Tanhan (2011) and Anoliefo *et al.* (2003) proposed that *Chromoleana odorata* could be used for phytoextraction of lead contaminated soil.

In a comparative assessment of the crude oil remediating potential of *Cynodon dactylon* and *Eleusine indica* in a screen house experiment with various dosage of crude oil concentrations, Oyedeji *et al.* (2012) reported that fresh and dry mass of *Eleusine indica* was significantly higher than *Cynodon dactylon*. Similarly, Lu *et al.* (2009) investigated the potential of Goose grass (*Eleusine indica*) for phytoremediation of soil

contaminated with TPH and noted that the level of contamination was reduced by 47% in planted soil and 11% in unplanted soil. Polycyclic Aromatic Hydrocarbon (PAHs) was removed by 32% in vegetative soil but only 5% was dissipated in the unvegetated pot. Merkl *et al.* (2005) attributed the increase in biomass and reduction in TPH/PAHs to microbial activities in the rhizophere which in turn helps in accelerating the degradation processes of hydrocarbon.

Meng *et al.* (2011) studied the effects of mono or mixed cultures of different plant species on petroleum aromatic hydrocarbon (PAH) phytoremediation and found that some multispecies mixtures facilitate the phytoremediation of PAH polluted soils over monocultures. Noori *et al.* (2012) examined some species of Fabacea family planted in different concentrations of oil polluted soil and recommended those species as potential phytoremediation plant.

Njoku *et al.* (2012) evaluated the comparative effects of *Abelmoschus esculentus* and *Corchorus olitorius* on soil contaminant with mixture of petroleum products and concluded that *A. esculentus* had better remediating potentials than *C. olitorius*. Ighovie and Ikechukwu (2014) observed that *Axonopus compressus* reduced the TPH by 66 % and raised the pH of crude oil impacted soils of Ubeji and Alesa Eleme in River States.

The suitability of *A. compressus* for the removal of petroleum hydrocarbon from contaminated soils was assessed for a period of 360 days. Stephen *et al.* (2013) observed that the TPH was reduced by 70% in fertilized vegetated soil and 40% in unfertilized unvegetated soils. The rate of degradation was significantly greater in vegetated plots than unvegetated plots due to microbial action and natural attenuation.

In a three months experiment to assess the efficacy of *Axonopus compressus* and nut sedge (*Cyperus rotundus*) in the management of hydrocarbon polluted soil, Efe and Okpali (2012) reported that, the combined effect of *A. compressus* and soil amendment accounted for 59 % reduction in hydrocarbon while *A. compressus* and nutsedge

TABLE 1

Plants with demonstrated potential to tolerate and phytoremediate hydrocarbon polluted soils.

Elephant grass	*(Pennisetum purpureum)*
Siam weed	*(Chromoleana odorata)*
Bermudia grass	*(Cynodon dactylon)*
Soybean	*(Glycine max)*
Switch grass	*(Panicum virgatum)*
Bush beans	*(Phaseolus vulgaris L)*
Vertiver grass	*(Chrysopogon zizanioides*
Guinea grass	*(Panicum maximum)*
Goose grass	*(Eleusine indica)*
Duck weed	*(Lemma gibba)*
Sudan grass	*(Sorghum vulgare L)*
Sorghum	*(Sorghum bicolor)*
Groundnut	*(Arachis hypogea)*
Maize	*(Zea mays L)*
Sunflower	*(Helianthus annus)*
Round sedge	*(Carex rotundata)*
Rock sedge	*(Carex rupestric)*
Water sedge	*(Carex aquatilis)*
Carrot	*(Daucus carota)*
Field pea	*(Pisum arvense)*
Big bluestem	*(Andropogon gerardi)*
Bell Rhodes grass	*(Chloris gayana)*
Verde klein grass	*(Panicum coloratum var. verde)*
Wheat	*(Triticuma estivum)*
Perennial rye grass	*(Lolium perenne L.)*
Blue grama	*(Bouteloua gracilis)*
Common buffalo grass	*(Buchloe dactyloides)*
Millet	*(Panicum miliaceum L)*
Couch grass	*(Agropyrum tenerum L)*
Barley	*(Hordeum vulgare)*
Smooth broom	*(Brompsi sinermis)*

Sources: Apill and Sim, 1990; Baily and McGill, (1999); Belford *et al.* (2009); Atagana (2011) Olatunji *et al.* (2014); Frick *et al.* (1999); Anoliefo *et al.* (2006); Reilly *et al.* (1999); Pradhan *et al.* (1998); Wild *et al.* (1992); Qiu *et al.* (1997); Ayotamuno *et al.* (2006); Nascimento *et al.* (2006); Njoku *et al.* (2008); Muratova *et al.* (2008); Otaraku *et al.* (2014); Lu *et al.* (2009); Agamuthu *et al.* (2010).

(*Cyperus rotundus*) accounted for 47 % and 48 % reduction in petroleum. Although the two plant species can be used successfully in phytoremediation trials for the reclamation of hydrocarbon impacted soils, the combined effects of *A. compressus* nutsedge (*Cyperus rotundus*) and soil amendment (organic and inorganic) fertilizer was the most effective method for reducing TPH in soils.

Basumatary *et al.* (2013) examined the potentials of two sedge weeds namely (*Cyperus rotundus (Linn)* and *Cyperus brevifolius (Rottb) Hassk* for enhanced degradation of petroleum sludge contaminated soil. The authors reported a 75% decreased in TPH in fertilized soils under *C. rotundus* and 64% under *C. brevifolius*. Total petroleum accumulation in root, shoot were higher in fertilized than unfertilized soil. In a net house study, Budhadev *et al.* (2012), found that crude oil degradation was higher in pots vegetated with C. rotundus than the unvegetated pots

Mathur *et al.* (2010) examined the rhizophere of *Terminalia arjuna* (L.) Druce, *Anogeissus latifolia* (L.) Wild and *Tecomella undulate* (L.) Wild. They found a greater reduction (26 %) in TPHs in the rhizophere soil of *T. arjuna* than that of *A. latifolia* and *T. undulate* respectively. In a short term ecological study to assess the phytoremediating potential of *L. leucocephala* and *Bauhinia monandra* at different levels of crude oil pollution, Edwin-Wosu and Albert (2010) found that *L. leucocephala* absorbed more of the TPH than *B. monandra*. Ekpo *et al.* (2012) suggested that cocoa pod husk due to its buffering capacity was a good bioremediating agent and should be used in the phytoremediation trials of crude oil polluted soils.

In a four (4) week greenhouse study to assess the phytoremediation potential of *Panicum maximum* (guinea grass) for selected heavy metal removal from contaminated soils, Olatunji *et al.* (2014) observed that *P. maximum* (guinea grass) generally accelerated the reduction in concentrations of lead (Pb), chromium (Cr) and cadmium (Cd).

Ayotamuno *et al.* (2006) examined the potential of corn and elephant grass for the phytoremediation of a petroleum hydrocarbon contaminated agricultural soil, and showed that an average hydrocarbon loss of 77.5% for *Zea mays* and 83% for *P. purpurum* within the first two weeks, with values decreasing to 67.5% and 55% after six weeks of remediation. However, the corn plus elephant grass treatment showed hydrocarbon losses of 62% and 74% for the two and six weeks of the study. Xia (2004) reported that ecological rehabilitation with *P. purpureum* (elephant grass) enhanced the phytoremediation of an oil shale mined land contaminated with heavy metals.

Agamuthu *et al.* (2010) studied the effectiveness of *Jatropha curcas* to remediate soil contaminated with used lubricating oil and amended with organic wastes. After 180 days, between 56.6and 67.3% reductions in waste lubricating oil was recorded in *Jatropha* remediated soil without organic amendment. However, addition of organic waste to *Jatropha* remediation rapidly increased the removal by 87 - 97 %. The *Jatropha* plant root did not accumulate hydrocarbon from the soil, but the number of heterotrophic utilizing bacteria were higher in the rhizophere of the *Jatropha*, suggesting that the mechanism of the oil degradation was via rhizodegradation.

Schwab *et al.* (1985) showed that mineralization of [14C] phenanthrene was significantly higher in the grass family, with sorghum (0.46 %) and bermudia grass (0.31 %) than the unplanted control pot with 0.11 %. Similarly, Merkl *et al.* (2005) found that leguminous plants died within six to eight weeks in heavily crude oil polluted soil, while grasses showed reduced biomass production. However, a positive correlation between root biomass and oil degradation was found. Muratova, (2008) observed that several plant species (Italian ryegrass, sorghum, maize, alfalfa, bermudia grass, rice, kudzu and begger tricks) caused a more significant decrease in the diesel contaminated soil.

Diab (2008) observed reductions in TPH in the rhizophere by 30 % for broad bean, 17 % for corn and 14 % for wheat. Peng *et al.* (2009) reported a 42 – 63 % removal of

TPHs by *Mirabilis jalapa*. *Cyperus laxus* Lam also yielded a significant reduction in TPH when raised on hydrocarbon contaminated soil (Lopez-Martinez *et al.*, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Greenhouse experiment

The experiment was carried out in the greenhouse of the Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus in OrukAnam Local Government Area.

3.2 Experimental materials / sources

The crude oil was obtained from Shell Petroleum Development Company (SPDC) Limited, Port Harcourt, Rivers State, Nigeria. Top soil was taken from the Teaching and Research Farm of the Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus.

Seeds of *Jatropha (Jatropha curcas)* and stems of water leaf (*Talinum fructicosum*) were purchased from the local market; grasses and legumes were transplanted within the experimental area while stem of Gliricidia sepium was collected from the Forestry Department of the University of Uyo, Akwa Ibom State. Other materials used were four plastic jerry cans, weighing scale and 152 perforated plastic buckets (5 litres each).

3.3 Soil sampling and processing

Composite surface soil samples (0-30cm) were randomly collected from the Teaching and Research Farm of the Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus. The bulk samples were air dried, crushed and sieved through a 2 mm mesh sieved. The sieved samples were used in the determination of particle size distribution and chemical analysis while the core samples were used for the determination of some physical characteristics.

3.4 Treatment application and planting

The potentials of some native plant species to remediate crude oil polluted soils were studied using perforated plastic buckets (5 litres capacity) filled with 5 kg of the 2 mm sieved soils. The potted soils were treated with various levels [2.5% (147.5ml) 5% (295ml), 7.5% (442.5 ml and 0 % (w/w) (control)] of crude oil. The oil was thoroughly mixed with the soil for even distribution and then watered to field capacity as and when necessary. One week after treatment, *Jatropha* seedlings, *Leuceana leucocephala* and water leaf stems averaging 5 cm in length were transplanted from the nursery with one seedling per pot to a depth of 5 cm. *Axonopus compressus, Pennisetum purpureum, Eleusine indica, Panicum maximum, Cyperus rotundus* and *legumes: Calapogonium mucunoides* and *Centrosema pubescens* were transplanted within the experimental area. *Gliricidia sepium* was planted by stem cuttings. The experiment was a factorial combination of 13 plant species and four levels of pollution (Table 2). The pots were irrigated on the day of sowing and at regular intervals to maintain soil moisture at field capacity. The duration of the pot experiment was four months. Soil samples were collected at 2 and 4 months for laboratory analysis.

3.5 Microbial Analysis

The following microbial parameters of the soils were determined at two (2) and four (4) months. These include:

3.5.1 Enumeration of total heterotrophic bacteria

Surface spreading technique was used to count the total number of heterotrophic bacteria in the contaminated soil sample (Antai and Mgbomo, 1989). Serial dilutions of the soil samples were prepared from 10^{-1} to 10^{-4} and 1ml of 10^{-6} dilution was plated out onto nutrient agar plates to which $50ugm^{-1}$ of nystatin was incoperated to inhibit fungal growth (William and Gay, 1973). The plates were prepared in duplicates and incubated

TABLE 2

Treatments for the pot experiment

Treatment code	-	Description
Plant species		
V_o	-	No planting (control)
V_1	-	Carpet grass (*Axonopus compressus*)
V_2	-	Elephant grass (*Pennisetum purpureum*)
V_3	-	Goose weed (*Eleusine indica*)
V_4	-	Guinea grass (*Panicum maximum*)
V_5	-	White leadtree (*Leuceana leucocephala*)
V_6	-	*Gliricidia (Gliricidia sepium*)
V_7	-	Waterleaf (*Talinum fructicosum*)
V_8	-	Siam weed (*Chromoleana odorata*)
V_9	-	Nut sedge weed (*Cyperus rotundus*)
V_{10}	-	Calapo (*Calapogonium mucunoides*)
V_{11}	-	Jatropha (*Jatropha curcas L*)
V_{12}	-	Centro (*Centrosema pubescens*)
Crude oil pollution		
P_o	-	No pollution (control)
P_1	-	Pollution at 2.5 % (w/w)
P_2	-	Pollution at 5.0 % (w/w)
P_3	-	Pollution at 7.5 % (w/w)

TABLE 3

Treatment combinations for the pot experiment

V_0P_0	V_oP_1	V_oP_2	V_0P_3
V_1P_0	V_1P_1	V_1P_2	V_1P_3
V_2P_0	V_2P_1	V_2P_2	V_2P_3
V_3P_0	V_3P_1	V_3P_2	V_3P_3
V_4P_0	V_4P_1	V_4P_2	V_4P_3
V_5P_0	V_5P_1	V_5P_2	V_5P_3
V_6P_0	V_6P_1	V_6P_2	V_6P_3
V_7P_0	V_7P_1	V_7P_2	V_7P_3
V_8P_0	V_8P_1	V_8P_2	V_8P_3
V_9P_0	V_9P_1	V_9P_2	V_9P_3
$V_{10}P_0$	$V_{10}P_1$	$V_{10}P_2$	$V_{10}P_3$
$V_{11}P_0$	$V_{11}P_1$	$V_{11}P_2$	$V_{11}P_3$
$V_{12}P_0$	$V_{12}P_1$	$V_{12}P_2$	$V_{12}P_3$

PLATE 1: Picture showing crude oil polluted soil creating anaerobic conditions.

0.1ml of 10^{-2} was plated onto mineral salt agar medium containing 15 $megml^{-1}$ of streptomycin to inhibit bacterial growth. After inoculation of the agar plates with the samples, a sterile filter paper (Whatman No.1) soaked with crude oil was aseptically placed onto the inside cover of the petri dishes to act as a source of carbon and energy for the growth of microorganisms through vapour pressure phase transfer. All plates were prepared in duplicates inverted and incubated at 28°C for 7days before the colonies were counted and expressed as colony forming units per gram of the soil ($CFUg^{-1}$). The isolates were sub-cultured onto freshly prepared sterile malt extract agar plates.

3.5.5 Purification and maintenance of microbes isolated

The bacterial and fungal isolates obtained from mineral salt medium were purified by repeated sub-culturing. The isolates were subjected to series of transfer unto fresh medium. The bacterial isolates were transferred onto fresh nutrient agar medium and incubated at 27°C for 24 hours. Fungal isolates were transferred onto fresh malt extract agar and incubated at 28°C for 3 days. Pure colonies of bacteria and fungi were maintained on slopes of nutrient agar and malt extract agar slant and stored in a refrigerator at 8°C.

3.5.6 Characterization and identification of the isolates

Standard inocula were prepared from the preserved stock culture by taking a loopful of the isolates and a septically inoculating onto sterile nutrient agar plates. The plates were incubated at 28°C for 24 hours. The characterization of the bacteria isolated was performed using gram staining reaction, spore staining oxidase test, methyl red vogesproskakeuer (MR-VP) test, indole test, citrate test, urease test, coagulase test and sugar fermentation test (Mac-Faddin, 1980). The fungal isolates were examined macroscopically and microscopically using the wet mount method (cotton – blue in lactophenol). Fungal identification was carried out using the method of Hunter and Bennett (1973).

3.6 Calculation for the Different Concentrations (2.5, 5.0 and 7.5 % w/w) of Crude Oil Used for the Study

100g = 118ml

$$\frac{x}{10{,}000g\ soil} \times \frac{100}{1} = \frac{5}{1}$$

Where x = amount of crude oil

5kg = of soil used for the experiment

$$\therefore \quad \frac{x100}{10{,}000} = 5$$

$100x = 10{,}000 \times 5$

$$\frac{10{,}000\ x\ 5}{100} = 500g$$

500g of crude oil = 5kg

∴ ½ of 500g = 250g

250g of crude oil +5kg = 5% w/w

If 100g = 118ml

$$\therefore 250\ g = \frac{250\ x\ 118}{100} = 295ml$$

∴ 295ml crude oil + 5kg = 5% w/w

147.5ml = 2. 5% w/w

442.5ml + 5kg of soil = 7.5% w/w

3.7 Laboratory analysis of soil samples

The soil samples collected before and after treatment application were analyzed following standard procedures as outlined in Udo *et al.* (2009). The following properties were determined:

3.7.1 Particle size distribution

Particle size distribution was determined by Bouyoucos hydrometer method, using sodium-hexametaphosphate as a dispersing agent. The soil texture was determined from percent sand, silt and clay using the USDA textural triangle.

3.7.2 Bulk Density

Bulk density was determined by core procedure as described by Blake, 1965. The core samples were dried in oven at 105°C to a constant weight (W2). The bulk density was determined using the equation:

$BD = \frac{W_2 - W_1}{V} = \frac{MS}{VS}$ (Blake, 1965)

Where:

Bd = Bulk density (g/cm^3)

W1 = Weight of empty core (g)

W2 = Weight of soil + core (g)

Ms = Mass of dry soil (g)

Vs = Volume of soil (cm^3)

3.7.3 Total porosity

Total porosity was calculated using the equation below:

$P = (\frac{1-Bd}{Bp}) \times 100\%$

Where P = total porosity (%) (m^3m^{-3})

Bd = bulk density (mgm^{-3})

Bp = particle density (2.65 mgm^{-3})

3.7.4 Soil reaction

Soil pH was determined using glass electrode pH meter in 1:2.5 soil to water ratio.

3.7.5 Organic carbon

Organic carbon was determined using Walkley Black wet oxidation method. The value was multiplied by 1.724 to obtain organic matter content.

3.7.6 Total nitrogen

This was determined by macrokjeldal digestion metho and distillation method as describe by Udo *et al* (2009)

3.7.7 Available phosphorus.

Available phosphorus was determined using Bray-lextraction method and the content of P was determined by the molybdenum blue color technique of Murphy and Riley (1962).

3.7.8 Base saturation

Base saturation was determined by the summation of total exchangeable base/ECEC x 100.

3.7.9 Exchangeable acidity

Exchangeable acidity was extracted with IM KCL solution and the acidity in the extracts was measured by titration with 0.01M NaOH.

3.7.10 Effective cation exchange capacity (ECEC)

Effective cation exchange capacity (ECEC) was calculated by the summation of total exchangeable cation and exchangeable acidity.

3.7.11 Exchangeable cations (Ca, Mg, K and Na)

These were determined with IN ammonium acetate (pH 7.0) using 1:10 soil/ liquid ratio, Ca^{++} and mg^{++} in the filtrate was determined with atomic absorption spectro-photometer (AAS) Perkin Elmer 403 while Na^{+} and K^{+} was determined with a flame photometer.

3.8 Determination of plant biomass

Plant biomass (shoot and root) was determined at the end of the experiment at four (4) months after pollution using the methods described in Edwin-Wosu and Kinako (2004). The fresh weight was obtained using a weighing balance. The entire plants of known fresh weight were oven dried at 75°C for 72 hrs for dry matter determination. The dried plants were reweighed to obtain the dry weight.

3.9 Determination of total petroleum hydrocarbons in the soil

The amount of crude oil in the soil sampls was determined using air- dried soils that were sieved through 1mm mesh. The crude oil in the soil was first extracted with n-hexane by shaking with a mechanicl shaker for 30 minutes as was described by Okolo, Amadi and Odu (2005). The soil crude oil n-hexane mixture was filtered into a beaker of known weight through a Whatman No.1 filter paper. The crude oil content of the filtrate was determined after heqating the beaker at 40 % C to a constant weight (Merk, Schutze-Kraft and Infante, 2005). The amount of crude oil lost from the soil was determined as the amount of crude oil added to the soil minus that in the soil at 2 and 4 months at the time of sampling.

3.10 Determination of total petroleum hydrocarbon in plant tissues

Whole plants were harvested from the experimental pot after 4 months of treatment and washed with distilled water to rid them of soil materials. The olants were separated into shoots and roots and chopped before homogenizing with a blender. The plants shoot and roots from the different treatments were then separately extracted in carbon tetrachloride. The extracts were then analyzed using IR Spectroscopy for TPH.

3.11 Statistical analysis

The data obtained were subjected to analysis of variance. Duncan's Multiple Range Test (DMRT) was used to compare differences between means at 5 % significant level.

3.12 Field Experiment

Two identified plant species with considerable potential for phytoremediation of crude oil polluted soil namely: *Pennisetum purpureum* and *Leuceana leucocephala* were further studied under field conditions at the Teaching and Research Farm of the Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus in Oruk Anam Local Government Area. Obio Akpa is situated between latitude $4^{o}30^{1}$ and $5^{o}30^{1}$N and longitude 7^{o} 31^{1} and $8^{o}0^{1}$E (Slus-AK, 1989). The mean annual temperature is 24°C - 30°C, while relative humidity is 75 – 79% (Slus-AK, 1989). Obio Akpa is located in the tropical rain forest belt of Nigeria with a bimodal annual rainfall range of about 2000 to 2500mm. The rainy season normally starts from March to late October following the dry season from November to late February. The soils are mainly acid sands with a pH of 4.9 – 6.1 with high buffering capacity in the order 2.0-10.0 meq/100g soil, low base saturation, high exchangeable aluminum and low nutrient status with severe leaching (Enwezor *et al.*,1981).

3.12.1 Experimental Materials used/ Sources

The experimental materials used were crude oil, and two plant species (*Pennisetum purpuruem* and *Leuceana leucocephala*). Organo-mineral fertilizer was obtained from John ker Company located in Ikot Ekpene Local Government Area, Akwa Ibom State. Plant growth hormone (Brassinolide) was obtained from Beijing CONSULT-TECH COMPANY LTD. PR. China.

3.12.2 Soil sampling and processing

Soil sampling and processing followed same procedure in the greenhouse.

3.12.3 Site preparation, treatment /planting

The experimental site was manually cleared, stumped, tilled and mound beds measuring 1 m x1m made. The treatments studied were:

T_1 = 2.5 % crude oil polluted soil + organomineral fertilizer (OF), no planting or brassinolide

T_2 = 5.0 % crude oil polluted soil + OF, no planting or brassinolide

T_3 = 7.5 % crude oil polluted soil + OF, no planting or brassinolide

T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*

T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*

T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*

T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under *P.purpureum*

T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*

T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*

T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*

T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*

T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*

T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*

T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*

T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

The experiment was laid out in a Randomized Complete Block Design (RCBD) with 15 treatments each replicated thrice. Blocks were separated from each other with 1m alleyways and an inter-plot spacing of 0.5m. Crude oil was applied to designated plots by sprinkling from perforated cans and the plots were covered with mulching materials to prevent volatilization of the oil. The aim was to simulate condition of a major oil spill. The plots were left undisturbed for one week before tilling and application of organo-mineral fertilizer to designated plots at 5 t/ha to provide nitrogen, a major limiting factor in crude oil-polluted soils and proliferation of soil microorganisms. The organomineral fertilizer (OF) contained 40 % organic matter, 2.8 % total N, 1.46 % P and 14% moisture. Two weeks after application of the fertilizer, seedlings of *Leuceana leucocephala* and *Pennisetum purpureum measuring 5 cm* were transplanted to the prepared beds to a depth of 5cm. Twenty eight (28) days after planting, the plant growth hormones (Brassinolide) was diluted at the rate of 1ml to 1000ml (1litre) of distilled

water and foliarly applied to designated plots at the rate of 250 ml/plant. Soil samples were collected from each plot at interval of 3 and 6 months after pollution for laboratory analysis.

3.14 Laboratory analysis

Microbial, soil and total petroleum hydrocarbon analyses in soils and plants followed same procedure in the greenhouse.

3.15 Chemical analysis of organo-mineral fertilizer

The organo-mineral fertilizer was produced by drying digestwd sewage- sludge cake at 80oC in a tumbling evaporator which produces sludge granules of between 3 and 6mm in diameter. Supplementary mineral nutrients (such as poultry droppings, cow dung, compost manure as source of nitrogen, phosphorus and potassium respectively were added to provide a higher proportion of available nutrients in the product. The chemical properties of the fertilizer was analysed using the method of Lynda *et al.*, (2013).

3.16 Determination of plant biomass

Plant biomas (shoot and root) were determined at 6 months after pollution using same procedure in the greenhouse

3.17 Determination of heavy metals concentrations in soil and plant tissue

Soil and plant tissues were analyzed for heavy metals at the end of the experiment at six (6) months. The heavy metals analyzed were those known to accumulate in plants affected by petroleum hydrocarbon. These were cadmium (Cd) lead (Pb) and nickel (Ni). Portions of the air-dried samples (10 g) of plants harvested from the experimental site were homogenized and digested in nitric acid before analyzing for lead, cadmium and nickel using the atomic absorption spectrophotometer as described by (Udo *et al.*, 2009).

(a). John Ker Organo-mineral Fertilizer

(b). Plant Growth Hormone (Brassinolide)

PLATE 3: Materials used for field experiment

PLATE 4: Field trials showing *Pennisetum purpureum* 3 months after crude oil pollution

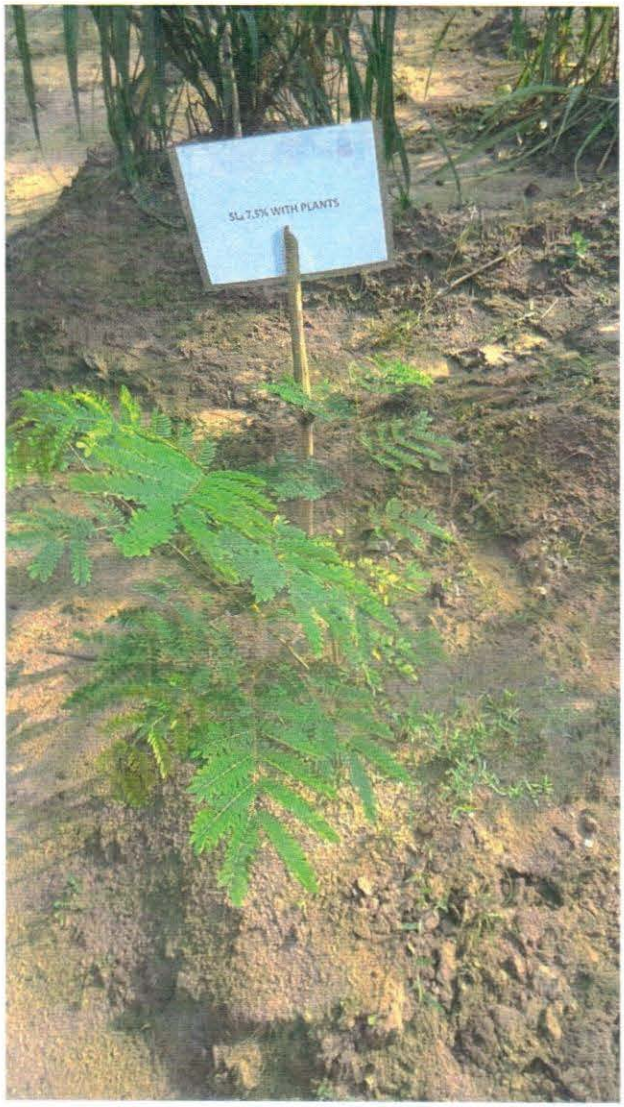


PLATE 5: Field trials showing *Leuceana leucocephala* 3 months after crude oil pollution

PLATE 6: Field trials showing *Pennisetum purpureum* 6 months after crude oil pollution

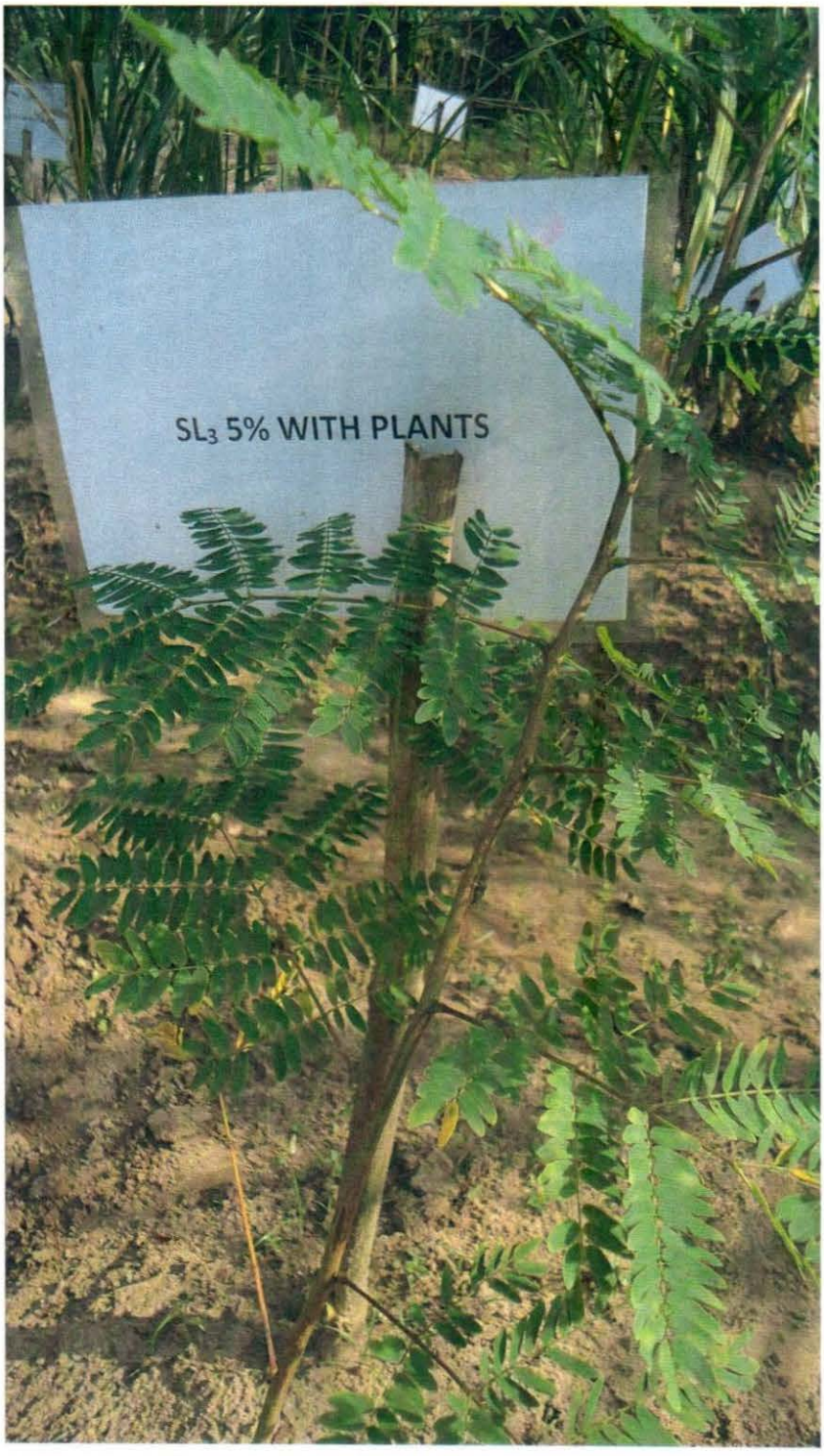


PLATE 7: Field trials showing *Leuceana leucocephala* 6 months after crude oil pollution

3.18 Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using Statistix 8.0 (2005); where significant differences exist between treatments, the differences were separated using Duncans Multiple Range Test (DMRT) at the 5% level of significance.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Greenhouse

Properties of the soil and crude oil

The physico-chemical properties of the soil before crude oil application are shown in Table 4. Particle size distribution was dominated by sand with texture being loamy sand. Soil pH was slightly acidic (6.1) while the organic matter (2.96%) and available phosphorous (41.29mg/kg) were high and total nitrogen (0.10%) was low as classified by Chude *et al.* (2012). Exchangeable calcium (6.4 cmol/kg) and magnesium (3.25cmol/kg) were high while exchangeable sodium (0.06cmol/kg) was low. Total exchangeable bases and effective cation exchange capacity (ECEC) were moderate while base saturation was higher than the 50% critical limit for crop production (Udo *et al.*, 2009).

Table 5 shows the physical and chemical properties of the crude oil used for the study. The crude oil had a specific gravity of 0.834 g/cm^3, viscosity (CP) of 0.28 and contained 85.6 % carbon, 12.61 % hydrogen, 1.48 % sulphur, 0.47 % nitrogen, 0.50 % oxygen, and 0.13 % trace metals with a gas-oil ratio of 88.1.

