PHYTOREMEDIATION OF SOME TROPICAL SOILS CONTAMINATED WITH PETROLEUM CRUDE OIL

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF PhD ENVIRONMENTAL SCIENCE DEGREE.

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DECLARATION

On my honour, I hereby humbly declare that with the exception of references cited in this study which have been duly acknowledged, this thesis is the result of a detailed and pain-taken research undertaken by me under effective supervision; and that neither part nor whole of it has been presented elsewhere in pursuance of the award of another degree.

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DEDICATION

This project is dedicated particularly to the plights of the people and environment of the Niger Delta in Nigeria and indeed, those elsewhere, who suffer neglect and depravity as a result of the exploration and exploitation of their natural endowments.

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ABSTRACT

This study was undertaken in three phases to identify (phase 1), screen (phase 11) and evaluate (phase 111) plants for their phytoremediation potential.

In Phase 1, 15 plant species made up of grasses and legumes namely: *Paspalum. vaginatum, Cynodon.dactylon, Pueraria. phaseoloides, Centrosema. pubescens, Panicum. maximum, Schrankia. leptocarpa, Eclipta. alba (Linn.), Cyperus. haspen (Linn.), Melastromastrum. capitatum, Acreceras. zizanoides Dandy, Pteridum aquilinum (Linn), Ludwigia.decurrens Walt,Setaria longiseta P.Beauv., Physalis angulata (Linn.),* and *Desmodium scorpiurus Desv.*were identified on sites previously polluted by crude oil spills in the Niger Delta Area of Nigeria. The first 6 species were used in phase 11 while the first four species were earmarked (rolled over) for phase 111. Responses to Questionnaire indicated that majority of residents in the selected sites/communities had lived in these areas for 10 or more years had mainly JHS/SHS education; were self employed – mainly farmers and fishers although most were unemployed in the public sector. Adverse effects of the operations of oil companies particularly oil spillage on the environment and local residents include: loss of vegetation and farmlands, soil and water body contamination, weak social and cultural institutions (disrespect by youth for elders and institutions), militancy and hostage taking among youth from the area. In phase 11, seeds of legumes among the six selected species were collected from Accra, Aburi environs and Kusi in the Eastern region of Ghana; they were scarified, cultured in growth medium and the seedlings which emerged from them were transplanted into experimental pots, each containing 2000g of either Alajo or Toje soil series. One week after transplanting, each pot was simulated with a corresponding serial crude oil concentration of 0% (control) 1 % (24ml), 3% (83ml), 5.5% (130ml) and 8% (189ml) or 10% (237ml) in three replicates. These concentrations were arrived at, using the fomular [(vol of crude = % X wt / 0.844) ml]. Similarly, three replicates of each selected grass species were

planted vegetatively into 2000g of either Alajo or Toje soil series using known numbers of propagules (stolons). After one week of planting, pots were treated with the above serial crude oil concentrations using the appropriate volume indicated. Germination rates were variable and results of percentage plant Survival Difference (PSD) indicated that *Paspalum vaginatum* and *Cynodon dactylon* were highly tolerant of crude oil concentrations at 3%, 5.5% and 8% on the Toje soil series and 3%, 5.5% and 10% on the Alajo soil series as compared to *Panicum maximum* and where therefore selected as high contaminant tolerant species for phase 111; whereas *Puraria phaseoloides* and *Centrosema pubescens* which had -45% & ±42% and -16% & -35%respectively, as PSD for Alajo and Toje soil series as compared to *Schrankia leptocarpa,* were also selected as low/poor contaminant tolerant species (at 1% & 2% - introduced in phase 111as upper contamination limit for the poor tolerant species) also, for the last stage (phase 111) of the study. For phase 111, *C. dactylon, P .vaginatum, P. phaseoloides* and *C. pubescens* were evaluated for phytoremediation potential at crude oil concentrations of 3%, 5.5% (added to determine mid-point tolerance for high contamination tolerant species) and 8% for Toje soil series, and going up to 10% for Alajo soil series for high contaminant tolerant species and 1% & 2% for low/poor tolerant species respectively, using the randomized complete block design. Results of plant biomass (fresh weight) decreased (between 0.5g/fw & 20g/fw) at low contaminant concentrations and increased (between 14g/fw & 122g/fw) at high contaminant concentrations.Values of plant enrichment were higher for *Cynodon dactylon* (0.9% & 4.49%) than for *Paspalum vaginatum* (1.2% & 3.12%) respectively for Toje and Alajo soil series. Therefore, both *Cynodon dactylon* and *Paspalum vaginatum* can be good plants species for phytoremediation having accumulated total hydrocarbons up to 33,270mg/kg at 5.5% on Toje soil series and 40,466mg/kg at 8% on Alajo soil series.

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CHAPTER ONE

1.0 INTRODUCTION

Crude Oil is defined as Petroleum in its natural state (Ukoli, 2003). Crude oil can be found in different forms ranging from very fluid volatile liquids to viscous semi-solid materials (Ojo and Adebusuyi, 1996). It also comes in a variety of colours ranging from black or green to light yellow. It varies considerably in density which ranges from heavy to average or light [American Petroleum Institute (API, as cited in Ojo and Adebusuyi, 1996)].

Globally, crude oil constitutes 33.5% of the energy requirements for human development (World Energy Consumption; Wikipedia, 2012) and the bulk of this is obtained from drilling activities either offshore or onland. Paramount among the problems associated with drilling for crude oil is pollution including soil contamination.

Crude oil pollution occurs whenever unrefined hydrocarbons spill into the environment where it is exploited, explored or handled. Pollution is an undesirable change in the physical, chemical, and biological characteristics of all the components of an environment, which can threaten human health and that of beneficial organisms (Aboribo, 2001). Whenever a spill occurs, the immediate natural environment which is affected may include farmlands, surface water bodies (eg. fish ponds, and lakes) and overtime, groundwater through leachates and ultimately also the ambient atmosphere becomes polluted with it through volatilization. More often than not, these affected /polluted areas are resources owned by peasant farmers and inhabitants of impacted areas whose source(s) of livelihood is /are temporarily or permanently denied them throughout the duration of the effect of the spillage.

Globally, the first major oil spill was experienced as far back as the 1960s when a giant oil tanker called *Torrey Canyon* wrecked off the Scilly Isles. On board were 118,000 tons of crude oil, and her ruptured tank released oil which had very serious consequences on marine life. Two years after *Torrey Canyon*, an offshore oil rig operated by Union Oil erupted in the Santa Barbara Channel in California. The disaster to wildlife was a repetition of the *Torrey Canyon* incident (Sheridan and John, 1965).

In Africa, Countries such as Nigeria, Cote d'vore, Gobon and Cameron have been involved in the commercial production of crude oil since the 1950s and have had incidents of oil spillages and pollution effects on the environment (UNCTD, 2000).

Ghana is the latest addition to the growing list of commercial oil producing countries on the West coast of Africa and has had a fair share of contamination by petroleum products and pollution from its processing, distribution and wastes disposal; although, no spillage large enough has occurred as the time of this study (Smith, 2010).

The major source of worry arising from every crude oil spill is the toxicity and hazardous nature of hydrocarbon elements to the soil, soil-borne organisms and man, as well as the possible contamination of the food chain/web. Idoniboye (2000) reported that crude oil pollution spells far more hazardous consequences for soil and soil borne organisms than air pollution because whatever is absorbed by plants would be rich in the content of water soluble constituents of petroleum oil some of which are toxic to plants. Crude petroleum oil production and its potential spillage unto the soil will alter the carbon-nitrogen ratio and lead to nitrogen deficiency which can in turn threaten the survival of soil biota (Jobson *et al.*, 1974). The presence of crude in pore spaces in soil has been reported to expel air, deplete oxygen reserves and impede all forms of gaseous exchange between the atmosphere and soil thus limiting the survival of soil biocene as they (biocene) will lack elements essential for their growth and development (Ayotamuno and Kogbara, 2006).

These developments amongst others have heightened the growing quest by man to find solutions to crude oil polluted environments with efficiency and effectiveness, resulting in the search for the most appropriate remediation method(s)/technique(s) to adopt.

Over the years, physico-chemical methods have been adopted to either disperse hydrocarbons through the use of "Detergents" which may add more chemicals to the soil, or scoop and burn contaminated soils by the excavation and burning of the waxy layers Idoniboye (2000). The other clean-up measures include the injection of gaseous oxygen into anaerobic zone of contaminated environment to stimulate biodegradation - soil vent, or sparging, and the use of more soluble electron acceptors such as nitrates or sulphates. However, oxidation was reported to be slow and hydrocarbon degradation incomplete (Amro, as cited in DreamEssays.com, 2009). Amro, further suggested that other methods of hydrocarbon removal from contaminated sites could include the construction of vertical and horizontal wells (commercial oil and gas technologies), the efficiency of which depends on the type of oil/soil, amount of oil spilled and depth of penetration, age and degree of contamination and prevailing weather conditions. However, these mechanical methods have been considered generally harmful to habitats not only because they strip soils of nutrients and micro-organisms, but also because they are very expensive and relatively inefficient and generate a lot of waste to be disposed of (DreamEssays.com. 2009). Another method involving the use of organic fertilizers has been found to be effective in enhancing the rate of soil recovery after pollution by crude oil (Ofori, 1998 and Kogbara, 2007). Again, the growing concern for all physico-chemical methods, border on their availability for large scale application at short notice.

Ofori (1998) noted that, although crude oil polluted soils have the potential of natural recovery; a very long period of time is required for total recovery. Oyibo (2005) also reported that, although organic materials may be cheap and locally sourced, they may not be readily available to cope with large scale applications in time and space. Results of previous phytoremediation studies on crude oil contaminated soils have shown that certain plants can contain, translocate and volatilize petroleum hydrocarbons from contaminated matrixes

(Cunningham *et al.*, 1996 and Wiltse *et al.*, 1998). Furthermore, the exact levels of contaminant concentrations at which tropical plants can tolerate and degrade crude oil contamination are poorly understood (Ayotamuno and Kogbara, 2007).

Therefore, this project has been designed to investigate the potential/effectiveness of the use of plants in developing a clean-up technogy for oil contaminated soils (phytoremediation). This choice is based on the fact that phytoremediation has been reported to be cheap, environmentally friendly and a ready organic alternative to the physico-chemical methods of remediation of polluted sites. It has been estimated that phytoremediation can cost $10-$100 per cubic meter whereas metal washing can cost $30-$300 per cubic meter (Blacky, as cited in DreamEssays.com, 2009). Furthermore, Blacky (as cited in DreamEssays.com, 2009) reported that even if contaminated plants are unusable, they can be burnt and the resulting ash will be approximately 20-30 tons per 5,000 tons soil (DreamEssays.com. 2009).

Ultimately therefore, Phytoremediation is considered to be suitable to tropical environments because climatic conditions favour microbial growth and activity, nutrient availability and high plant biomass production (Merkl *et al.*, 2005).

Consequently, in order to fulfill the first plant selection criteria in the phytoremediation process which requires that test plants must be indegenious to zones of contamination, the search for appropriate plants for use in this study led investigation in the first phase of this project to the Niger Delta area of Nigeria where incidents of crude oil spills abound since no major spillage has occurred in Ghana up till the time of this research. Also, Kobgara (2007) has reported that petroleum contamination in the Niger Delta has become a common occurence.

1.1 Problem Statement

Crude oil pollution appears inevitable wherever hydrocarbons are explored, exploited and handled as afore stated. Its spill and pollution effects do not only cause immense ecological damage (both edaphic and aerial) wherever they occur, but also incredible distortion of the livelihoods of inhabitants of the affected area particularly of peasant/small scale farmers and hunters in rural areas of developing countries. Alongside the devastating problems of ecological, economic and human, is the huge cost of current clean-up techniques necessitating the search for relatively less expensive and more useful options such as phytoremediation.

Furthermore, existing physico-chemical methods of clean-ups are not only environmentally unfriendly, but also, their equipment is very expensive to acquire, operate and maintain. Manpower needs are technical, high in cost as well as in training. Operations of these techniques in the long run are inefficient, time consuming and therefore, may not readily be available for large scale applications.

1.2 Justification of Study

Crude oil exploration, exploitation and handling have been associated with frequent oil spills resulting from oil pipeline vandalization/rupture, equipment failures, tanker accidents and indiscriminate disposal of products. Tanee & Kinako (2008) have reported that despite more stringent environmental regulations, the risk of an oil spill affecting ecosystems, devastating biodiversity and stripping soils of nutrients is still high and must be accepted as inevitable; necessitating the choice of this project (phytoremediation) which is less distructive and less expensive. Therefore, this project has also been selected for the following additional reasons

i) *Biodiversity challenges: - i.e the potential problems of adaptation, competition, new pests and diseases that may arise from the introduction of species into different environments/habitats.*

ii) *The need for each country/region to identify indigenous plants species that can potentially be used for phytoremediation (Norman, 2003).*

iii) *Phytoremediation is generally considered a promising technology for the tropics because tropical climates have been reported to support microbial growth and activity, nutrient availability and biomass production (Merkl et al., 2005).*

iv) *The paucity of information on tropical species that could serve for the cleanup of oil contaminated soils (Ogbo et al., 2009).*

1.3 Hypotheses

Null Hypothesis (H_O): None of the selected plant species for this study has the potential to tolerate and extract petroleum hydrocarbons from soil contaminated with crude oil.

Alternate Hypothesis (H_1): All or some of the selected plants species for this study have the potential to tolerate and extract petroleum hydrocarbons from soil contaminated with crude oil.

1.4 Objectives of the study

1.4.1 Main objective

To identify, screen for tolerance and evaluate/assess selected test plants for their potential for use in the phytoremediation of selected crude oil contaminated tropical soils; in line with **Plant Selection Criteria** adopted from literature as follows:

i) Plants must be indigenous to site/zone of contamination (Gudin and Syratt, 1975 and Banks *et al*., 2003).

ii) Plants must be tolerant to contaminant stress (Kirk *et al.*, 2002).

iii) Plants must be able to stimulate microbial growth/activity, as well as reduce contaminant concentration in the soil (Hegde and Fletcher, 1996, Liste and Alexander, 1999 and Ogbo *et al*., 2009).

1.4.2 Specific objectives

Phase 1: Pilot survey in the Niger Delta Area of Nigeria.

i) *Determine textural class (es) of soil(s).*

ii) *Collect and identify plant species found to have regenerated on selected spilled sites.*

iii) *Assess the impact of spills on socio-economic and ecological parameters using the questionnaire.*

Phase 11: Screening of experimental plants for tolerance to hydrocarbons in Ghana.

i) *Screen the selected plants species identified in phase one (1) for tolerance to hydrocarbons contamination/toxicity.*

Phase 111: Evaluation of experimental plants for phytoremediation potential.

i) *Evaluate/assess selected plants species screened for tolerance to hydrocarbons contamination and establish their remediation potential as well as suitability for use in the phytoremediation trials/process*.

1.5 Anticipated benefits of this study to stakeholders

i) *Results from this research project would serve a useful guide and provide data base to the oil production industry, academia, host/impact communities as well as the general public concerning the phytoremediation mechanism.*

ii) *Potential selected plants could be used in ameliorating the effects of hydrocarbon toxicity and retore affected soils back to pre spill quality in the event of a spill.*

iii) *To popularize and institutionalize phytoremediation technology as the best complementary remediation practice for the oil industry in West Africa and even beyond.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Nature of Petroleum oil

The word petroleum takes its origin from the Latin word "Petra" meaning Rock, and the Greek word "Oleum" meaning Oil (Clark and Clark, as cited in Hydrocarbons Wikipedia, 2010). It is formed from the partial decomposition of organic matter (plants and animals remains), over geologic time (Clark and Clark, as cited in Hydrocarbons Wikipedia, 2010). Its constituents are separated by distillation into fractions of light colour, low density and low viscosity. Total Petroleum Hydrocarbon (TPH) is a term used to describe a large family of several hundreds of chemical compounds that originally come from crude oil. In general, all crude petroleum is made up of a combination of carbon and hydrogen in the approximate proportions of 84-86% carbon and 12-14% hydrogen (hydrocarbons Wikipedia, 2010).

The hydrogen and carbon atoms are bonded with non-polar covalent bonds and because of its lack of polar covalent bonds, hydrocarbons cannot create hydrogen bonds with water and are hydrophobic (Hydrocarbon Wikipedia, 2010).Petroleum oils are of varied kinds; examples include gasoline, kerosene, fuel oils, lubricating oils etc (USEPA, 2001). Also, the Nigerian National Petroleum Corporation (NNPC, 2000 as cited in Agagu and Fumi, 2008) reported that the high grade, high API Specific gravity (low specific gravity) coupled with the low corrosivity to refinery infrastructure, as well as low environmental impact of refinery effluent, place high premium value on the Nigerian Bonny Light crude oil at the global market. However, records of the major hydrocarbon molecules (C, H and N), not provided for by the NNPC specification and even else where in literature, could also not be attained owing to the absence of appropriate equipment in Nigeria and Ghana. This was in spite of all efforts made at contacting major refineries and laboratories for analysis- for which reason therefore, a more detailed characterization of the crude oil used in this study could not be made. Instead the study relied on secondary data from the API (Table 2.1) and the NNPC's 1983 official technical specification for Bonny light crude oil (Paragraph 2.1.1).

Table 2.1: Crude petroleum oil characterization: the American Petroleum Institute (API) Classification of light crude hydrocarbons

Compounds	**Percent (%)**	**Description**
Carbon	83 – 87	
Hydrogen	10 – 14	
Sulphur	2 – 6	
Nitrogen	1.5 – 2	
Oxygen	0 – 1.5	
Molecules	**Chains of C/H atoms**	
Paraffins	C_nH_2 (2_n + 2)	Also known as alkanes; contain linear carbon chains with rings b/w carbon atoms, and isoalkanes when chains are branched.
Naphthenes	C_nH_2 (n + 1 – g): g=no. of rings	Also called cycloalkanes with one or more single ring structures b/w atoms of carbon.
Aromatics	C_6 H	Contains one or more rings of six carbon atoms and double bonds b/w carbon atoms.
Asphaltics		Occurs as solid crude oil in a carbon to hydrogen ratio of approximately 1:1.2. Has got high molecular weight. & is insoluble in light alkanes.
Specific Gravity	**Degree (^{0})**	
Light Crude	31.1 and above	Has higher paraffins to naphthenes and aromatics content
Medium crude	22.3 & 31.1	
Heavy crude (Bitumen)	8 – 18	Consist of higher aromatics and naphthenes to paraffins. It does not flow unless either heat or dilution is applied.
Sour oil		Contains sulphur compounds especially sulphide.

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2.1.1: Nigerian National Petroleum Corporation (NNPC) 1983 official technical specifications for Bonny Light crude oil.

1. *Specific gravity @ 60F/15.55^{O}C: 0.8387 – 0.8498*
2. *API Specific Gravity @ 60F/15.55^{O}C:35.00 – 37.00max.*
3. *Density @ 60F/15.55^{O}C Cg-CM-MAC: 0.85ma*
4. *Pour Point: <40F/4.44^{O}C*
5. *Sulphur Content wt., Pct (%): 0.14max.*
6. *Colour: Dark Brown*
7. *Salinity: TB @ 0.10% BS7W47.0max.*
8. *Acid Number: 0.39max.*
9. *Reid Vapour Pressure: 6.52p sig max.*
10. *Water & Sediment Content: Pct (%) 11.00% max*
11. *Iron wt, Ppm: 1.00 max*
12. *Nickel wt, Ppm: 4.00 max.*

Furthermore, the NNPC technical specification aptly captured very key elements that served useful guides to this study. For example, the low specific gravity of (0.8387^{0}-0.8498^{0}) and the high American Petroleum Institute (API) specific gravity of 35-37^{0} were indications of a substance very low in viscosity, high in fluidity with a potentially high permeability factor for medium to high porosity matrixes. This development imply grave toxicity effects for soils, soil-born organisms and aquifer. Also, low porosity media would naturally experience high surface gradient flow from logging in the event of heavy substance spill, and a possibility of contamination for adjoining land masses. These inferences conform to the reports of Spectrum Analytic Incorporated (2005).

Also, the NNPC (2010) reported that it's very low metals' content (1ppm. for Iron and 4ppm. for Nickel), has the potential to reduce likely pollution effects on refining infrastructure, the environment and organisms as well.

2.1.2 Pollution

As earlier stated, Aboribo (2001) defined pollution as an undesirable change in the physical, chemical, and biological characteristics of all the components of an environment which can threaten human health and that of beneficial organisms.

2.1.3 Sources of crude oil pollution and their potential impacts on the environment

Crude oil pollution of the environment may arise from oil-well drilling, production operations, transportation and storage in the upstream industry and refining, transportation, and marketing in the downstream industry. It could also result from anthropogenic sources (Oberdorster and Cheek, 2000). Other sources may also include accidental spills and spills from ruptured oil pipelines, human activities (either intended or otherwise) such as waste management of oil and vandalization of oil pipe lines (Wyszikowaska *et al*., 2002). The various kinds of petroleum oil pose varying degrees of toxicity to the natural environment such as risk of fire outbreak and explosion possibilities because of their high volatility and flammability. They are persistent in the environment and the low molecular weight aliphatic compounds of oil can have anaesthetic properties. Also, the volatile components of oil can burn the eyes, skin, and irritate or damage sensitive membranes in the nose, eyes and mouth etc (USEPA, 2001).

Pollution from spent motor oil is one of the major environmental problems in the world and is more widespread than crude oil pollution (Odjegba and Sadiq, 2002). The disposal of spent engine oil into gutters, water drains, open vacant plots and farms in tropical Africa and indeed anywhere crude petroleum oil is explored and exploited is a common practice by auto mechanics as they ply their trade. This product also referred to as spent lubricant or waste engine oil is usually obtained by draining during routine servicing of automobile, outboard and generator engines amongst others (Anoliefo, 2001). The resultant oil usually, is indiscriminately disposed off onto either soil or water surfaces with the potential to contaminate underground water over time.

2.1.4 The effects of oil contamination on the environment

Adverse effects of crude oil contamination of the natural environment include the poisoning of animals and plants, altering of ecosystems, and the potential risk to human health if the contaminated product is ingested. Again, owing to their chemical structure, many synthetic organic compounds could be extremely resistant to natural breakdown processes. Therefore, when released into the environment, they may persist for years and even decades (McGuinness and Dowling, 2009).

Volatile organic compounds (VOCs) are vapours emitted by various petroleum products, cleaning supplies and adhesives many of which have short- and long-term adverse health effects.The volatilization from contaminated materials can release substantial amounts of hydrocarbons up to 30 ppm (Akutam, 2012) and contribute significantly to environmental degradation. The aromatic compounds which can induce carcinogenic and mutagenic reactions are particularly problematic because they pose serious health challenges for the population (Akutam, 2012)

Groundwater is one of the many media through which human beings, plants and animals come into contact with petroleum hydrocarbon pollution (Joel and Amajuoyi, 2009). The major concern therefore is its contamination potential and the challenge posed to clean-up. The poor miscibility of crude oil accounts for the accumulation of free oil on the surface of ground water and this may migrate laterally over a wide distance to pollute other zones very far away from the point of pollution.

In environments that are completely aquatic, oil sometimes floats on water surfaces where it is dispersed to shorelines by wind and wave actions invariably affecting the mangrove floor (Osuji *et al.*, 2006). Further more, due to the release of mineral oil hydrocarbons (MOH), phenol, and polycyclic aromatic hydrocarbons (PAHs) often rich in large amounts of tar; drinking water supplies have been endangered and often polluted.

Crude oil spills have been reported to affect soil fertility but the scale of impact depends on the quantity and type of oil spilled. Spent engine oil also, when present in the soil, creates an unsatisfactory condition for organic life in the soil due to the poor aeration condition established therein, immobilization of soil nutrients, and the lowering of soil pH (Atuanya, 1987).

Petroleum hydrocarbons contamination has also been reported to affect plants by retarding seed germination and reducing shoot height, stem density, photosynthetic rate and over all biomass yield (in the short run), and resulting in complete mortality in the long run, (Mendelssohn *et al*., 1990; Lin and Mendelssohn, 1996; Pezeshki *et al*., 2000).

On land, crude oil spills have caused great negative impact on food production. For example, an appreciable percentage of oil spills that occurred on the dry land between 1978 and 1979 in Nigeria, affected farmlands in which crops such as rice, maize, yams, cassava, plantain and other staples were cultivated and since this infraction has persisted till date, anticipated yields from harvest have remained largely illusive and so is food security in the zone of contamination. This invariably has shot up commodity prices and has also reduced living standards (Onyefulu and Awobajo, 1979).

Again, because many toxic synthetic organic compounds are persistent in nature, when ingested by organisms at higher levels of the food chain, they tend to be stored in the fatty tissues due to their hydrophobic properties resulting in bioaccumulation (McGuiness and Dowling, 2009). These researchers also reported that PAHs and BTEX components of petroleum hydrocarbons have also been observed to affect the liver, lungs, kidneys and nervous system leading to cancer as well as immunological, reproductive, feto-toxic and geno-toxic effects or

Biomagnifications. This may arise as a result of the physiological effects of the toxins in higher organisms. Again, at the highest level of the food chain, i.e. humans, these toxic organic compounds can be passed from mother to child either in uterus via the placenta or post-natally via breast milk if ingested (McGuiness and Dowling, 2009).

Crude oil spilled into mangrove environments under the tidal influences that characterize the marine ecosystem provides for wider dispersal and distribution in the intertidal flats (zones) resulting in the deposition of crude oil and or other petroleum products on the aerial roots and sediments (Baker *et al.,* 1981). Thus, crude oil covers the breathing roots and pores, thereby asphyxiating the sub-surface roots that depend on the pores for oxygen transfer and in turn impairs the normal salt exclusion process resulting in the accumulation of excess salt in the plant thereby contributing to an enhanced stress condition of the plant and ultimately, to death/loss of mangrove plants, habitat destruction and degradation (Akutam, 2012).

2.1.5 The Composition of various oils and effect on other living organisms

Crude petroleum oil and associated products contain heavy metals, polycyclic aromatic hydrocarbons and chemical additives including amines, phenols, benzenes, Ca, Zn, Pb, Ba, Mn, P and S. These chemicals have been reported to be dangerous to the health of living organisms when ingested or metabolized (Meinz, as cited in Akutam, 2012).

2.1.6 The effects of petroleum- hydrocarbons on soils

A soil is defined as a natural body of loose, unconsolidated material several centimeters deep that forms a thin layer of the Earth’s surface (Brady and Duncan, 1990). This corresponds also to the zone referred to as “Agricultural Soil” which in this context describes the top layer of the soil rich in organic matter and soil nutrients such as carbon, nitrogen, phosphorous, calcium, iron, magnesium, etc. Layers beneath the top agricultural soil are described as engineering soil (George, 2005). Since the top soil is home to soil biota, soil water, and air, the proper balance of these and soil nutrients become necessary conditions for the proper growth and development of plants and animals life. Therefore, the presence of petroleum-hydrocarbon via spill would necessarily upset the balance of these nutrients and ultimately, living organisms in the soil. It is for this reason that the degradation of petroleum – hydrocarbon is impeded when large amounts of hydrocarbon cause soil nutrients to be limiting (George, 2005).

Petroleum is a complicated mixture of hydrocarbons, as a result there are hundreds of individual compounds in every crude oil and the compositions vary with the origin of the product (George, 2005); that is why its fate in the soil environment is also complex. The susceptibility of petroleum-hydrocarbon to degradation is determined by the structure and molecular weight of the hydrocarbon molecule. Thus increase in alkane chain length brings about a corresponding increase in resistance to degradation (Atlas and Bartha, as cited in George, 2005). Furthermore, the nature of crude oil is such that it has some compounds which are readily degraded by bacteria and others that are not. Consequently, petroleum oil in the environment changes its chemical composition only overtime and therefore tends to persist in any medium it contaminates for as long as it takes the natural decomposition process to occur.