4.2 Physicochemical properties of the soils

4.2.1 Main effects of different concentrations of crude oil on some physicochemical properties of the soil at 2 and 4 months after pollution in the green house

The main effects of different concentrations of crude oil on some physicochemical properties of the experimental soil at 2 and 4 months after pollution (MAP) are shown in Table 6. Successive increases in the concentration of crude oil had no significant ($P > 0.05$) effect on the soil texture as reported previously (Abosede, 2013; Marinescu *et al.* (2001). The soil was loamy sand across samples. This may be attributed to the nature of the parent material from which they were formed. Gray and Murphy (2002) reported that the coarser the grain size of the parent material, the coarser will be the particle size of the soil,

TABLE 4

Physicochemical properties of soil before crude oil pollution

Parameter	Value
Particle size:	
Sand (mg/kg)	88.60
Silt (mg/kg)	4.50
Clay (mg/kg)	6.90
Textural class	Loamy sand
Bulk density (g/cm)	10.78
Total porosity (%)	32.5
pH (H_2O)	6.1
Organic carbon (%)	0.29
Organic matter (%)	0.499
Total nitrogen (%)	0.97
Available phosphorus (mg/kg)	41.29
Exchangeable bases (cmol/kg) :	
Ca^{++}	6.4
Mg^{++}	3.28
Na^{+}	0.06
K^{+}	0.12
Total TEB	9.86
Exchange acidity	2.72
ECEC (cmol/kg)	12.58
Base saturation (%)	78.38

TABLE 5

Characteristics of crude oil used for the study

Parameter	Specific value
Specific gravity(g/cm^3)	0.834
Viscosity(CP)	0.28
Carbon (%)	85.5
Hydrogen (%)	12.61
Sulphur (%)	1.48
Nitrogen (%)	0.47
Oxygen (%)	0.50
Trace metals (%)	0.13
Gas : oil ratio	88.1

TABLE 6

Main effects of different concentrations of crude oil on some physico-chemical properties of the experimental soil at 2 and 4 months after pollution in the screen house

Treatment	Sand (mg/kg)	Silt (mg/kg)	Clay (mg/kg)	Texture	Soil pH	Org. C. (%)	Total N (%)	Avail. P mg/kg	Exchangeable cations Ca	Mg	K	Na cmol/kg	Exchange acidity Al	H	ECEC cmol/kg	Base Sat. (%)
2 months after pollution																
Control	88.33a	5.07a	6.60a	LS	5.13a	1.30d	0.11d	31.35a	5.35a	1.25a	0.10a	0.07a	0.17a	0.98a	7.92a	85.47a
2.5	88.00a	5.70a	6.30a	LS	5.04b	2.28c	0.19c	23.64b	5.07b	1.22a	0.09b	0.07a	0.05d	0.85b	7.35b	87.75a
5.0	79.69a	5.70a	6.83a	LS	4.97c	2.71b	0.23b	18.88c	4.39c	1.15ab	0.09b	0.06b	0.08b	0.81b	6.58c	86.47a
7.5	86.51a	3.89a	9.60a	LS	4.96c	4.01a	0.29a	15.38d	4.31c	1.12b	0.08c	0.07a	0.06c	0.74c	6.86b	81.40a
4 months after pollution																
Control	85.95a	3.50a	10.55a	LS	5.23a	0.62d	0.06b	41.70a	3.67a	2.06a	0.12a	0.14a	0.01a	0.54a	6.54a	91.59b
2.5	86.50a	3.90a	9.60a	LS	5.10b	0.92c	0.07b	38.65b	2.92b	1.78b	0.08b	0.08b	0.01a	0.36c	4.94c	91.67a
5.0	85.40a	4.90a	9.62a	LS	5.01c	1.19b	0.12a	33.46c	2.63c	1.82b	0.08b	0.07b	0.01a	0.37b	5.29b	92.05a
7.5	85.90a	4.55a	9.55a	LS	4.97d	1.39a	0.12a	23.69d	2.57c	1.53c	0.06c	0.08b	0.01a	0.34d	4.59d	92.37a

Means in the same column followed by same letter (s) are not significantly different at 5% probability level
Org. C = Organic carbon, T/N = Total nitrogen, Avail. P = Available phosphorus, Exchangeable magnesium, Exch. Na. = Exchangeable sodium, Exch. K = Exchangeable potassium, ECEC = Effective cation exchange capacity, EA = Exchangeable acidity, Base Sat. = Base saturation, P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

especially of the surface soil. The coastal plain sand soils of the area have the coarser and much resistant quartzite as their dominant mineral.

At 2 months after pollution, there was a significant reduction ($P<0.05$) in the soil pH across pollution levels (Table 6). The pH was highest in the control pot where there was no pollution. This was followed by the soil polluted with 2.5 % crude oil which was significantly higher than the soils polluted with 5.0 and 7.5 % crude oil. The same trend was observed at 4 months after pollution. Although the soils were strongly acidic (Udo *et al.*, 2009), at 2 and 4 months after pollution, the pH decreased by 34 and 35% respectively as the concentration of crude oil increased. Ijah *et al.* (2008) also observed a decrease in pH in crude oil polluted soils. This acidity may be associated with the acidic nature of the oil or the leaching of basic cations in soil solution which is typical of soils of the south eastern part of Nigeria.

At 2 months after pollution, the organic carbon content was higher at the 7.5 % pollution level followed by 5.0 % and then 2.5 % pollution levels. A similar trend was also observed at 4 months after pollution. Generally,the organic carbon increased by 14 and 18% at 2 and 4 months after pollution respectively. The increases in the organic carbon content of the polluted soil were attributed to microbial mineralization of crude oil in the soil (Ijah *et al.*, 2008; Eneje and Abomotei, 2011; Ogboghodo *et al.*, 2004b). Based on soil fertility rating (Chude *et al.*, 2012), the percent organic carbon was high irrespective of the pollution level but low in the control pots.

Total nitrogen (N) was significantly higher in the 7.5 % polluted soil (Table 6), followed by 5.0 and 2.5 % pollution at 2 months. However, at 4 months after pollution, the total N under 7.5 % and 5.0 % pollution levels was the highest. There was no significant ($P>0.05$) change in the N content of soils polluted with 2.5 % crude oil.

and 45% at 2 and 4 months respectively.This result is consistent with that of Bello and Inobeme (2015) but contradicts those of others (Onyeike *et al.*, 2002; Akubugwo *et al*, 2009; Ezeigbo *et al.*, 2013) who observed an increase in exchangeable calcium in crude oil polluted soils.

Exchangeable Mg was significantly higher in the control pot and pots with 2.5 % pollution while there was little difference between 5.0 and 7.5 % pollution levels whether at 2 or 4 months after treatment.The respective decreased were 36 and 40%.

Successive increases in soil pollution significantly decreased the content of exchangeable potassium. At 2 and 4 months after pollution, the exchangeable potassium was higher in the control than the oil impacted soils. At 4 months after pollution, the highest level of exchangeable potassium was in the control soil while soil polluted with 7.5 % oil had the lowest concentration.The respective decreased were 38 and 50%.

At 2 months after pollution, the exchangeable Na did not significantly differ ($P<0.05$) between the control pot and soil polluted with 2.5 and 7.5 % crude oil but was significantly different from soil polluted with 5.0 % crude oil. At 4 months, the control pot had the highest content of exchangeable Na, significantly exceeding levels in all the polluted soils. Generally, exchangeable Na decreased by 35 and 61% at 2 and 4 months after pollution.

Exchangeable aluminum value was significantly decreased by oil pollution at 2 months after pollution (MAP) but at 4 MAP, there was little difference among treatments. At 2 MAP, the exchangeable hydrogen was highest ($P<0.05$) in the control soil followed by soil polluted with 2.5, 7.5 and 5.0 %. At 4 MAP, the concentration varied in the order control > 5 % > 2.5 % and 7.5 pollution levels. (Table 6).

Generally, the total exchangeable bases were higher in the control than the oil impacted soils. The reduction in Ca, Mg, K and Na may be due to nutrient immobilization arising from the formation of complexes in the soil after uptake by plant.

Base saturation (BS) values were little affected by pollution at 2 MAP but not at 4 MAP when the polluted soils had higher concentration than the unpolluted soil (Table 6). Base saturation was increased by 33% at 2 and 4 months.The base saturation was generally high across treatments based on criteria in FDALR (1990) and Landon (1991) considering BS greater than 60 percent to be high.

The ECEC in the control soil substantially exceeded that in the polluted soils whether at 2 or 4 MAP. The respective decreased were 38 and 43%. Based on established criteria (Landon 1991; FDARL 1990) in which ECEC less than 10 cmol/kg was considered low, our studied soils had low ECEC.

4.2.2 Phytoremediation effects of different plant species on crude oil polluted soils based on some physico-chemical properties

Data on the effects of different plant species grown on crude oil polluted soils on some physico-chemical properties are presented in Table 7. The soil pH under the different plant species varied significantly ($P \leq 0.05$) at 2 and 4 MAP. At 2 MAP, soils planted with *Eleusine indica*, *Talinum fructicosum*, *Calapogonium mucunoides* and *Jatropha curcas* significantly had higher pH values than those planted with other species or not planted (control) excepting those under *Cyperus rotundus* and *Centrosema pubescens*. Generally, all the plant species significantly increased the soil pH relative to the unplanted soil.

At 4 MAP, all the plants except *P. maximum* significantly ($P \leq 0.05$) increased the soil pH. Soil planted with *Pennisetum purpurem* had the highest pH followed by those under *Axonopus compressus*, *Leuceana leucocephala*, *Calapogonium mucunoides*, and *Centrosema pubescens*. The pH of all the planted soils was rated as strongly to

TABLE 7

Phytoremediation effects of different plant species on crude oil polluted soils based on some physicochemical properties

Plant species	Soil pH (H_2O)	Org. C. (%)	Total N (%)	H Avail. P (mg/kg)	Exchangeable cations Ca	Mg	K	Na	Exchange acidity Al	H	Base Sat. (%)	ECEC (cmol/kg)
					← cmol/kg →							
2 months after pollution												
V_o	4.450g	3.005c	0.2150e	21.90e	5.233bc	1.550a	0.1075a	0.0775a	0.0475f	0.7925cd	88.75a	7.808a
V_1	4.975d	2.455h	0.1925f	25,43c	4.233g	1.008cd	0.0850gh	0.0666d	0.1200b	0.9558b	83.08a	6.468e
V_2	5.050cd	2.670f	0.2225de	27.78b	5.000de	1.600a	0.0825h	0.0666d	0.0933de	0.7383f	83.41a	7.581c
V_3	5.250a	2.140k	0.1675ghi	19.54g	3.725h	1.033c	0.0900fg	0.0683cd	0.1058bcd	0.9900a	81.77a	5.022f
V_4	4.833e	2.180j	0.1575i	22.02e	3.842h	1.042c	0.0908ef	0.0675d	0.09991cde	1.0100a	80.73a	5.152f
V_5	5.000d	1.913i	0.1692ghi	33.70a	5.200bcd	1.050c	0.0983bed	0.0750ab	0.1608a	0.8075c	83.33a	7.392b
V_6	4.650f	2.435i	0.1850f	21.05f	5.27b	1.575a	0.0941def	0.0733abc	0.0500f	0.7975cd	83.86a	7.859ab
V_7	5.225a	2.540g	0.1950f	15.85i	5.033cde	1.542a	0.0891fg	0.0758ab	0.0475f	0.7983cd	76.98a	7.586ab
V_8	5.075bcd	2.735e	0.2325cd	15.94i	4.500f	0.825d	0.0908ef	0.0675d	0.1566a	0.7592cf	84.00a	6.504e
V_9	5.150abc	2.148k	0.1515hi	19.69g	4.35fg	1.033c	0.1008bc	0.0716bcd	0.0850e	0.9917a	82.72a	6.841d
V_{10}	5.250a	3.088b	0.2617b	25,75c	5.658a	0.900cd	0.1000bc	0.0766ab	0.0483f	0.7767de	84.83a	6.778d
V_{11}	5.250a	2.977d	0.2358c	18.44h	4.817e	0.992cd	0.0958cde	0.0775a	0.1100bc	0.7808cde	86.13a	7.493b
V_{12}	5.175ab	3.203a	0.2775a	22.97d	5.267b	1.258b	0.1016b	0.0683cd	0.0466f	0.7867cde	88.93a	7.528ab
4 months after pollution												
V_o	4.958e	1.026d	0.0925a	34.58bcd	2.600c	1.075d	0.1600a	0.2550a	0.0125a	0.4550a	89.22g	4.220f
V_1	5.317b	1.012de	0.1042a	40.96a	2.950bc	2.175ab	0.1800a	0.0775b	0.0125a	0.3822d	92.47bc	5.730c
V_2	5.550a	1.315a	0.1075a	40.00a	3.300ab	2.325a	0.1100a	0.0825b	0.0125a	0.3723d	93.81a	6.202a
V_3	5.083d	0.830h	0,0875a	36.93b	2.933bc	2.150ab	0.1700a	0.0775b	0.0100a	0.3724d	93.55a	5.650c
V_4	5.017de	0.797i	0.0825a	35.93bc	3.000bc	2.150ab	0.1000a	0.0775b	0.0100a	0.3723d	93.07ab	5.730c
V_5	5.317b	0.980fg	0.0800a	28,84fg	3.600a	1.775bc	0.1100a	0.0825b	0.0100a	0.4311b	92.75abc	6.000ab
V_6	5.092d	1.130e	0.0925a	34.68bcd	2.700c	1.133d	0.0999a	0.0750b	0.0125a	0.4550a	89.75fg	4.380f
V_7	5.100d	0.997ef	0.1475a	28.07g	2.700c	1.425cd	0.1100a	0.0725b	0.0150a	0.4100c	90.42ef	4.751e
V_8	5.242bc	0.965g	0.0850a	28.79fg	3.533a	1.725c	0.1100a	0.0775b	0.0075a	0.4100c	92.12cd	5.862bc
V_9	5.133cd	0.782i	0.0775a	33.97cd	2.725c	2.125ab	0.0999a	0.0750b	0.0125a	0.3724d	93.00ab	5.402d
V_{10}	5.267b	1.317a	0.1050a	31.24ef	2.750c	2.275a	0.1000a	0.0825b	0.0125a	0.3811d	90.72e	5.733c
V_{11}	5.225bc	1.005e	0.0850a	32.22de	2.975bc	1.713c	0.1000a	0.0800b	0.0100a	0.4311b	91.50d	5.365d
V_{12}	5.275b	1.51b	0.0967a	40.65a	2.550c	1.300d	0.0999a	0.0725b	0.0150a	0.4451ab	89.72fg	4.451f

Means in the same column followed by same letter (s) are not significantly different at 5% probability level.

V_o=*No plant*, V_1=Carpet grass (*Axonopus compressus*), V_2=Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4=Guinea grass (*Panicum maximum*), V_5=White leadtree(*Leuceana leucocephala*), V_6=Gliricidia (*Gliricidia sepium*), V_7=Waterleaf (*Talinum fructicosum*), V_8=Siam weed (*Chromoleana odorata*), V_9=Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

moderately acid. The low soil pH may be due to the coarse textured nature of these soils permitting extensive leaching of basic cations under the usually high rainfall in the area.

At 2 MAP, the total nitrogen contents of the soil differed significantly among species. The soil under *C. pubescens* had the highest N content followed by that under *C. mucunoides*. Generally, soils planted with *C. pubescens*, *C. mucunoides*, *J. curcas* and *C. odorata* had much more N than the control soil while the reverse was the case for the other plant species, excepting *P. purpureum* that had similar N content with the control. At 4 MAP, there was little change in the N content among the plant species but soil organic carbon differed significantly between control and planted pots whether at 2 or 4 MAP (Table 7).

At 2 MAP, the soil under *C. pubescens* had the highest ($P<0.05$) content of organic carbon, followed by that under *C. mucunoides* but the soils supporting the other plant species had lower organic carbon than the bare soil . At 4 MAP, the soil under *P. purpureum* and *C. mucunoides* had much higher organic carbon than those planted to other plant species. Generally, the soils under *P. purpureum*, *C. mucunoides*, *C. pubescens* and *G. sepium* had more organic carbon than the control soil. The organic carbon content across soils whether at 2 or 4 MAP were high to low according to the ratings of soil fertility (FAO, 1976; Udo *et al.*, 2009).

The soil available phosphorus differed significantly ($P<0.05$) among the plant species at 2 and 4 MAP (Table 7). At 2 MAP, the soil under L. *leucocephala* had the highest available phosphorus. Generally, soils under *L. leucocephala*, *P. purpureum*, A. *compressus*, *C. mucunoides* and *C. pubescens* had much higher available P than the bare soil (control). At 4 MAP, soils under *A. compressus*, *P. purpureum* and *C. pubescens* had much higher available phosphorus, while the reverse was the case for those under *L. leucocephala*, *T. fructicosum*, *C. odorata* and *C. mucunoides*. There was no significant change in the available phosphorus content of soils under the other 5 plants species. The

content of available phosphorus in all the soils studied was rated medium to high according to FMANR (1990).

The exchangeable calcium content of soil under *C. mucunoides* was much more than the other species at 2 MAP while at 4 MAP, soils planted to *L. leucocephala* and *C. odorata* had the highest content than the control.

At 2 MAP, soils under *P. purpureum*, *G. sepium* and *T. fructicosum* had slightly more exchangeable magnesium while those under the other 8 species had much less than the control soil (Table 7). At 4 MAP, soil under *P. purpureum* and *C. mucunoides* had higher content of exchangeable magnesium. This was followed by those under *A. compressus*, *E. indica*, *P. maximum* and *C. rotundus* while those under the other 4 species had lower or similar contents.

All the soils planted with different plant species significantly reduced the content of exchangeable potassium at 2 MAP but not at 4 MAP (Table 7).

The exchangeable sodium content of soils under *L. leucocephala*, *J. curcas*, *T. fructicosum* and *C. mucunoides* was not significantly ($P > 0.05$) different from the control at 2 MAP (Table 7). The other 8 plant species significantly ($P > 0.05$) reduced the exchangeable sodium content of the soil. At 4 MAP, all the planted soils had much lower exchangeable sodium than the bare soil. The exchangeable cations were generally low at 2 and 4 MAP according to FMANR (1990).

The exchangeable acidity (EA) is a combination of H^+ and Al^{3+}. At 2 MAP, soils planted with *L. leucocephala* and *C. odorata* had the highest content of exchangeable aluminum (Table 7). This was followed by those planted with *A. compressus*, *E.indica* and *J. curcas*. At 4 MAP, no significant difference was observed among plant species when compared with the control. At 2 MAP, the soils under *E. indica*, *P. maximum* and *C. rotundus* had the highest content of exchangeable hydrogen. This was followed by soil under *A. compressus* while the other 7 species had lower content of exchangeable

hydrogen, excepting *P. purpureum* and *C. odorata* which were significantly lower than the control. At 4 MAP, the control soil and that under *G. sepium* had the highest content of exchangeable hydrogen. This was followed by *C. pubescens*, *L. leucocepha* and *J. curcas*, while the other species significantly ($P<0.05$) reduced the content of exchangeable hydrogen. The different plant species decreased the exchange acidity of the soil.

There was no significant change in base saturation among treatments at 2 MAP, soils under *E. indica* and *P. purpureum* had the highest base saturation at 4 MAP (Table 7). Generally, the base saturation was rated medium to high across soils (Esu, 1991; Enwezor *et al.*, 1981).

At 2 MAP, the ECEC was highest in the control soil, followed by soil under *C. pubescens*, *G. sepium* and *T. fructicosum*; the other 9 plant species significantly ($P<0.05$) reduced the ECEC contents of the soil. At 4 MAP, the soil planted with *P. purpureum* had the highest ECEC followed by that under *L. leucocephala* while soils under the other species had lower ECEC. There was no significant change in the ECEC of soils under *C. pubescens* and *G. sepium* but overall, the ECEC of all the soils was generally low. This could be attributed to the leaching of basic cations due to continuous watering of the soils. Excessive watering results in the leaching of basic cations, which are then replaced at the exchange sites by H^+.

4.2.3 Interactive effects of crude oil pollution and plant species on some soil physico-chemical properties in the greenhouse

The interactive effects of crude oil pollution and different plant species on some soil properties at 2 and 4 months after pollution are shown in Figure 3. There was no significant change in the sand, silt and clay content or textural class of the soil. This may be due to the nature of the parent material from which the soil was formed. Abosede, (2013) reported that pollution of soil with crude oil had no significant effect on the

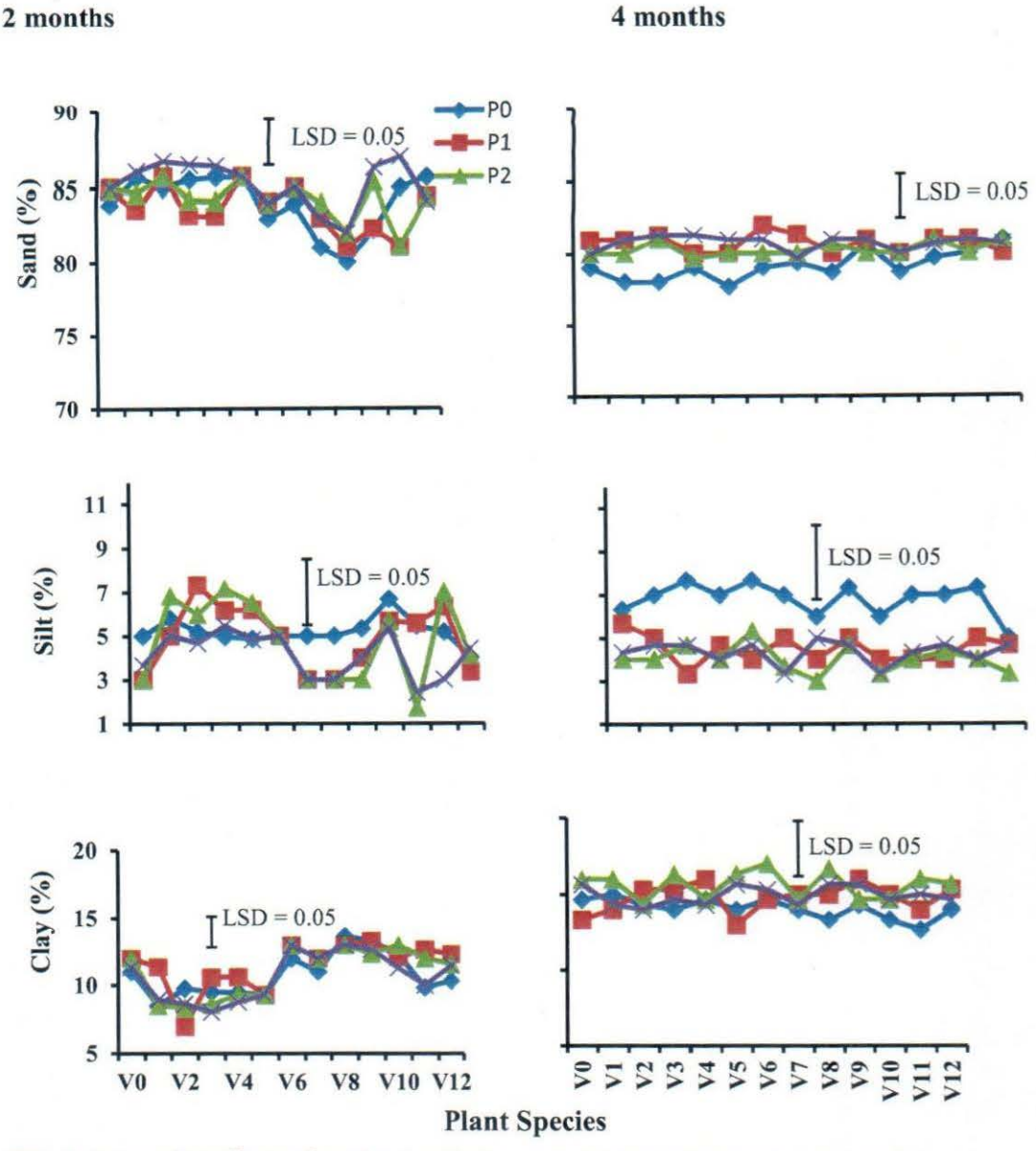


FIG. 3: Interactive effects of crude oil pollution and plants species on sand, silt and clay fractions at 2 and 4 months after pollution in the greenhouse.

V_o=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=*Goose* weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5= White leadtree (*Leuceana leucocephala*), V_6=Gliricidia (*Gliricidia sepium*), V_7=Waterleaf (*Talinum fructicosum*), V_8=Siam weed (*Chromoleana odorata*), V_9=Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).
P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

textural classes. Similarly, Marinescu *et al.* (2001) found no significant effect of crude oil pollution on the granulometric fraction of the soil.

Significant interactive effects were observed between crude oil pollution and the different plant species on soil pH at 2 MAP (Figure 4). The control pots (P_0) had higher pH than other treatments and the lowest PH was in soils polluted with 7.5% crude oil under *P. maximum.*

At 4 MAP, the pH of unpolluted soils under *C.odorata*, *C.rotundus*, *C. mucunoides* and *J.curcas* increasesd significantly. The lowest pH was in soils polluted with 5.0 and 7.5% crude oil without planting followed by soils treated with 2.5 and 5.0 % crude oil under *G. sepium.* Generally, the polluted soils were more acidic than the control or background soil. The relatively lower pH in soils polluted with crude oil may be attributed to the acidic nature of the oil. Osuji and Nwoye (2007) reported that the soil pH was reduced in the presence of hydrocarbons that produce organic acids when acted upon by microorganisms. Ijah (1998) also observed decreases in pH values of polluted soils.

The organic carbon content of the soil differed significantly ($P<0.05$) among the different levels of pollution (Figure 5) with the polluted soils having higher values and increasing significantly as the level of pollution increased.

The increase in organic carbon content may be due to the fact that carbon is the major component of crude oil. Similar increases reported previously (Ogboghodo *et al.*, 2004a; Ijah *et al.*, 2008; Eneje and Ebomotei, 2011) were attributed to microbial mineralization of crude oil in the soil.

The interactive effects of crude oil polluted soil and plant species on soil nitrogen are shown in (Figure 6). At 2 MAP, the total nitrogen was much ($P<0.05$) higher in soils contaminated with 7.5% crude oil under *J. curcas*, *while at* 4 MAP, the highest value was also obtained in soil polluted with 7.5% crude oil but under *C. mucunoides*. There

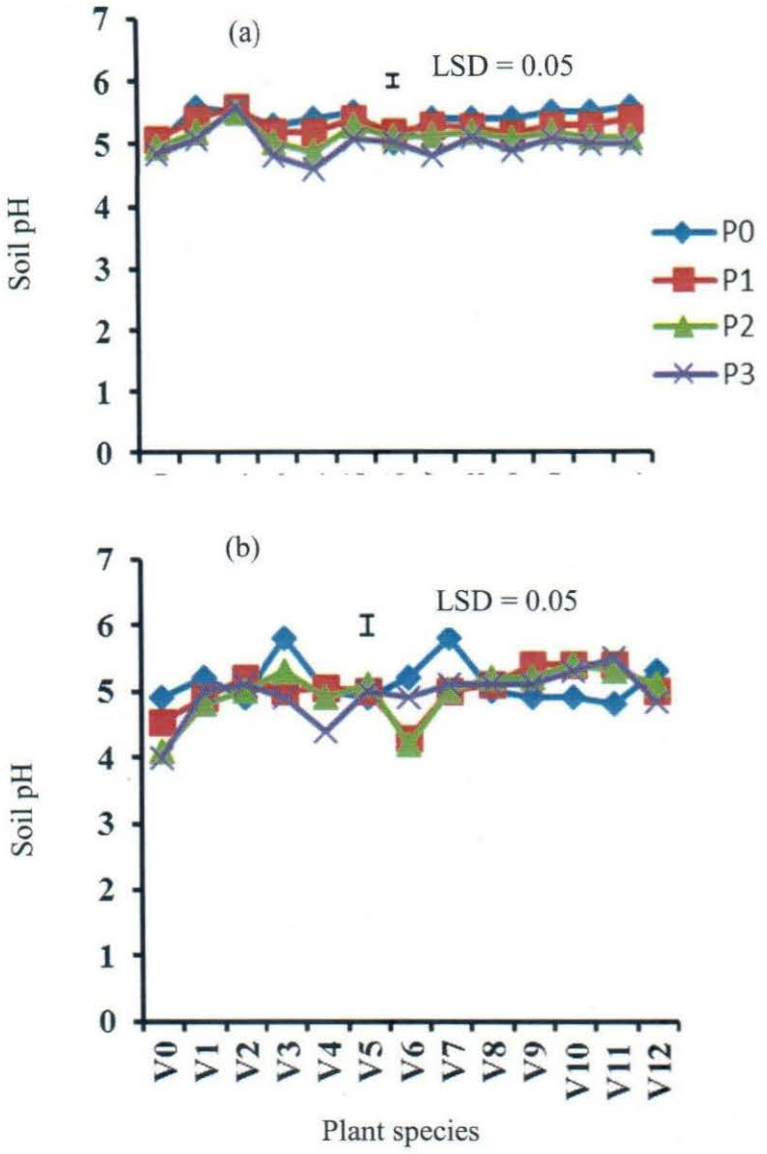


FIG. 4 : Interactive effects of crude oil pollution and plants species on soil pH at 2 (a) and 4 (b) months after pollution in the greenhouse.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*)

P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

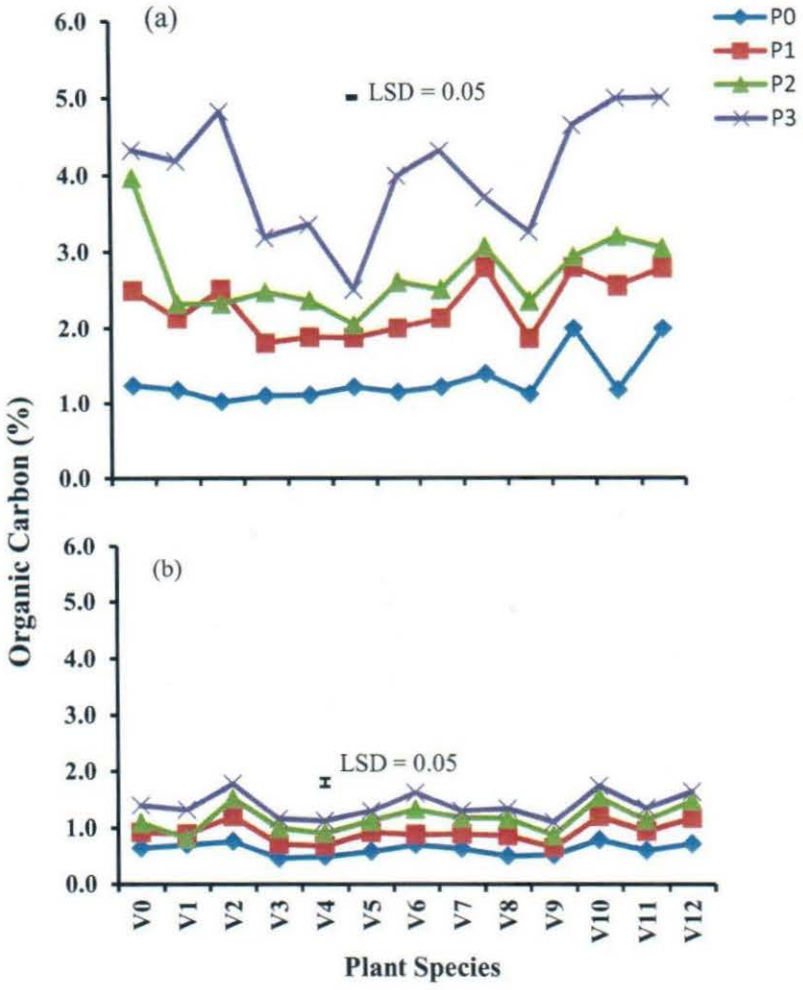


FIG. 5: Interactive effects of crude oil pollution and plants species on soil organic carbon at 2 (a) and 4 (b) months after pollution in the greenhouse.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).
P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

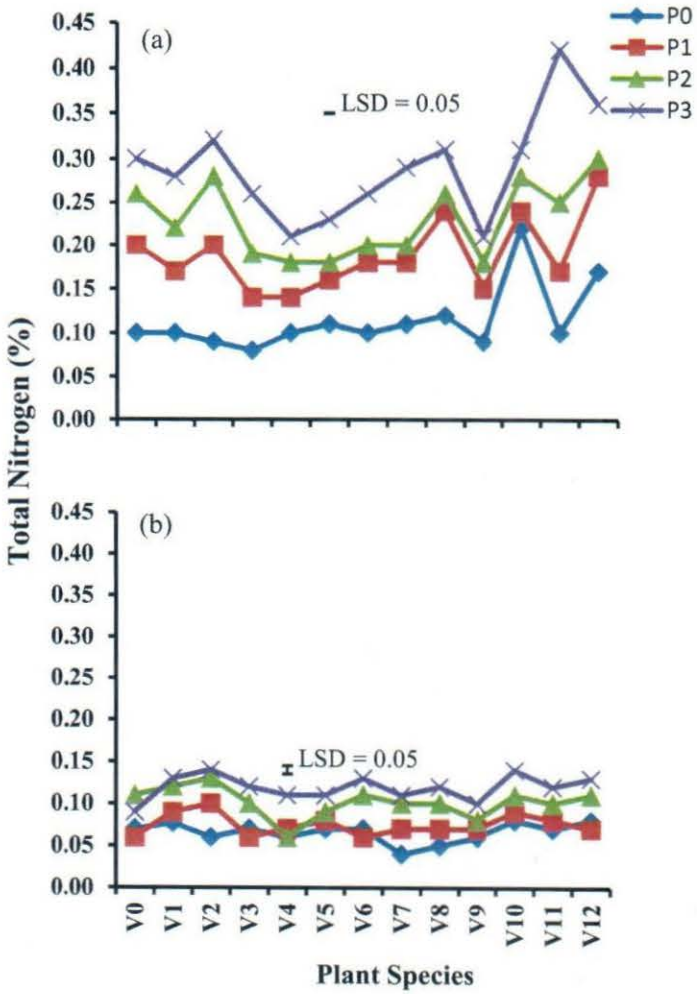


FIG. 6 Interactive effects of crude oil pollution and plants species on soil total nitrogen at 2 (a) and 4 (b) months after pollution in the greenhouse.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}= Calapo (*Calapogonium mucunoides*), V_{11}= Jatropha (*Jatropha curcas*), V_{12}= Centro (*Centrosema pubescens*).