The carbon-hydrogen mixture of petroleum-hydrocarbon causes its spill on soil to upset the carbon-nitrogen balance, and ultimately limit plants and animals life. Therefore, for the effective metabolism, growth and development of living organisms in the soil, a relatively high carbon-nitrogen ratio and a carbon-phosphorous ratio are ideal (Dibble and Bartha, 1979). Also, the high carbon content of crude petroleum oil was reported by Jobson *et al*., (1974) to create a carbon-nitrogen ratio of about 100:1 or 1000:1 when large quantities of crude oil are introduced into the soil. Under similar soil conditions, soil oxygen reserves are equally depleted causing limitation to the growth of soil organisms. These facts were corroborated in the work of Jobson *et al*. (1974), where it was reported that in addition to nitrogen deficiency in crude oil soaked soils, other nutrients like phosphorous may become growth rate limiting. These growth limitations however, clearly manifest in the stunted growth of plants, leaf sclerosis and other plant diseases associated with nitrogen and phosphorous deficiency.

2.1.7 The effects of crude oil on soil chemical properties

The mineral content of soil can be affected whenever there is an increase in the concentration of petroleum crude oil (George, 2005) and this may affect the intended use of the soil. Crude oil in the soil up to 100% could be degraded by natural processes of rehabilitationsince it has been reported to have the potential to increase organic matter contents and improve the fertility, physical and chemical properties of the soil (Odu, as cited in George, 2005).

Furthermore, available nitrogen was reported by Aman (as cited in Worji, 2007), to have decreased with increase in applied crude oil concentration. It was argued that this may have been due to the immobilization of crude oil and the resultant increase of microbial activities in the soil as well as its attendant demand for more nitrogen. This trend accounts for the decrease of available nitrogen in the soil each time crude oil is introduced [(Dibble and Bartha, 1979 and Aman (as cited in Worji, 2007)]. These researchers reported that nitrate production was lowered by fresh oil in coarse and fine soils. Suzanne (as cited in Worji, 2007) also noted that carbon mineralization was stimulated by crude oil in coarse and fine soils whereas Dibble and Bartha (1979) argued that the decrease in available nitrogen with increase in crude oil level may be due to the immobilization of hydrocarbons resulting from the use of carbon materials as an energy source by microbes.

The pH of the soil increased with increase in crude oil concentration (Aman, as cited in Worji, 2007). The highest pH values of 5.8 and 5.9 respectively were recorded at 4% treatment of soil with crude oil. The increase in pH of 5.5 – 6.0 was observed to have increased phosphorous availability, but beyond this pH range, phosphorous availability started to decrease, probably due to carbon dioxide evolution (Dalyan *et al*., as cited in Worji, 2007). The application of crude oil to soil according to Aman (as cited in Worji, 2007) did not show any effect on soil electrical conductivity. That is to say that there was no increase in salinity when soil was contaminated with crude oil.

2.1.8 Effect of petroleum crude oil on selected physical properties of soils

Whenever there is an oil spill, the crude oil moves into the pore spaces of the soil to displace the air in the void and form oil films around the soil particles. According to Rowell, (as cited in Worji, 2007), crude oil in soil causes insufficient aeration of soil due to displacement of air from the pore spaces between the particles of the soil. The presence of crude oil within the pore spaces of the soil suggests that the soil is partially filled. Therefore, its porosity must change and since water and air exist in the same pore spaces, an increase in water content may be expected to reduce the air content by about the same amount. In the same vein, oil will physically displace both air and water, with the tendency to promote anaerobic conditions in soil (Hocks, as cited in Worji, 2007). Also, the production of poor soil structure, contamination of crust and water logging, together have the potential to reduce exchange of gas in the soil and promote anaerobiosis (Worji, 2007).

Atuanya (1987) reported waste water oil processing to have caused a breakdown of soil texture which is followed by soil depression. Gill and Nyawuame (as cited in Worji, 2007) also observed that fresh crude oil rather showed a coagulatory effect on the soil and that seeds sown in such soil failed to germinate and the structures laid will not be firmly erected. The destruction of soil texture affects other physical properties and function of soil.

Crude oil in soil does not only affect soil structure, but also affect the wet-ability of soil. Therefore, a complete breakdown of structure and dispersion of soil particles was jointly reported by Plice (1948) and Odu (1972). They argued further that oil entering the soil displaces air and water from the soil pores while the more volatile soil components and much of the water, with time, evaporate leaving a dry soil containing aggregates of coated thin layer of the heavier oil fractions. Surface wetting may also be slow since capillary water from water table is reduced. Plice (1948) has noted that once a heavy oil soil is wet, it remains wet. This may be due to the fact that the water only fills the macro pores between the soil aggregates and a hydrophobic layer which prevents the water from wetting the centre of aggregates. Consequently, when soil particles disperse, there is an expansion of the double layer of soil colloid which renders the structure of soil weak (Worji, 2007). Thereafter, the density of oil contaminated soil increases with the increase in mass, but without a corresponding increase in volume. Porosity of the soil is also affected as the fluids are now substituted by solid mass of crude oil that blocks up the interstices of the particles. Also, decrease in soil porosity leads to decrease in permeability and increase in moisture content (Plice, 1948).

Other effects of crude oil on soil were reported by Puri (as cited in Worji, 2007) as follows:

i. *That soil has higher weight due to reduction in friction.*
ii. *That soil is subjected to decreased value of peak shear stress as their saturation increased.*
iii. *That increased magnitude of horizontal displacement is required to mobilize peak shear stress as oil saturation increased.*
iv. *That there is a decreased angle of internal friction based on total stress due to oil in the materials pore spaces.*
v. *That there is a greater vertical settlement.*

2.1.9 Attempts at cleaning crude oil polluted sites

Cleaning up of contaminated sites after crude oil spillage has posed great economic challenges to developed countries as well as developing countries. It is estimated that traditional global remediation costs are in the range of $US25-50 billion annually (DreamEssays.com. 2009). Unfortunately, this high cost of remediation contributes to the abandonment worldwide of a large number of polluted commercial sites or brownfields (DreamEssays.com 2009). It is as a result of this challenge and the inadequate knowledge on the potential of tropical plants to serve in clean-up operations that appropriate and alternative remediation technique are sought in phytoremediation.

2.2 Phytoremediation.

The generic term phytoremediation consists of the Greek prefix ‘phyto’-meaning plant, and the Latin root ‘remedium’ meaning; to remove an evil (Cunningham *et al*., 1996). Therefore, phytoremediation mechanisms consist of the processes mediated by plants in treating pollutants in the environment.

According to the United States Environmental Protection Agency (USEPA, 1999) phytoremediation is generally defined as the direct use of vascular plants, algae, and fungi for *in-situ* remediation of contaminated soil, sludge, sediments and ground water by plant.

The use of phytoremediation as a clean-up option may not only degrade contaminants but could enhance habitat recovery through the stimulation of vigorous vegetative plant growth (Lee *et al*., 2001). It also has the added advantage of being applied without the need for removal and transport of contaminated soil. Also, it has the ability to remediate a broad spectrum of contaminants in various environments. It is less expensive, less labour-intensive, relies on solar energy to give lower carbon footprint, and has got a high level of public acceptance (McGuiness and Dowling, 2009). It can be used at sites with low to moderate levels of contamination. It entails more than just planting and allowing the foliage to grow. The site must be constructed to prevent erosion and flooding in order to maximize pollutant uptake (DreemEssays.com 2009).

Currently, most of research in this field is concentrated on determining the best candidate plant species to deploy for phytoremediation trials, and also, quantifying the mechanisms by which the plants interact with the pollutants to bring about degradation or remediation. Therefore, as more plants species are subjected to site studies, phytoremediation is being established as a promising technology for a variety of contaminants. Merkl *et al*., (2005) and Ogbo *et al.*, (2009) have observed that the potential for the technology is high in the tropics due to the prevailing climatic conditions which favour growth of plants, and stimulate microbial activities. They added that the low cost component of the technology is advantageous to tropical environments where requisite funds for alternative clean-up measures may be lacking.

Furthermore, most researchers such as April and Simms (1990), Gunther *et al.*, (1996), Qui *et al.*, (1997), Merkl *et al.*, (2005), Kirkpatrick *et al.*, (2006) and Schwab *et al.*, (2006) have employed grasses (Poaceae) and legumes (Leguminosae) to clean-up contaminants from the environment. Also, others have concluded that grasses and legumes are the best candidates for the phytoremediation process owing to their multiple root ramifications (grasses), improved rhizhospheral zone (Adam and Duncan, as cited in Adams and Duncan, 2002, Merkl, *et al.*, 2004 and 2005), and they argued further that these twin potentials offer increased rhizosphere zone and encourage microbial growth and activity. It is to be noted also that the ability of legumes to fix atmospheric nitrogen could potentially correct for its limitation in soil during spills.

In the Niger Delta area of Nigeria, and other tropical African climes or even beyond, incidents of crude oil spills have led to the pollution of agricultural lands many of which have been abandoned in the aftermath of the effects of spill (Ayotamuno and Kogbara, (2007). Crude oil pollution has been shown to have adverse effects on growth of plants ranging from morphological aberrations to reduction in biomass and stomata abnormalities (Jumbo, 2007). Udo and Fayemi (1975) have reported significant difference in growth between treated and untreated plants (with crude petroleum oil) due to the adverse effects of applied crude oil. Also, a positive relationship was observed between the extent of retardation in plant growth and concentration of crude oil applied to the soil (Atuanya, 1987). Although, in majority of studies, grasses have been singled out for their potentials to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbons (Reilly *et al.,* 1996 and Qui *et al*., 1997), there is however, paucity of information on the restoration of oil- impacted soils using tropical plants species solely (i.e, not in synergy with other micro organisms/chemicals i.e bacteria/fungi).

2.2.1 The Processes and mechanisms of phytoremediation

Various types as defined by Schwitzguebel (2000) include:

a) ***Phytoextraction****: The use of pollutant-accumulating plants to remove pollutants like metals or organics from soil by concentrating them in the harvestable plant parts.*

b) ***Phytotransformation:*** *The degradation of complex organic molecules to simple molecules or the incorporation of these molecules into plants tissues.*

c) ***Phytostimulation:*** *Plant-assisted bioremediation; the stimulation of microbial andfungal degradation by release of exudates/enzymes into the root zone (rhizosphere).*

d) ***Phytovolatilization:*** *The use of plants to volatilize pollutants or metabolites.*

e) ***Rhizofiltration:*** *The use of plants roots to absorb/adsorb pollutants, mainly metals, but also organic pollutants, from the environment.*

f) ***Pump and tree (Dendroremediation):*** *The use of trees to evaporate water and thus to extract pollutants from the soil.*

g) ***Phytostabilization:*** *The use of plants to reduce the mobility and bioavailability of pollutants in the environment, thus preventing their migration to groundwater or their entry* *into the food chain.*

h) ***Hydraulic control:*** *The control of the water table and the soil field capacity by plant canopies.*

The potential of a plant to serve as a phytoremediation plant is dependent on the condition that it meets the general characteristics of phytoremediation plants. The most effective of phytoremediation plants are those classified as hyperaccumulators, while accumulators are also used (Lasat, 2002 and Wei *et al*., 2009).

i. ***Hyperaccumulators*** *are characterized based on four features:*

ii. ***Hyperaccumulation****- the concentrations of contaminants in the shoots (stems and leaves) must be very high (Baker and Brooks, 1989 and Srivastava et al., 2006). Depending on the pollutant involved, the range of concentration is between 1,000 – 10,000 mg/kg of dry weight (mhtl: file://k:\phytoremediation-Wikipedia).*

iii. ***Translocation factor-*** *The concentrations of contaminants in the shoots of a plant should be higher than those in the roots by a factor greater than one [> 1 (Baker and Brooks, 1989)]*

iv. ***Enrichment factor-*** *The concentrations of contaminants in the plant should be higher than those in the habitat by a factor greater than one [> 1 (Wei et al., 2009)].*

v. ***Tolerance factor-*** *A hyperaccumulator should have high tolerance to toxic contaminants than accumulators (Baker and Brooks, 1989).*

The first feature is a unique character particular to hyperaccumulators, while the other three are shared with accumulators. Baker and Brooks (1989), and Zhou *et al*., (2006), reported that although shoots concentration of contaminants depend on the increase in the metal/contaminant concentration in the habitat, accumulators display consistently and relatively lower levels than hyperaccumulators.

Phytoremediation plants remove harmful chemicals from the ground when their roots take in water and nutrients from the polluted soil, stream, and ground water and can only clean-up chemicals as deep as their roots can grow. Tree roots grow deeper than those of smaller plants, so they are used to reach pollution deeper in the ground (USEPA, 2001).

According to USEPA (2001), phytoremediation occurs even if the pollutants are not taken into the plant roots. For example, pollutants can stick or *sorb* to the roots of the plants or they can be changed into less harmful pollutants by bugs or *microbes* that live near plants roots. The plants are allowed to grow and take in or sorbs pollutants afterward they are either harvested and destroyed or recycled if pollutants stored in the plants can be reused. Usually, trees are left to grow and are not harvested (USEPA, 2001).

2.2.4 Safety measures in phytoremediation process

Before phytoremediation starts, the United State Environmental Protection Agency (USEPA) recommends a study to determine whether plants grown to clean-up pollutants, can or cannot be harmful to people. Plants should be tested together with air to make sure that the plants do not release harmful gases into the atmosphere. Plants with known gas emission status are exempted from this test. In general, as long as plants are not eaten, they are not harmful to humans.

2.2.5 Duration of the phytoremediation process

The time taken to clean-up a contaminated site using phytoremediation technology depends on several factors:

a. *Type and number of plants in use*

b. Type and amounts of harmful pollutants present

c. Size and depth of the polluted area

d. Type of soil and weather/climatic conditions present

These factors vary from site to site. Plants may have to be replaced if they are destroyed by

considerations such as contaminated media characteristics, contaminant characteristics, plant characteristics as well as site characteristics. Utilizing plants to absorb, accumulate and detoxify contaminants in the growth substrate through physical, chemical or biological processes is a widespread practice (White *et al.*, 2006 and Jilani and Khan, 2006). However, because phytoremediation may be limited by the growth rate of plants, more time probably in years may be required to completely remediate a site. Therefore, for sites that pose immediate acute risks for human beings and the environment, phytoremediation may not be the remediation technique of choice (USEPA, 2000).

Furthermore, despite the rather extensive studies that have been carried out worldwide regarding phytoremediation of oil polluted soils, some contradictory results have been reported regarding the efficiency, performance and effectiveness of this technology in removing contaminants from soil (Akutam, 2012). For example, studies have shown that crested wheat grass [*Agropogon desertorum* (Fisher ex Link) Schultes] had no effect on the rate or extent of mineralization of phenanthrene when planted and unplanted systems were compared (Ferro *et al.*, 1997). In another study, alfalfa (*Medicago sativa* Mesa, var. Cimarron VR) planted in artificial loamy sands had no effect on either the rate or extent of mineralization of benzene compared to unplanted soils (Ferro *et al.*, 1997). One of the most important reasons for this development is that phytoremediation is more of a site-specific remediation method than generic. Thus selecting and employing new native plant species that are tolerant to high concentrations of oils in soil is a key factor in the successful application and outcome of phytoremediation. The plant selection process should involve the selection of plant species with appropriate characteristics for growth under site conditions and this can be facilitated by (a) looking through handbooks for a list of plants available for phytoremediation under specific environmental conditions. (b) Using native crop, forage and other types of plants that can grow under regional conditions or zone of contamination, and (c) plants can also be proposed based on those observed to have grown at sites previously spilled by hydrocarbons (Akutam, 2012).

2.2.7 The selection of tropical plants species in a phytoremediation study

Although, there is very little debate on the wealth of tropical crops/plants species diversity within the tropical zone of West Africa, research has been so far mostly restricted to the temperate zone (Merkl *et al*., 2004). To this extent, little is known about tropical species that could serve as phytoremediation tools for the cleanup of petroleum crude oil contamination.

It should be noted that the efficiency of this technology called phytoremediation depend on the type of contaminant (nature and property of contaminant), bioavailability (of contaminant for absorption by plants) and soil properties (Cunningham and Ow, 1996). But the mechanism believed to be responsible for most of the degradation of petroleum hydrocarbons in vegetated soil is the stimulation of growth and activity of degrading microorganisms in the rhizosphere (Frick *et al*., 1999). From the foregoing therefore, the screening of plants for their ability to grow and establish in crude oil contaminated soil should be one of the first steps in the search/selection of species for phytoremediation in the tropics, followed by the evaluation of their influence on the degradation of petroleum hydrocarbons in the soil (Merkl *et al*., 2005).There are therefore several approaches to selecting candidate plants for phytoremediation of soils contaminated with organic pollutants. These approaches have been based on:

i. *The occurrence of plants under specific conditions (Gudin and Syratt, 1975 and Banks et al., 2003). Plants should be indigenous to the zone of contamination.*
ii. *Their resistance or tolerance to pollutant toxicity (Kirk et al., 2002).*
iii. *The presence of phenolic compounds in the plant root exudates (Hedge and Fletcher,*

1996 and Liste and Alexander, 1999) or their capability to reduce the pollutant concentration in the soil (Merkl et al., (2004).

2.2.8 Uptake and movement of chemical elements from soil by plants

It has been established that of all the components of waste/contaminants applied to soil, chemical elements have the greatest environmental implications because most of them, in their varying amounts, are necessary for the growth of plants; just as most are toxic and therefore, inhibitive to the growth of plants and development. Isirimah (2001), confirmed that plants differ in their capacity to absorb nutrients from the soil. Some of these differences are genetic in nature and are associated with physical distribution and chemical characteristics. Almost any element in soil solution is taken up by the plant to some extent, whether needed or not. An ion in the soil goes from the soil particle to the soil solution, through the solution into the plant root, and moves from the root to the location where it is either used or retained. The process of material uptake by plants is complex and knowledge of it is incomplete. But some of the key points to note include:

i. *The process is not the same for all plants concerning one or more minerals/contaminants.*
ii. *The complete process occurs in a healthy root system.*
iii. *The necessary mineral/contaminant must be available in the rhizhosphere in Suitable amounts.*
iv. *Uptake varies from one mineral/contaminant to another (Isirimah, 2001)*

Although effluents and sludge have been applied on many kinds of grasses, vegetables, legumes, field crops and woody plants, grasses seem to be the most effective in neutralizing wastes. Grasses have superior biological pumps (Isirimah, 2001). Many species have high water use factor combined with abundant root production. Examples could be found in grapes, alfalfa, sugar beets, hops and cotton. Legumes with broad leaves are less effective in contaminant removal than grasses like *Cynodon* spp. (Bermuda grass). This was reported in a study of the effects of field crops, vegetables and legumes in neutralizing waste by Isirimah, (2001).

Again, on the complementary role of plants and micro organisms, Sung *et al*., (2004) reported that the most important factor affecting contamination of plants is bioavailability and that, as bioavailability increased, the concentrations in the root and shoot compartments of the plants were predicted to increase; and also that microbial activities and contamination of plants are closely related, suggesting that both can have complementary roles in phytoremediation. In a related study using *Vigna unguiculata*, Isikhuemhen *et al*., (2001) stated that significant improvements were achieved in the percentage germination, plants' height and root elongation of the experimental plants, as well as decreased hydrocarbon degradation in soil. The result of the germination study on eight months post crude oil treatment on soils planted with *Zea mays* revealed that seeds could not sprout at 10g level of contamination due to the high hydrocarbon content of the soil (Ofori, 1998). This result corroborated the work of Amakiri and Onofeghara (1983) who stated that crude oil pollution inhibited seed germination. Whereas Rowell (as cited in Worji, 2007) attributed the lack of seed germination mainly to rot from oil soaked soil. McGill in the report by Isaac (2008) was of the view that this was due to poor wet-ability, aeration in the soil and the toxic effect of oil.

Furthermore, Muratova *et al*., (2009), working on "Phytoremediation of oil-sludge-contaminated soil" screened fifteen (15) plant species consisting of a mix between grasses and legumes.Results of this work showed that the estimation of oil-sludge degradation in the root zone of the tested plants revealed that rye plants accelerated cleanup most effectively, degrading the entire main contaminant fraction in the oil sludge by a total of 52%.

In a work titled "Screening of Australian native grasses for rhizoremediation of aliphatic hydrocarbon-contaminated soil", by Sharyn *et al.* (as reported by Isaac, 2008), it was reported that seedling emergence was not adversely affected by the presence of hydrocarbon contamination for all but one grass. *Brachiaria decumbens, Cymbopogonambiguous*, and *Microlaena stipoides* var. Griffin, were considered promising having survived for 120 days in the contaminated soil and in some instances, produced considerably more root biomass in the presence of contamination. In a similar development, the assessment of local tropical plants for phytoremediation of petroleum contaminated soil by Isaac, (2008) results obtained showed a considerable reduction in the TPH level of the soil samples compared to the unplanted control soil sample.

In the work of Ogbo *et al*., (2009), titled Phytoremediation of soil contaminated with used motor oil, results obtained revealed that *S. rhombifolia* and *M. alternifolius* successfully extracted over 60% of total petroleum hydrocarbons from respective sites, and were therefore considered suitable for use in phytoremediation of oil contaminanted soil. However, for all the plants species used in this study, reduction in shoot length, leaf area, root length and total chlorophyll content, were observed. In similar studies, Chaineau *et al*., (1997) reported growth rate reductions of beans and wheat by 80%. Molina-Barahona *et al*., (2005), also reported reduction in the elongation of stems and roots due to immobilization of nutrients caused by contamination of the test plants used. On roots interaction with contaminant, Nature and Science (2009) quoted Grasset and associates to have observed in the year 1993 that reduction in root length is a sensitive response of plants to exposure to chemical substances. Test crops also exhibit sensitivity to toxicity of contaminants by poor shoot growth, particularly soils contaminated by long chain alkanes (Palmroth *et al.*, 2002 and Trap *et al*.,1994).

The effectiveness of phytoremediation crop - *Vigna unguiculata* in the remediation of crude oil-polluted soil for cassava cultivation was investigated and the effects of petroleum hydrocarbon contamination on plants included retarded seed germination, reduction in height, stem density; photosynthetic rate and biomass or in complete mortality (Mendelssohn *et al*., 1990, Lin and Mendelssohn, 1996, Pezeshkl *et al*., 2000, Njoku (as cited in Njoku *et al*., 2009) and Chronopoulos *et al*., (1997).

Tanee and Anyanwu (2007) reported that cassava cannot tolerate post- planting crude oil pollution at concentration as low as $500 ml/m^2$ probably due to the reduction in available nutrients for plants in the soil as observed by (Xu and Johnson, 1997 and Akonye and Onwudiwe, 2004), or phytotoxicity of the oil (Siciliano and Germida, 1998). Consequently,results from their efforts concluded that the use of *Vigna unguiculata* as a phytoremediation plant is a good remedial option in crude oil-polluted soil for cassava cultivation as phytoremediation caused increased “fresh weight yield” and above/below ground weights respectively, of cassava varieties used in this experiment (Tanee and Akonye, 2009). Test plants yield may not be the focus of the current work but above/below ground weights and their analytical techniques served as very useful guides.

The exploration of crude petroleum oil in Nigeria has led to the pollution of land and waterways. Agricultural lands have become less productive and the creeks as well as the fishing waters have become more or less dead (Okpokwashili and Odokuma, 1990 and Odokuma and Ibo, 2002). Several civil unrests have also been witnessed due to environmental degradation owing to crude oil exploration in the Niger Delta region of Nigeria. Consequently, the need to effect clean-up operations on polluted sites have resulted in physicochemical and thermal processes dominating common techniques that have been involved in the cleaning up of crude oil contaminated sites (Frick *et al*., 1999).

These techniques however have some adverse effects on the environment and are also very expensive as previously stated (Frick *et al*., 1999 and Lundstedt, 2003). Njoku *et al*. (2009) evaluated the growth of *Glycine max* on the physical chemistry of soils polluted with crude petroleum oil and the results indicated that the growth of *G. max* on crude oil contaminated soils affected the physical chemistry of the soil enhancing the degradation of crude oil and helped to restore polluted soils back to agricultural use. It was further reported that the high acceptability of *G.max* due to its high nutritional value, adaptability, and ease of propagation will make it an easy species for remediation of soil contaminated with crude oil (Njoku *et al.*, 2009).

The work of Foluso *et al.*, (2009) on the phytoremediation using *Eichornia crassipes* in metal- contaminated coastal water was reviewed and in the study, Lasat (2002) and Wei *et al*. (2009), revealed that *E. crassipes* was found to accumulate metals in both its roots and shoots in a high degree (enrichment factor-EF), and also capable of transferring the metals absorbed into the shoots to give higher translocation factors- TF (Foluso *et al*., 2009).

In the determination of the tolerance level of *Zea mays* (maize) to a crude oil polluted agricultural soil, Ayotamuno and Kogbara (2007) reported that the increasing dependence of humanity on fossil fuels, especially petroleum- hydrocarbons has led to the pollution of agricultural lands, owing to spillage of crude oil during extraction and processing operations in many petroleum crude oil producing countries.

In Nigeria, the situation is unique as it is on record that between 1976 and 1980, a total of about 784 incidents of oil spills led to the release of about 56.1 million barrels of crude oil into aquatic and terrestrial ecosystem (Awobajo, 1981). Since then, crude oil contamination of farmlands has become common experience in the country, and many of these farmlands have been abandoned in the aftermath of the effects of spill. Therefore, in the adoption of a suitable remediation technique, it must be noted that the success and or failure of the phytoremediation techniques is/are a function of the totality of events which occur at the rhizosphere. This is why Merkl *et al*. (2006), in a study captioned "Phytoremediation in the tropics-influence of heavy crude oil on root morphological characteristics of *Brachiaria brizantha, Cyprus aggregatus* and *Eleusine indica*, reported that *Brachiaria brizantha* and *Cyprus aggregatus* showed coarser and increased average root diameter (ARD) in polluted soil as compared to the control. Additionally, a shift of specific root length (SRL) and surface area per diameter class towards higher diameters was found. The presence of oil contaminant in soil led to significantly smaller SRL and surface area in the finest diameter class of *C. aggregatus*. Furthermore, it was reported that the root structure of *Eleusine indica* was not significantly affected by crude oil, whereas higher specific root surface area was not only recorded but related to higher degradation of petroleum hydrocarbons as reported in previous studies.

In another related study captioned; "Petroleum hydrocarbon contamination and impact on soil characteristics from oilfield Monoge Wetland", Xiao-yu *et al*. (2009), reported that all contaminated areas had significantly higher contents of TPH than those of the control sites ($p<0.5$). A significantly positive correlation between TOC and TPH contents in oilfield was observed ($r= 88$, $p<.05$).

The presence of oil contaminant caused a decrease of TN and TP and the maximum decline were 33% and 28% respectively. Contaminated sites also exhibited significantly higher ($p<.05$) pH values, C: N and C: P ratios. Soil petroleum contamination also resulted in the increase of EC. However, the impact of TPH on EC were not significant ($p>.05$). Collectively, it was concluded that petroleum hydrocarbon pollution had caused some major changes in soil properties in Monoge Wetland.

2.3 Causes and ecological effects of oil spills in the Niger Delta area of Nigeria

Prior to commencement of oil exploitation and exploration in the Niger Delta, the region was a peaceful place with farming and fishing as means of livelihood. However, since the commencement of exploitation prio to 1958 and exploration of crude petroleum oil in the area from 1958, the traditional means of livelihood have been truncated and the region, environmentally degraded while the inhabitants suffer from grave deprivations. Oil exploration started for several decades in the Niger Delta before attention was paid to the issue of pollution and environmental degradation. Thus, as observed by Soremekun and Obadare (as cited in NNPC, 1983), “Although the shipment of oil from the Nigerian coast in commercial quantities started as far back as 1958, it was not until some thirty years later that attention was drawn to the havoc being wrecked on the environment by the oil companies.”

Oil spills in the Niger Delta occur due to a number of causes which include: corrosion of pipelines and tankers (accounting for 50% of all spills), sabotage (28%), and oil production operations (21%), with 1% of the spills attributed to inadequate or non-functional production equipment (NNPC, 1983). Corrosion of pipes and tanks contribute the largest quota to the oil spill total due mainly to the rupturing or leakage of production infrastructures (infrastructure/equipment failure) that were described as, “very old and lack regular inspection and maintenance”.