P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

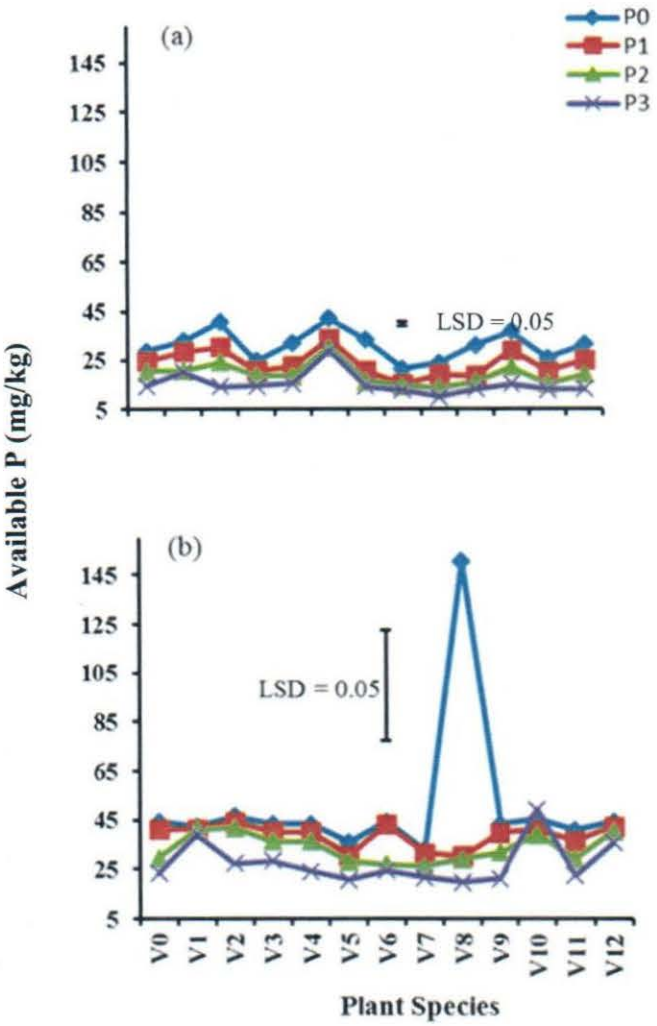


FIG. 7: Interactive effects of crude oil pollution and plants species on available phosphorus at 2 (a) and 4 (b) months after pollution in the greenhouse.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).
P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

was a general reduction in total nitrogen level as the growth period prolonged, indicating leaching effect or uptake of this element by the plants. Generally, it was observed that successive increases in crude oil pollution significantly ($P<0.05$) increased soil nitrogen. The higher content of total nitrogen in the crude oil polluted soils may be because crude oil initiates soil reactions that result in the availability of nutrients in the polluted soil (Odu, 1972; Udo, 2008; Eneje and Abomotei (2011).

There was significant ($P<0.05$) interactive effects of crude oil pollution levels with plant species on available phosphous (P) content. At 2 MAP, soils treated with 7.5 % crude oil under *C. odorota* and *A.compressus*, significantly ($P<0.05$) decreased the available P (figure 7). The soil under *L. leucocephala* showed remarkable increases in available P across pollution levels, indicating that this plant enhanced phosphorus availability.

At 4 MAP the soil available P also decreased with increases in pollution levels as reported previously (Isirimah *et al.* 1989; Ogboghodo *et al.* 2004b; Eneje and Abomotei, 2011). This could be associated with phosphorous fixation in the polluted soil.

Generally, there were considerable reductions in exchangeable calcium (Ca) in the oil impacted soils (Figure 8), possibly due to uptake by plants and temporal immobilization by soil microbes. This result is consistent with those of Obasi *et al.* (2013) and shukry *et al.,* (2013) who reported a decrease in exchangeable calcium (Ca) in crude oil polluted soils.

At 2 MAP, there was no significant difference in exchangeable Mg between soil treated with 2.5 % crude oil under G. *sepium*, *T. fructicosum* and soil polluted with 7.5 % crude oil under *P. purpureum* relative to control but the values were significantly higher than those in other treatments.

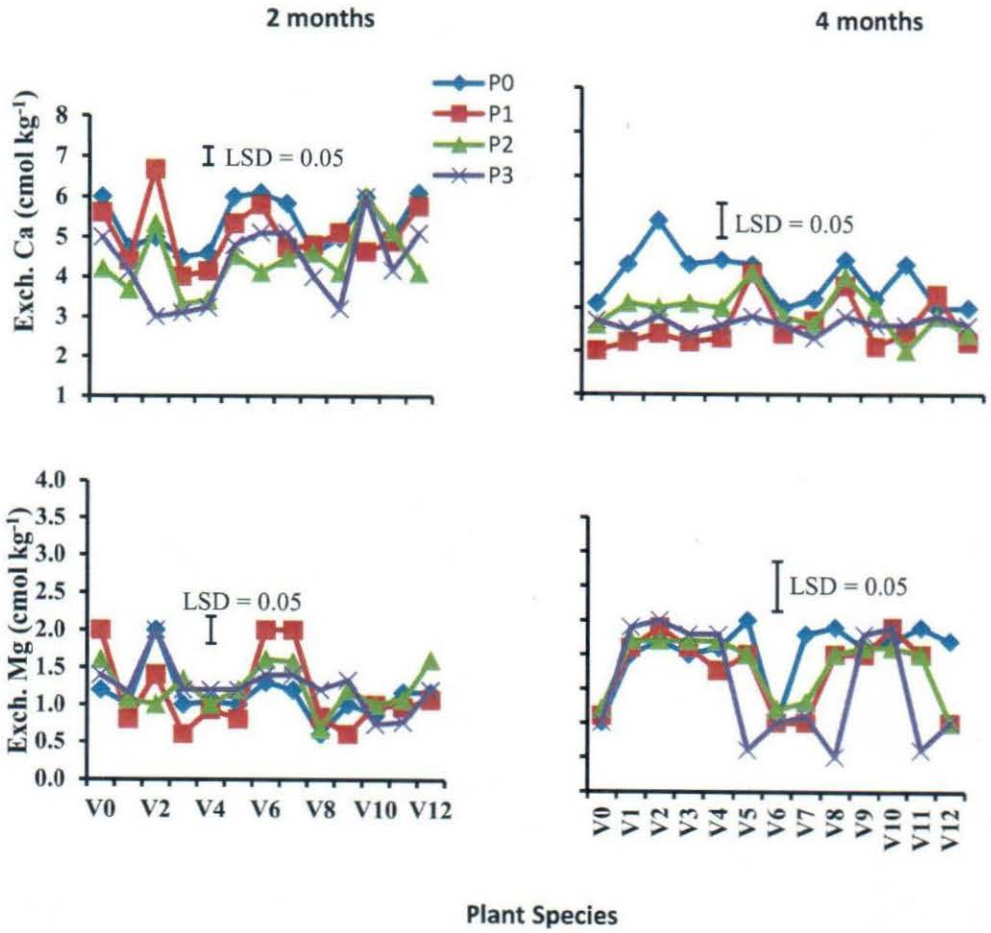


FIG. 8: Interactive effects of crude oil pollution and plants species on soil exchangeable calcium and magnesium at 2 and 4months after pollution in the green house.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

The lowest exchangeable Mg was in soil treated with 2.5 % crude oil under *E. indica*. At 4 MAP, there were no significant change in the polluted pots but the lowest content of exchangeable Mg was in soils treated with 7.5 % crude oil under *L.leucocephala*, *C. odorota* and *J.curcas* .The decrease in exchangeable Mg in some of the treated soils may be attributed to uptake by the plants as well as leaching losses.

At 2 MAP, the exchangeable K changed little but at 4 MAP, soils polluted with 2.5 and 5.0% crude oil under *A. compressus* and *E. indica* showed significant increases compared with other treated soils and the control (Figure 9). This confirms earlier reports (Onyeike *et al*., 2002) on potassium (K) dynamics in polluted soils.

There were no significant differences in exchangeable Na across treatments (Figure 9). Generally, the exchangeable bases were lower in the polluted soils than the unpolluted soils.

The lower levels may be due to nutrient immobilization or complexation in the soil. This contracdicts the findings of Akubugwo et al., (2009) who reported increases in exchangeable bases as a result of crude oil pollution.

Exchangeable Al in the polluted soils was significantly reduced when compared with the unpolluted soils except in soils polluted with 2.5% and 5.0 % crude oil under *C. odorota* and *J. curcas* (Figure 10) However, polluted soils especially at higher pollution levels had more exchangeable aluminium than the control. Exchangeable H in the polluted soils was higher in soils polluted with 7.5 % crude oil under *A. compressus, E. indica, P. maximum and C. rotundus* and the lowest was in soils polluted with 2.5% crude oil under *P. purpureum* and *C. odorata* at 2 MAP (Figure 10). At 4 MAP, the unpolluted soils irrespective of the plant species had much higher exchangeable H than the polluted soils.

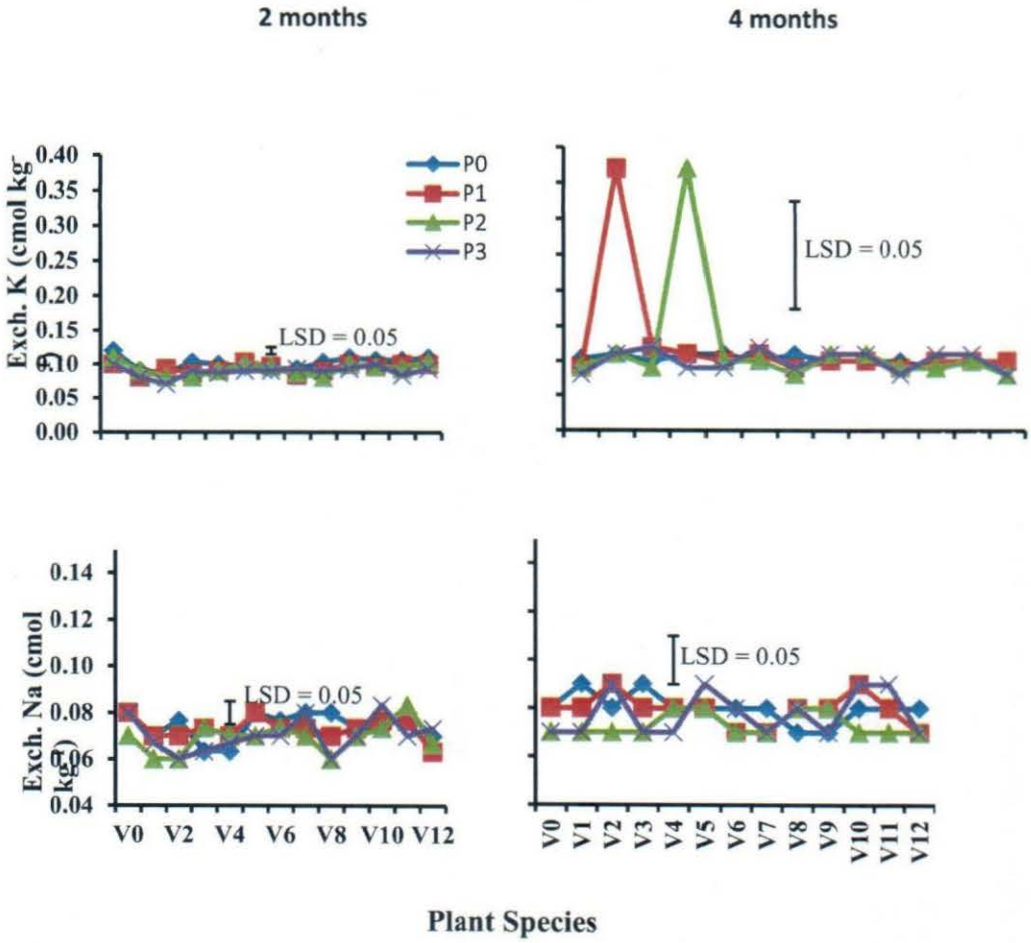


FIG. 9: Interactive effects of crude oil pollution and plants species on soil exchangeable potassium and sodium at 2 and 4 months after pollution in the green house

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

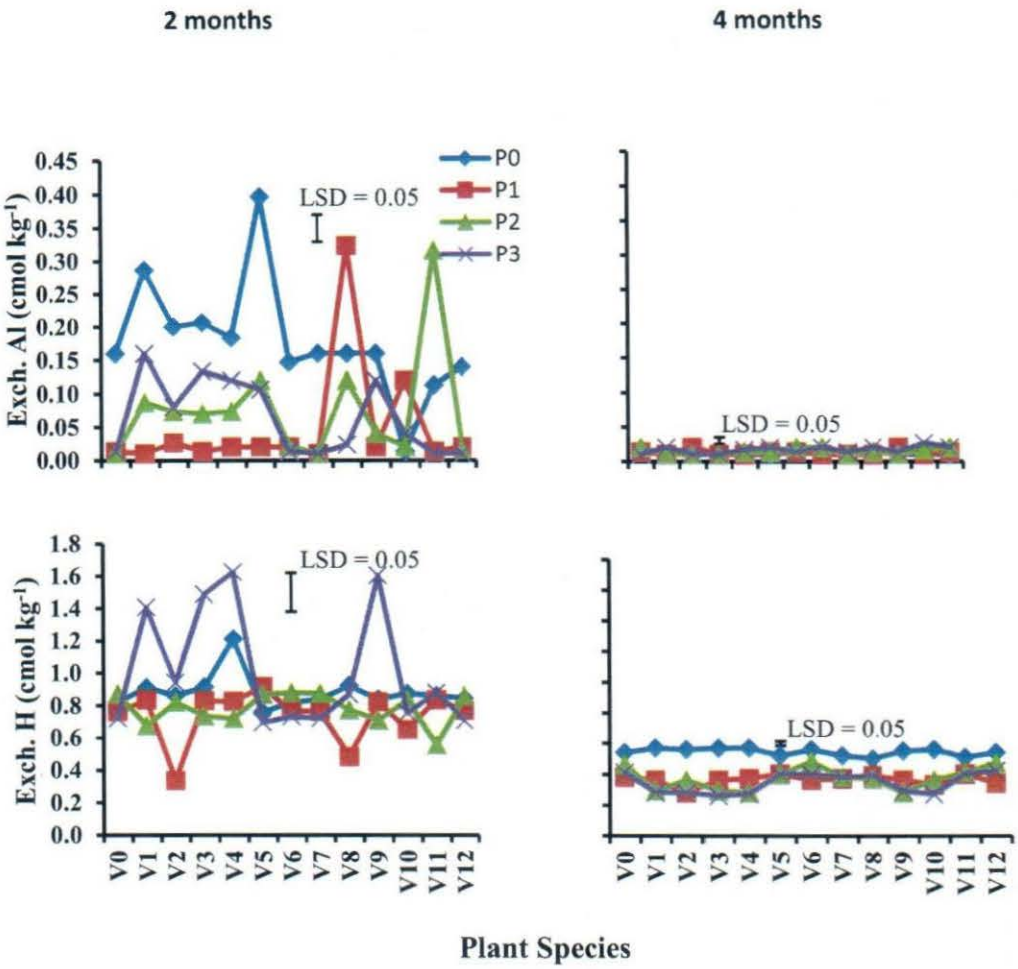


FIG. 10: Interactive effects of crude oil pollution and plants species on soil exchangeable Al and H at 2 and 4 months after pollution in the greenhouse.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

The ECEC at 4 MAP was higher in the unpolluted soils than the polluted soils, except for soils under *G. sepium* (Figure 11). Generally, the effective cation exchange capacity was low irrespective of the pollution level and plant species.

Base saturation was higher in the polluted than the unpolluted soils (Figure 12). The highest at 4 MAP was in the 7.5% polluted soil under *C. mucunoides*. This agrees with the report of Eneje and Abomotei (2011) who observed higher base saturation in polluted soils.

4.3 Main effects of concentration of crude oil on microbial properties

4.3.1 Total heterotrophic bacteria count (THB)

The main effects of concentration of crude oil on total heterotrophic bacteria count (THB) at 2 and 4 months after pollution are shown in Table 8. The count was significantly reduced in the polluted soils. This low count under crude oil pollution was also reported in Ijah and Antai (2003a) and Ekpo and Ebeaguru (2009), who attributed the reduction in microbial biomass to the anaerobic condition created by crude oil pollution which automatically damaged most of the aerobic organisms.

The bacteria species isolated from both polluted and unpolluted soils using biochemical test (Table 9) were identified to include: *Bacillus subtilis, Eschericha coli, Actinomycetes spp., Staphylococcus aureus, Spingomonas spp., Enterobacter cloacae, Pseudomonas aeruginosa, Protus vulgaries, Acinetobacter spp, Achromobacter xylosidens, Enterobacterium spp., Mycobacterium spp., Micrococcus spp., Corynebacterium spp., Chromobacterium spp., Rhodococcus spp.* and *Flavobacterium spp.*

4.3.2 Total heterotrophic fungi count (THF)

The main effects of concentration of crude oil on total heterotrophic fungi count (THF) at 2 and 4 MAP in the greenhouse are shown in Table 10.

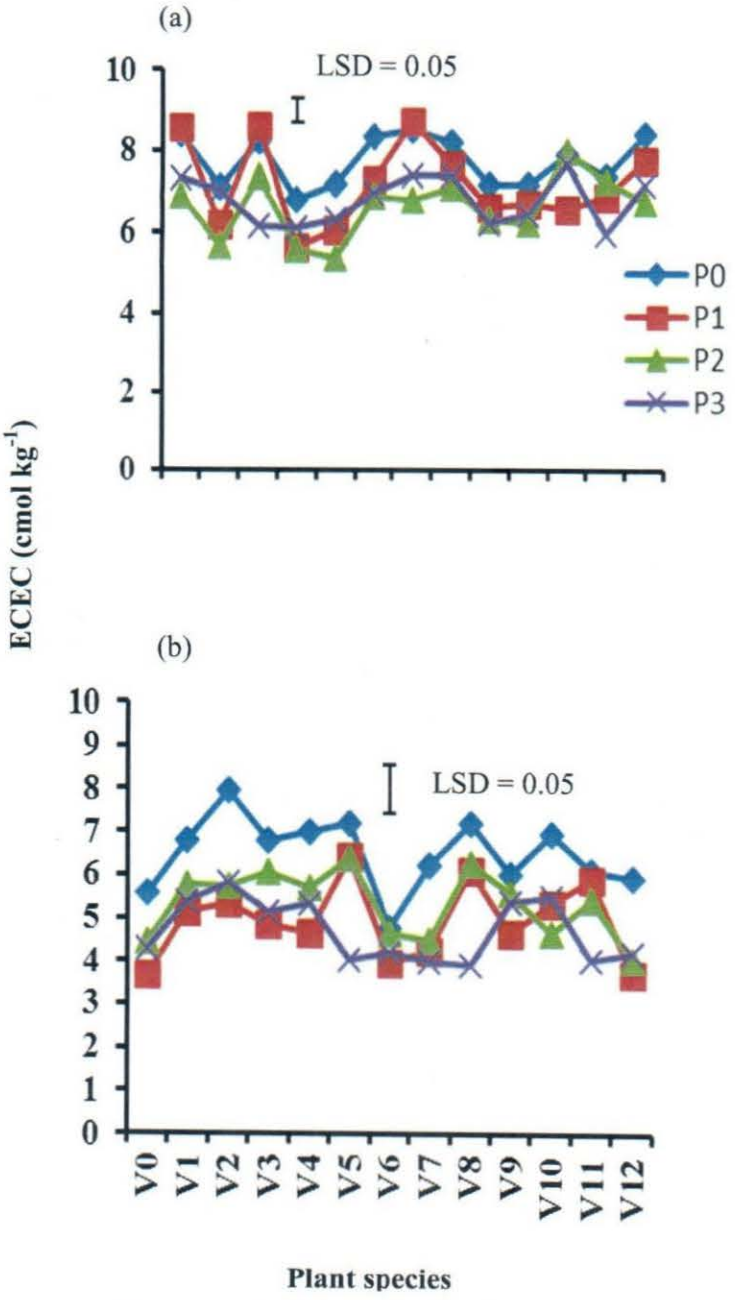


FIG. 11:Interactive effects of crude oil pollution and plants species on effective cation exchange capacity (ECEC) at 2 (a) and 4 (b) months after pollution in the greenhouse.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed(*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha(*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

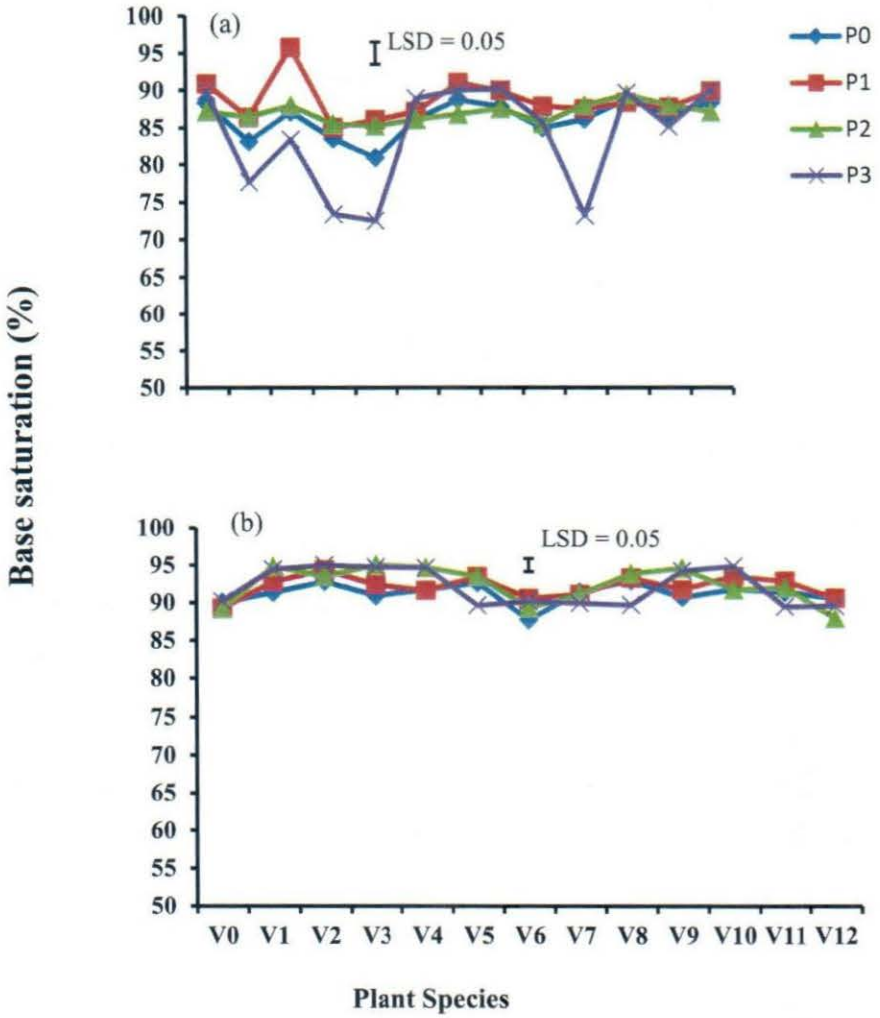


FIG. 12 Interactive effects of crude oil pollution and plant species on base saturation at 2 and 4 months after pollution in the greenhouse.

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

P_o = No pollution, P_1 = 2.5 %, P_2 = 5.0 %, P_3 = 7.5 % (w/w) pollution.

TABLE 8

Total heterotrophic bacterial count (THB) (cfu/g) at two and four months after pollution in the green house

Treatment	Bacterial isolate	2 months after pollution		Bacterial isolate	4 months after pollution	
		TBH (cfu/g)	Mean count (cfu/g)		TBH(cfu/g)	Mean count (cfu/g)
P_0V_0	*Bacillus subtilis*	6.1×10^5		*Bacillus subtilis*	5.0×10^5	
P_0V_1	*Eshericha coli*	2.1×10^5		*Stresptococcus feacalis*	5.0×10^5	
P_0V_2	*Actinomcetes spp.*	3.7×10^5		*Escherica coli, Streptococcusfaecalis*	6.0×10^5	
P_0V_3	*Staphylococcus aureus, Spinngonomas spp.*	3.0×10^5		*Pseudomonas aeruginosa*	4.0×10^5	
P_0V_4	*Enterobacter cloacae*	2.1×10^5		*Bacillus spp.*	3.0×10^5	
P_0V_5	*Bacillus spp,Enterobacter cloacae*	3.5×10^5		*Pseudomonas aeruginosa, Streptococcus aureus*	8.0×10^5	
P_0V_6	*Bacillus spp., Pseudomonas aeruginosa*	2.3×10^5		*Eshericha coli, Enterobacter cloacae*	5.0×10^5	
P_0V_7	*Actinomycetes spp., Proteus vulgar*	2.6×10^5		*Micrococcus spp.*	4.1×10^5	
P_0V_8	*Pseudomonas aeruginosa*	2.3×10^5		*Bacillus anthrax*	4.1×10^5	
P_0V_9	*Pseudomonas aeruginosa, Bacillus spp.*	2.2×10^5		*Pseudomonas aeruginosa*	4.0×10^5	
P_0V_{10}	*Achinombacter xylosoxidens,Bacillus spp.*	2.7×10^5		*Protus vulgaris, Actinomycetes*	3.4×10^5	
P_0V_{11}	*Enterobacter cloacae*	7.1×10^5		*Protus vulgaris, Corynebacterium pyogenes*	4.0×10^5	
P_0V_{12}	*Eshericha coli*	2.1×10^5	**2.84×10^5**	*Serratia marcescens*	5.0×10^5	**4.66×10^5**
P_1V_0	*Bacillus spp.*	1.8×10^5		*Pseudomanas spp.*	2.5×10^5	
P_1V_1	*Enterobacterium spp.*	1.8×10^5		*Micrococcus leuteus, Bacillus spp.*	2.0×10^5	
P_1V_2	*Mycobacterium sphingomona, Micrococcus arthrobacter*	2.2×10^5		*Bacillus spp., Anthrobacter micrococcus, P. aeruginosa*	6.0×10^5	
P_1V_3	*Bacillus subtilis*	1.8×10^5		*Staphylococcus aureus, Bacillus subtilis*	3.0×10^5	
P_1V_4	*Streptococcus faecalis, Mycobacterium spp.*	1.8×10^5		*Achromobacter xylosixidens*	3.1×10^5	
P_1V_5	*Bacillus anthrax, Mycobacterium spp., Corynebacterium spp.*	2.6×10^5		*Achromobacter spp., Mycobacterium spp.*	5.1×10^5	
P_1V_6	*Micrococcus leuteus*	1.7×10^5		*Streptococcus faecalis, Bacillus subtilis, Micrococcus spp.*	4.0×10^5	
P_1V_7	*Chromobacterium spp.*	1.4×10^5		*Eschericha coli, Chromobacterium spp.*	3.1×10^5	
P_1V_8	*Staphylococcus aureus*	1.8×10^5		*Staphylococcus aureus*	2.0×10^5	
P_1V_9	*Actinetobacter spp.*	1.7×10^5		*Actinobacter spp.*	3.1×10^5	
P_1V_{10}	*Enterobacter cloacae*	1.8×10^5		*Enterobacter cloacae, P. aeroginosa*	3.1×10^5	
P_1V_{11}	*Streptococcus faecalis, Macrococcus spp.*	1.4×10^5		*Micrococcus luteus*	3.0×10^5	
P_1V_{12}	*Staphylococcus spp., P. aeruginosa*	2.2×10^5	**1.85×10^5**	*Corynebacterium dyogenes, P. aeroginosa*	3.3×10^5	**3.30×10^5**

TABLE 8 CONTD.

Treatment	Bacterial isolate	2 months after pollution TBH(cfu/g)	Mean count (cfu/g)	Bacterial isolate	4 months after pollution TBH(cfu/g)	Mean count (cfu/g)
P_2V_0	*Pseudomonas aeruginosa*	1.3×10^5		*Chromobacterium*	2.3×10^5	
P_2V_1	*P. aeruginosa*	1.5×10^5		*Actinomycetes spp.*	3.0×10^5	
P_2V_2	*Flavobacterium spp, P. aeruginosa, Baccillus subtilis*	2.0×10^5		*Acinobacter spp.*	5.3×10^5	
P_2V_3	*P. aeruginosa*	1.7×10^5		*Bacillus anthrax*	3.1×10^5	
P_2V_4	*P. aeruginosa, Actinonycetes*	1.5×10^5		*Chromobacterium spp., Actinomycetes spp.*	2.2×10^5	
P_2V_5	*Actinomycetes spp, Acinetobacter, Bacillus subtilis*	2.1×10^5		*Bacillus anthrax*	4.3×10^5	
P_2V_6	*Chromobacterium spp*	1.3×10^5		*Pseudomonas aeruginosa*	5.0×10^5	
P_2V_7	*Micrococcus luteus, Actinomycetes spp.*	1.6×10^5		*Pseudomonas spp., Bacillus anthrax*	3.0×10^5	
P_2V_8	*Bacillus spp.*	1.5×10^5		*Actinomycetes spp.*	2.1×10^5	
P_2V_9	*Actinomycetes spp., Bacillus spp.*	1.6×10^5		*Flavobacterium spp.*	3.0×10^5	
P_2V_{10}	*Flavobacterium spp.*	1.4×10^5		*Flavobacterium spp.*	2.0×10^5	
P_2V_{11}	*P. aeruginosa*	1.2×10^5		*Enterobacter spp.*	3.1×10^5	
P_2V_{12}	*P. aeruginosa, Escherichia coli*	1.6×10^5	**1.56×10^5**	*Flavobacterium spp., Enterobacter spp.*	3.0×10^5	**3.18×10^5**
P_3V_0	*Bacillus spp.*	1.1×10^5		*Bacillus spp.*	1.5×10^5	
P_3V_1	*Chromobacterium spp.*	1.2×10^5		*Chromobacterium spp.*	2.1×10^5	
P_3V_2	*Bacillus spp., Micrococcus spp.*	1.5×10^5		*Corynebacterium spp.*	3.0×10^5	
P_3V_3	*Micrococcus luteus, Acinetobacter spp*	1.3×10^5		*Acinotobacter spp.*	2.1×10^5	
P_3V_4	*Bacillus spp.*	1.4×10^5		*Escherica coli*	2.0×10^5	
P_3V_5	*P. aeruginosa, Bacillus subtilis*	1.6×10^5		*Enterobacter cloacae, Bacillus subtilis*	4.0×10^5	
P_3V_6	*Eschericha coli*	1.0×10^5		*Flavobacterium spp., Escherica coli*	4.0×10^5	
P_3V_7	*Bacillus anthrax, Spinnogomonas spp.*	1.4×10^5		*P. aeruginosa, Bacillus spp.*	2.1×10^5	
P_3V_8	*Bacillus anthrax, Staphylococcus spp.*	1.3×10^5		*Staphylococcus spp.*	1.0×10^5	
P_3V_9	*Flavobacterium spp., Bacillus spp.*	1.5×10^5		*Bacillus spp., Chromobacterium*	2.1×10^5	
P_3V_{10}	*Chromobacterium spp., Rhodococcus spp.*	1.1×10^5		*Chromobacterium spp.*	2.1×10^5	
P_3V_{11}	*Eschericha coli, Mycobascterium*	1.1×10^5		*Escherica coli*	2.0×10^5	
P_3V_{12}	*Bacillus spp. (Pseudomonas aeruginosa)*	1.2×10^5	**1.28×10^5**	*Bacillus subtilis*	2.4×10^5	**2.34×10^5**

Table 9

Characterization and identification of bacterial isolates

Isolates codes	Gram reaction	Cultural characteristics	Oxidase	Catalase	Lactase	Sucrose	Glucose	H_2S	Gas	Motility	ORN	Indole	Urease	Citrate	MR	VP	Cell morphology	Confirmed organism
A	+	Large, dry, irregular flat, colony	+	+	-	-	+	+	-	+	+	-	-	+	-	+	Bacillus rod	*Bacillus anthrax*
B	-	Smooth, raised, round and greenish colony	+	+	-	-	-	-	-	+	-	-	-	+	+	-	Curved rod	*Pseudomonas aeroginosa*
C	+	Bright, yellow, convex & raised colony	+	+	-	-	+	-	-	-	+	-	-	+	+	-	Cocci in clusters	*Micrococcus luteus*
D	-	Red pigmented, mucoid and spreading	-	+	+	+	-	-	+	-	-	-	+	+	-	+	Rod in pairs	*Acinetobacter spp.*
E	+	Smooth, raised, glistering and spherical	-	+	-	-	+	-	-	-	+	-		-	-	+	Cocci in clusters	*Staphylococcus aureus*
F	-	Convex, circular mucoid and smooth	-	+	+	+	+	-	+	+	+	+	-	-	+	-	Single short red	*Escherica coli*
G	-	Purple pigmented, circular, smooth, convex	+	+	+	+	+	-	-	+	-	-	+	+	+	-	Rod	*Chromobacterium spp.*
H	+	Circular, yellow, pigmented, wax & large	-	-	-	-	+	-	-	-	-	-	+	+	-	+	Branched rod	*Actinomycetes spp.*
I	+	Flat, irregular, swarming growth, smooth	-	+	+	+	+	+	-	+	-	-	+	+	+	-	Small rod	*Proteus vulgaris*
J	-	Smooth, moist, circular, raised and gray	-	+	+	+	+	-	+	+	+	-	-	+	-	+	Small rod single	*Enterobacter coacae*
K	-	Yellow, circular, smooth, shining colony	+	+	-	-	+	-	-	-	-	+	-	-	+	-	Long slender rod	*Flavobacterium spp.*
L	-	Pale yellow, smooth and glistering colony	+	+	-	-	-	-	+	+		-	+	+	-	-	Slender rod	*Achromabacter xylosoxiden*
M	+	Spherical, flat, large & mucoid colony	-	-	+	+	+	-	+	-	-	-	-	-	-	-	Cocci in chain	*Streptococcus facalis*
N	+	Round white, flat and dry surface colonies with rhizoidal edge	-	+	-	-	+	+	-	+	-	-	-	+	-	+	Rod shape in chain	*Bacillus subtilis*

TABLE 10

Total hydrocarbon fungi count (THF) (cfu/g) at two and four months after pollution in the green house

Treatment	Fungal isolate	2 months after pollution		Fungal isolate	4 months after pollution	
		THF(cfu/g)	Mean count(cfu/g)		THF(cfu/g)	Mean count(cfu/g)
P_0V_0	*Penicillum spp., Trichoderma viride*	3.5×10^3		*Fusarium spp., Aspergillus spp.*	3.2×10^3	
P_0V_1	*Rhizopus spp., Mucor indicus*	2.2×10^3		*Penicillium spp., Mucor indicus*	3.1×10^3	
P_0V_2	*Fusarium oxysoporium, Mucor indicus*	2.1×10^3		*Verticillium spp., Fusarium spp., Mucor indicus*	4.0×10^3	
P_0V_3	*Pennicillum spp., Fusarium oxysporium*	2.2×10^3		*Penicillium spp.*	2.1×10^3	
P_0V_4	*Rhizopus spp.*	1.9×10^3		*Trichoderma viride, Rhizopus indicus*	3.5×10^3	
P_0V_5	*Mucor indicus*	2.2×10^3		*Rhizopus spp., Mucor indicus*	5.0×10^3	
P_0V_6	*Mucor indicus*	2.3×10^3		*Mucor indicus, Chrysoporium tropicum*	4.0×10^3	
P_0V_7	*Trichoderma viride, Mucor indicus*	1.7×10^3		*Fusarium spp., Mucor indicus*	3.1×10^3	
P_0V_8	*Trichoderma viride*	1.8×10^3		*Rhizopus spp., Trichoderma spp.*	2.1×10^3	
P_0V_9	*Rhizopus spp*	2.3×10^3		*Fusarium spp., Rhizopus spp.*	3.4×10^3	
P_0V_{10}	*Penicillum spp., Fusarium spp.*	2.3×10^3		*Fusarium spp., Pencillium spp*	3.0×10^3	
P_0V_{11}	*Aspergillus fumigatus, Rhizopus indicus, Verticillium spp.*	4.5×10^3		*Trichoderma viride, Aspergillus spp.*	3.1×10^3	
P_0V_{12}	*Fusarium oxysaporium*	2.2×10^3	**2.40×10^3**	*Mucor indicus, Fusarium spp.*	3.0×10^3	**3.27×10^3**
P_1V_0	*Aspergillus spp., Penicillium spp.*	1.5×10^3		*Aspergillus spp.*	1.8×10^3	
P_1V_1	*Mucor indicus*	1.6×10^3		*Mucor indicus, Fusarium spp.*	1.2×10^3	
P_1V_2	*Pencillium spp., Rhizopus indicus*	1.7×10^3		*Yeast spp., Rhizopus indicus*	3.5×10^3	
P_1V_3	*Mucor indicus*	0.6×10^3		*Verticillium spp., Mucor indicus*	2.0×10^3	
P_1V_4	*Mucor indicus*	1.5×10^3		*Rhizopus spp., Mucor indicus*	2.0×10^3	
P_1V_5	*Rhisopus spp.*	1.8×10^3		*Pencillium spp., Rhizopus spp.*	4.0×10^3	
P_1V_6	*Rhisopus spp., Mucor indicus*	1.5×10^3		*Mucor indicus*	3.0×10^3	
P_1V_7	*Penicillum spp.*			*Aspergillus spp., Penicillum spp.*	2.0×10^3	
P_1V_8	*Verticillium spp.*	1.4×10^3		*Trichodema spp., Verticillum spp.*	3.0×10^3	
P_1V_9	*Fusarium spp.*	1.5×10^3		*Mucor indicus, Fusarium spp.*	3.0×10^3	
P_1V_{10}	*Aspergillus niger*	1.4×10^3		*Pennicillium spp., Aspergillus spp.*	2.1×10^3	
P_1V_{11}	*Pencillium spp.*	1.7×10^3		*Verticillium spp., Penicillium spp.*	3.0×10^3	
P_1V_{12}	*Aspergillus fumigatus*	1.6×10^3	**1.36×10^3**	*Verticillium spp., Aspergillus spp.*	3.0×10^3	**2.58×10^3**

TABLE 10 CONTD

Total hydrocarbon fungi count (THF) (cfu/g) at two and four months after pollution in the green house

Treatment	Fungal isolate	2 months after pollution		Fungal isolate	4 months after pollution	
		THF(cfu/g)	Mean count (cfu/g)		THF(cfu/g)	Mean count (cfu/g)
P_2V_0	*Penicillium spp.*	0.6×10^3		*Aspergillus spp., Penicillium spp.*	1.5×10^3	
P_2V_1	*Aspergillus fumigatus, Rhizopus spp.*	1.3×10^3		*Rhizopus spp., Mucor indicus*	2.0×10^3	
P_2V_2	*Aspergillus fumigatus, Pencillium spp.*	1.5×10^3		*Aspergillus spp., Pencillium spp.*	3.0×10^3	
P_2V_3	*Rhizopus spp.*	1.6×10^3		*Aspergillus spp., Rhizopus spp.*	3.0×10^3	
P_2V_4	*Verticillium spp.*	1.2×10^3		*Mucor indicus, Verticillum spp.*	4.0×10^3	
P_2V_5	*Aspergillus fumigatus*	1.4×10^3		*Yeast, Aspergillus fumigatus*	3.1×10^3	
P_2V_6	*Penicillium spp.*	1.4×10^3		*Penicillium spp.*	3.1×10^3	
P_2V_7	*Verticillium spp.*	0.7×10^3		*Mucor inicus, Verticillium spp*	3.0×10^3	
P_2V_8	*Mucor indicus*	1.1×10^3		*Fusarium spp., Mucor indicus*	2.0×10^3	
P_2V_9	*Penicillium spp., Mucor indicus*	1.2×10^3		*Rhizopus spp., Penicillium spp.*	2.1×10^3	
P_2V_{10}	*Verticillium spp.*	1.1×10^3		*Trichoderma viride, Verticillium spp.*	2.0×10^3	
P_2V_{11}	*Trichoderma viride*	1.3×10^3		*Trichoderma viride, Mucor spp.*	3.1×10^3	
P_2V_{12}	*Mucor indicus*	1.3×10^3	**1.21×10^3**	*Aspergillus spp., Mucor indicus*	2.0×10^3	**2.60×10^3**
P_3V_0	*Penicillium spp.*	1.3×10^3		*Penicillium spp.*	1.2×10^3	
P_3V_1	*Verticillium spp.*	1.0×10^3		*Aspergillus spp., Verticillium spp.*	3.0×10^3	
P_3V_2	*Mucor spp.*	1.2×10^3		*Rhizopus spp., Mucor spp.*	2.0×10^3	
P_3V_3	*Aspergillus fumigatus*	1.0×10^3		*Aspergillus spp., Fusarium spp.*	2.1×10^3	
P_3V_4	*Aspergillus fumigatus*	1.2×10^3		*Penicillium spp., Aspergillus spp.*	3.1×10^3	
P_3V_5	*Penicillium spp.*	1.6×10^3		*Aspergillus spp., Penicillium*	3.0×10^3	
P_3V_6	*Trichoderma viridi*	1.0×10^3		*Fusarium spp., Trichoderma viride*	3.0×10^3	
P_3V_7	*Mucor indicus*	1.4×10^3		*Penicillium spp., Mucor indicus*	3.0×10^3	
P_3V_8	*Rhizopus spp., Mucor indicus*	1.3×10^3		*Mucor indicus, Verticillium spp.*	2.1×10^3	
P_3V_9	*Trichoderma viride*	1.0×10^3		*Aspergillus spp., Trichoderma spp.*	2.0×10^3	
P_3V_{10}	*Rhizopus spp.*	1.1×10^3		*Penicillium spp., Rhizopus spp.*	1.1×10^3	
P_3V_{11}	*Mucor indicus*	1.3×10^3		*Rhizopus spp., Mucor indicus*	2.1×10^3	
P_3V_{12}	*Penicilliumspp., Fusarium spp*	1.1×10^3	**$1.19sx \times 10^3$**	*Penicillium spp., Fusarium spp.*	2.1×10^3	**$2.29x \times 10^3$**

Total heterotrophic fungi count in the control soil was significantly ($P < 0.05$) higher than that in the polluted soils. The possible reason for the low microbial population under pollution is the anaerobic soil condition created by the crude oil. Ijah and Antai (2003a), Adesina and Adelasoye (2014) also observed a decrease in total heterotrophic fungi count in crude oil polluted soils.The fungi isolates using cultural characteristics (Table 11) identified were: *Penicillium spp., Trichoderma viride, Mucor spp., Fusarium oxysporium, Aspergillus spp., Rhizopus spp., Verticillium spp.*

4.3.3 Hydrocarbon utilizing bacteria (HUB)

The main effects of concentration of crude oil on hydrocarbon utilizing bacteria (HUB) at 2 and 4 months after pollution are presented in Table 12. The HUB were significantly higher in the polluted than the control (unpolluted) soil. At 4MAP, soil treated with 5.0% crude oil had significantly ($P<0.05$) more population ($5.25x10^5$) than the other polluted soils and the control.

Evidently, the hydrocarbon in the polluted soil serves as a nutrient source for the proliferation of microbes. This is consistent with Ijah *et al.* (2008) who reported a higher population of HUB in crude oil polluted soils. The hydrocarbon utilizing bacteria identified were: *Bacillus spp., Pseudomonas spp., Micrococcus spp., Mycobacteria spp., Enterobacter spp., Acinetobacter spp.* and *Flavobacterium spp.*

4.3.4 Hydrocarbon utilizing fungi (HUF)

The main effects of concentration of crude oil on hydrocarbon utilizing fungi (HUF) at 2 and 4 MAP are presented in Table 13. At 2 months after treatment, the HUF were significantly ($P < 0.05$) higher in soil contaminated with 7.5 % crude oil than the control soil. At 4 MAP, soil treated with 2.5 % crude oil had significantly ($P < 0.05$)more population ($2.47x10^3$) than the other polluted soils and the control. This is in agreement with the reports of Saadoun (2002) that old contaminated soils showed greater numbers

TABLE 11
Characterization and identification of fungal isolates

Colony code	Microscopic features	Cultural characteristics	Confirmed organisms
A	Septate hyphae, conidiophores developed into branches phalides bearing chains of conidia and brush like appearance	Moderate, round, greenish and velvety colony	*Penicillium spp.*
B	Non-septate hyphae, long and erect	Hairlike, white to dark gray and fast growing colony	*Rhizopus spp.*
C	Narrow spherical, head that is entirely covered with phalides bearing chains of conidia and septate hyphae	Rapid growing colony, woolly in appearance and flat on the agar surface and fast spreading. Later appeared colony as colony grows old.	*Fusarium oxysporium*
D	Aseptate broad hyphae, large spherical head produced by the conidiasphore	Fast growing colony, initially white in appearance and fluffy white in appearance as culture grows older	*Mucor indicus*
E	Narrow spherical head that is entirely covered with phalides bearing chains of conidia and septate hyphae	Brownish velvety colony that grows rapidly	*Aspergillus spp.*
F	The conidiosphere is vertically arranged, the phalidesappears like flask-shape and clustering conidia	Wolly colony and rapid grow	*Trichoderma viride*
G	Cardisphere are branched when observed, the phalides are long with septate hyphae	Moderate grain colony, velvety and woody like in appearance. Initially colony appears whitish and later yellowish as it became old.	*Verticillium rubrium*
H	Oval and elongated yeast with buds	White to creamy like colony soft with bacteria like appearance	*Yeast spp.*

TABLE 12

Total Hydrocarbon utilizing bacteria (THUB) (cfu/g) at two and four months after pollution in the green house

Treatment	Bacterial isolate	2 months after pollution		Bacterial isolate	4 months after pollution	
		THUB (cfu/g)	Mean count (cfu/g)		THUB (cfu/g)	Mean count (cfu/g)
P_0V_0	*Bacillis spp*	1.6×10^5		*Bacillus spp.*	1.9×10^5	
P_0V_1	*Escheria coli, Flavobacterium spp.*	3.9×10^5		*Flavobacterium spp.*	3.2×10^5	
P_0V_2	*Bacillis spp., Actinomycetes spp*	2.3×10^5		*Bacillis spp.*	2.5×10^5	
P_0V_3	*Stapyllococcus aureus, Sphingomonas spp.*	1.4×10^5		*Shingomonas spp.*	1.2×10^5	
P_0V_4	*Proteus vulgaris, Enterobacter cloacae*	1.9×10^5		*Enterobacter spp.*	1.7×10^5	
P_0V_5						
P_0V_6	*Bacillis spp., P. aeruginosa*	1.4×10^5		*Bacillis spp.*	1.3×10^5	
P_0V_7	*Bacillis subtilis, Proteus vulgaris*	2.0×10^5		*Bacillis spp.*	1.9×10^5	
P_0V_8	*P. aeruginosa, Acinotobacter*	1.7×10^5		*Acinetobacter spp.*	1.8×10^5	
P_0V_9						
P_0V_{10}	*Bacillis spp., Acinotobacter*	1.8×10^5		*Acinotobacter*	2.0×10^5	
P_0V_{11}	*Bacillis subtitis*	0.9×10^5		*Bacillis substilis*	1.2×10^5	
P_0V_{12}			**1.45×10^5**			**1.44×10^5**
P_1V_0	*Acinotobacter spp., Bacillis spp.*	2.8×10^5		*Bacillis spp.*	3.1×10^5	
P_1V_1	*Arthrobacter, Micrococcus spp.*	5.2×10^5		*Micrococcus luteus*	6.2×10^5	
P_1V_2	*Mycobacterium, Micrococcus spp.*	4.4×10^5		*Micrococcus spp., Bacillus subtilis*	5.1×10^5	
P_1V_3	*Sphingomonas spp., Bacillus subtilis*	3.4×10^5		*Bacillus subtilis*	3.9×10^5	
P_1V_4	*Mycobacterium spp.*	4.7×10^5		*Mycobacterium spp.*	5.0×10^5	
P_1V_5	*Bacillus spp., Corynebacterium spp.*	5.2×10^5		*Corynebacterium micrococcus*	6.1×10^5	
P_1V_6	*Micrococcus luteus*	4.1×10^5		*Bacillus subtilis, Micrococcus spp.*	5.8×10^5	
P_1V_7	*Chromobacterium spp.*	3.0×10^5		*Chromobacterium spp.*	4.1×10^5	
P_1V_8	*Staphylococcus aureus*	5.1×10^5		*Staphylococcus aureus*	5.9×10^5	
P_1V_9	*Acinetobacter spp.*	5.4×10^5		*Acinetobacter spp.*	5.8×10^5	
P_1V_{10}	*Enterobacter spp.*	3.3×10^5		*P. aeruginosa*	4.3×10^5	
P_1V_{11}	*Micrococcus spp.*	4.0×10^5		*Micrococcus luteus*	4.9×10^5	
P_1V_{12}	*P. aeruginosa spp.*	4.7×10^5	**4.25×10^5**	*Corynebacterium spp., P. aeruginosa*	5.2×10^5	**5.03×10^5**

TABLE 12 CONTD.

Total hydrocarbon utilizing bacteria (THUB) (cfu/g) at two and four months after pollution in the green house

Treatment	Bacteria isolate	2 months after pollution		Bacteria isolate	4 months after pollution	
		THF(cfu/g)	Mean count (cfu/g)		THF(cfu/g)	Mean count (cfu/g)
P_2V_0	*Bacillus spp.*	5.1×10^5		*Bacillus spp.*	4.0×10^5	
P_2V_1	*P. aeruginosa*	4.7×10^5		*P. aeruginosa spp., Bacillus subtilis*	5.5×10^5	
P_2V_2	*Bacillus subtilis, Flavobacterium spp.*	6.8×10^5		*Acinobacter spp.*	7.1×10^5	
P_2V_3	*P. aeruginosa spp*	5.0×10^5		*Bacillus spp.*	6.3×10^5	
P_2V_4	*P. aeruginosa spp*	5.4×10^5		*Chromobacterium spp.*	5.8×10^5	
P_2V_5	*Acinetobacter, Bacillus subtilis*	4.2×10^5		*Bacillus subtilis, Acinetobacter*	5.7×10^5	
P_2V_6	*Chromobacterium spp.*	5.0×10^5		*P. aeroginosa*	5.5×10^5	
P_2V_7	*Micrococcus spp.*	7.0×10^5		*Micrococcus spp.*	7.3×10^5	
P_2V_8	*Bacillus spp.*	4.3×10^5		*Bacillus spp.*	5.1×10^5	
P_2V_9	*Bacillus spp.*	5.1×10^5		*Flavobacterium*	5.9×10^5	
P_2V_{10}	*Flavobacterium*	4.1×10^5		*P. aeruginosa spp.*	5.2×10^5	
P_2V_{11}	*P. aeruginosa*	5.0×10^5		*P. aeruginosa spp.*	4.9×10^5	
P_2V_{12}	*P. aeruginosa*	4.1×10^5	**5.06×10^5**			**5.25×10^5**
P_3V_0	*Bacillus spp.*	5.7×10^5		*Sphingomonas spp.*	5.9×10^5	
P_3V_1	*Chromobacterium spp., Acinetobacter*	5.1×10^5		*Acinetobacter*	5.3×10^5	
P_3V_2	*Bacillus subtilis, P. aeruginosa spp.*	7.2×10^5		*Bacillus spp., P. aeruginosa spp.*	7.6×10^5	
P_3V_3	*Micrococcus luteus, Bacillus subtilis*	8.0×10^5		*Arthrobacter, Bacillus spp.*	8.2×10^5	
P_3V_4	*Bacillus spp.*	4.3×10^5		*Bacillus spp.,*	4.6×10^5	
P_3V_5	*Bacillus spp., P. aeruginosa spp.*	5.1×10^5		*Bacillus spp., P. aeruginosa spp.*	5.4×10^5	
P_3V_6	*Mycobacterium*	5.3×10^5		*Flavobacterium, Bacillus subtilis*	5.8×10^5	
P_3V_7	*Bacillus spp.*	8.1×10^5		*Sphingomonas, spp., Mycobacterium*	8.8×10^5	
P_3V_8	*Bacillus spp.*	5.6×10^5		*Bacillus spp.*	5.9×10^5	
P_3V_9	*Flavobacterium*	5.7×10^5		*Micrococcus spp., Bacillus spp.*		
P_3V_{10}	*Acinetobacter, Chromobacterium*	5.0×10^5		*Acinetobacter, Corynebacterium*	5.3×10^5	
P_3V_{11}	*Mycobacterium*	1.8×10^5		*Mycobacterium*	5.0×10^5	
P_3V_{12}	*Bacillus spp., P. aeruginosa spp.*		**5.14×10^5**	*Bacillus spp., Micrococcus spp.*		**5.22×10^5**

TABLE 13
Total hydrocarbon utilizing fungi (THUF) (cfu/g)at two and four months after pollution in the green house

Treatment	Fungi isolate	2 months after pollution THUF (cfu/g)	Mean count (cfu/g)	Fungi isolate	4 months after pollution THUF (cfu/g)	Mean count (cfu/g)
P_0V_0	*Penicillium spp*	0.71×10^3		*Fusarium spp.*	1.7×10^3	
P_0V_1	*Mucor spp*	0.71×10^3		*Penicillium spp*	1.5×10^3	
P_0V_2	*Mucor spp*	0.71×10^3		*Fusarium spp, Mucor indicus*	1.9×10^3	
P_0V_3	*Penicillium spp*	0.71×10^3		*Pencillium spp*	1.3×10^3	
P_0V_4	*Penicillium spp*	0.71×10^3		*Rhizopus indicus*	1.4×10^3	
P_0V_5	*Mucor spp*	0.71×10^3		Mucor indicus	1.4×10^3	
P_0V_6	*Mucor spp*	0.71×10^3		*Mucor indicus*	1.7×10^3	
P_0V_7	*Mucor spp*	0.71×10^3		*Rhizopus spp.*	1.1×10^3	
P_0V_8	*Fusarium spp*	0.71×10^3		*Fusarium spp.*	2.7×10^3	
P_0V_9	*Fusarium spp*	0.71×10^3		*Fusarium spp*	1.6×10^3	
P_0V_{10}	*Penicillium spp*	0.71×10^3		*Penicillium spp.*	1.6×10^3	
P_0V_{11}	*Mucor spp*	0.71×10^3		*Aspergillus spp.*	1.4×10^3	
P_0V_{12}	*Fusarium spp*	0.71×10^3	**0.71×10^3**	*Fusarium spp.*	1.4×10^3	**1.59×10^3**
P_1V_0	*Penicillium spp*	0.71×10^3		*Aspergillus spp.*	0.71×10^3	
P_1V_1	*Aspergillus niger*	1.4×10^3		*Mucor indicus*	2.9×10^3	
P_1V_2	*Rhizopus indicus, Asperillus fumigatus*	1.9×10^3		*Aspergillus niger, Penicillium spp., Rhizopus indicus*	3.3×10^3	
P_1V_3	*Mucor indicus*	0.71×10^3		*Mucor indicus*	2.7×10^3	
P_1V_4	*Penicillium spp.*	0.71×10^3		*Rhizopus indicus, Penicillium spp.*	2.3×10^3	
P_1V_5	*Aspergillus fumigatus*	0.71×10^3		*Aspergillus fumigatus, Rhizopus indicus*	2.3×10^3	
P_1V_6	*Mucor indicus*	1.4×10^3		*Mucor indicus*	1.9×10^3	
P_1V_7				*Aspergillus niger*	2.3×10^3	
P_1V_8	*Penicillium spp.*	0.71×10^3		*Penicillium spp.*	2.7×10^3	
P_1V_9	*Fusarium spp.*	0.71×10^3		*Mucor indicus, Fusarium spp.*	3.1×10^3	
P_1V_{10}	*Asperillus niger*	0.9×10^3		*Penicillium spp., Aspergillus fumigatus*	2.3×10^3	
P_1V_{11}	*Pencillium spp.*	0.9×10^3		*Penicillium spp, Rhizopus indicus*	3.1×10^3	
P_1V_{12}	*Rhizopus spp.*	1.1×10^3	**0.91×10^3**	*Aspergillus niger*	2.5×10^3	**2.47×10^3**

TABLE 13 CONTD.

Total hydrocarbon utilizing fungi (THUB) (cfu/g) at two and four months after pollution in the green house

Treatment	Fungi isolate	2 months after pollution		Fungi isolate	4 months after pollution	
		THF(cfu/g)	Mean count (cfu/g)		THF(cfu/g)	Mean count (cfu/g)
P_2V_0	*Penicillium spp*	0.71×10^3		*Penicillium spp.*	1.2×10^3	
P_2V_1	*Rhizopus spp.*	0.71×10^3		*Rhizopus indicus*	2.6×10^3	
P_2V_2	*Penicillium spp.*	0.71×10^3		*Aspergillus niger, Penicillium spp.*	3.1×10^3	
P_2V_3	*Aspergillus niger*	1.6×10^3				
P_2V_4	*Rhizopus indices*	1.7×10^3				
P_2V_5	*Aspergillus fumigatus*	1.4×10^3		*Penicillium spp.*	1.9×10^3	
P_2V_6	*Penicillium spp.*	1.6×10^3		*Aspergillus fumigatus*	2.1×10^3	
P_2V_7	*Fusarium spp.*	1.1×10^3		*Aspergillus niger, Rhizopus indicus*	3.4×10^3	
P_2V_8	*Mucor indicus*	1.4×10^3		*Penicillium spp., Fusarium spp.*	3.6×10^3	
P_2V_9	*Penicillium spp.*	1.2×10^3				
P_2V_{10}	*Penicillium spp.*	0.71×10^3				
P_2V_{11}	*Fusarium spp.*	0.71×10^3		*Aspergillus niger, Mucor indicus*	3.7×10^3	
P_2V_{12}	*Aspergillus niger*	0.71×10^3	**1.09×10^3**	*Aspergillus niger*	2.6×10^3	**1.86×10^3**
P_3V_0	*Fusarium spp.*	0.71×10^3		*Penicillium spp.*	1.3×10^3	
P_3V_1	*Penicillium spp.*	1.7×10^3				
P_3V_2	*Aspergillus niger*	1.6×10^3		*Fusarium spp.*	2.5×10^3	
P_3V_3	*Aspergillus fumigatus*	1.5×10^3		*Aspergillus fumigatus*	2.6×10^3	
P_3V_4	*Aspergillus fumigatus*	1.5×10^3		*Penicillium spp., Aspergillus niger*	2.6×10^3	
P_3V_5	*Penicillium spp.*	0.71×10^3		*Aspergillus niger*	2.9×10^3	
P_3V_6	*Mucor indicus*	1.6×10^3		*Fusarium spp., Rhizopus indicus*	3.5×10^3	
P_3V_7	*Fusarium spp.*	2.0×10^3				
P_3V_8	*Rhizopus indicus*	1.3×10^3				
P_3V_9	*Mucor indicus*	1.1×10^3				
P_3V_{10}	*Rhizopus spp.*	0.71×10^3				
P_3V_{11}	*Fusarium spp.*	0.71×10^3		*Rhizopus spp., Aspergillus fumigatus*	3.1×10^3	
P_3V_{12}	*Penicillium spp*	0.71×10^3	**1.22×10^3**	*Penicillium spp.*	2.1×10^3	**1.58×10^3**

of microorganisms, while fresh contaminated soils showed lower numbers. The hydrocarbon utilizing fungi identified were: *Penicillium spp., Aspergillus niger, Aspergillus fumigatus, Fusarium spp, Mucor spp,* and *Rhizopus spp* (Table 13).

4.4 Effects of plant species on total heterotrophic bacteria counts

4.4.1 The effects of different plant species on total heterotrophic bacteria at 2 and 4 months after soil pollution

The population density of heterotrophic bacteria (THB) at 4 months was significantly ($P > 0.05$) higher than that at 2 months (Figure 13). The heterotrophic bacteria were preponderance in the order *L. leucocephala* > *G. sepium* > *P. purpureum* > *C. pubescens* > *A. compressus* > *E. indica* > *C. rotundus* > *J. curcas* (control) > *P. maximum* > *T. fructicosum* > *C. odorata.*

This implies that *L. leucocepha, G. sepium* and *P. purpureum* would be more tolerant of crude oil pollution and are the best fitted for phytoremediation of crude oil polluted soils.

4.4.2 Effects of plant species on total heterotrophic fungi at 2 and 4 months in the greenhouse

At 2 MAP, the soil under *J. curcas* had a higher population of hydrocarbon fungi than those under other species and the control while soil under *T. fructicosum* significantly reduced the population (Figure 14). At 4 MAP, the soil under *L. leucocephala* had significantly more population than other treatments. Generally, total hydrocarbon fungi was much ($P < 0.05$) higher in soil at 4 MAP than at 2 MAP.