Another reason corrosion accounts for such a high percentage of total spills is because of the small sizes of the oilfields in the Niger Delta. It is also for this reason that there exist an extensive network of pipelines between the fields and the numerous small networks of flowlines (the narrow diameter pipes that carry oil from wellheads to flowstations), allowing many opportunities for leaks. In onshore areas of the Niger Delta, most pipelines and flowlines were laid above ground. Pieplines, which have an estimated life span of about fifteen years, are old and susceptible to corrosion. Many of the pipelines are as old as twenty to twenty-five years, and are still in use in the region (NNPC, 1983). This trend is however changing as all above ground pipes are currently being buried for obvious safety and security reasons. It was estimated that it will be unlikely that pipes would still be found above ground as the year 2010 comes to an end in most oil producing areas of the Niger Delta.

The Nigerian National Petroleum Corporation places the quantity of oil jettisoned into the environment yearly at 2,500 cubic meters with an average of 300 individual spills annually. However, since this quantity does not take into account “minor” spills, the World

Bank argues that the true quantity of oil spilled into the environment of the Niger Delta could be as much as ten times the officially claimed amount. Moffat and Linden (1995), projected that the total amount of oil in barrels spilled between 1960 and 1997, was in the neighborhood of 100 million barrels.

The report of the Niger Delta Committee (NDC) indicated that oil spillage has been widely reported to have a devastating impact on the ecosystem into which it is released and that immense tracts of the mangrove forests which are especially susceptible to oil, have been destroyed due mainly to the storage of oil in the soil after spill, which in turn is released annually with inundation.

Spills in populated areas, according to them, often spread out over a wide area, contaminating farmlands, aquacultures as well as groundwater through soils. Therefore, consumption of dissolved oxygen by bacteria feeding on the spilled hydrocarbons also contributes to the death of marine lives. In agricultural communities, often a year's supply of food can be destroyed by only a minor leak, debilitating the farmers and their families who depend on the land for their livelihood. Drinking water is also often contaminated, and films of hydrocarbons are usually visible in many localized water bodies. In cases of water contamination, even if no immediate health effects are apparent, the numerous hydrocarbons and chemicals present in oil are highly carcinogenic and people often do manifest sickness following consumption of polluted water. Also, offshore spills, which are usually much greater in scale, contaminate coastal environments and cause a decline in local fish production.

2.4 The Niger Delta Area of Nigeria: Ecology and climate

The Niger Delta ecological region falls within the tropical rainforest zone of Nigeria, covering a total land mass of about 14,400 square kilometers (Willinks' Commission Report, as cited in Kuperberg *et al.*, 1999). This geographical region is currently occupied by Rivers, Bayelsa, fourteen (14) Local Government Areas (LGAs) of Delta, and two (2) Local Government Areas (LGAs) of Edo States respectively.

The climate is characterized by a long rainy season from March to October. Precipitation increases from the North of the delta with an average of 2,500mm, to the coastal area where mean annual rainfall is around 4,000mm making it one of the wettest areas in Africa. Relative humidity rarely dips below 60% and fluctuates between 90% and 100% for most of the year. During most of the rainy season, cloud cover is nearly continuous resulting in 1,500 mean annual sunshine hours and an average annual temperature of approximately $28^{o}C$ (Akutam, 2012).

2.5 Climate of Accra

The climate of Accra is typically warm of two rainy seasons with mean annual rainfall of about 800 mm (Table 3.1). The main rainy season spans between March and mid-July, followed by a short dry spell that runs till the end of August. A minor rainy season commences from September and terminates in mid-November. The Accra plains consist of about 71 to 80 annual rainy days. Also, the main dry season begins from November and ends in February and it is during this season that the harmattan haze is felt the most. Temperatures are relatively high all year round with February, March and April having the highest average temperature of about $29^{0}C$, while July and August have the lowest temperatures. The mean annual temperature is about $27^{0}C$. Relative humidity varies from 55% to 65% during the day and falls to about 40% within the main dry season; it could go up to about 100% at night all year round (Akutam, 2012).

2.6 Vegetation of sampling sites

The vegetation of the sampling sitestogether with the rest of the Accra Plains consists mainly of the savanna type vegetation which is comprised essentially of grassland laced with dense short thickets often less than 5m high, together with a few tree Species. The thickets consist of dense shrubs often made of small trees and termitaria. Dorminant tree species include *Antiaris africana*, *Ceiba pentandra* and *Milicia excelsa;* while shrubs include *Baphia nitida*, *Dialium guineense*, *Diospyros mespiliformis* and *Hymennostegia afzeli* as dorminant species (Akutam, 2012).

Table 2.2: Mean seasonal rainfall (mm), and temperature (^{0}C) for Accra plains over 30 years.

	Rainfall (mm)		Rainny Days	Temp (^{o}C)
Major rainy season	March	60.3	5	28.4
	April	96.8	6	28.3
	May	131.3	10	27.6
	June	207.0	13	26.4
Minor dry season	July	66.0	7	25.3
	August	27.2	5	25.2
Minor rainy season	Sept	67.8	7	24.9
	Oct	63.9	8	26.7
	Nov	25.5	4	27.6
Major dry season	Dec	18.1	2	27.5
	Jan	10.9	2	27.7
	Feb	21.8	2	28.4
	Annual Total	**796.5**	**27.0**	Annual avg.**27.0**

Source:Ahenkorah *et al*., as cited in Akutam (2012).

CHAPTER THREE

3.0: MATERIALS AND METHODS

3.1 Phase I (Pilot survey; March –April 2010)

As earlier stated in the introductory section, the Questionnaire administered in study was functionally structured to address three major issues: a) biometric information of respondents, b) agricultural practices/occupation of the people and c) the socio-cultural practices of respondents and also to confirm the reality of spill incidents and effects on the environment. A total of twenty copies of questionnaire were administered on each community. There were five (5) communities in all; two (2) each in Rivers and Bayelsa States, and one (1) in Delta State. A minimum of three days was spent in every community visited (Appendices IA and 1B for questionnaire details).

3.2 Phase II: The screening of experimental plants for crude oil contamination tolerance using soils from the catena of the Accra Plains. (March – May 2011).

3.2.1 Design of experiment

The experiment was a randomized complete block design comprised of six (6) plant blocks (used in the study to denote the different plants species used in experiment, each complete with control and THC treatment options) in the first instance (phase II), and four (4) plant blocks in the second instance (phase III), each complete with control and treatment options. Pristine soils from the catena locations were augered at random and bulked together to form composite samples. Collected soil samples were air dried in the green house and homogenized. Thereafter, 2kg of soil each was introduced into plastic buckets. Conditions of a minor spill were simulated by sprinkling unto known weights of soil samples, serial crude oil concentrations of 0% (control) 1%, 3%, 8% and 10%. Contaminated soils in pots were then left undisturbed in the green house for 1 week to assume the time taken for contingency arrangements/plans before cleanup/remediation and to allow for better infiltration and percolation of contaminant. Each of the six plant blocks was replicated thrice. The experiment spanned for 106 days in phase 11 (March to May 2011) and 120 days in phase III (November 2010 to January 2011) respectively. These periods coincided with the time between soil preparation and harvesting of the experimental/plants sampling of experimental soil. The placement of pots of selected plants in the greenhouse was done in no preferential order.

3.2.2 Experimental soils: sampling and preparation

Soils used in this study were from grid and profile samples collected from two sites along the catena between the Legon hill of the University of Ghana and Ashale-Botwe (Plates 7 and 8) based on textural classes of soils sampled in the Niger Delta and the zone of oil exploitation in Ghana.

Surface samples were collected at depths of 0-20cm and at intervals of 2m^2 on a grid layout. Profile horizons were identified using observed morphological characteristics, delineated and described (Appendices IV-I and V-I). Thereafter, both disturbed and undisturbed sub-soil samples were collected from each horizon. The undisturbed soil samples were obtained using the core samplers of 5.0cm (circumference) x 5.0cm (height) for bulk density determination, while the disturbed samples were air dried, ground and sieved through the 2mm mesh to obtain fine sample fractions which were used for laboratory determinations (Tables 4.3 and 4.4 for details). Finally, the locations of sample sites were determined with the aid of a Global Positioning System (GPS). There were a total of 92 samples: 72 surface samples (36 per site) and 20 sub-surface samples (10 each of disturbed and undisturbed per site). Soil samples used as growth matrixes were collected independently of the above.

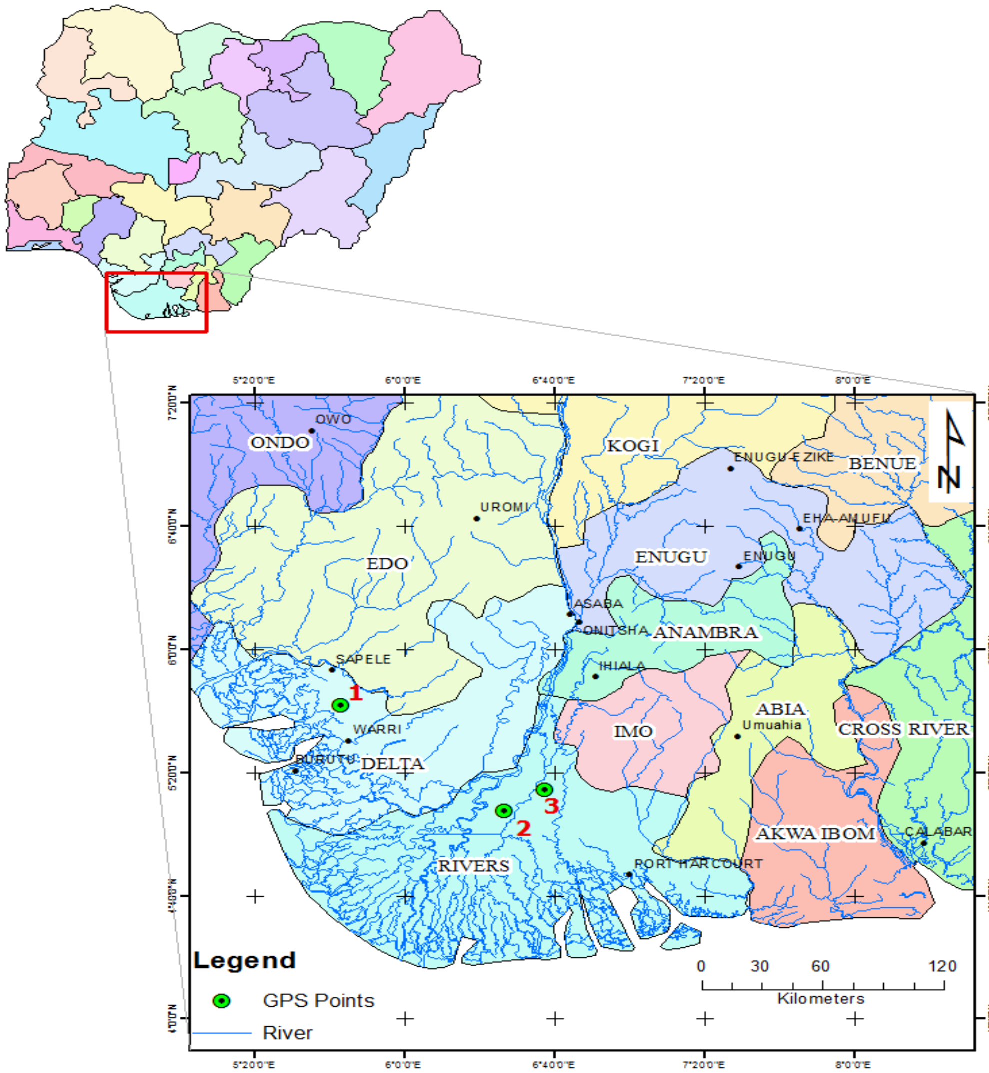


Fig 3.1: Map of Nigeria indicating GPS locations 1, 2 and 3 as sampling points of soils and plant species in Delta, Bayelsa and Rivers States respectively.

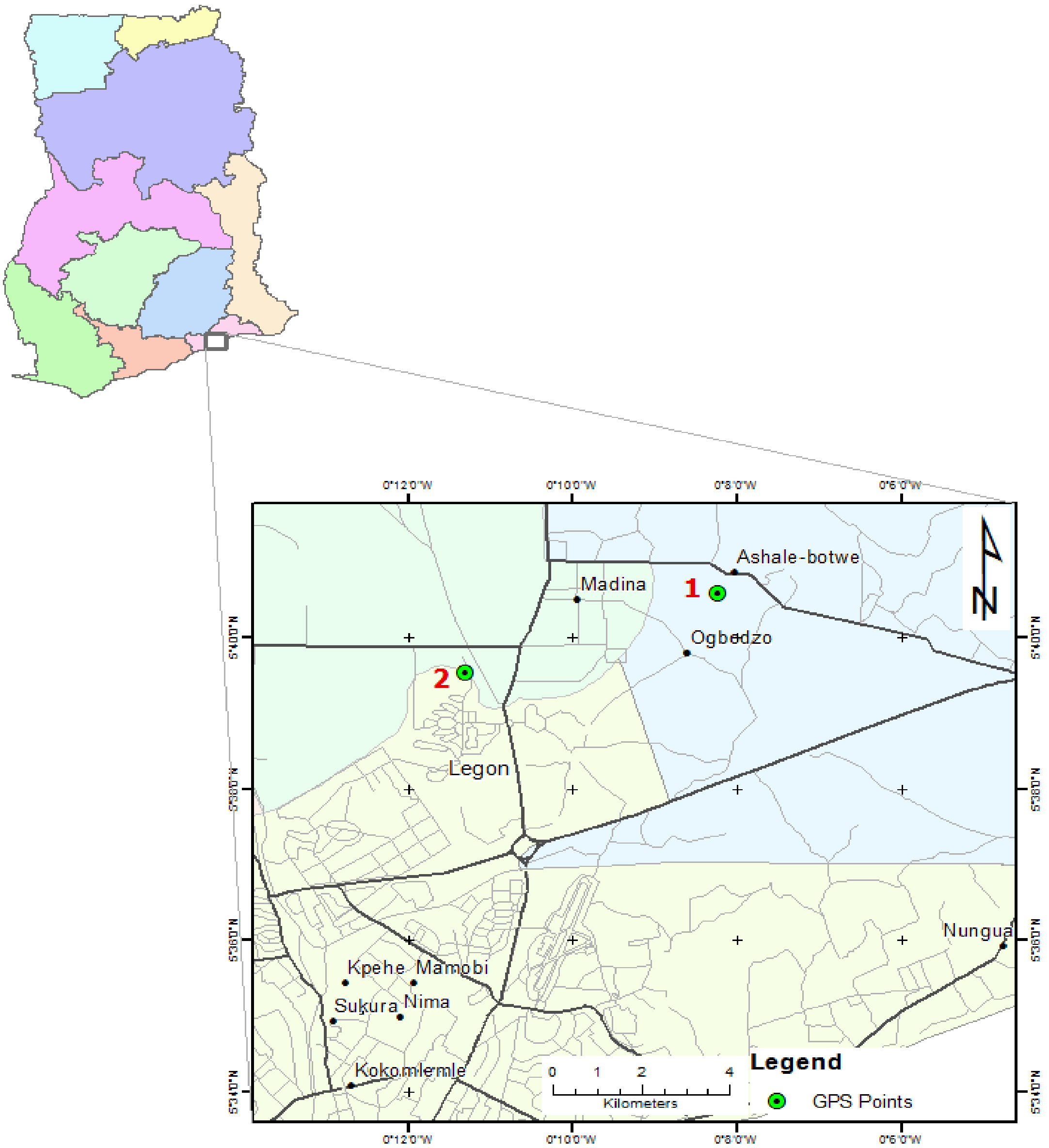


Fig. 3.2: Map of Ghana showing GPS locations1 and 2 representing sampling sites of Alajo and Toje soil series of the Accra plains.