4.4.3 Effects of plant species on hydrocarbon utilizing bacteria at 2 and 4 months in the greenhouse

The effects of plant species on hydrocarbon utilizing bacteria at 2 and 4 MAP in the green house are presented in Figure 15. At 2 MAP, the soil under *P. purpureum* had more hydrocarbon utilizing bacteria than other treatments and the control. This was closely followed by soil under *T. fructicosum*. At 4 MAP, the soil under *P. purpureum*

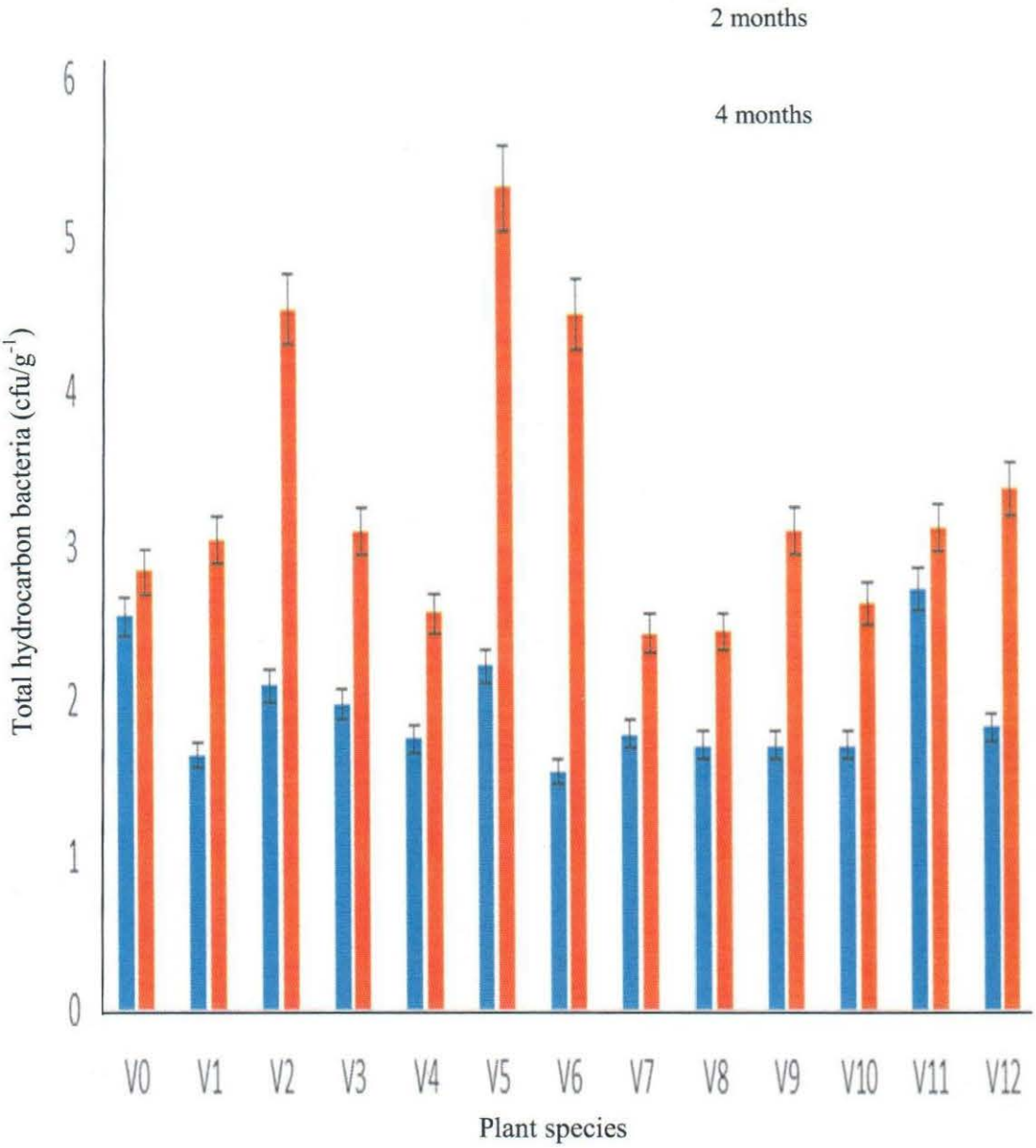


FIG. 13: Effect of different plant species on total hydrocarbon bacteria at 2 and 4 months in the greenhouse

Treatments

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

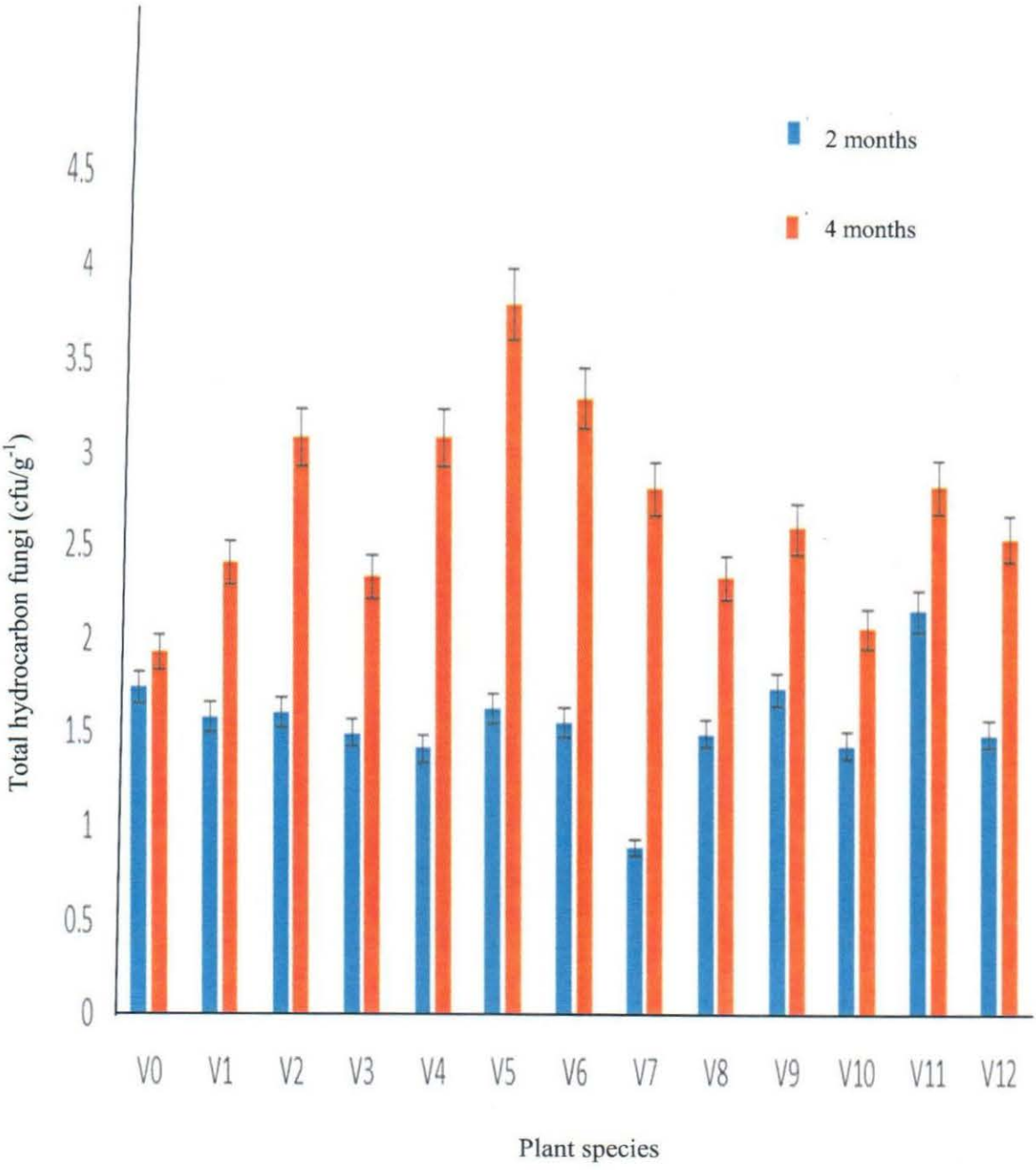


FIG. 14: Effects of plant species on total hydrocarbon fungi at 2 and 4 in the green house

Treatments

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

also had the highest population of hydrocarbon utilizing bacteria, followed by soil under *C. pubescens*.

4.4.4 Effects of plant species on hydrocarbon utilizing fungi at 2 and 4 months in the greenhouse.

At 2 MAP, there was no significant effect of plant species on the population of hydrocarbon utilizing fungi (Figure 16). At 4 MAP, soils under *P. purpureum* and *J. curcas* had much higher population than other treatments. The population of hydrocarbon utilizing fungi was generally higher at 4 MAP than 2 MAP.

4.5 Main effects of crude oil on growth parameters of plant species in the greenhouse

4.5.1 Plant Biomass

The main effects of crude oil on fresh weight of roots are shown in Figure 17. Successive increases in crude oil pollution decreased the fresh weight of plant, possibly due to the alteraction in soil properties, leading to nutrient immobilization and non-availability. Similar observation have been reported in Benka-Coker and Ekundayo (1995).

4.5.2 Fresh weight of roots of different plant species at 4 months after crude oil pollution in the greenhouse

The variation in the fresh weight of roots of different plant species across pollution levels are shown in Figure 18. *Axonopus compressus* and *P. purpureum* produced significantly heavier fresh roots than other plant species. The increase in fresh

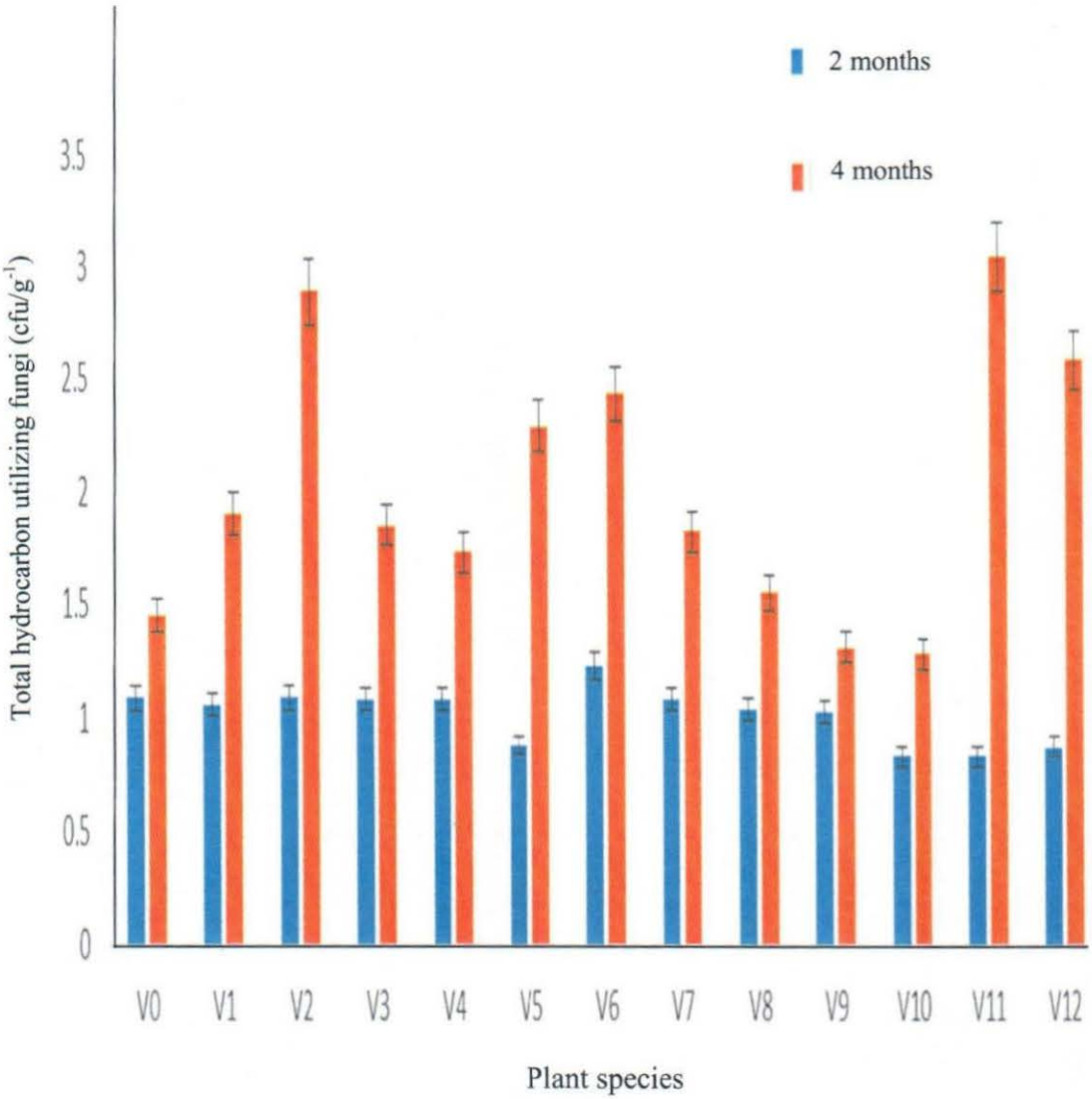


FIG. 16: Effects of plant species on hydrocarbon utilizing fungi at 2 and 4 months after crude oil pollution in the greenhouse

Treatments

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

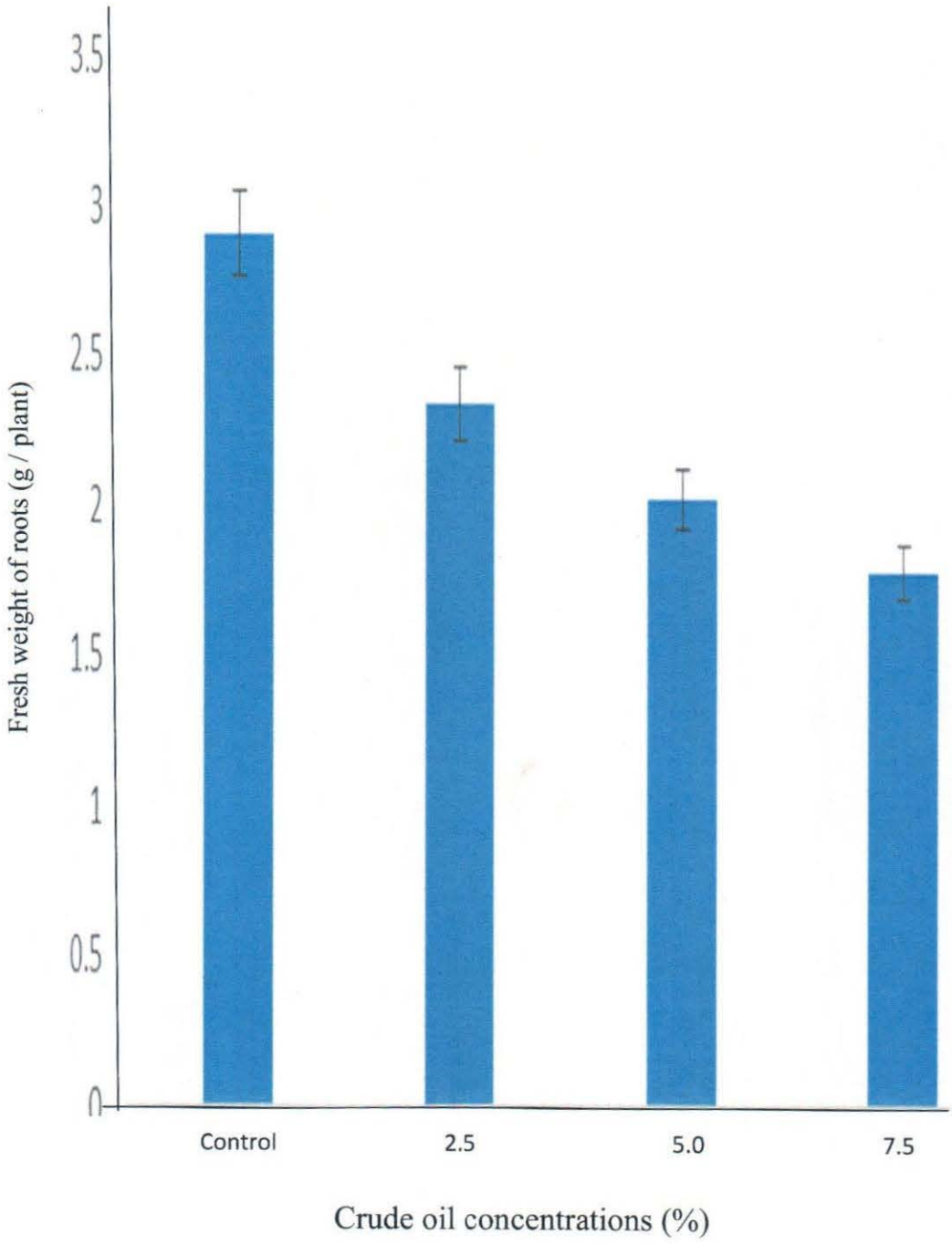


FIG. 17: Main effects of different concentrations of crude oil on fresh weight of roots of plant species

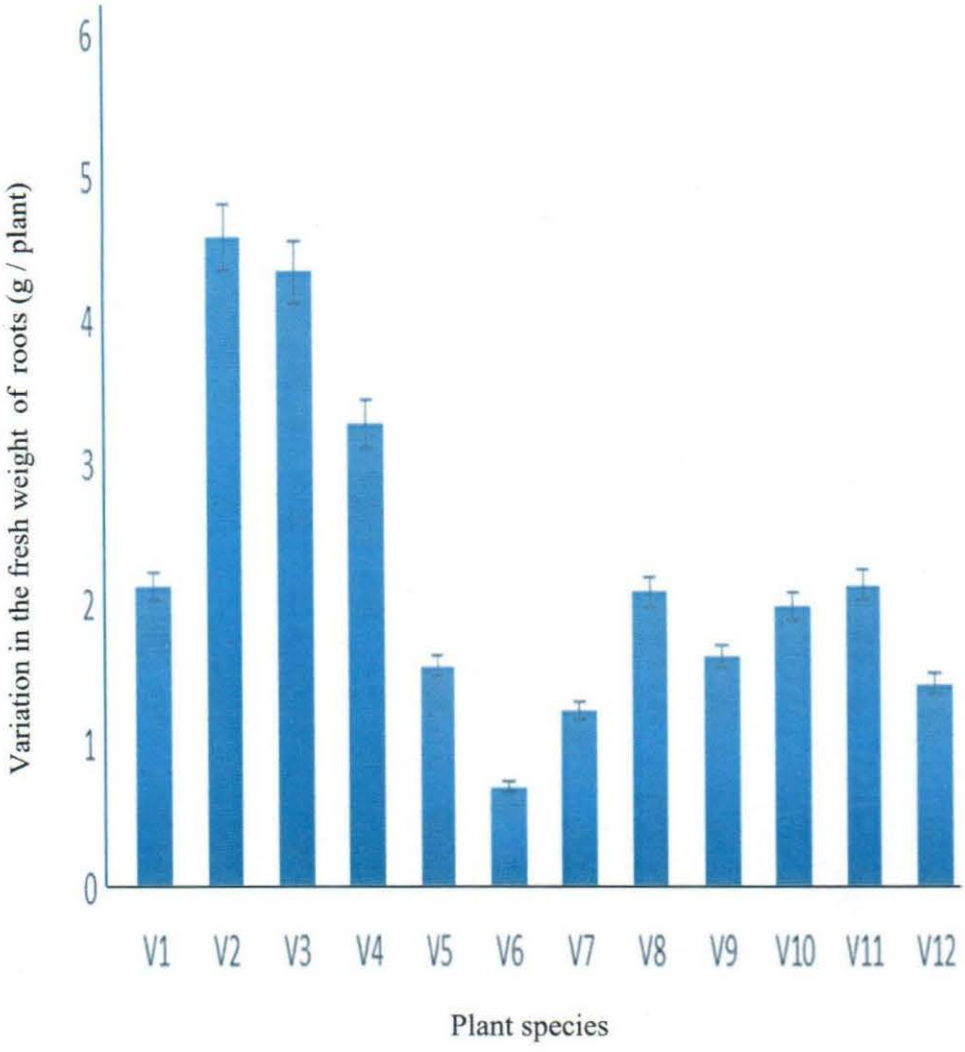


FIG.18: Variation in fresh weight of roots of different plant species

Treatments

V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}= Calapo (*Calapogonium mucunoides*), V_{11}= Jatropha (*Jatropha curcas*), V_{12}= Centro (*Centrosema pubescens*).

weight of roots in this study may be due to the agronomic advantages such as adaptability to various soil types and their rapid growth rate.

4.5.3 Dry weight of root of plant species

The main effects of crude oil on dry weight of roots of plant species at 4 MAP in the greenhouse are shown in Figure 19. Increased in crude oil pollution decreases the dry weight of root of plant species when compared with the control.

4.5.4 Variation in dry weight of roots of different plant species in the greenhouse

The soil under *P.purpureum* significantly had the highest dry weight of roots among treatments (Figure 20). Soils under *G. sepium, T. fructicosum, C.mucunoides, J. curcas, C. pubescens, C. odorata, C. rotundus* and *L. leucocephala* produced much lower dry weight of root than the control.

4.5.5 Fresh shoot biomass

Data for fresh shoot biomass of the species at different levels of pollution are shown in Figure 21. Successive increases in crude oil pollution decreased the shoot fresh weight of the plants, possibly due to interference in nutrient availability and uptake. A similar observation has been reported in Benka-Coker and Ekundayo (1995).*Pennisetum purpureum* had the highest ($P<0.05$) fresh weight than all other treatments, including the control (Figure 22). The lowest fresh weight was in *Gliricidia sepium.*

4.5.6 Dry weight of shoots of different plant species

The dry weight of shoots of different plant species across pollution level are shown in Figure 23. *Pennisetum purpureum* significantly had the highest dry weight relative to other treatments and the control while the other species had much lower dry weight than the control.

4.6 Total petroleum hydrocarbon content in soil

4.6.1 Total petroleum hydrocarbon content in soil under different plant species

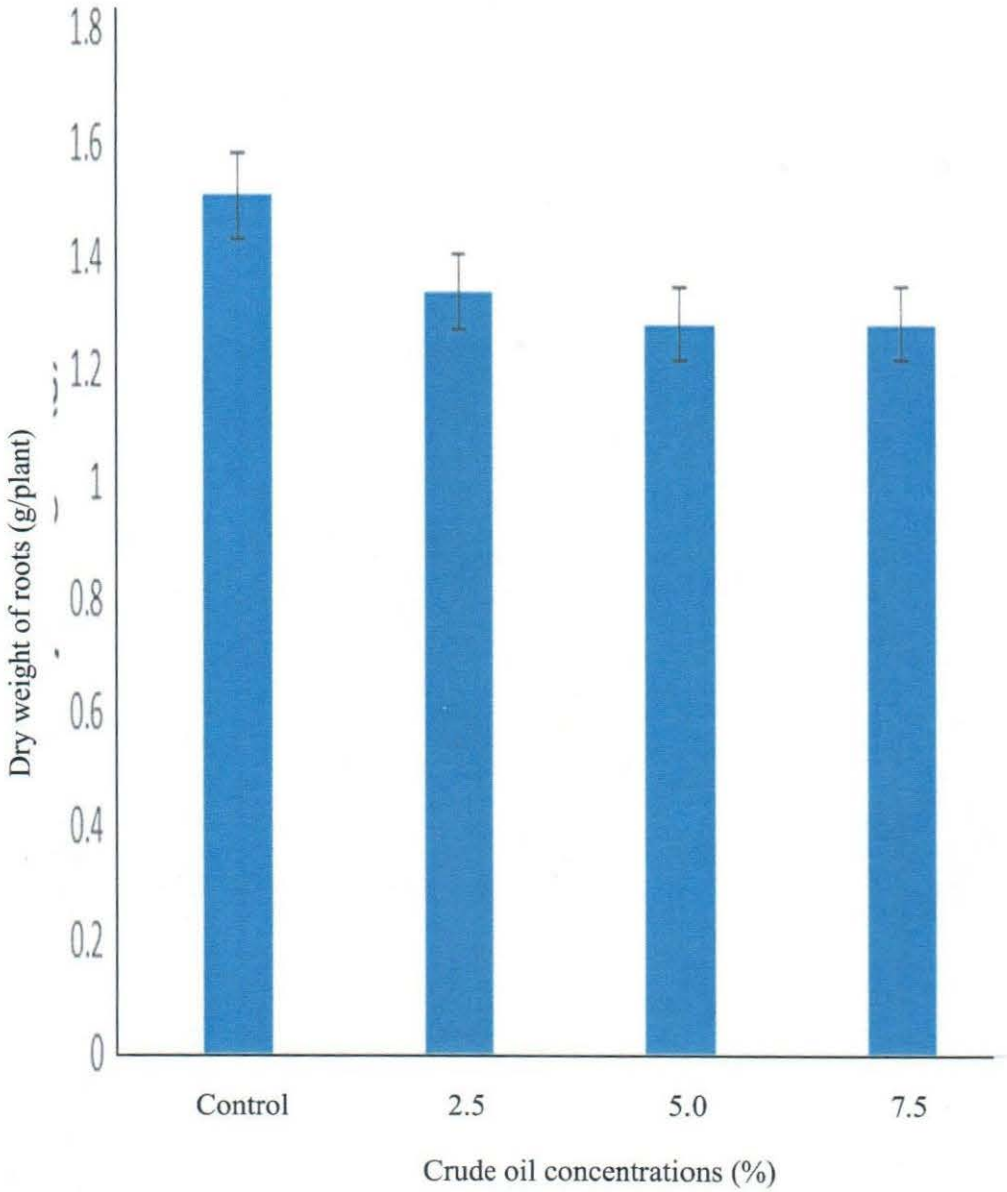


FIG. 19: Variations in the dry weight of root of plant species

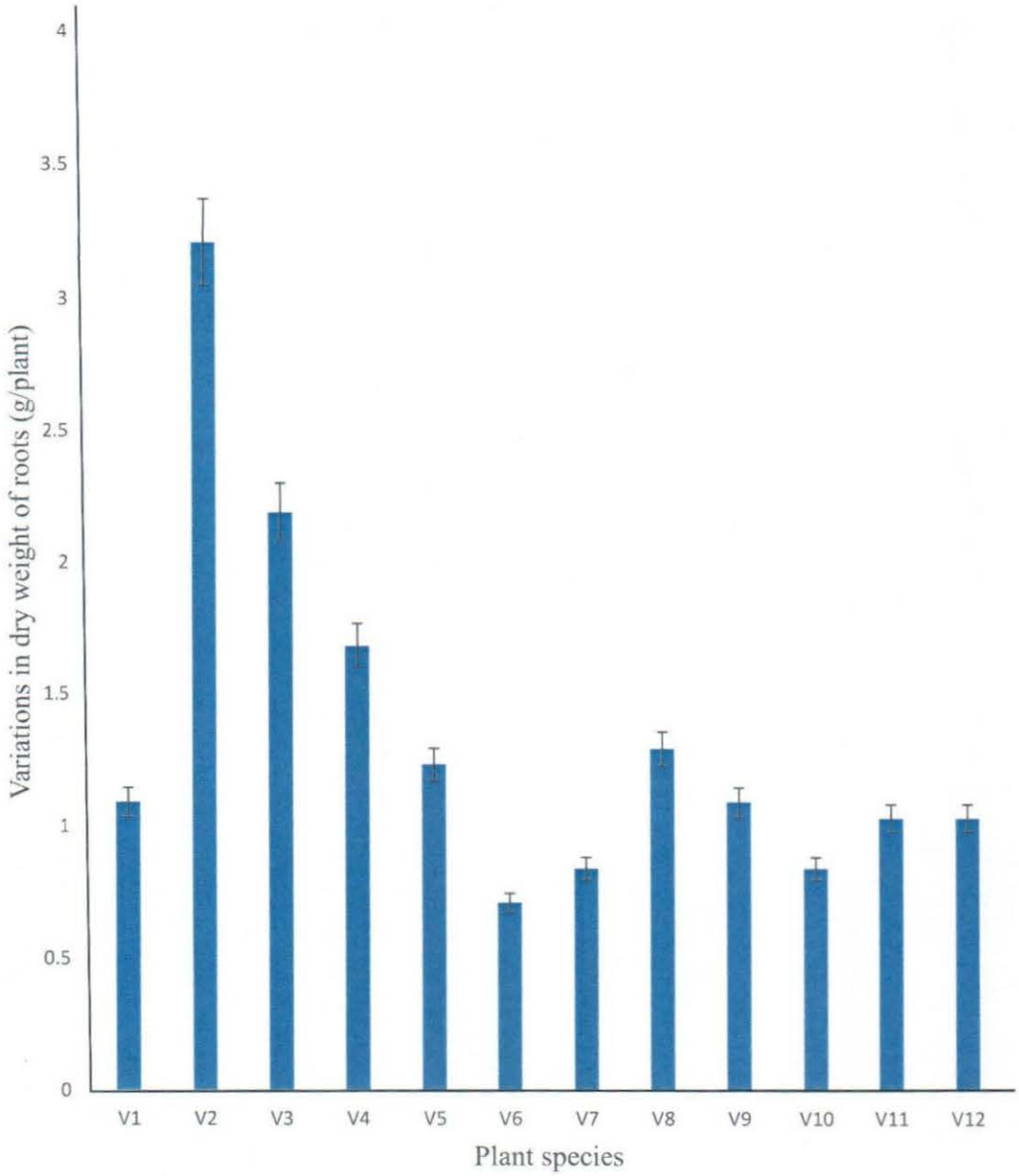


FIG. 20: Variations in dry weight of roots of different plant species in the greenhouse

Treatments

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

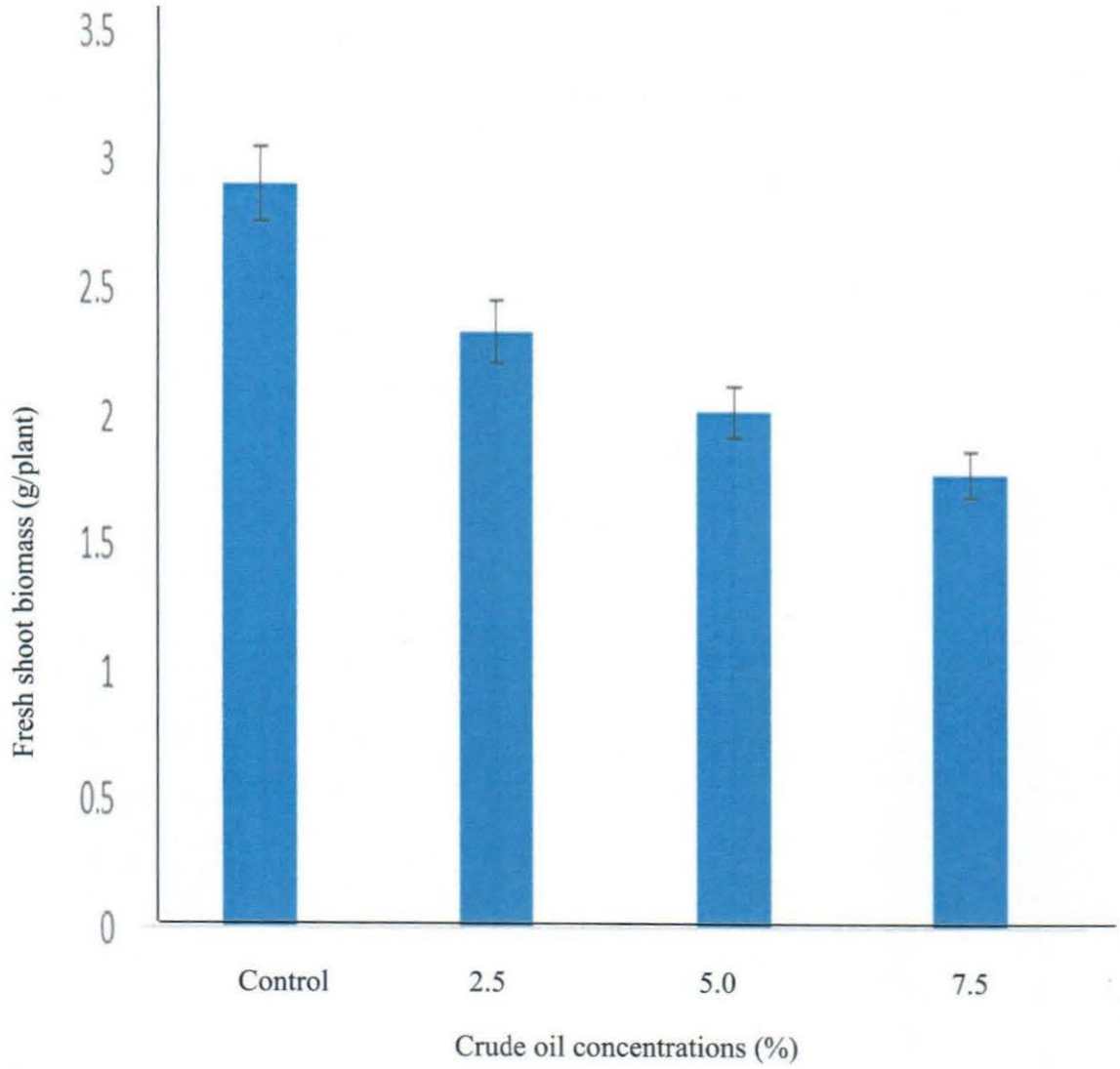


FIG. 21: Effect of crude oil pollution on fresh shoot biomass

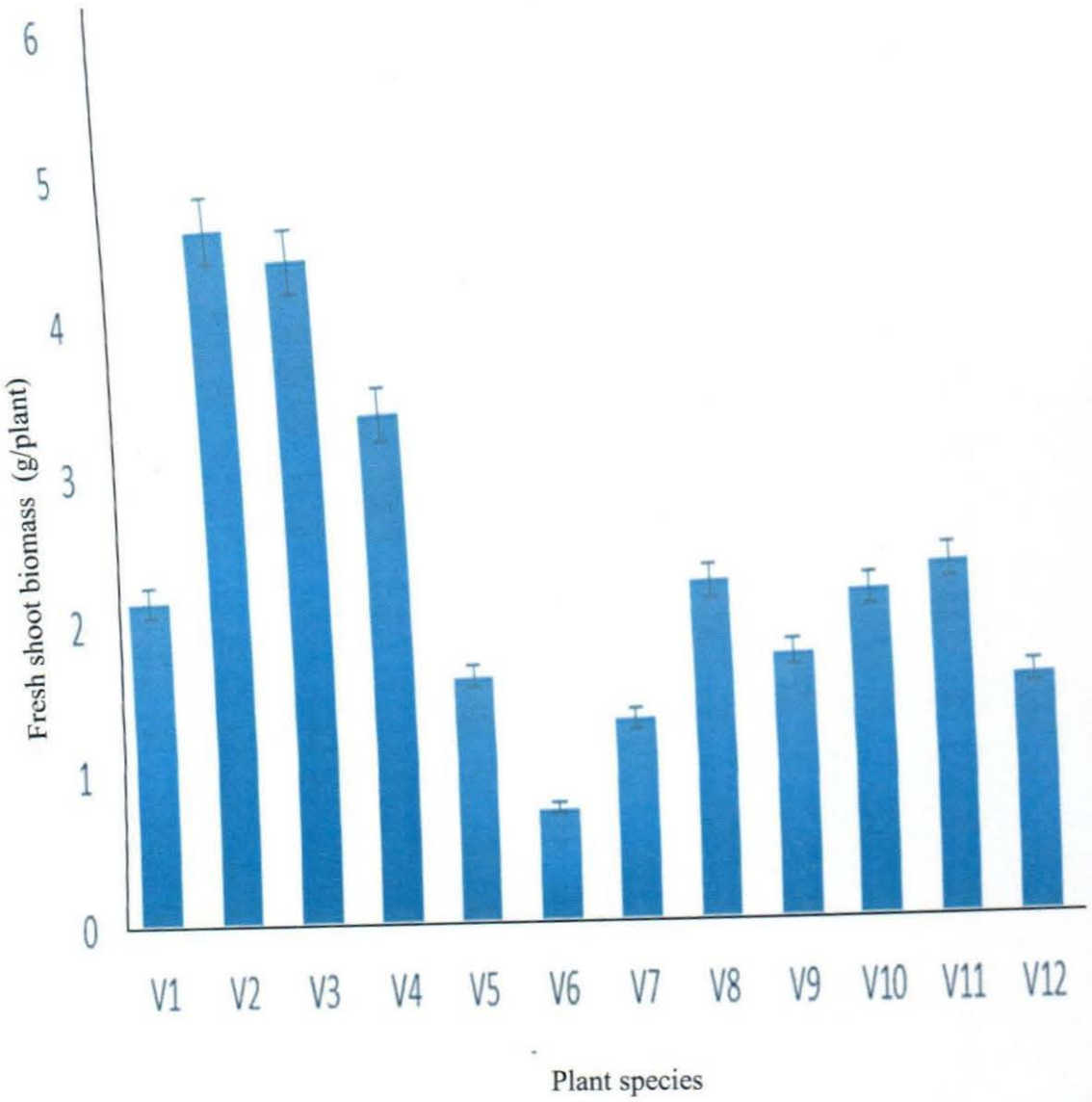


FIG. 22: Variations in shoot biomass among species grown on crude oil polluted soils

Treatments

V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}=Calapo (*Calapogonium mucunoides*), V_{11}=Jatropha (*Jatropha curcas*), V_{12}=Centro (*Centrosema pubescens*).