Plate 1: Vegetation at the upper slope of the Toje soil series on the Catena of the Accra Plains.

Plate 2: Vegetation at the bottom slope of the Alajo soil series on the Catena of the Accra Plains.

3.2.3 Sources of propagation materials

Six plants species; three (3) grasses (*Panicum maximum*, *Paspalum vaginatum* and *Cynodon dactylon*) and three (3) legumes (*Puereria phaseoloides*, *Schrankia leptocarpa* and *Centrosema pubescens*), from the pool collected and identified from the pilot survey were selected for phytoremediation (green house) trial on the basis of commonality/accessibility, ease of propagation and acceptability.

All plant materials used in this study were obtained from the wild with the exception of *Centrosema pubescens* which was supplied by the Ghana Oil Palm Research Institute-Kusi. *Puereria phaseoloides* and *Panicum maximum* were collected from bush fallows around the University of Ghana main campus while *Schrankia leptocarpa* was collected from the road sides/bush fallows of the Legon Campus of the University of Ghana. *Paspalum va ginatum* and *Cynodon dactylon* were collected from roadsides at Okoh-Dome, all within the Greater Accra Region of Ghana. Apart from the grasses which were all transported under moist root conditions, this batch of seeds used for propagation were put in desiccators and stored in the refrigerator until the time for sowing.

3.2.4 Crude oil sample

The crude oil used (1420ml of Bonny Light) was obtained from the Etelebu flow station in Bayelsa State of Nigeria and was provided by the kind courtesy of Shell Petroleum Development Corporation (SPDC). It was conveyed by road in a 25L. PVC (photo proof) container from Nigeria to Ghana.

3.2.5 Determination of crude oil application levels by weight to volume (ml).

Volume(s) of crude oil used in the study to simulate experimental soil was arrived at using the mathematical equation which evaluated for the milliliter (ml) equivalents of contaminant for all treatment options as follows:

Vol. of Crude = (% x wt_s */ 0.844) ml*

Where % = percentage of treatment option.

Wt_s *= weight of soil in g.*

0.844 = conversion factor from g to ml. and the mean weight (g) of 1ml of Bonny light crude oil.

Therefore, in applying this equation, agreed percentages of crude to be used in the experiment are multiplied by the known or constant weight of the experimental soil to establish a weight in grams which is in turn converted to volume(ml) using the conversion factor (0.844).

3.2.6 Germination Study

All seeds sown were scarified using boiled water prior to planting. Thirty (30) seeds were introduced into every pot whereas observation for germination commenced three (3) days after planting (3DAP). Sprouts were then thinned down to 10 seedlings per pot 1 week after planting. Consequently, records of germination were taken one (1) week after planting (1WAP), and two (2) weeks after potted plants were simulated with appropriate concentrations of crude petroleum oil.

3.2.7 Determination of the rate of irrigation applied to soil [at constant weight (ml/g)]

The determination of the rate of irrigation was undertaken volumetrically to establish appropriate irrigation rates for experimental soils *in-situ.* A constant weight of a perforated plastic bucket was obtained (W_2). Thereafter, 2000g of soil was introduced into each bucket (W_1), and irrigated to water log point. Irrigated soil was then allowed to drain for a minimum of three days before a final weight of bucket + soil + left over water, was taken (W_3). Subsequently, field capacity was determined as follows:

Field Capacity = (W3-W2) - W1

Where, W_1 *= weight of soil sample*

W_2 *= constant weight of plastic bucket*

W_3 *= weight of soil + bucket + left over water*

The resultant weight (g) equivalent of water (ml) was determined volumetrically.

3.2.8 Propagation

All seeds sown in the study had their dormancy broken to facilitate sprouting. This was achieved by brief immersion of the seeds of each species into boiled water for approximately one (1) to two (2) minutes before sowing. Grasses were propagated as propagules by burying entire portions with established roots and leaving three nodes on stem portions above ground.

Seeding rate (i.e, number of seeds per pot) for the leguminous species was done at the rate of ten seeds per pot, while propagules for grasses were sewn at the rate of three per pot, with the exception of *Cynodon dactylon*, which was sown at four propagules per pot due to its morphological features, for purposes of achieving the critical mass required for laboratory analysis.

3.2.9 Installation of thermo-hygrograph

A thermo- hygrograph was installed in the green house as shown in Appendix VIII, to record the ambient temperature and relative humidity of the green house throughout the duration of the experiment.

3.2.10 Assessment of experimental plants for growth performance (cm).

Plants were assessed for attained heights using metric (calibrated meter rule and tape) measurements for the above ground portions (shoots). The short to medium shoots were measured with the ruler whereas tall and the very tall shoots were measured with the tape.

3.2.11 Tolerance of the selected plant species to Total Hydrocarbons' contamination (% Plants Survival Difference - PSD)

Tolerance of the selected plants to THC contamination was evaluated on the basis of the Plants Survival Difference (% PSD) as follows:

$$\% \, PSD = \Delta \text{ in plants growth} = \frac{(wk_1 - wk_5)}{wk_1} \times 100$$

Where Δ *= change in tolerance of the selected plant to THC between weeks 1 and 5 after planting.*

wk_1 *= germination count*

wk_5 *= survival count*

3.3 Phase III (Extraction phase): The evaluation of tolerant species for phytoremediation potential (November 2010 – January 2011).

3.3.1 Experimental design

Details of the experimental design are contained in phase II, but additional THC treatment options of 2% and 5% were introduced in this phase to determine species tolerance limit for low/poor tolerant plants and mid-point performance for high tolerant plants respectively.

3.3.2 Evapovolatilization (%)

All surface losses of crude oil fractions were determined through the direct weight measurements in the green house using a suspended scale. This was done fortnightly whereas readings were taken before and after every irrigation process in each case to evaluate for changes in weight caused by evaporation (soil surface loss), evapo-transpiration (shoots surface loss) and volatilization (due to photolysis and chemical oxidation). The mean of all data sets was determined before final calculations for percentage (%) evapo-volatilization. This was arrived at as follows:

$$Wt \, loss = total \, wt - \Delta wt$$

Where total wt = constant wt of plastic bucket + soil wt + wt of crude oil applied + wt of irrigation water.

While ∆wt (change in wt) = wt attained due to evaporation + evapo-transpiration + volatilization.

Percentage (%) Evapo-volatilization of crude oil therefore = (wt of sample crude oil / ∆wt) x 100/1.

3.3.3 Determination of fresh weight of test plants [wet weight (g)]

Samples of plants used for the determination of fresh weight were thoroughly washed clean of debris and soil particles immediately after harvest and weighed.Subsequently, each plant sample was separated into shoots and roots, weighed and oven dried separately in paper envelopes before storage awaiting total hydrocarbons (THC) analysis.

3.3.4 Soil sampling and handling

Soil properties such as particle size/texture, bulk density, organic carbon and total nitrogen were analyzed using methods adapted from relevant laboratory manuals (Rayment and Higginson, 1992 and Black, as cited in Ayotamuno and Kogbara, 2007). Soil samples were obtained after crude oil simulation, air dried, sieved and stored for laboratory analyses.

Subsequently, the total hydrocarbons content (THC) in the various plant pots was determined so as to establish the true concentration of the contaminant in milligram per kilogram of soil (mg/kg). Lastly, as experiment progressed, soil samples were taken fortnightly with an improvised hand auger to minimize impact on pots using an auger shaped ½ inch PVC pipe. Sample sets were subjected to the same routine handling procedure, put into polyethylene bags and stored in the laboratory waiting analysis.

The Total Hydrocarbons (THC) analytical procedure involved the use of toluene to extract the hydrocarbon content. The concentration was then obtained at 420nm using a

Spectronic 45 Lamba spectrophotometer. Thereafter, the THC of the soil was determined from standard curves of known concentrations of petroleum fractions as contained in the work of Osuji and Nwoye (2006). Also, indices of performance of the sampled plants, and the THC of the roots and shoots of each replicate plant were evaluated in order to determine the tolerance/extraction level/potential of each plant to contaminant.

3.4 Laboratory determinations

3.4.1 Soil morphological (physical) properties

Details of Profile samples are contained in Tables 4.3 and 4.4 and Appendices IV-I&II and V-I&II. It was done adopting the procedure contained in Globe Soil Characterization Protocol (2005) with reference to the guidelines for soil Profile description of the Food Agriculture Organization (FAO as cited in FDALR, 1990). The colour of the soil was determined using the Mussel colour chart.

3.4.2 Particle size and textural class determination

Particle size distribution for texture determination was carried out using the Bouyoucous hydrometer method. 40g of 2mm sieved soil was weighed into an extraction bottle before 100ml of 5% calgon (sodium hexametaphosphate) solution was added to the bottle. The suspension was mechanically shaken for 30 minutes and thereafter transferred into 1L graduated sedimentation cylinder. Distilled water was added to make up the 1 liter mark after which a plunger was inserted into the cylinder to stir the suspension vigorously by moving the plunger up and down several times. Finally, hydrometer readings were taking at 45minuites in the first instance, then after 5 minutes (for silt) and later at 5hours (for clay). The sand component was obtained by decanting the suspension from the sedimentation cylinder through 0.2mm sieve, washed with pipe-borne water and the left over fraction was oven dried at 105^{0}C over a 24 hour period. The oven dried samples were retrieved and kept in desiccators for cooling and weight measurement subsequently.

The sand, silt and clay proportions of the soils were then calculated for as follows:

$$Silt\ (\%) + clay\ (\%) = \frac{corrected\ hydrometer\ reading\ @\ 5mins}{Sample\ weight\ (g)} \times 100 \qquad (1)$$

$$Clay\ (\%) = \frac{corrected\ hydrometer\ reading\ @\ 5hrs}{Sample\ weight\ (g)} \times 100 \qquad (2)$$

$$Silt\ (\%) = (1) - (2) \qquad (3)$$

$$Sand\ (\%) = 100 - (1) \qquad (4)$$

The obtained percentages of silt, clay and sand components respectively, were read off on the textural triangle (USDA) for texture classifications.

3.4.3 Bulk Density

The bulk density was determined on clod soil samples taken by driving a metal corer into the soil horizon of investigation. The sand samples were then taken to the laboratory where they were oven dried and weighed. Data on bulk density was obtained by derivation using the formula:

$BD = (Ms/Vt)\ g/cm^3$

Where BD = Bulk density

Ms = Mass of oven dried soil and

Vt = total volume of core sampler.

3.4.4 Porosity

Porosity has an inverse proportionality to bulk density and was therefore arrived at by calculation as follows:

$P\ (\%) = 1 - (BD/PD) \times 100$

Where P = Porosity

BD = Bulk Density

PD = Particle Density

[(Normal particle density=2.65 (Birkeland, 1984; Wikipedia)]

3.5 Chemical properties

3.5.1 Soil pH

The soil pH was determined by preparing a 1:2 soil/water suspension. 10g of sieved air dried soil was weighted into a beaker and added 20ml of distilled water. The mixture was mechanically stirred (end-over-end) for about 20-30 minutes at laboratory room temperature and allowed to stand for another ten (10) minutes to enable solution to settle.

The pH meter was standardized using a buffer solution at pH 4.0 and 7.0 before the pH electrode was inserted into the suspension and readings recorded as pH for water. The same procedure was carried out on 0.01 M $CaCl_2$at a 1:1 ratio of soil solution.

Thorough washing of the electrodes was done between measurements of buffer solutions and between buffer solutions and soil solutions (Rayment and Higginson, 1992). The difference in pH (ΔpH) was then calculated for as follows:

$\Delta pH = [(pHw - pHs)]$

Where pHw = pH in water

pHs = pH in $CaCl_2$.

The pH reading was obtained using the Metrohn (Bie & Beinstsen) 691 meter at 25.8^0C.

3.5.2 Soil Electrical Conductivity (EC)

Electric conductivity was determined by preparing a 1:2 soil/water suspension. 10g of sieved air dried soil was weighted into a beaker and added 20ml of distilled water. The mixture was mechanically stirred (end-over-end) for about 20-30 minutes at laboratory room temperature and allowed to stand for another ten (10) minutes to enable solution to settle. The

Bie & Berntsen A-S E587 Conduct meter was calibrated to 1000µscm^{-1}at 25.8^{O}C. The conductivity cell was dipped into the supernatant, moving it up and down slightly without disturbing the slurry and corresponding readings taken when the system had stabilized (Rayment and Higginson, 1992).

3.5.3 Total nitrogen

Total Nitrogen was determined by weighing 1g of sieved air dried soil sample into 250ml digestion tube. 2.0ml of deionized water was added and swirled for a few minutes, allowed to stand for 30minutes. 2.2g of catalyst mixture (Selenium) and 8ml of conc. H_2SO_4 were added and heated in a fume digestion chamber. Inside the digestion chamber, samples were heated until they became bleached. They were removed and allowed to cool under laboratory temperature.

The Markham distillation apparatus was prepared by adding 5mlof 40% of H_3BO_3 to each 100ml of borosilicate beaker/flask. Approximately 25ml 60% solution of (400g) of NaOH was carefully add in each flask and attached to the distillation unit; The resultant mixture was distilled for about 5minutesafter which 2% Boric acid indicator was added to 5ml of collected distillate (ie.2g of Boric acid in 100ml. of distilled water). The distillate was titrated with standardized 0.01m Hydrochloric acid (HCl). The end point was indicated by a colour change from pale green to faint pink. These were in turn used to evaluate for percentage soil nitrogen (% N) as follows:

$\% N = (a - b) \times 0.01 \times 14 \times v \times 100 / 1000 \times w \times al$

Where, v = final vol. of digestion

w = weight of the sample taken in grams.

al = aliquot of the solution taken for analysis- 5ml.

0.01 = Molarity of acid (0.014)

14 = molar weight of nitrogen.

(Rayment & Rayment and Higginson, 1992)

3.5.4 Exchangeable Potassium, Calcium, Magnesium and Sodium

These parameters were determined by extracting 10g of sieved air-dried soil sample with 20ml of each cation and sieved through Whatman filter paper. The extract was mixed with 77.08g of Ammonium acetate dissolved in a 100ml beaker and stirred mechanically for about an hour. Thereafter, samples were taken for flame photometer reading (meq/100g). The resultant reading for individual parameter was further subjected to calculation to ascertain actual values for nutrients, as follows: % = (aas reading/1000) x (vol.of extract/1000) x (100/wt of soil)

The Cation exchange capacity was determined by calculation using the values obtained (in meq/100g) in an equation formulated by "Spectrum Analytic Incorporation" as follows:

(Aas = Atomic absorption spectrophotometer and Meq= mil-equivalent).

METHOD 1*: Use if a buffer pH (BpH) is available.*

*C.E.C. = (lb K ÷ 780) + (lb Mg ÷ 240) + (lb Ca ÷ 400) + [12 × (7 - BpH)]**

If buffer pH is 7.0 or greater, use a 0 value as the remainder...Example: (7.0 - 7.1) = 0

METHOD 2: *Use if Buffer pH is not available.*

C.E.C. = [(lb K ÷ 780) + (lb Mg ÷ 240) + (lb Ca ÷ 400)] × Factor

Table 3.1 Multiplication factors to use for CEC in method 2

If pH is	>7.3	7.2	7.1	7.0	6.9	6.8	6.7	6.6	6.5	<6.4
Use factor	1.00	1.05	1.10	1.15	1.17	1.20	1.22	1.25	1.28	Use Mthd.1

The multiplication factor accounts for other cations. Note also that in converting meq/100g to lb, multiply by 20

Source: ***Spectrum Analytic Inc. (2005)***

3.5.5 Total Organic Carbon / Organic Matter Determination

Total organic carbon was determined by weighing 0.5g of air-dried sieved soil into a 250ml flask wetted with 10ml of dichromate solution. 20ml of concentrated H_2SO_4 was added to the flask and swirled to achieve a homogenous mixture before allowing stand for about 30minutes. Subsequently, 200ml of distilled water, 10ml of orthophosphoric acid and 2ml of barium diphenylamine sulphate indicator were added in that sequence and titrated with the ferrous ammonium sulphate solution from its blue colour to a green end point.

Titer values were subsequently used to calculate for percentage carbon as follows:

% Carbon = [10 – (X N) x 0.3] / W

Where 10 = dichromate solution (ml)

X = ml. of ferrous ammonium sulphate solution required for the titration

N = normality of ferrous ammonium sulphate solution

W = weight of soil sample in gram.

Organic Matter content (%) of soil was calculated for, by multiplying % carbon with 1.724. where 1.724 is the conversion factor (Blacky et al., as cited in Spectrum Analytic, 2005).

3.5.6 Carbon-Nitrogen Ratio

The carbon- nitrogen ratio (C: N) was obtained by dividing percentage organic carbon with percentage nitrogen.

C: N = OM (%) / N (%)

Where OM = Organic mater (%)

N = Nitrogen (%)

3.6 Biological property of soil samples

3.6.1 Enumeration of Total microbial population (TMP)

The total microbial population count in the soil samples was done by the pour plate technique using diluents prepared with a 10^5 factor in a 10g soil to 200ml 1% sodium carboxymethyl cellulose (CMC). The mixture was allowed to oscillate for about 20 minutes, before transferring 1ml aliquot of it into 9ml CMC diluents in 4oz bottle. Again, this was allowed to oscillate for another 4 minutes after which another 1ml aliquot was transferred into 49ml of CMC for yet another two (2) minutes of shaking and finally, 1ml of aliquot was transferred to each of the 5 sterile petri dishes for each soil sample; followed by an additional 17ml of appropriately pre-cooled medium and dispensed inoculums which was subjected to circular and side by side motion in petri dishes. Dishes/plates were subsequently inverted after agar had hardened to make room for bacterial growth. The inoculated plates (of TMP) were incubated for 48 – 72 hours before enumeration for MP colonies. The mean values of the number of colonies which appeared were multiplied by the dilution factor of 10^5 to give the number per gram of soil (Hornby, 1998).

3.7 Data analyses

A multivariate analyses of variance (MANOVA), Least Significance Difference (LSD) as well as correlation tests were performed on the data obtained in order to establish variations from the mean values of all dependable variables from the various treatment options. The purpose of these analyses was to ascertain whether the differences in the mean values for the various indices of plants' performance between treated options, and within or between plant blocks were significant, real and due to the different treatment applications or due to experimental errors; and to also ensure that results and conclusions in this study arescientifically and statistically valid (objective) rather than judgmental. These analyses followed the procedure contained in the Statistical Package for Social Sciences (SPSS) Version 16.0.

CHAPTER FOUR

RESULTS

4.1 Phase 1: Pilot survey

4.1.1 Biometric data on respondents

Biometric data are contained in fig 4.1a-e. Results from respondents' inputs and the oral interviews conducted during site tours revealed that in all Questionnaire retrieved, 90% of respondents were males whereas only ten were females. Majority of the respondents were married adults (66%) while single youths and adolescents constituted about (34%). Educational qualification also indicated that 30%, 27% and 22% were senior school certificate holders, tertiary institution and junior school certificate holders respectively. 27% however, were primary school holders.

Farming and fishing were identified as the chief/primary occupations of the residents of the study area.Majority of respondents (83%) have lived in communities for 10 years and above whereas 75% (Fig. 4.2) have experienced crude oil spill on several occasions.

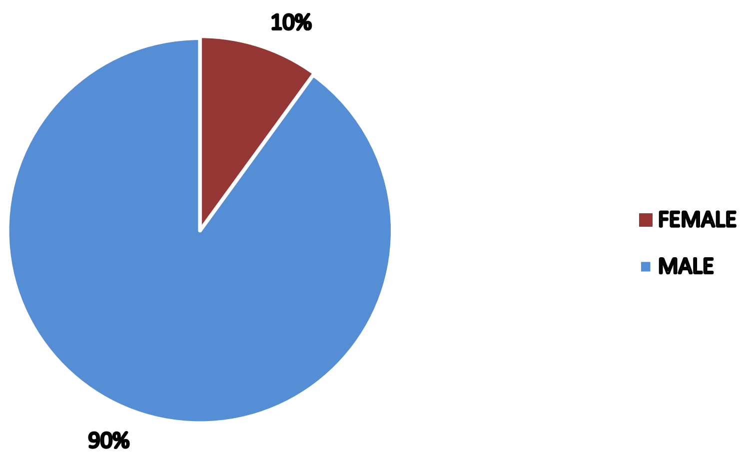


Fig 4.1a: Gender composition of respondents

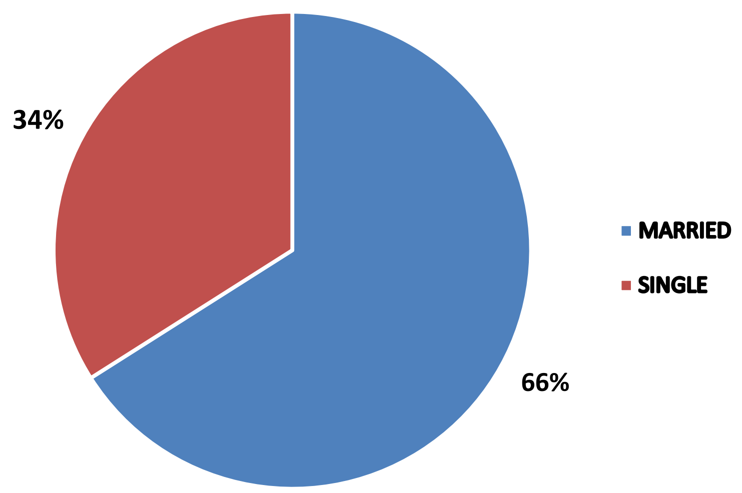


Fig 4.1b: Distribution of respondents by marital status

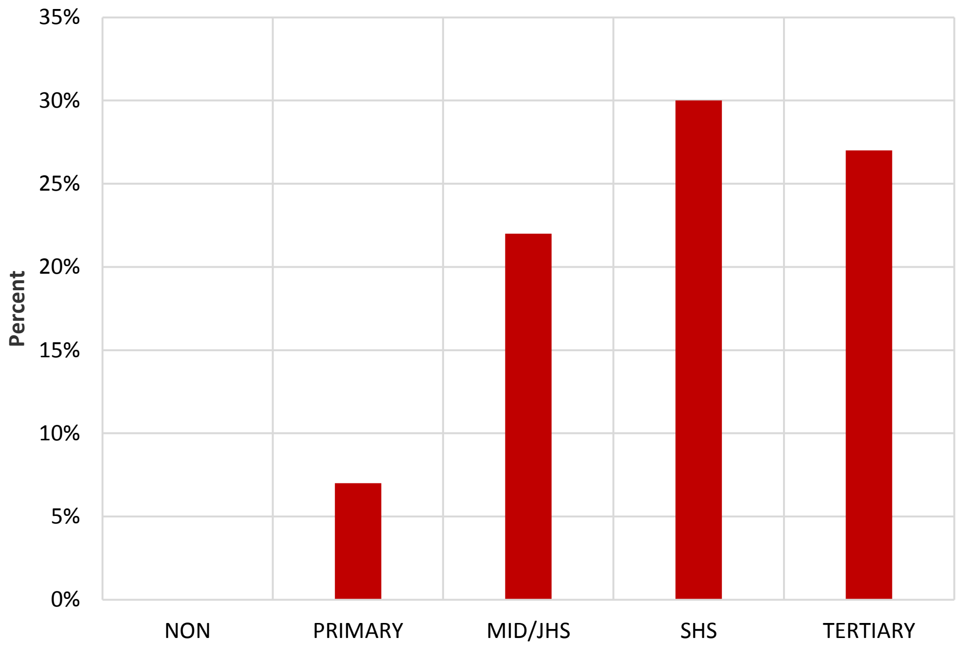


Fig 4.1c: Distribution of respondents by educational qualification

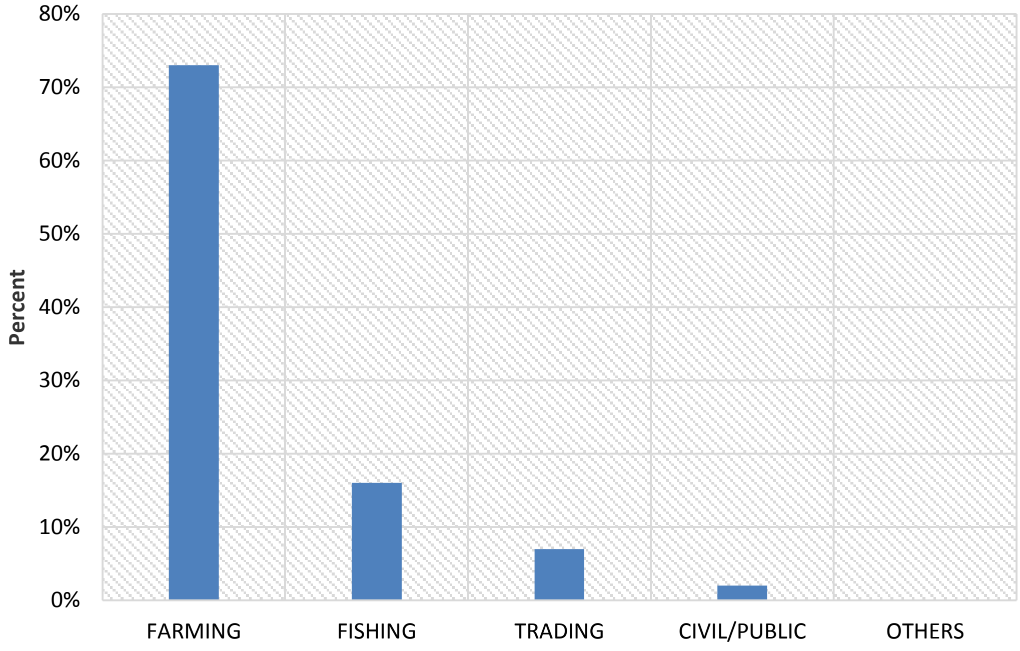


Fig 4.1d: Proportion of respondents according to their occupation

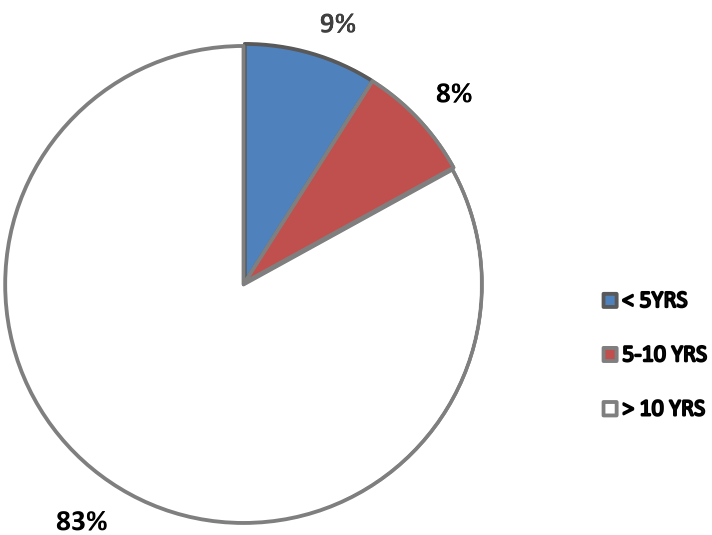


Fig4.1e: Proportion of respondents by duration of residency in the locality

4.1.2 Particle size distribution, textural class determination and GPS locations of sites.

Data on particle size distribution and textural class determination are presented in Table 4.1. Clay was the predominant textural class of soil in parts of the region surveyed, but laced with varying mixtures of silt and sand.