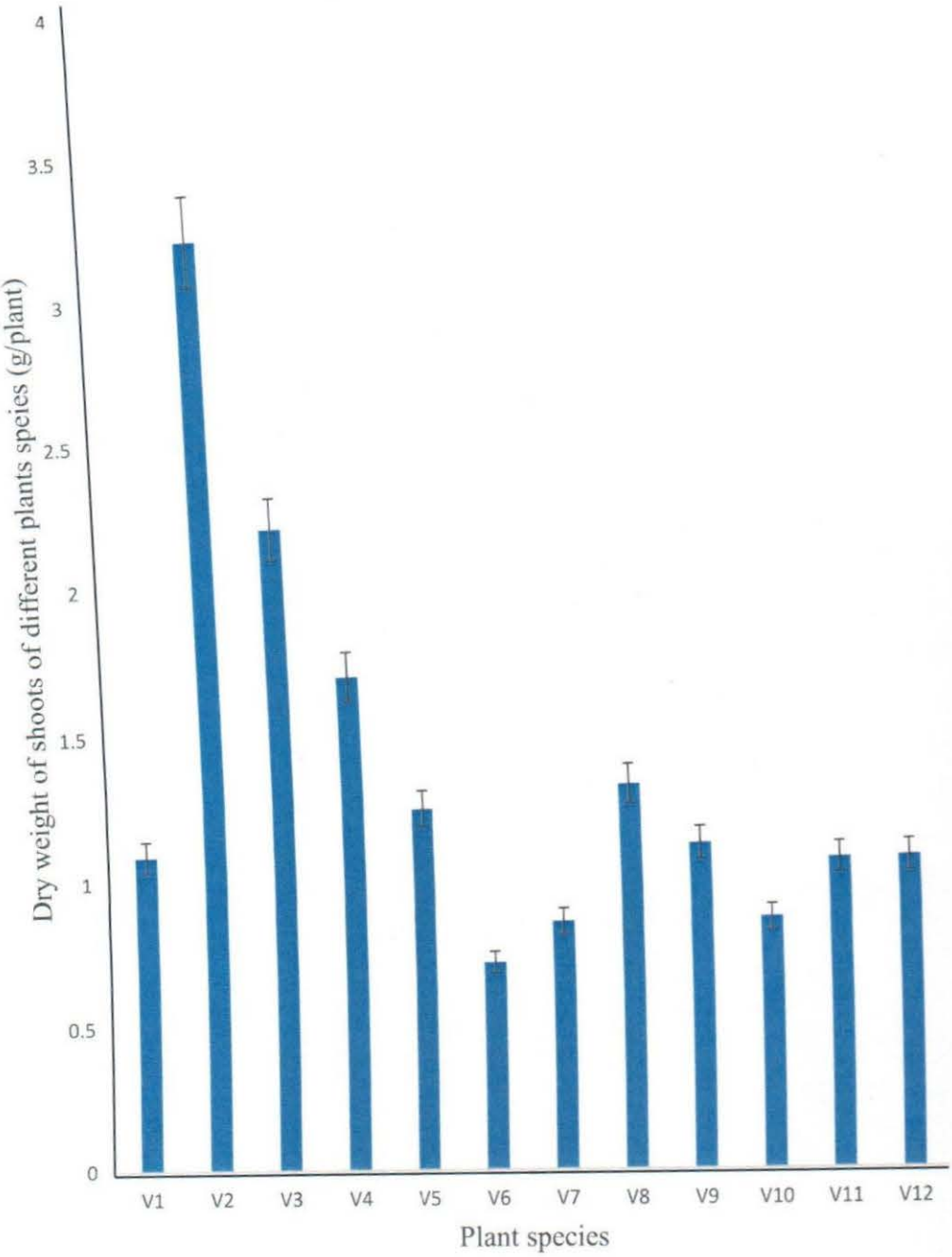


FIG. 23: Dry weight of shoots of different plant species across pollution levels in the greenhouse

Treatments

V_0=No plant species, V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}= Calapo (*Calapogonium mucunoides*), V_{11}= Jatropha (*Jatropha curcas*), V_{12}= Centro (*Centrosema pubescens*).

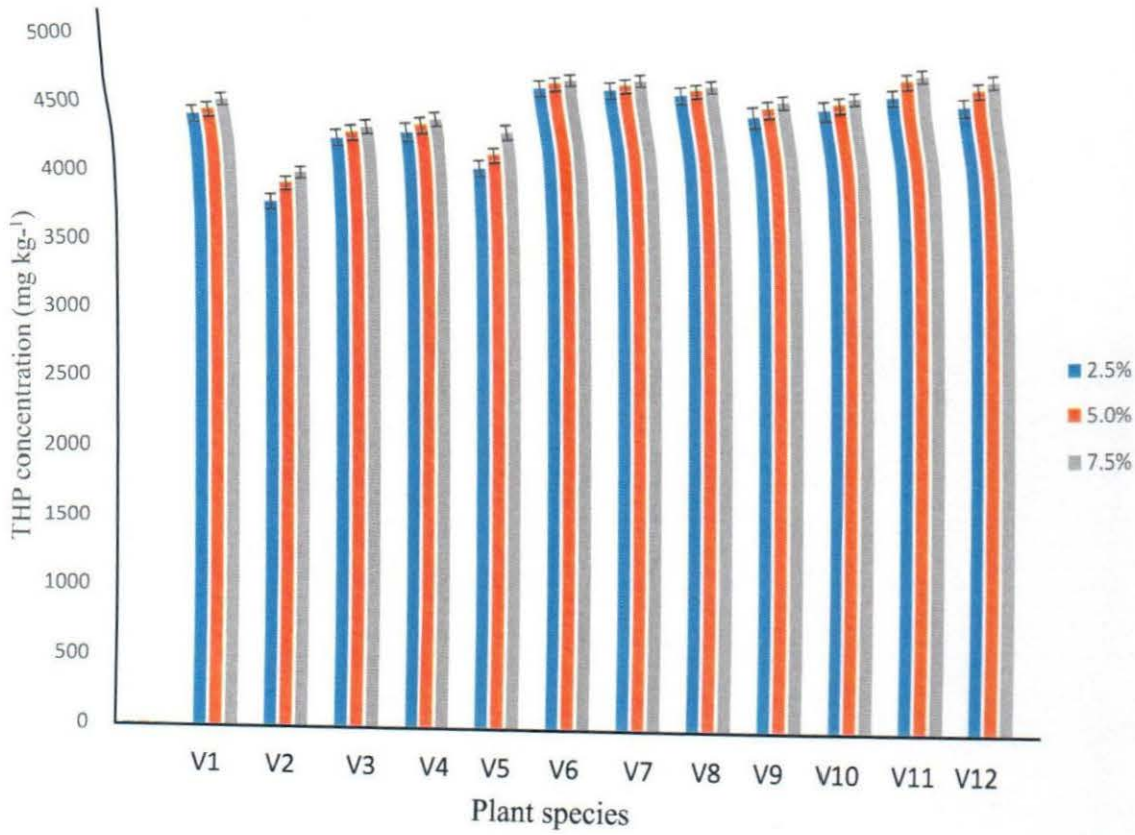


FIG. 24. Total petroleum hydrocarbon (TPH) in soils at 2 months after crude oil pollution in the greenhouse

Treatments

V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leatdtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}= Calapo (*Calapogonium mucunoides*), V_{11}= Jatropha (*Jatropha curcas*), V_{12}= Centro (*Centrosema pubescens*).

At 2 MAP, there was no significant change in TPH among soils under the different plant species (Figure 24). However, the level of TPH was lowest in soils under *P. purpureum* and *L. leucocephala*. Although both species reduced the soil TPH, they had demonstrable potentials as shown by the lesser amount of residual crude in the soil compared with other soils that received the same amount of crude oil.

At 4 MAP, the reduction in TPH was much more under *P. purpureum* and *L. leucecophala* (Figure 25). The reduction was attributed to fast growth rate of these plants and high population of hydrocarbon degraders in the soil.

4.6.2 Total petroleum hydrocarbon in plant tissues

There was a great difference in the removal rate of total petroleum hydrocarbon into the tissues of the twelve plants species studied (Figure 26). The amount of total petroleum hydrocarbon in *P. purpureum* and *L. leucocephala* in soil polluted with 2.5 % crude oil was significantly ($P < 0.05$) higher than the other plant species. This was followed by soil with 5.0 % and 7.5% pollution.The lowest TPH was in *A. compressus, P. maximum, G. sepium, T. fructicosum, C. odorata, A. compressus, C. cyperus, C. mucunoides, J. jatropha and C. pubescens.* This contradicts earlier reports (Agamuthu *et al.*, 2010; Atagana, 2011; Efe and Okpali, 2012; Budhadev *et al.*, 2012, Basumatary *et al.*, 2013; Ighovie and Ikechukwu, 2014) on accumulation of TPH in these species on crude oil polluted soils.

The high accumulation of TPH in *P. purpureum* and *L. leucocephala* may be attributed to their agronomic advantages such as adaptability to various soil types, rapid growth rate and higher root biomass which increased the secretion of exudates such as phenol to stimulate microbial activity in the soil. Besides, these two species were shown to maintain a large number of soil microorganisms such as petroleum hydrocarbon

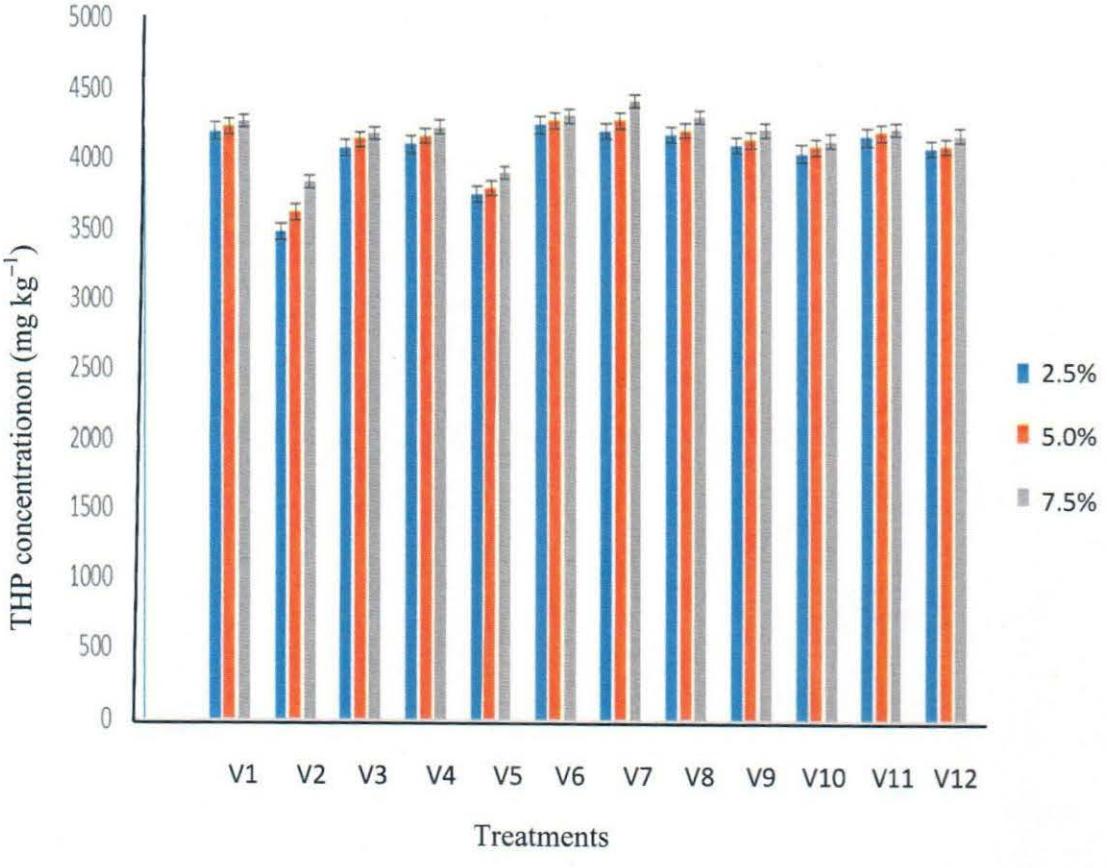


FIG. 25: Total petroleum hydrocarbon (TPH) content in soils at 4 months after pollution in the greenhouses

Treatments

V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia (*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}= Calapo (*Calapogonium mucunoides*), V_{11}= Jatropha(*Jatropha curcas*), V_{12}= Centro (*Centrosema pubescens*).

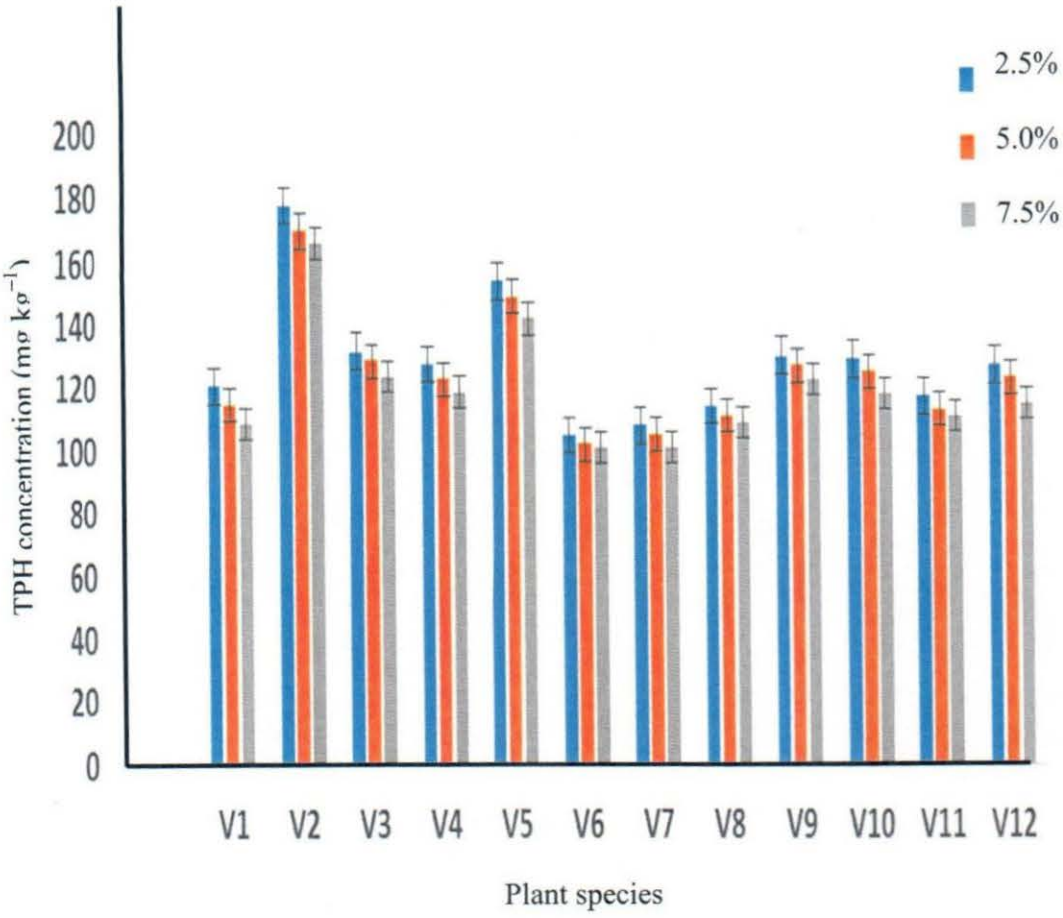


FIG. 26 Total petroleum hydrocarbon content in plant tissues at 4 months after pollution in the greenhouse

Treatments

V_1= Carpet grass (*Axonopus compressus*), V_2= Elephant grass (*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass (*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia(*Gliricidia sepium*), V_7= Waterleaf (*Talinum fructicosum*), V_8= Siam weed (*Chromoleana odorata*), V_9= Nut Sedge weed (*Cyperus rotundus*), V_{10}= Calapo (*Calapogonium mucunoides*), V_{11}= Jatropha (*Jatropha curcas*), V_{12}= Centro (*Centrosema pubescens*).

degraders. This result is in consonance with previous reports (Xia, 2004; Ayotamuno, 2006; Edwin-Wosu, 2010 and Noori *et al.*, 2012) of a higher reduction in TPH in soils planted with *P. purpureum*, *L. leucocephala* and some species of the fabaceae family.

Given the result obtained from the pot experiment, *P. purpureum* and *L. leucocephala* were selected for field evaluation based on their potential to remediate crude oil polluted soil.

4.7 Field experiment

4.7.1 Chemical properties of organomineral fertilizer used for the field experiment

The chemical characteristics of the organo-mineral fertilizer used for the field experiment are shown in Table14. The fertilizer contained 2.8 % N, 1.2 % P, 2.2 % K, 40 % total organic matter and 14 % moisture.

4.8 Physicochemical properities

4.8.1 Soil properties 3 months after pollution

The effect of *Pennisetum purpureum, Leuceana leucocephala,* organo-mineral fertilizer (OF), brassinolide and crude oil pollution on some soil characteristics at 3 months after pollution in the field are shown in Table 15. Neither soil pollution nor soil amendments had a significant effect on the textural class. The texture was loamy sand in all the treatments.

The pH of the 2.5 % polluted, amended with organo-mineral fertilizer and brassinolide, under *P. purpureum* was the lowest. The treated soils generally ranged from very strongly acidic to slightly acidic. Generally, at 2.5, 5.0 and 7.5% pollution, there were 25, 26 and 27% increase in soil pH respectively

The organic carbon was significantly higher in the 7.5 % polluted soil, without organo-mineral fertilizer, brassinolide or planting. The organic carbon content was generally higher across treatments, possibly due to the application of organo-mineral

TABLE 14

Chemical analysis of organo-mineral fertilizer used for the field experiment

Properties	Values
N (%)	2.8
P (%)	1.2
K (%)	2.2
Moisture (%)	14
Total organic matter (%)	40

TABLE 15
Soil propertiesat 3 months after pollution in the field

Treatment	Sand (ml/kg)	Silt (ml/kg)	Clay (ml/kg)	Texture	Soil pH	Org. C. (%)	Total N (%)	Avail. P mg/kg	Exchangeable cationsExchange acidity Ca	Mg	K	Na	Al	H	ECEC	Base Sat. (%)
									← cmol/kg →							
T_1	84.3c	9.00b	6.70a	LS	5.5bc	3.40cde	0.27g	65.21c	2.0a	1.4d	0.07bc	0.05abc	0.12d	0.56a	4.28c	83.8c
T_2	85.3a	9.00b	5.70b	LS	5.4cd	3.53bcd	0.30f	60.24d	3.0a	17ab	0.08ab	0.06ab	0.94ab	0.52a	6.18a	87.0b
T_3	82.7e	10.60a	6.70a	LS	5.3cd	3.84a	0.38e	58.40e	2.0a	1.6bc	0.07bc	0.06ab	1.11a	0.51a	5.08f	88.0ab
T_4	82.7e	10.60a	6.70a	LS	5.2de	2.92g	0.19jk	33.71i	2.1a	1.0f	0.09a	0.04c	0.12d	0.52a	4.50h	70.9f
T_5	82.7e	10.60a	6.70a	LS	5.1e	3.60abcd	0.23i	30.03j	2.2a	1.0f	0.08ab	0.06ab	0.19cd	0.56a	4.09i	81.66a
T_6	82.7e	10.60a	6.70a	LS	5.0f	3.72ab	0.25h	25.61k	2.3a	1.0f	0.06c	0.05ab	0.19cd	0.58a	4.52g	79.6d
T_7	84.6b	10.70a	4.60c	LS	5.8a	3.11fg	0.42c	53.34f	2.1a	1.7ab	0.08ab	0.05ab	0.82ab	0.46a	5.21d	89.0a
T_8	83.6d	10.70a	5.70b	LS	5.6bc	3.20efg	0.45b	46.17g	1.7b	1.5cd	0.08ab	0.06ab	0.81ab	0.51a	4.66e	71.67ab
T_9	82.6e	10.70a	6.70a	LS	5.5bc	3.33def	0.49a	38.00h	2.1a	1.5cd	0.08ab	0.06ab	1.11a	0.48a	5.33f	70.01ab
T_{10}	84.3c	9.00b	6.70a	LS	5.2de	3.10fg	0.18k	29.42j	2.1a	1.0f	0.08ab	0.05ab	0.33bcd	0.04b	4.45j	71.8f
T_{11}	84.3c	10.70a	5.70b	LS	5.1e	3.52bcd	0.20j	26.12k	2.1a	1.2e	0.08ab	0.05ab	0.33bcd	0.33a	5.43a	62.5g
T_{12}	84.3c	10.70a	5.70b	LS	5.0f	3.66abc	0.22i	21.21i	2.2a	1.0f	0.09a	0.05ab	0.67abcd	0.03b	4.44a	82.67a
T_{13}	84.3c	10.70a	5.70b	LS	5.4cd	2.34i	0.40d	69.02a	2.0a	1.8a	0.06c	0.06ab	0.56abcd	0.48a	5.32c	88.0ab
T_{14}	84.3c	9.00b	6.70a	LS	5.2de	2.64h	0.43c	68.45a	1.8b	1.7ab	0.07bc	0.06ab	0.74abc	0.30a	5.30c	89.0a
T_{15}	84.3c	10.70a	5.70b	LS	5.3d	2.93g	0.45b	66.41b	2.0a	1.6bc	0.09a	0.07a	0.77abc	0.48a	5.01c	75.04a

Means in the same column followed by same letter (s) are not significantly different at 5% probability level

T_1 = 2.5 % crude oil polluted soil + organomineral fertilizer (OF), no planting or brassinolide
T_2 = 5.0 % crude oil polluted soil + OF, no planting of brassinolide
T_3 = 7.5 % crude oil polluted soil + OF, no planting or brassinolide
T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under*P. purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under*P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under*P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under*P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under*P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under*P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under*L. leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under*L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under*L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under*L. leucocephala*

fertilizer or crude oil. The respective increased were 30, 27 and 28% at 2.5, 5.0 and 7.5% pollution. .The total nitrogen content in the soil was generally low in all the soils as reported previously (Shukry *et al.*, 2013) for crude oil polluted soils amended with organic manure. At 2.5, 5.0 and 7.5% pollution, there were 23, 21 and 27% decreased in total nitrogen respectively. The decrease in total nitrogen concentration in the polluted soils may be due to temporal immobilization of the nutrient by soil microorganisms. Also the reduction in nitrogen content suggests that the nitrification rate might have reduced following oil pollution. According to Odu *et al.*, (1986), after oil spillage, hydrocarbon utilizing microbes like *Azotobacter spp.* normally become more abundant while nitrifying bacteria such as *Nitrosomonas spp.* become less.

The available phosphorus (P) content of the soil treated with 2.5 and 5.0 % crude, amended with organo-mineral fertilizer and brassinolide, under *L. leucocephala* was significantly ($P < 0.05$) higher than other treatments (Table 14). The available phosphorus increased by 35 and 39% at the different levels of pollution .Soil contaminated with crude oil at 7.5 %, without organo-mineral fertilizer and brassinolide under *L. leucocephala* had the lowest P content. There was no significant difference between the 7.5 % polluted soil without amendment under *P. purpureum* and the unamended 5% polluted soil under *L. leucocephala*. The increase in available phosphorus could be as a result of inputs of this nutrient from the organomineral fertilizer since organic manure could boost soil nutrients by supplementing the limiting nutrients (Mbah *et al.*, 2009, 2006; Tanee and Kinako, 2008).

There were significant increases in the basic cations (Ca, Mg and K) on addition of the organomineral fertilizer (Table 14). The increase in these cations could be attributed to the input from the organomineral fertilizer. The addition of these basic cations to soil would improve the fertility of the soil.

The organo-mineral fertilizer and brassinolide reduced the exchangeable acidity across the treated soils. At 2.5, 5.0 and 7.5% pollution, there were 22, 51 and 36% decreased in exchangeable acidity. The base saturation of all the treatments was high except in the unamended soil polluted with 7.5 % under *L. leucocephala* which had a saturation of only 60.7 %, which is considered low according to the rating of soil chemical properties (FDALR 1990; and Landon 1991). The effective cation exchange capacity of all the treated soils at 3 months after pollution were generally low. The respective decreased were 27 and 28%.

4.8.2 Soil properties 6 months after pollution

At 6 months after pollution, there was no significant change in soil texture (Table 16). Soil treated with 2.5 % crude oil, plus organomineral fertilizer and brassinolide, under *P. Purpureum* significantly had higher pH ($P < 0.05$) than other treatments. Generally, soil pH increased by 27% in all the treated soils. This increase in soil pH might be probably due to ion exchange reactions which occur when terminal Al and Fe^{2-} hydroxyl oxides are replaced by organic anions which are products of decomposition of organic manures (Bell and Besho, 1993). The ability of the organic manure to increase soil pH can be attributed to the enrichment of the soil through mineralization of cations, particularly calcium. Natsher and Schwetmann (1991) observed that such basic cations are released upon microbal decarboxylation. Narambuye and Haynes (2006) attributed the short term effects of manure in reducing potentially toxic Al^{3+} in solution to both an increase in pH and a complexing effect by soluble organic matter. The results obtained from this study are in consonance with those by Ijah *et al.*, (2008). Ijah and Antai (2003a) reported that organic manure (for example, chicken droppings) have a buffering effect on crude oil polluted soil. This rise in pH of soil amended with organo-mineral fertilizer and brassinolide may favour oil degradation by micro-organisms as observed in similar studies elsewhere (Tanee and Kinako, 2008; Atlas and Bartha, 1992).

TABLE 16
Soil properties in the field at 6 months after crude oil pollution

Treatment	Sand (%)	Silt (%)	Clay (%)	Texture	Soil pH	Org. C. (%)	Total N (%)	Avail. P mg/kg	Exchangeable cations Exchange acidity Ca cmol/kg	Mg	K	Na	Al	H	ECEC	Base Sat. (%)
T_1	80.0b	14.0ab	6.0ab	LS	6.4bc	1.34g	0.14a	55.56a	1.7cd	0.6f	0.07b	0.06bc	0.00b	0.60ab	3.03h	80.19k
T_2	80.0b	15.0a	5.0b	LS	6.4bc	1.52ef	0.11b	50.03c	2.1bcd	0.8e	0.08b	0.06bc	0.00b	0.64ab	5.05g	68.2i
T_3	82.0ab	13.0ab	5.0b	LS	6.2c	1.69cd	0.10bcd	47.22d	2.8ab	1.2c	0.10b	0.08a	0.00b	0.80a	4.78i	85.0cd
T_4	80.0b	14.0ab	6.0ab	LS	5.2de	1.86ab	0.08ef	23.39h	2.6ab	1.2c	0.37a	0.04d	0.33a	0.14cd	5.40e	72.9g
T_5	80.0b	13.0ab	7.0a	LS	5.2de	1.88a	0.06gh	20.61i	2.3bcd	0.8e	0.09b	0.04d	0.66a	0.03d	5.34f	61.6i
T_6	84.0a	13.0ab	7.0a	LS	5.0e	1.95a	0.07fgh	16.81k	1.7cd	0.6f	0.10b	0.05cd	0.66a	0.06cd	3.17b	77.3jk
T_7	80.0b	13.0ab	7.0a	LS	6.7a	1.34g	0.11b	42.54e	2.6ab	1.2c	0.08b	0.06bc	0.00b	0.62ab	3.96k	84.0d
T_8	80.0b	14.0ab	6.0ab	LS	6.5b	1.40fg	0.10bcde	32.37f	4.0a	1.6b	0.08b	0.05cd	0.00b	0.78a	5.71d	86.0c
T_9	84.0a	10.0c	6.0ab	LS	6.5b	1.64de	0.08def	28.57g	3.2a	1.5b	0.09b	0.07ab	0.00b	0.68ab	5.04g	86.0c
T_{10}	81.0ab	13.0ab	6.0ab	LS	5.2de	1.71bcd	0.09cde	18.98j	2.5abc	1.2e	0.10b	0.04d	0.33a	0.36bc	5.94a	64.9j
T_{11}	81.0ab	14.0ab	5.0b	LS	5.2de	1.72bcd	0.07fgh	17.71jk	2.3bcd	1.0d	0.09b	0.05cd	0.34a	0.49ab	4.27c	80.6h
T_{12}	81.0ab	14.0ab	5.0b	LS	5.0de	1.81abc	0.06h	13.41il	2.3bcd	1.1cd	0.09b	0.04d	0.00b	0.56ab	4.60j	87.6b
T_{13}	82.0ab	12.0c	6.0ab	LS	6.4bc	1.32g	0.10bc	56.38a	2.2bcd	0.8e	0.09b	0.07ab	0.00b	0.70ab	3.89i	81.2e
T_{14}	81.0ab	13.0ab	6.0ab	LS	6.4bc	1.37fg	0.09cde	52.31b	4.0a	1.8a	0.08b	0.06bc	0.00b	0.64ab	5.34f	89.1a
T_{15}	81.0ab	14.0ab	5.0b	LS	6.4bc	1.40fg	0.09cde	48.89c	1.6d	0.6f	0.10b	0.08a	0.00b	0.62ab	3.00m	79.0f

Means in the same column followed by same letter (s) are not significantly different at 5% probability level.

T_1 = 2.5 % crude oil polluted soil + organomineral fertilizer (OF), no planting or brassinolide
T_2 = 5.0 % crude oil polluted soil + OF, no planting of brassinolide
T_3 = 7.5 % crude oil polluted soil + OF, no planting or brassinolide
T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under*P. purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under*P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under*P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under*P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under*P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under*L. leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under*L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under*L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under*L. leucocephala*

The organic carbon content of the soil at 6 months after pollution was significantly reduced across treatments (Table16). At 2.5, 5.0 and 7.5% pollution, there were 22, 24 and 25% decreased in organic carbon content respectively. The possible reason for the reduction might be the mineralization of the organic carbon in the soil. Also, the lower organic carbon observed in this study could be due to biological ageing (delayed senescence) of older tissues (both root and shoot) in the soils polluted with crude oil and uptake of hydrocarbons by the plant. A similar observation was reported in Osuji and Nwoye (2007). Crude oil pollution have been reported by Merkl *et al.* (2005) to lead to the alteration in plant development and a postponement of senescence. Besides, soil microbes, especially the hydrocarbon utilizing bacteria do feed exclusively on hydrocarbon. Njoku *et al.*, (2009) reported similar observation of lower organic matter contents in crude oil polluted soils under *Glycine max*.

The total nitrogen (N) content of soil treated with 2.5 % crude oil, amended with organomineral fertilizer with neither brassinolide nor planting was significantly higher than all other treatments. Generally, total N was low across soils, even with organo-mineral fertilizer application (Table 16). The respective decreased were 37, 34 and 33%.This contradicts the reports of Njoku *et al.* (2008) that total nitrogen increased with the application of fertilizers. The low content of nitrogen in this study might be due to high bacterial activity in the soil. Fitzpatrick (1986) and Ibia (2012) reported that in addition to biological uptake, nitrogen could be rapidly lost from soils by leaching of ammonia and by denitrification. Brady and Weil (2002) also noted that during biodegradation, nitrogen may be lost to the atmosphere when nitrate ions are converted to gaseous forms of nitrogen by a series of widely occurring biochemical reduction reactions induced by the activities of denitrifying bacteria such as *Pseudomonas, Bacillus* and *Micrococcus,* especially when low oxygen exist within soil aggregates. The soil microbes isolated from the present studies could have been the reason for this trend.

The continuous utilization of available nitrogen implies that the nitrogenous nutrient supplied favoured phytoremediation.

The available phosphorus (P) content of soil treated with 2.5% crude oil plus organo-mineral (OF), with neither brassinolide nor planting and soil treated with 2.5 % crude-oil plus *L. leucocephala*, amended with OF and brassinolide were significantly higher than all other treatments (Table 16). The respective increased were 39, 41 and 44% at 2.5, 5.0 and 7.5% crude oil pollution. The increase in available phosphorus may be as a result of anthropogenic inputs of nutrients from the organomineral fertilizer. Mbah *et al.*,(2009) and Tanee and Kinako(2008) reported that organic manure increased soil nutrients by supplementing the limiting nutrients. Similarly, Odokuma and Ibor (2002) and Lee *et al.*,(1995) observed that the application of phosphorus fertilizer enhanced biodegradation of crude oil polluted soil. Lee *et al.*, (2007) observed that when pH increased near 6.5, phosphorus availability occured in most soils. This may be the possible reason that organo-mineral amended soil with pH of 6.0 – 6.7 (observed in this study) increased the available P in the soil.

The results also showed a slight increase in calcium content (Ca) by 17 and 32% while Mg and K were generally low across the treated soils (Table 16). The respective decreased for Mg was 14, 15 and 32% while K was 11, 24 and 26%. Mbah *et al.*(2006) observed similar trend. The possible cause of the increment in Ca may be due to the application of organo-mineral fertilizer. However, the application of organo-mineral fertilizer and brassinolide had no effect on sodium content.

The application of organo-mineral and brassinolide reduced the exchangeable acidity in crude oil polluted soil by 33 and 42%. Similar observations were reported in Ekpo *et al.* (2012) who found that the exchangeable acidity of crude oil polluted soils was reduced in the soil amended with cocoa pod husk and plantain peels. A significant increase in the base saturation of all the treated soils by 21, 22 and 27% was also

observed (Table 16). However, soils treated with 5.0 % crude oil, amended with OF and brassinolide under *L. leucocephala* significantly increased base saturation relative to other treatments.

The effective cation exchange capacity was higher in soils polluted with 2.5% crude oil without amendment under *L. leucocephala* than other treatments. Generally, ECEC was low irrespective of the treatment. The respective decreased were 26, 23 and 27% at 2.5, 5.0 and 7.5% pollution. According to FDARL (1990), soil with ECEC less than 10 cmol/kg were considered low. Generally, all the chemical properties in the soils irrespective of the treatments were generally higher at 3 months than 6 months. The possible reasons for the low content of these nutrients at 6 months could be attributed to the uptake by plant, leaching losses and denitrification.

4.9 Microbial count

4.9.1 Total heterotrophic bacteria count (TBH) in the field at 3 and 6 months after crude oil pollution

At 3 and 6 months after pollution, soil polluted with 7.5% crude oil, amended with organo-mineral and brassinolide under *P. purpureum* had higher heterotrophic bacteria count($6.6x10^5$) and ($8.6x10^5$) than other treatments (Table 17). This was followed by soils polluted with 2.5 and 5.0% crude oil, amended with organomineral fertilizer and brassinolide and under *P. purpureum*.

Generally total heterotrophic bacteria was higher in all the treatments excepting treatment T_4, T_{10}, T_5, T_{11}, T_{12} and T_6 which significantly reduced heterophic bacteria count . The bacteria isolates identified were: *Bacillus subtilis, Eschericha coli, Actinomycetes spp., Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter spp, Enterobacterium spp, Micrococcus spp, Corynebacterium spp, Chromobacterium spp* (Table 17).

4.9.2 Total heterotrophic fungi (THF) count at 3 and 6 months after crude oil pollution in the field.

TABLE 17

Total heterotrophic bacteria count in the field at three and six months after crude oil pollution

Treatment	Bacterial isolates	3 months after pollution THB (cfu/g)	Mean count (cfu/g)	Bacterial isolate	6 months after pollution THB (cfu/g)	Mean count (cfu/g)
T_1	*Bacillus subtilis, Pseudomonas aeruginosa*	4.0×10^5		*Pseudomonas aeruginosa, Micrococus luteus*	5.1×10^5	
T_2	*Bacillus spp., Eshericha coli, Micrococus luteus*	4.5×10^5		*Bacillus spp., Chromobacterium spp., Micrococus luteus*	6.1×10^5	
T_3	*Enterobacter cloacae, P. aeuroginosa*	5.1×10^5		*P. aeruginosa, Mycobacterium spp., Escherica coli*	6.6×10^5	
T_4	*Chromobacterium spp., Bacillus spp.*	3.2×10^5		*Achromobacter xylosoxiden, Bacillus spp., Enterobacter cloacae*	3.6×10^5	
T_5	*Pseudomonas aeruginosa, B. subtilis*	2.3×10^5		*P. aeruginosa, Protus vulgaris*	2.8×10^5	
T_6	*Esherica coli, Bacillus subtilis*	1.8×10^5		*Escherica coli, P. aeuroginosa*	2.1×10^5	
T_7	*Pseudomonas aeruginosa, B. subtilis, Flarobacterium spp.*	6.1×10^5		*Bacillus subtilis, Flavobacterium, Chromobacterium spp.*	6.8×10^5	
T_8	*Bacillus spp., Mycobacterium spp., Micrococusluteus*	6.5×10^5		*Bacillus Spp., Mycobacterium Spp., Pseudomonas aeruginosa*	7.4×10^5	
T_9	*Bacillus spp., E. coli, Psedomonas aeruginosa*	6.6×10^5		*Bacillus spp., Micrococus luteus, Pseudomonas aeruginosa*	8.6×10^5	
T_{10}	*Micrococus luteus, P. aeruginosa, Bacillus subtilis*	2.4×10^5		*P. Aeruginosa, Chromobacterium Spp., Bacillus subtilis*	3.2×10^5	
T_{11}	*Chromobacterium spp., B. subtilis*	2.1×10^5		*Bacillus subtilis, Micrococus luteus*	2.4×10^5	
T_{12}	*Pseudomonas aeruginosa, Mycrobacterium spp.*	1.9×10^5		*P. aeruginosa, Microbacterium spp.*	2.1×10^5	
T_{13}	*Escherica coli, Bacillus subtilis*	5.2×10^5		*Bacillus subtilis, Mycrobacterium spp. Escherica coli*	6.6×10^5	
T_{14}	*Bacillus subtilis, P. aeruginosa*	5.4×10^5		*Bacillus subtilis, P. aeruginosa, Acinetobacter spp.*	7.1×10^5	
T_{15}	*Bacillus subtilis, P. aeruginosa, Micrococus spp.*	5.3×10^5	**4.20×10^5**	*Bacillus spp., Flavobacterium spp., P. eruginosa*	7.6×10^5	**4.58×10^5**

TABLE 18

Total heterotrophic fungi count in the field at three and six months after crude oil pollution

Treatment	Fungi isolate	3 months after pollution THF (cfu/g)	Mean count (cfu/g)	Fungi isolate	6 months after pollution TBF (cfu/g)	Mean count (cfu/g)
T_1	*Aspergillus spp., Penicillium spp.*	2.2×10^3		*Penicillium spp., Rhizopus indicus*	2.5×10^3	
T_2	*Aspergillus spp., Fusarium oxysporium*	2.6×10^3		*Aspergillus spp., Mucor spp.*	3.1×10^3	
T_3	*Rhizopus indicus, Penicillium spp.*	2.7×10^3		*Penicillium spp., Trichoderma viride, Rhizopus*	3.4×10^3	
T_4	*Penicillium spp., Aspergillus spp.*	1.6×10^3		*Aspergillus spp., Verticillium rubrium*	1.7×10^3	
T_5	*Aspergillus spp., Rhizopus indicus*	1.5×10^3		*Rhizopus indicus, Aspergillus spp.*	1.7×10^3	
T_6	*Penicillium spp., Fusarium oxysporium*	1.2×10^3		*Fusarium oxysporium, R. indicus*	1.4×10^3	
T_7	*Aspergillus spp., Penicillium spp.*	3.3×10^3		*Aspergillus spp., F. oxysporium*	3.5×10^3	
T_8	*Rhizopus indicus, Penicillium spp., Aspergillus spp.*	3.3×10^3		*Rhizopus indicus, Penicillium spp.*	3.5×10^3	
T_9	*Fusarium oxysporium, R. indicus*	3.5×10^3		*Fusarium oxysporium, Rhizopus indicus Aspergillus spp.*	3.8×10^3	
T_{10}	*Penicillium spp., Mucor spp.*	1.7×10^3		*Penicillium spp., Fusarium oxysporium*	2.0×10^3	
T_{11}	*Penicillium spp., Fusarium oxysporium*	1.4×10^3		*Fusarium oxysporium, Aspergillus spp.*	1.6×10^3	
T_{12}	*Aspergillus spp., Penicillium spp.*	1.2×10^3		*Aspergillus spp., Mucor indicus*	1.4×10^3	
T_{13}	*Pencillium spp., Aspergillus spp.*	2.2×10^3		*Penicillium spp., Fusarium oxysporium*	2.7×10^3	
T_{14}	*Fusarium oxysporium, Penicillium spp.*	2.4×10^3		*Aspergillus spp., Pencillium spp.*	2.9×10^3	
T_{15}	*Penicillium spp., Mucor spp.*	2.6×10^3	**2.2×10^3**	*Mucorindicus, Rhizopus spp., Penicillium spp.*	3.4×10^3	**2.6×10^3**

At 3 and 6 months after crude oil pollution, soil polluted with 7.5 % crude oil, amended with organo-mineral fertilizer and brassinolide under *P. purpureum* had significantly higher population of heterotrophic fungi ($3.5x10^3$) and ($3.8x10^3$) count than other treatments. The lowest population was in treatment T10, T4, T5, T11, T6 and T13 at 3 months and T4, T5, T11, T6 and T12 at 6 months after pollution.

Fungi isolated using cultural biochemical characteristics were *Pennicilium spp., Aspergillus spp, Trichoderma viride, Rhizopus indicus, Verticillium rubrium, Fusarium oxysporium* and *Mucor spp.* (Table 18).

4.9.3 Hydrocarbon utilizing bacteria at 3 and 6 months after crude oil pollution in the field.

The effects of *Pennisetum purpureum*, *Leuceana leucocephala*, organo-mineral fertilizer, brassinolide and crude oil pollution on hydrocarbon utilizing bacteria at 3 and 6 months after pollution are presented in Table 19. At 3 and 6 months after pollution, soils polluted with 7.5 % crude oil, amended with organomineral fertilizer and brassinolide under *P. purpureum* had higher population of hydrocarbon utilizing bacteria ($5.4x10^5$) and ($9.0x10^5$) relative to other treatments. The lowest population was in treatment T5, T11, T12, and T6 at 3 months and T11, T6 at 6 months after crude oil pollution. This is similar to the finding of Ijah and Antai, (2003b) who observed an increase in hydrocarbon utilizing bacteria in crude oil amended soils.

Successive increases in crude oil pollution significantly ($P<0.05$) increased the hydrocarbon utilizing bacteria, possibly due to the availability of sufficient nutrients from organo-mineral fertilizer in the amended soils. Frick *et al.*, (1999) and Singh and Ward (2003) reported that bacteria were useful in the degradation of crude oil. This suggests that planting of *P. purpureum* and *L. leucocephala* plus the application of organo-mineral fertilizer and brassinolide can enhance the bacterial population in crude oil polluted soil and thereby lead to higher degradation of crude oil in the soil. The

TABLE 19

Hydrocarbon utilizing bacteria count in the field at three and six months after crude oil pollution

Treatment	Bacterial isolate	3 months after pollution THB(cfu/g)	Mean count (cfu/g)	Bacterial isolate	6 months after pollution TBH(cfu/g)	Mean count (cfu/g)
T_1	*Bacillussubtilis, Pseudomonas aeruginosa*	3.6×10^5		*Bacillus spp., Flavobacterium spp., Pseudomonas aeruginosa*	5.7×10^5	
T_2	*Acinetobacter spp., Flavobacterium spp.*	2.9×10^5		*Acinetobacter spp., Bacillus spp., Flavobacterium spp.*	6.5×10^5	
T_3	*Bacillus subtilis, Staphylococcus aureus*	4.0×10^5		*Bacillus subtilis, Staphylococcus aureus*	7.0×10^5	
T_4	*P. aeruginosa, Flavobacterium spp.*	2.3×10^5		*P. aeruginosa, Flavobacterium spp., Acinetobacter spp.*	3.8×10^5	
T_5	*Acinetobacter spp., S. aureus*	1.8×10^5		*Staphylococcus aureus, Acinetobacter spp., Bacillus subtilis*	3.0×10^5	
T_6	*Bacillus subtilis, Chromobacterium spp.*	1.2×10^5		*Bacillus subtilis, Chromobacterium spp Pseudomonas aeruginosa*	2.4×10^5	
T_7	*Pseudomonas aeruginosa, B. subtilis*	4.2×10^5		*Bacillus spp., P. aeuroginosa, Acinetobacter spp.*	7.3×10^5	
T_8	*Bacillus subtilis, A.xylosoxiden*	5.1×10^5		*Bacillus subtilis, A. xylosoxiden, P. aeruginosa*	7.7×10^5	
T_9	*Bacillussubtilis, Flavobacterium spp.*	5.4×10^5		*Bacillus subtilis, Flavobacterium spp., Acinetobact7er spp.*	9.0×10^5	
T_{10}	*Micrococusluteus, Proteus vulgaris*	2.0×10^5		*Micrococcus luteus, Protus vulgaris*	3.9×10^5	
T_{11}	*Micrococcus luteus, Flavobacterium spp.*	1.6×10^5		*Flavobacterium spp., M. luteus, Protus vulgaris*	2.8×10^5	
T_{12}	*Chromobacterium spp., Streptococcus faecalis*	1.4×10^5		*Chromobacterium spp., B. subtilis*	3.2×10^5	
T_{13}	*Bacillus subtilis, Acinetobacter spp.*	3.9×10^5		*Bacillus subtilis, P. aeruginosa, Achromobacter xylosoxiden*	7.3×10^5	
T_{14}	*P. aeruginosa, Acinetobacter spp.*	4.5×10^5		*P. aeruginos, Acinetobacter spp., Flavobacterium spp.*	7.8×10^5	
T_{15}	*Bacillus subtilis, P. aeruginosa,*	4.8×10^5	**3.2×10^5**	*Bacillus subtilis, Mycobacterium spp., Pseudomonas aeuroginos*	8.5×10^5	**5.7×10^5**

temporal increase in soil pH suggest a prevalence of favourable conditions for soil bacteria and biodegradation (Dibble and Bartha, 1979). The utilizing bacterial isolates identified were *Bacillus spp., Pseudomonas aeruginosa, Flavobacterium spp., Acinetobacter spp., Chromobacterium spp., Achromobacter, Xylosiden, Protus vulgaris* and *Micrococcus luteus* (Table 19).

4.9.4 Hydrocarbon utilizing fungi at 3 and 6 months after crude oil pollution in the field

At 3 months after crude oil pollution, a significantly higher population of hydrocarbon utilizing fungi ($2.6x10^3$) was observed in soils contaminated with 7.5 % crude oil, amended with organo-mineral fertilizers, without planting and brassinolide. This was followed by soil contaminated with 5.0 % crude oil amended with organomineral fertilizer and brassinolide under *P. purpureum*.

At 6 months after pollution, soils polluted with 7.5 % crude oil, amended with organo-mineral fertilizer and brassinolide under *P. purpureum* increased the population of hydrocarbon utilizing fungi ($4.1x10^3$) relative to other treatments. The following utilizing fungi were identified: *Pennicillium spp, Fusarium spp, Aspegillus niger, Aspergillus fumigatus, Mucor spp,* etc. (Table 20).

4.10 Growth parameters in the field

4.10.1 Plant Biomass

The fresh and dry weights of shoot and root of the two plant species at 6 months after pollution in the field are presented in Figures 27 and 28. Soils polluted with 2.5 % crude oil amended with organo-mineral fertilizer and brassinolide under *P. purpureum* had significantly greater fresh and dry weights of shoot and root than other treatments. The polluted soils under *L. leucocephala* without organo-mineral amendment and *brassinolides* had the lowest fresh and dry weights of shoot and root.

TABLE 20

Hydrocarbon utilizing fungi in the field at three and six months after crude oil pollution

Treatment	Fungal isolate	3 months after pollution THF(cfu/g)	Mean count (cfu/g)	Fungal isolate	6 months after pollution TBF(cfu/g)	Mean count (cfu/g)
T_1	*Penicillium spp., Rhizopus spp.*	1.9×10^3		*Rhizopus spp., Penicillum spp., Mucorindicus*	2.9×10^3	
T_2	*Fusariumoxysporium, Mucorindicus*	2.1×10^3		*Fusarium oxysporium, Mucorindicus, Trichoderma viride*	3.0×10^3	
T_3	*Penicillium spp., Mucorindicus*	2.7×10^3		*Penicillium spp., Aspergillus spp. Rhizopus indicus*	3.5×10^3	
T_4	*Aspergillus spp., Rhizopus spp.*	1.3×10^3		*Aspergillus spp. Fusarium oxysporium*	1.8×10^3	
T_5	*Aspergillus spp., Trichodermaviride*	1.3×10^3		*Aspergillus spp., Rhizopus indicus*	1.8×10^3	
T_6	*Fusariumoxysporium, Rhizopus spp.*	1.1×10^3		*Fusarium oxysporium, Mucor indicus*	1.5×10^3	
T_7	*Aspergillus spp., Penicillium spp.*	2.4×10^3		*Penicillium spp., Aspergillus spp., Fusarium oxysporium*	3.6×10^3	
T_8	*Penicillium spp., Mucorindicus*	2.6×10^3		*Penicillium spp., Aspergillus spp., Rhizopus spp.*	3.7×10^3	
T_9	*Aspergillus spp., Rhizopus spp.*	2.5×10^3		*Aspergillus spp., Fusarium oxysporium*	4.1×10^3	
T_{10}	*Penicillium spp., Trichodermaviride*	1.4×10^3		*Penicilium spp., Verticillium rabium*	2.2×10^3	
T_{11}	*Mucorindicus, Verticilliumrabium*	1.2×10^3		*Aspergillus spp., Mucor indicus*	1.7×10^3	
T_{12}	*Verticilliumrabium, Rhizopus spp.*	1.1×10^3		*Fusariumo xysporium, Rhizopus spp.*	1.6×10^3	
T_{13}	*Fusariumoxysporium, Pencillium spp.*	2.1×10^3		*Fusarium oxysporium, Penicillium spp. Rhizopus spp.*	2.9×10^3	
T_{14}	*Penicillium spp., Fusariumoxysporium*	2.1×10^3		*Penicillium spp., Aspergillus spp., Verticillium rabium*	3.1×10^3	
T_{15}	*Aspergillus spp., Fusariumoxysporium*	2.4×10^3	**1.9×10^3**	*Fusarium oxysporium, Aspergillus spp., Trichoderma viride*	3.5×10^3	**2.7×10^3**

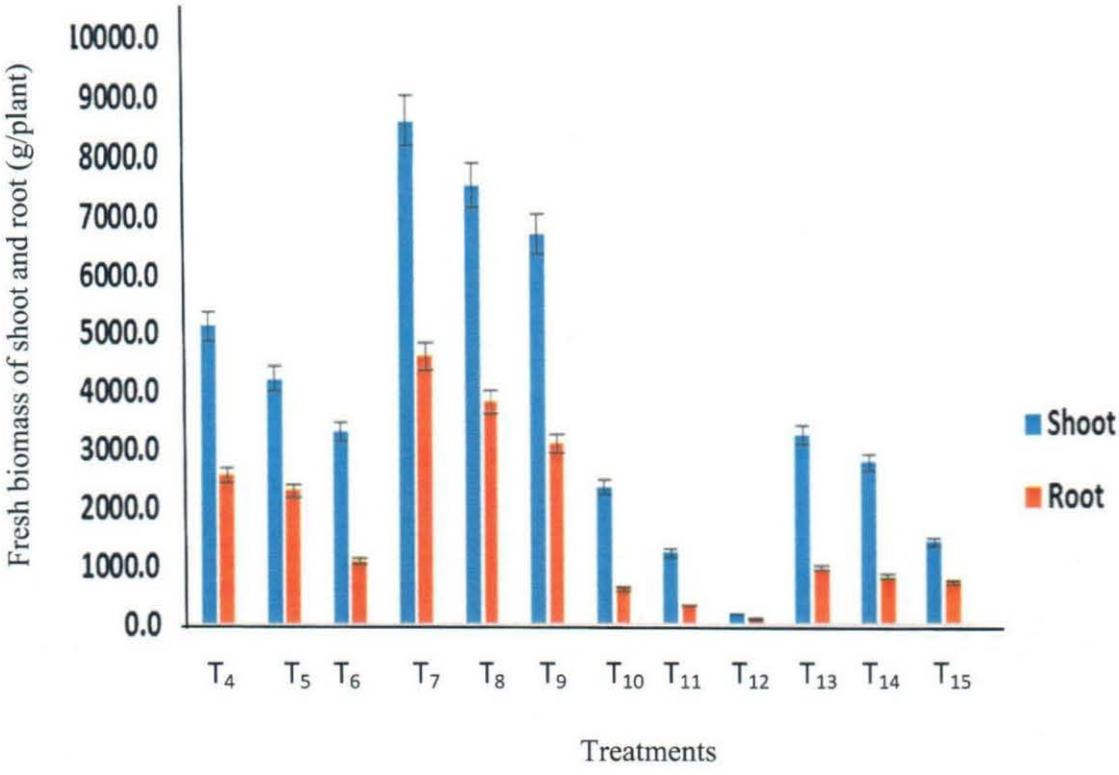


FIG. 27: Fresh weight of shoot and root of *Pennisetum purpureum* and*Leuceanaleucocephala* at 6 months after pollution in the field.

T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide unde r*P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

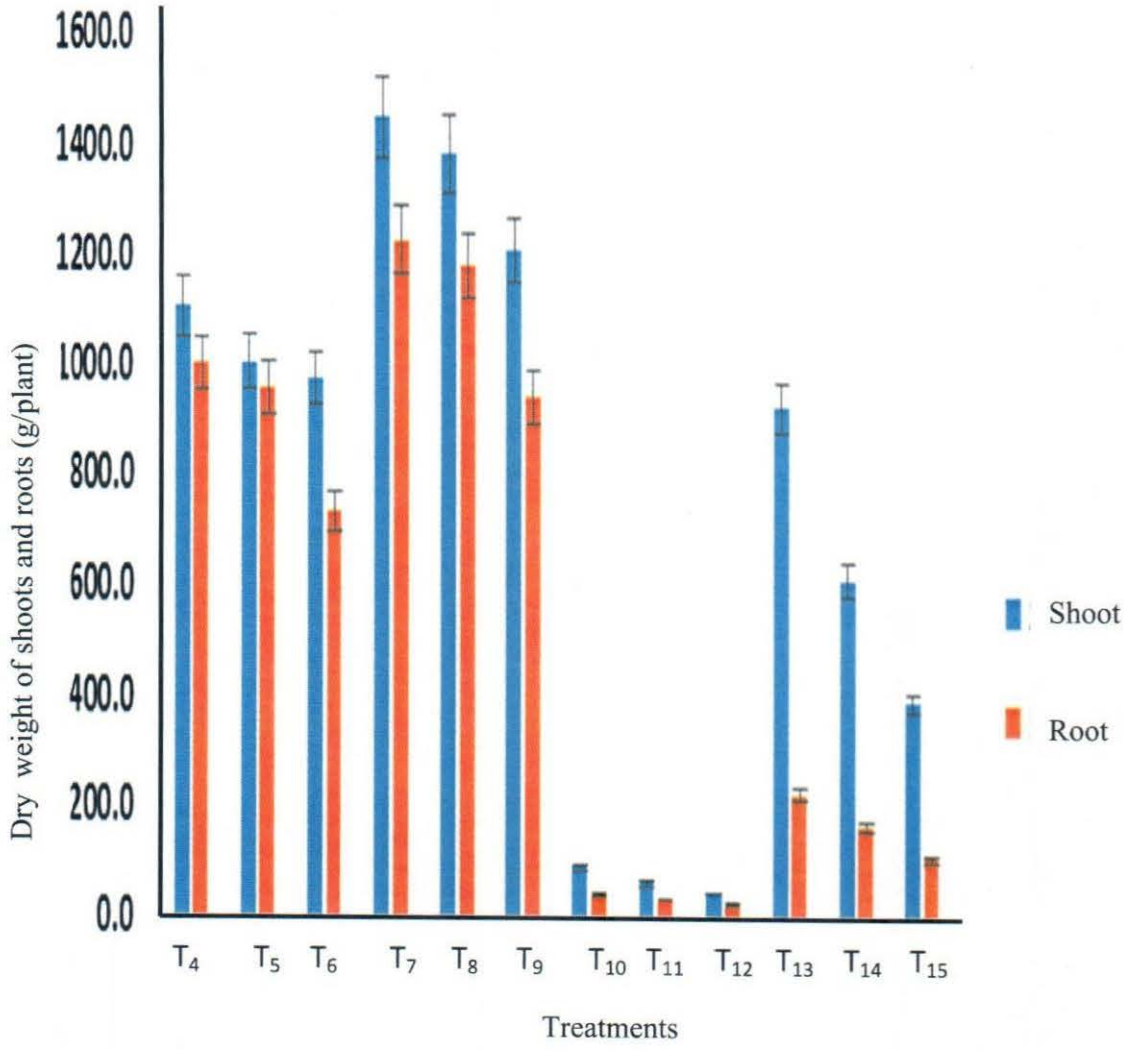


FIG. 28: Dry weight of shoot and root of *Pennisetum purpureum* and*Leuceanaleucocephala* at 6 months after pollution in the field.

T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

The increase in biomass in soil amended with organo-mineral fertilizer and brassinolide may be attributed to nutrient and growth factors from the organomineral fertilizer and brassinolide. It may also be due to the different organic compounds exuded by plants through roots which affected the density, diversity and activity of some specific soil microbes in the surrounding rhizophere. However, the low biomass in soils polluted without amendment and brassinolide may be due to the toxic effect of the crude oil on plants and microorganisms. Such a low plant biomass has been observed for different crops treated with petroleum hydrocarbon and diesel oil (Kulakow *et al.*, 2000; Tesar *et al.*, 2002). Agbogidi (2011) reported a significant reduction in biomass accumulation in *Jatropha curcas* seedlings raised in oil impacted soils. Ali *et al.* (2009) reported a reduction in total dry weight of olive *(Olea europacea Linn)* as a result of crude oil pollution.

4.11 Heavy metal contents in soils

4.11.1 Content of heavy metals [Lead (Pb), Nickel (Ni) and Cadmium (Cd)] in soils at 3 months after crude oil pollution

The content of heavy metals Lead (Pb), Nickel (Ni) and Cadmium (Cd) in soils under different treatments in the field at 3 month after crude oil pollution are presented in Table 21. The unplanted soil polluted with 7.5 % crude oil, amended with organo-mineral fertilizer without brassinolide had a significantly ($P<0.05$) higher content of Pb than other treatments. The lowest content of Pb was in planted soil polluted with 2.5 % crude oil, amended with organo-mineral fertilizer and brassinolide.

Generally, the content of Pb across the treated soils significantly exceeded the permissible limit of 85 mg/kg in soils (WHO, 1996). The increase in Pb content may be due to the pollution of the soil with crude oil. Hinojosa *et al.*, (2004) and Udo (2008) reported an increase in Pb content as a result of crude oil pollution.

TABLE 21

Content of heavy metals (Pb, Ni, Cd) in the soil at 3 months after crude oil pollution

Treatment	Lead (Pb)	Nickel (Ni) mg/kg	Cadmium
T_1	253.33de	55.67ef	4.26c
T_2	379.00b	62.67cd	5.01b
T_3	500.67a	72.33a	5.33a
T_4	220.00ef	51.33f	3.13f
T_5	280.00cd	58.0de	3.80e
T_6	294.33cd	65.33bc	3.98d
T_7	126.67h	36.33h	2.13j
T_8	148.00gh	40.67h	2.41i
T_9	165.00gh	45.33g	2.61h
T_{10}	227.00ef	53.67ef	3.23f
T_{11}	291.67cd	62.33cd	3.99d
T_{12}	317.33c	67.67b	4.36c
T_{13}	135.00gh	38.00h	2.21j
T_{14}	136.67gh	40.00h	2.550h
T_{15}	183.33fg	46.67g	2.78g

Means in the same column followed by same letter (s) are not significantly different at 5% probability level

T_1 = 2.5 % crude oil polluted soil + organomineral fertilizer (OF), no planting or brassinolide
T_2 = 5.0 % crude oil polluted soil + OF, no planting of brassinolide
T_3 = 7.5 % crude oil polluted soil + OF, no planting or brassinolide
T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide unde r*P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide unde r*P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

The highest level of nickel content was in the unplanted soil polluted with 7.5 % crude oil, amended with organo-mineral fertilizer, without brassinolide while the lowest was in soils polluted with 2.5 or 5.0 % crude oil, amended with organo-mineral fertilizer and brassinolide, under *P. purpureum* and *L. leucocephala*. Vwioko *et al*., (2006) and Hinojosa *et al*. (2004) also reported a high accumulation of nickel due to the presence of crude oil. The Ni content in the treated soils was higher than the permissible limit of 35 mg/kg (WHO, 1996). Cadmium (Cd) content in the soil was significantly higher in the unplanted soil polluted with 7.5 % crude oil, amended with organo-mineral fertilizer without brassinolide than all treatments. The lowest content of cadmium was in planted soils polluted with 2.5 % crude oil, amended with organo-mineral and brassinolide. The cadmium content in all the soils exceeded the permissible limit of 0.8 mg/kg (WHO, 1996).

The marked reduction in the content of heavy metals, especially in planted soils polluted with crude oil and amended with organo-mineral fertilizer and brassinolide may be attributed to complexation with organic molecules from the fertilizer or the uptake of these metals by the plant.

4.11.2 Content of Heavy Metals [Lead (Pb), Nickel (Ni) and Cadmium (Cd)] in soils at 6 months after crude oil pollution

The contents of lead (Pb), nickel (Ni) and cadmium (Cd) in soils at 6 months after crude oil pollution are presented in Table 22. The unplanted soil polluted with 7.5 % crude oil and treated with organo-mineral fertilizer but not with brassinolide had the highest ($P<0.05$) contents of lead and nickel among treatments.

The lowest content of nickel was in soils polluted with 2.5 or 5.0 % crude oil, amended with organo-mineral fertilizer and brassinolide under *P. purpureum* and soil polluted with 2.5 % crude oil, amended with organo-mineral fertilizer and brassinolide under *L. leucocephala*.

TABLE 22

Content of heavy metals Lead (Pb), Nickel (Ni) and Cadmium (Cd) in the soil at 6 months after crude oil pollution

Treatment	Lead (Pb) mg/kg	Nickel (Ni) mg/kg	Cadmium (Cd) mg/kg
T_1	240.00d	45.67de	3.23c
T_2	370.00b	56.67b	4.08b
T_3	440.00a	63.00a	4.23a
T_4	197.67e	41.33ef	2.84d
T_5	244.67d	48.33cd	3.01cd
T_6	273.33c	51.67bc	3.15cd
T_7	108.00h	29.67h	1.17h
T_8	124.67gh	34.33gh	1.31ef
T_9	139.00fg	36.67fg	1.50g
T_{10}	204.67e	43.67de	2.96d
T_{11}	233.67d	48.67cd	3.29c
T_{12}	258.67cd	53.33bc	3.23c
T_{13}	119.67gh	33.33gh	1.25ef
T_{14}	130.33fgh	37.33fg	1.49g
T_{15}	154.33f	41.00ef	1.60e

Means in the same column followed by same letter (s) are not significantly different at 5% probability level

T_1 = 2.5 % crude oil polluted soil + organomineral fertilizer (OF), no planting or brassinolide
T_2 = 5.0 % crude oil polluted soil + OF, no planting of brassinolide
T_3 = 7.5 % crude oil polluted soil + OF, no planting or brassinolide
T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide unde r*P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

Generally, the content of nickel exceeded the WHO (1996) permissible limits of 35mg/kg in soils except for soils treated with 2.5, 5.0 % crude oil, amended with organ-mineral fertilizer and brassinolide under *P. purpureum* and soil polluted with 2.5 % crude oil, amended with organo-mineral fertilizer and brassinolide under *L. leucocephala*.

Cadmium concentration followed a similar trend as Ni (Table 31). Polluted soils under *P. purpureum* and *L. leucocephala* amended with organo-mineral fertilizers had the lowest content of Cd. The content of cadmium across soils, irrespective of the treatment, was higher than the permissible limit of 0.8 mg/kg in soil (WHO, 1996).

4.12 Heavy metal concentrations in plants

The concentration of lead (Pb) in the root, stem and leaf of the two plant species across treatments is presented in Figure 29. *Pennisetum purpureum* raised in soils treated with 7.5 % crude oil amended with organo-mineral fertilizers and brassinolide had the highest ($P \leq 0.05$) concentration of Pb in the roots compared with other treatments. Generally, the roots of *P. purpureum* and *L. leucocephala* retained the highest concentration of Pb with tissue abundance in the order root > stem > leaf. This shows that the plants are hyper-extractors of lead (Pb) in crude oil polluted soil and can be used for phytoremediation. Olatunji *et al.* (2014) and Cho-ruk *et al.*, (2006) reported that most plants ordinarily accumulate heavy metals mainly in the root and less in the leaves or in the edible parts.

The accumulation of nickel (Ni) in the vegetative organs of the plants differed significantly (Figure 30). *Pennisetum purpureum* grown on soil

contaminated with 7.5 % crude oil and amended with organo-mineral fertilizer and brassinolide significantly ($P < 0.05$) had the highest content of Ni in the roots. The lowest content was observed in *L. leucocephala* in unamended polluted soils and *P. purpureum* under 2.5 % pollution without organo-mineral fertilizer and brassinolide. *Pennisetum*

purpureum planted in soil polluted with 7.5 % crude oil, amended with organo-mineral fertilizer and brassinolide had the highest content of Cd in the root (figure 31). A similar trend was also observed for soils under *L. leucocephala*. The concentration of Cd in the two plants were in the order root > stem >leaf. The high accummulation of Cd in the roots of both plants implies that Cd translocation from the soil to the root was substantially higher and the roots acted as a sink for Cd accumulation. Generally, the content of heavy metals was higher in the soil at 3 months than at 6 months. The possible reason for the low content of these metals in the soil at 6 months could be attributed to the uptake of the metals by the plant species.

Peer *et al*., (2005) reported that plants used for phytoremediation must have the ability to accumulate heavy metals and must be adaptable to the environment. Similarly, Abdel-Salem (2012) reported that *P. purpureum* was an efficient phytoremediator plant for Cd uptake. According to Ebbs et al., (1997), some plant species have the capacity to absorb and accumulate certain metals in their shoot and roots at levels that are toxic to ordinary plants. Likewise Schnoor *et al*., (1995) noted that plants grown in soil with high metal concentration were likely to have an elevated metal uptake and accumulation in tissues. The low accumulation of the trace elements in plants grown in unamended soils may be due to several factors such as the toxic effect of the crude oil, lack of nutrients and presence of competing ions (Prasad *et al.*1999).

4.13 Total petroleum hydrocarbon content in soil

The total petroleum hydrocarbon content of soils amended with organo-mineral fertilizer and brassinolide under *P. purpureum* and *L. leucocephala* was significantly

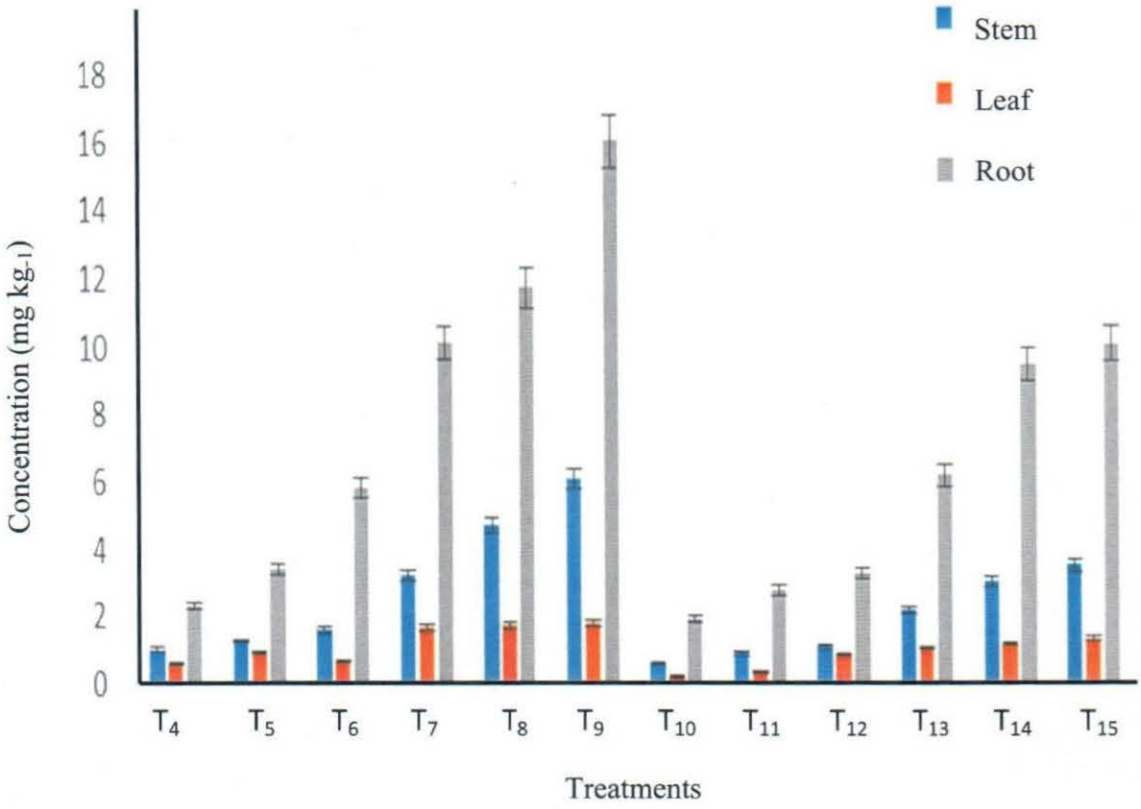


FIG. 29: Concentration of Pb in the stem, root and leaf of *Pennisetum purpureum* and *Leuceana leucocephala* under different soil treatments in the field after 6 months of pollution

T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under*Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under*P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

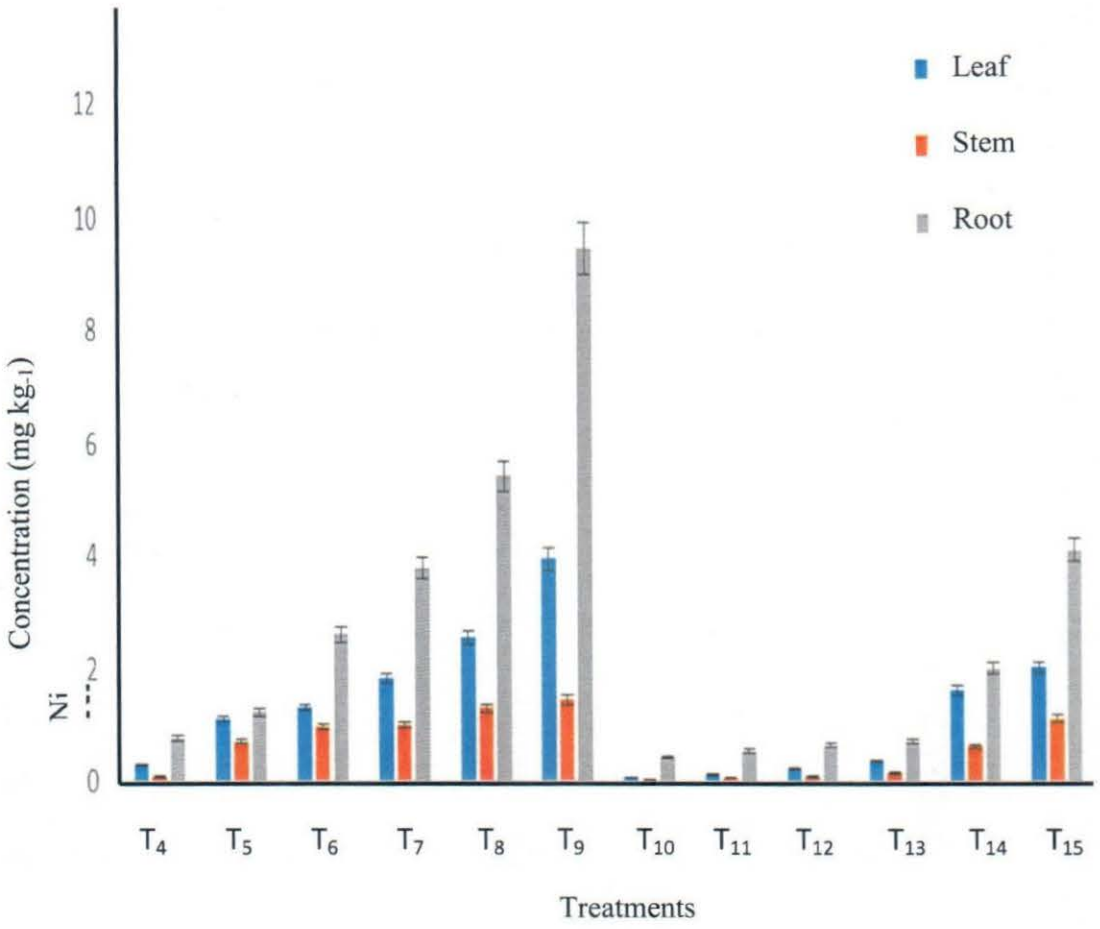


FIG. 30. Concentration of nickel (Ni) in the stem, root and leaf of *Pennisetum purpureum* and *Leuceana leucocephala* in the field 6 months after pollution

T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

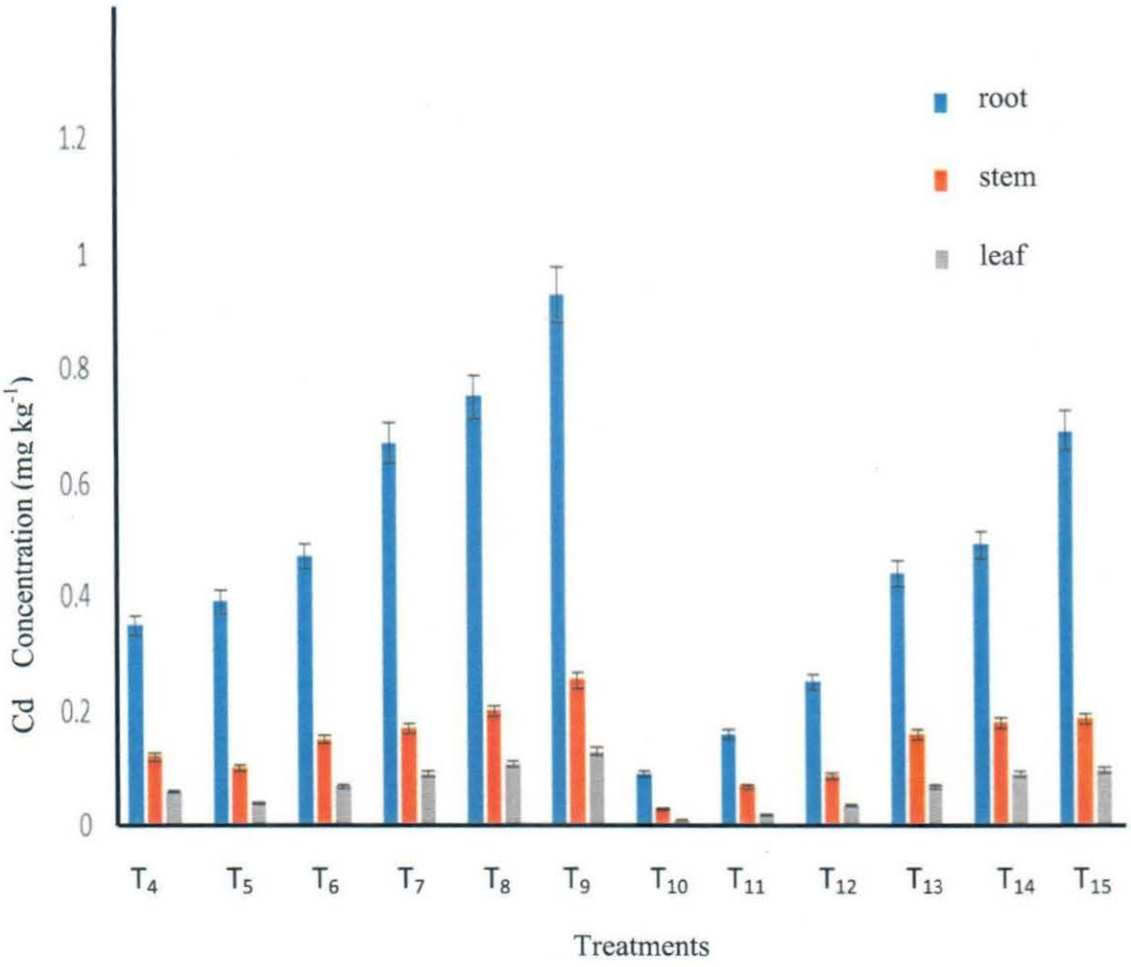


FIG 31: Concentration of Cadmium (Cd) in the stem, root and leaf of *Pennisetum purpureum* and *Leuceana leucocephala* in the field 6 months after pollution

T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide unde r*Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

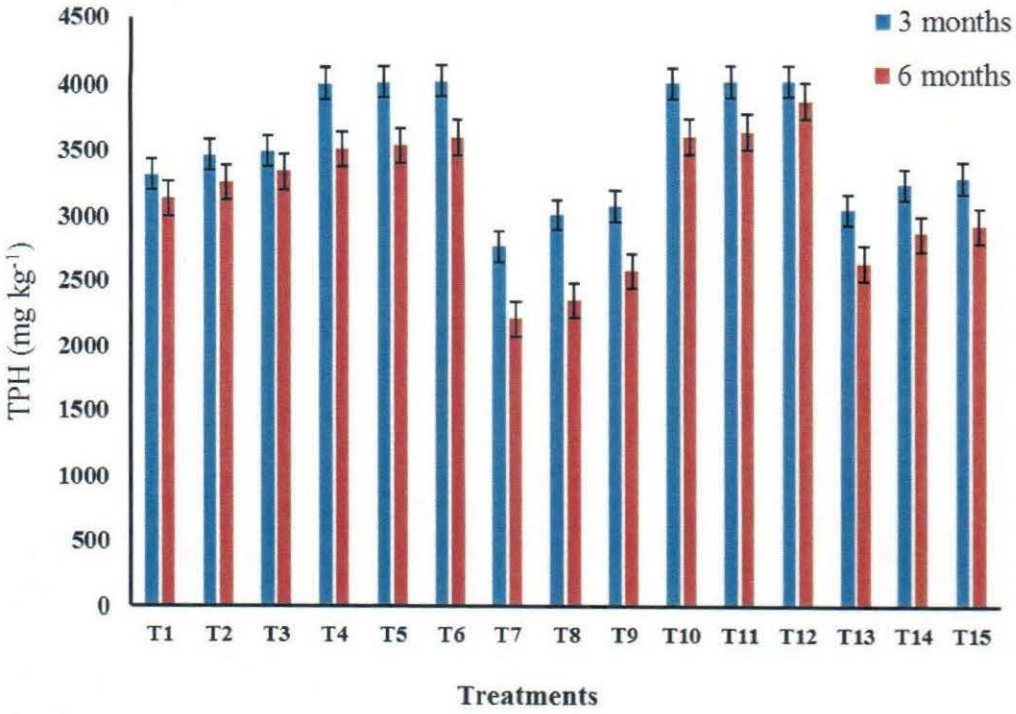


FIG. 32: Total Petroleum hydrocarbon (TPH) content in soil at 3 months after crude oil pollution in the field

T_4 = 2.5 % crude oil + *P. purpureum*, no OF or brassinolide
T_5 = 5.0 % crude oil + *P. purpureum*, no OF or brassinolide
T_6 = 7.5 % crude oil + *P. purpureum*, no OF or brassinolide
T_7 = 2.5 % crude oil + *P. purpureum* + OF + brassinolide
T_8 = 5.0 % crude oil + *P. purpureum* + OF + brassinolide
T_9 = 7.5 % crude oil + *P. purpureum* + OF + brassinolide
T_{10} = 2.5 % crude oil + *L. leucocephala*, no OF or brassinolide
T_{11} = 5.0 % crude oil + *L. leucocephala*, no OF or brassinolide
T_{12} = 7.5 % crude oil + *L. leucocephala*, no OF or brassinolide
T_{13} = 2.5 % crude oil + *L. leucocephala* + OF + brassinolide
T_{14} = 5.0 % crude oil + *L. leucocephala* + OF + brassinolide
T_{15} = 7.5 % crude oil + *L. leucocephala* + OF + brassinolide

($P < 0.05$) lower than other treatments at 3 months after pollution (Figure 32). The decrease in TPH in soils planted and amended with organo-mineral fertilizer and brassinolide could be attributed to ample supply of essential nutrients for microbial growth and degradation of TPH.

At 6 months after pollution, the highest reduction in TPH across pollution levels was in soil amended with organo-mineral fertilizer and brassinolide under *P. purpureum* or *L. leucocephala*. The possible reason for the low content of TPH in the polluted but amended and planted soils might be due to such mechanisms as rhizodegradation which involves the interaction effect of plant and soil microorganisms that favoured a greater reduction in total hydrocarbon.

Also the ability of the treatments (organo-mineral fertilizer and brassinolide) to supply the soil microbes and plants with nutrients such as nitrogen and carbon for their growth and development might also be a possible reason for the low content. This result is in agreement with that of White *et al.* (2006) who reported lower total petroleum hydrocarbon in vegetated fertilized plots than non-vegetated non-fertilizer plots.

4.13.1 Total petroleum hydrocarbon content in plants 6 months after crude oil pollution in the field

Pennisetum purpureum planted in soil polluted with 2.5 and 5.0 % crude oil amended with organo-mineral fertilizer and brassinolide had similar TPH levels which were higher than other treatments (Figure 33). Also *L. leucocephala* planted in soils polluted with 2.5 % crude oil, amended with organo-mineral fertilizer and brassinolide had high uptake of TPH. During this study, more weeds were observed sprouting from the polluted soils, amended with organomineral fertilizer, brassinolide under *P. purpureum* and *L. leucocephala* than the unamended soil. This indicated that the toxicity of crude oil in the vegetated and amended soils reduced to the extent of allowing

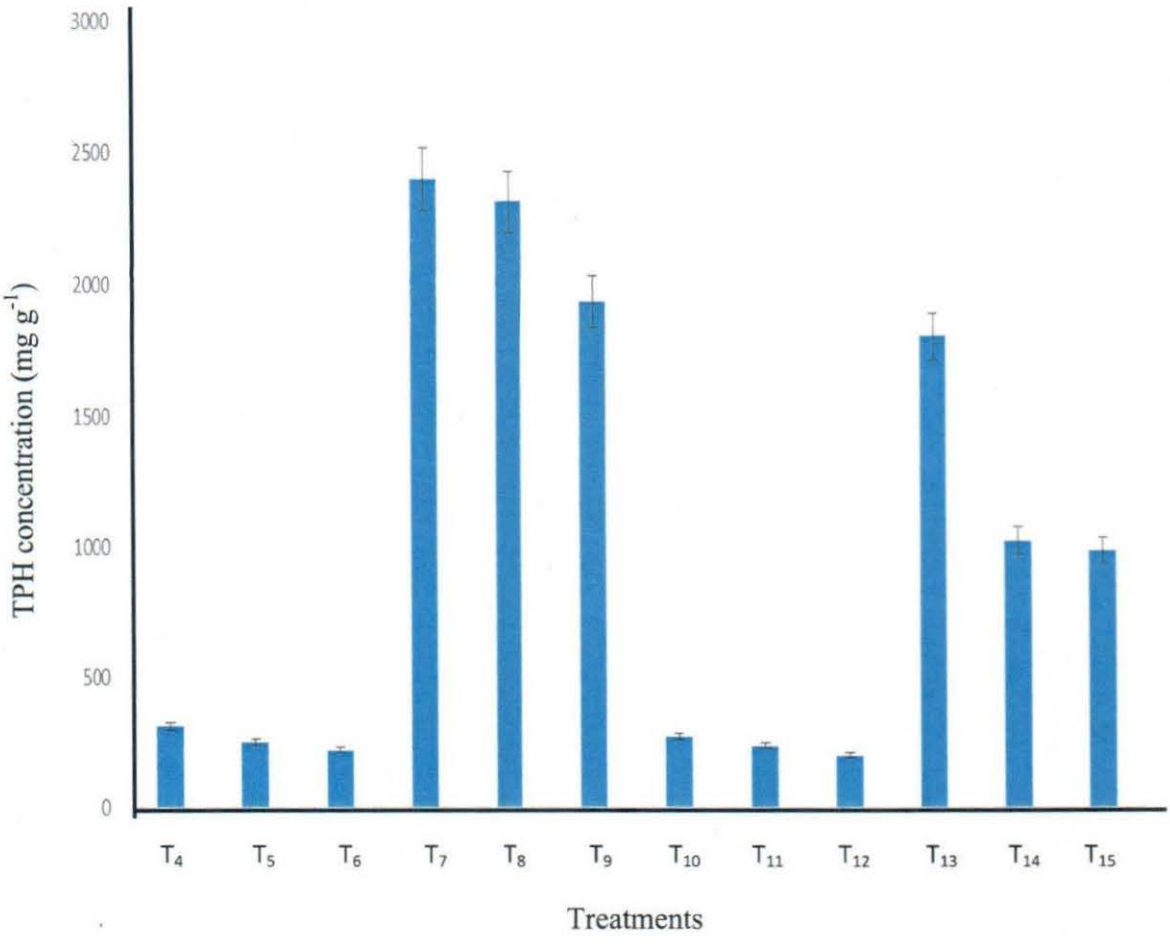


FIG. 33: Total petroleum hydrocarbon content in plants 6 months after crude oil pollution in the field

T_4 = 2.5 % crude oil plluted soil + no OF or brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + brassinolide under *L. leucocephala*

for the growth of volunteer weeds in the soil (Njoku, *et al.*, 2008). Previous studies (Xia 2004; Yateem *et al.*, 2000; Aprill and Sims 1990 and Muratova *et al.* 2008) have shown higher reductions of TPH in vegetated amended soils than in non-vegetated and unammended soil. As confirmed in this study, Edwin-Wosu (2000) and Xia (2004) listed *P. purpureum* and *L. leucocephala* as plants that can cleanup petroleum hydrocarbon polluted soils.

The accumulation of TPH by these plants may be attributed to several mechanisms of phytoremediation, including rhizodegradation [interaction of the plant with bacteria and fungi] (McCutcheon *et al.*, 2003; Siciliano *et al.*, 2003), phytoextraction (process in which metal accumulating plants are used to transport and concentrate metals from the soil into the harvestable parts of roots and above ground shoots) (Morikawa and Erkin, 2003; Sinha *et al.*, 2004) and phytovolatilization (McCutcheon *et al.*, 2003). An evidence of phytovolatilization was the leaf burn (leaf chlorsis) observed in the plants during the first few weeks of remediation. This suggests that violatile organic compounds were taken up by the roots of the plants, translocated within plants and transpired via the stems and leaves (Wiltse *et al.*, 1998). The leave burn gradually disappeared in the course of remediation, meaning that so many volatile petroleum hydrocarbons were lost to the atmosphere. Also the production of root exudates and plant materials acted as source of nutrient for hydrocarbon degrading microbes (Ernst, 1996; U.S. EPA, 2001). This increased the ability of the plants to remove the pollutants from the contaminated soil. Finally, the release of root-associated enzymes capable of transforming organic pollutants by catalyzing chemical reactions in soil and the physical and chemical effects of plants and their root systems on soil condition contributed to the phytoremediation.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

Advances in technology coupled with natural disaster have contributed to soil degradation. Contamination of agricultural soils by crude oil is one of the most prevalent problems associated with exploration and processing of petroleum hydrocarbon. Many techniques such as soil excavation, soil washing/flushing, chemical immobilization, stabilization and electrokinetics have been proposed and used to clean-up crude oil polluted soil. These techniques are rather expensive and further degrade the valuable components of the soil. Thus, there is need for a more cost-effective and environmentally friendly method such as phytoremediation. Phytoremediation refers to the use of green plants and their associated microorganisms to degrade, extract, contain or render harmful substances harmless in the soil. Plant and soil microbes clean up hydrocarbon contaminated soils through seven processes and these include; rhizodegradation, phytoremediation, phytovolatilization, phytostabilization, rhizofiltration, phytosalinization and hydraulic containment. Factors affecting phytoremediation are: choice of plant, soil type, weather, water availability and oxygen requirement.

Various plant species have been recognized for their effectiveness in remediating soils contaminated with petroleum hydrocarbons. In a number of studies, grasses and legumes have been preferred relative to others due to their potential to clean-up hydrocarbon contaminated soils.This research was carried out to evaluate the potential and associated mechanisms of some native species to phytoremediate crude oil polluted soil using greenhouse and field trials.

In the green house, the treatment consisted of twelve species of plants with four levels of crude oil pollution while the field experiment used two selected plant species with phytoremediation potentials from the greenhouse experiment, four levels of crude oil, organo-mineral fertilizer and plant growth hormones (brassinolide).

The results from the greenhouse revealed that crude oil pollution had no significant effect on the texture of the soil. Successive increases in pollution significantly ($P < 0.05$) decreased the soil pH, available phosphorus, exchangeable bases and effective cation exchange capacity while organic carbon, total nitrogen and base saturation were significantly ($P<0.05$) increased. Total heterotrophic bacteria and fungi count as well as plant biomass were significantly ($P<0.05$) reduced in polluted soils while hydrocarbon utilizing bacteria and fungi increased substantially.

Data from the field trials showed that soil pH, organic carbon, available phosphorus, exchangeable bases, plant biomass and microbial population were significantly increased by the organo-mineral fertilizer and plant growth hormones (brassinolide). The concentration of lead (Pb), nickel (Ni) and cadmium (Cd) in plant tissues were in the order: root > stem > leaf with the application of organo-mineral fertilizer and brassinolide.

Whether in the field or greenhouse, there was a significant ($P<0.05$) reduction in total hydrocarbon content of the soils following planting. Polluted soils amended with organo-mineral fertilizer and brassinolide, under *P. purpureum* and *L. leucocephala* had the highest removal efficacy of about 79.8 % and 61 % of total petroleum hydrocarbon compared with 41 % in non-amended and 38.6 % in non-vegetated soils.

5.2 Conclusion

In conclusion, *Pennisetum purpureum* and *Leuceana leucocephala* showed the ability to clean up crude oil polluted soils. This is confirmed by the higher concentration of total petroleum hydrocarbon in plant and greater reduction in the soil. *Pennisetum*

purpureum and *L. leucocephala* had higher levels of total petroleum hydrocarbon in plant tissues and low in soil indicating a superior remediating potential over the other species. Further, they favoured considerable microbial proliferation in the rhizosphere, leading to better crude oil degradation. Since these two plant species are widely distributed and have proved successful in phytoremediation of crude oil polluted soils, they can be of benefits for many tropical countries facing the problem of crude oil pollution.

5.3 Recommendations

Pennisetum purpureum and *Leuceana leucocephala* are common tropical plants considered as feed supplements for small ruminants and also for the control of soil erosion but from this experiment, they have shown considerable potential for remediation of crude oil polluted soil.

1. Based on the results obtained from this study, phytoremediation activities will benefit from use of organo-mineral fertilizers and plant growth regulation (brassinolide) as they could help to contain the adverse effect of crude oil pollution on soil properties and microbial population.
2. Having ascertained the performance and efficacy for remediation of crude oil polluted soil by *Pennisetum purpureum* and *Leuceana leucocephala,* these two plants are suitable for phytoremediation of hydrocarbon contaminated soils and should be studied further.
3. Findings from this study should be used for future remediation works on crude oil polluted soils, especially where cost effectiveness and eco-friendly methods are of paramount consideration.
4. Further screening of more native plant species should be explored.

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APPENDIX 1

Biochemical Test

The procedures, principle and purpose of the different biochemical tests used in this study are presented below. Description of stains, reagents and tests used for the identification of organisms.

(i) Gram stain reaction

A drop of distilled water was added to each of the slides and pure culture were transferred on these slides. Then wire loop was flamed (sterilized) and allowed to cool. Then, it was used to pick a small amount of the pure culture and places on the slide with proper labeling. The wire lop was used to spread the culture in order to produced a smear (thin film). It was then placed on the bunsen flame for fixing. The reason for this was to sterilize the organisms, coagulates the protein causing the cells to adhere to the slides with proper labeling. The slide was stained by flooding it with crystal violet for one minute and washed off gently with water after 20 seconds. The gram's iodine was added to the smear for 60 seconds and decolorized with 95% alcohol for 30 seconds. It was gently rinsed again with water at the end of the 30 seconds to stop the decolonization and then counter stain with 0.28% safranin for 30 seconds and washed with distilled water for 2 seconds. It was then blot dry by fanning and waving to dry. The smear on the slide were later examined on microscope with magnification (x) 100 using immersion oil.

ii) Spore Stain

This was carried out to distinguish endospore forming bacteria from non-formers. A smear of the test organism was prepared on a grease free slide and fixed by heating over a burnsen flame. The fixed smear was flooded with 50% malachite green solution and allowed to react for 5 minutes and thereafter washed off with water slowly. The stained specimen was counter stained with 1% safrànin and allowed for 30 seconds, then washed with water , air dried and examined under oil immersion on microscope with

magnification (x1 00). The microscope. Spore bearing organisms stained green while red stain was shown on vegetative cells.

iii) Catalase Test

Uses the catalase test is primarily use to distinguish among gram –positive *Staphyloccus aerus* from non- catalase "*Streptococcus* and *Enterococcus* spp. Catalase test is also used to differentiate acrotolerant strains of *Clostridiums* spp are catalase negative from *Bacillus* spp. which are positive. Catalase test can be used as an aid to the identification of Enterobacteriaceae spp. which are catalase positive.

Procedure of catalase test

A small amount of bacterial colony was added to a surface of clean, dry glass slide using a loop or sterile wooden stick. Then a drop of 3% H_2O was added to the slide and mixed thoroughly.

Result

Catalase positive reactions showed immediate effervescence (bubble formation) while catalase negative reaction did not showed bubble formation (no catalase enzyme hydrolyze the hydrogen peroxide).

iv) Oxidase Test

Use: The oxidase test is used to identify bacterial that produce C oxidase, an enzyme of the bacterial electron transport chain. When present the cytchrome C oxidizes the reagent *(tetramethyl-P-pheflylefflediamine)* to (*indophenols)* purple colour as end product.

Procedure of oxidase Test

A filter paper was soaked with *tetramethyl-P-phenylenedi-aminedihydrochloide.*

The paper was moisten with sterile distilled water. The colonies to be tested were picks from an oven night cultures and smeared on the filler paper and observed for colour change.

Result

It was observed that "inoculated area of paper had for a colour change to deep blue or pimple within 10-30 seconds".

v) Triple sugar lron Agar (TSI) Test

Triple sager lion Agar (TSI) is a test which has three sugar (lactose, sucrose and glucose) and iron it was also contains Agar- agar as solidifying agent TSI is a semi media having slant and built).

Procedure for triple sugar iron Agar (TSI) Test

A well-isolated cologne of grain negative bacilli were picked with a sterilized needle and inoculated into the medium by first stabling through the medium and finally streaking the surface of the slant and the cap were left loosely by incubating the tube at 35°C in ambient air for 18 to 24 hours.

Interpretation of triple sugar iron agar test

When the red phenol indicator tuned yellow (both butt and slant) showed that lactose (or sucrose) was fomented, a large amount of acid was produced by showing cracks /bubbles on the medium.

If the butt changed to yellow, it was seen to ferment glucose because the butt had more glucose and acid then Slant which was red in colour which indicated the sign of alkaline or neutral pH.

When the butt and slant turned red, it was an indication of no sugar fermentation.

It when the medium was completely dark in colour, it showed the production of H_2S

vi) Motility Tests

Purpose

This is a three-in-one test medium which was used for the differentiation of gram negative bacterial based on their motility, production of indolent form tryptophan and decarboxylation of amino acid ornithine.

Procedure

The medium was prepared by following the manufacturer's specification. Thirty one-grams (31 grams) of the medium was dissolved in 100ml of purified water, mixed and boiled for minutes before sterilization at 121°C for 15 minutes at 15 1bs and allowed at cool at 45°C. All the test tubes were arranged on a test rack and 15ml each of the liquor was dispensed into the tubes.

Result

a) Motility: Result was recorded as motility positive when diffusion of bacteria grew beyond the line of stabs and turbidity of the medium was observed and negative result was vice versa.

b) Indole: Indole result was read after the addition of few drops of kovac's regent positive result was indicative of red ring on the surface of the medium and negative result showed no change in colour after few seconds.

c) Ornithine decarboxylate: When omithine decarboxyalse is produced, the ornithine is decarboxylated to putrescine which caused a rise in the pH and corresponding colour change of the bromocresol from yellow to purples seen throughout the medium. This indicated a positive result for ornithine and negative when otherwise.

vii) Methyl Red Test

Purpose

It is used for differentiating the gram negative Enterobacteriaceae organisms.

One gram each of peptone and glucose were dissolved in 100ml of sterile distilled water and 8m1 of the broth dispensed into test tubes and autoclaved at 121°C for 15 munities. After autoclaving, tubes were allowed to cool before inoculated with the test organisms and incubated at 37°C for 24 hours.

APPENDIX 2

Calculations for the different concentration of crude oil 2.5 (5.9L), 5 .0 (11.8L), 7.5%w/w (17.7L) in the field.

$10,000m^2$ (1ha) contains 2,000,000kg soil.

$\therefore 1m^2$ land will contain $\frac{1m^2 \ x \ 2{,}000{,}000kg \ soil}{10{,}000m^2}$ = 200kg soil

100g of crude oil = 115ml

500g of crude oil in 10,000g (10kg) of soil = 5% w/w pollution

$\therefore$ If 10g soil = 500g crude oil

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100g of crude oil = 115ml

500g of crude oil in 10,000g (10kg) of soil = 5% w/w pollution

$\therefore$ If 10g soil = 500g crude oil

200kg soil = $\frac{200kg \ x \ 0.5kg}{10kg}$ = 10kg

200kg soil needs 10kg of oil

0.1kg (100g) of soil = 118ml

10kg of oil = $\frac{10kg \ x \ 118ml}{0.1}$ = 11.800ml

= 11.8 litres

$\therefore \ \frac{11.8}{2}$ = 5.9 litres

2.5% w/w = 5.9 litres

5.9 x 2 = 11.8 litres (5.0% w/w)

5.9 x 3 = 17.7 litres (7.5% w/w)