Table 4.1: Particle Size distribution, textural class determination and GPS Locations of pilot survey Sites

	% Silt	% Clay	% Sand	Texture	GPS Location
Iriama	**36**	**32**	32	Clay loam	NE: 000^0 Alt. 5^0 41' 54" long. 5^0 42' 43" Lat.
Kalaba	51	47	1.2	Silty clay	NE: 000^0 Alt. 5^0 7' 48" Long. 6^0 26' 33" Lat.
Ogbogu	51	46	2.1	Silty clay	NE: 000^0 Alt. 5^0 14' 29" long. 6^0 37' 21" Lat.

4.1.3 Effects of crude oil spills on the environment.

Data on Socio-economic and cultural implications of exploitation and exploration activities arepresented in Fig. 4.2. Results indicate that 100% of respondents voted negative for prevailent vices such as high levels of unemployment, disrespect by youths for elders, customs and institutions, sex trade and teenage pregnancy amongst teenage girls, seduction and harassment of women by security operatives and high rates of divorce amongst others.

Furthermore, evidence of the effects of crude oil spill on the environment are illustrated in Fig. 4.2 and Plates 3-6. Results indicated that biogeophysical impacts were real and included vegetation distortion and loss, land degradation (surface cracking), contamination of water bodies and loss of human and other living organisms.

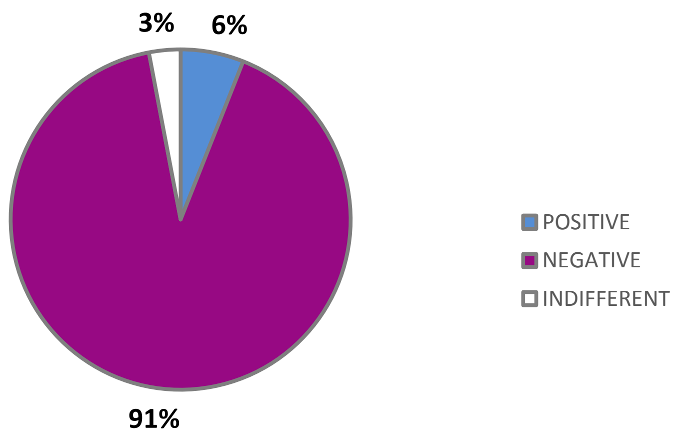


Fig 4.2: Impacts of crude oil spill on socio-cultural and biogeophysical factors of the Environment.

Plate 3: Vegetation Loss at Kalaba site from oil spill

Plate 4:Cracked unremediated land surface at Kalaba community due to oil spill impact.

Plate 5: *Eichornia crassipes* showing signs of stress from oil spill impact.

4.1.4 Evidence of tolerance of plant at the site of crude oil contamination.

Evidence of fresh growths of some plant species were manifest at site in Warri where clean-up measures had been undertaken (Plate 6). There was no sign of vegetative recovery at sites where clean-up measures had not been carried out as earlier indicated.

Plate 6: Evidence of fresh regrowths of *Comelina* sp. after remediation work at a site visited in Warri.

4.1.5 Land restoration

Results on land restoration are presented in Fig 4.4. They showed that 81% of respondents indicated that the commonly adopted clean-up measure was Dig and Burn. Whereas 18% opted for Mechanical measure, 63% indicated that spilled lands were not restored to pre-incident status (Fig.4.5). Also, 84% of respondents claimed that they where ignorant about any phytoremediation process either in the past or present that had been carried out on their lands (Fig 4.7). Mainwhile, 43% of respondents were willing to be receptive of it should this be the case in the future (Fig 4.5).

Finally, 84% (Fig.4.6) of respondents indicated that they where not aware of the use of plants to clean-up hydrocarbons contamination from spilled sites in their communities.

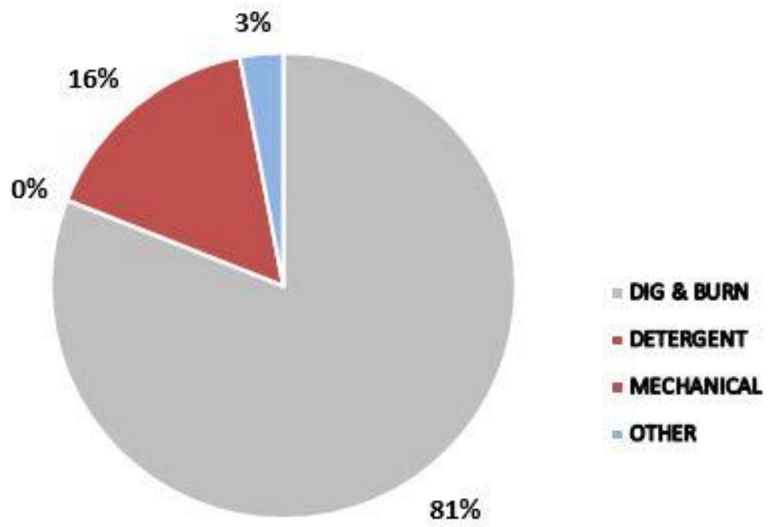


Fig 4.3: Relative proportions of different clean-up measures adopted by Concessionaires

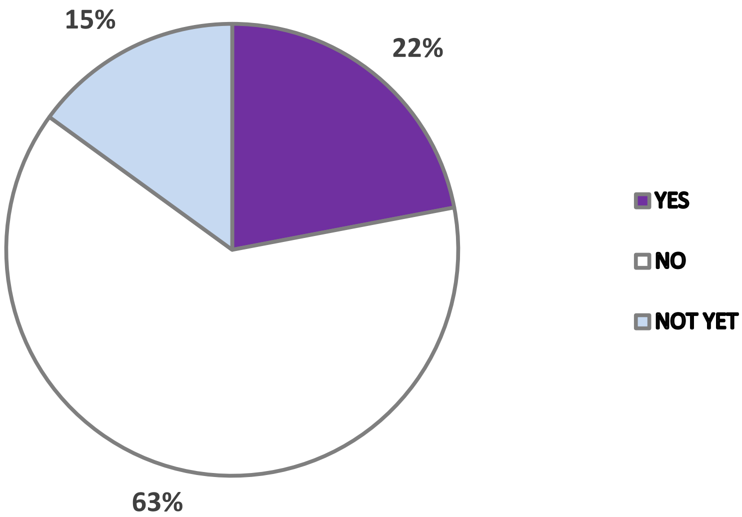


Fig. 4.4: Proportion of spilled land restored to pre-spill status

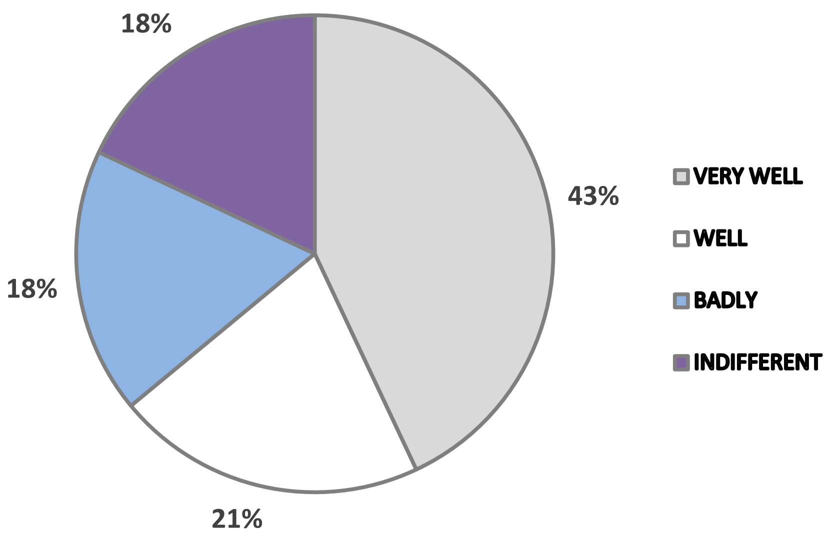


Fig 4.5: Relative acceptance by respondents of the use of local plants for the clean-up of oil spills.

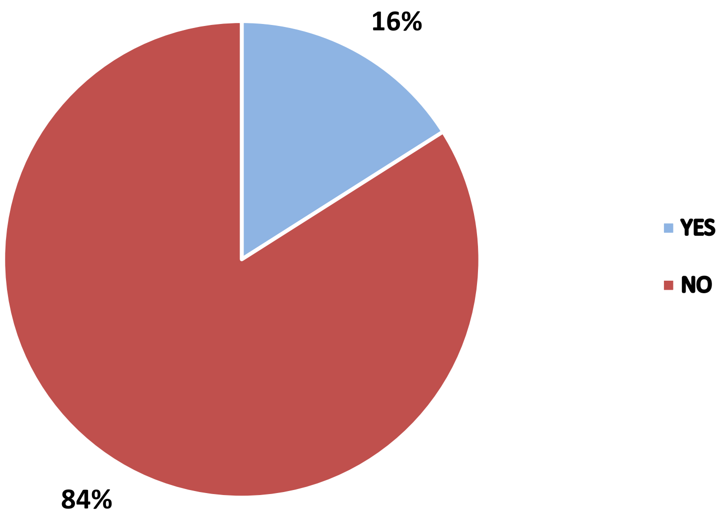


Fig 4.6: Awareness among respondents on the use of local plants for the clean-up of oil spill

4.1.6 Identification of collected plants

The list of plants collected from the fields surveyed and subsequently identified (using Akabundu and Agyakwa, 1998) is presented in Table 4.2, whereas Appendix II shows photographs of the plants species. There were fifteen (15) plants in all.

4.1.7 Questionnaire administration

The sample of the Questionnaire administered and the details of respondents' inputs are contained in Appendix I (i and ii). Eighty-three (83) questionnaires were retrieved, analyzed and processed into statistical models as evident in earlier results/illustrations.

Table 4.2: List of plant species collected and identified from fields in the Niger Delta of Nigeria.

Family name	Botanical name	Common name
Asteraceae	*Eclipta alba* (Linn.)	-
Cyperaceae	*Cyperus haspen* (Linn.)	-
Melastomataceae	*Melastomastrum capitatum*	Fern
Poceae	*Acroceras zizanoides Dandy*	
Dennstaeditiaceae	*Pteridum aquilinum (Linn.)*	Brackenfern
Poceae •	*Panicum maximum*	Guinea grass
Onagraceae	*Ludwigia decurrens* Walt.	Water primrose
Poaceae	*Setaria longiseta* P. Beauv.	Foxtail
Poaceae •	*Cynodon dactylon* (Linn) P.	Bahama grass
Solanaceae	*Physalis angulata* (Linn.)	Wildcape
Poaceae•	*Paspalum vaginatum*	Gooseberry
Leguminosae-Mimosoideae •	*Schrankia leptocarpa*	-
Leguminosae •	*Centrsema pubescence*	-
Leguminosae •	*Puereria phaseoloides*	Kudzu
Leguminosae-Papilionoideae	*Desmodium scorpiurus* Desv.	

All plants bearing asterisk represent species selected and used in the study.
Identification source: Akabundu and Agyakwa (1998).

4.2.4 Irrigation rates

Details of the appropriate rate of irrigation used in the study are indicated in Table 4.3. These weights were 260g and 314g, the equivalents of 175ml and 125ml of water respectively for the Toje and Alajo soil series.

Table 4.3: Data on particle size distribution, textural class and irrigation rate applied to Soil

Parameters	Alajo series	Toje series
Particle size (%)		
Silt	13.9	5.1
Clay	61.2	32.9
Sand	24.9	62.1
Texture	Clay	Sandy clay loam
Irrigation rate (ml)	125	175

4.2.5 Crude oil characteristics

The detailed characteristics (benchmarked) of Bonny light crude oil used, as given by American Petroleum Institute (API) and the Nigerian National Petroleum Corporation (NNPC), is shown in Table 2.1.

The American Petroleum Institute revealed that light crude oil has a Specific gravity at $31.1^0$and above and has higher proportions of Paraffin to Naphthene as well as Aromatics than the medium to heavy crude oil grades respectively. Its major compounds are Carbon and Hydrogen with corresponding percentages of 83 – 87% and 10 – 14% respectively. However, minor elements were Oxygen and metals which had a combined percentage range of 0 – 1.5%. Other compounds include Sulphur and Nitrogen. They both constitute a percentage range of 1.5 – 6%.

4.2.6 Crude oil application rates (%)

The complete data on percentage (%) crude oil concentrations with which soils were simulated is contained in Table 4.4. Mean serial volume of crude oil applied to experimental pots was in the range of 0.649 to 48.881mg/kg on Alajo series and 2.694 to 68.338mg/kg on Toje series.

4.2.7 Ambient temperature and relative humidity conditions

Data on temperature and relative humidity regimes inside the green house are shown in Table 4.5. The period covered November, 2010 to January, 2011for phase 1 and March - May, 2011 for phase 11 respectively. The mean temperature within the period was in the range of 25 to 40^{o}c and above; whereas the mean Relative Humidity rangewas between 44 and 95%.

4.2.8 Germination and sprouts count for propagules and seeds

The results of total germination of propagules and sprots of seeds are shown in Appendix VII and Table 4.6 (Figs. 4.8 & 4.9). Alajo soil series indicated that *Paspalum vaginatum* had 83% germination, followed by *Centrosema pubescens* and *Panicum maximun* with 68% sprouts count and 67% germination respectively. *Cynodon dactylon* had 63% while *Schrankia leptocarpa* and *Puereria phaseoloides* recorded 43% and 38% respectively.

Toje soil series indicated that *Cynodon dactylon* recorded 79% sprout count followed by *Panicum maximum* with 72% germination count. *Pureria* and *Paspalum* came next with sprouts of 67% and germination of 61% respectively. *Schrankia* and *Centrosema* species had sprouts of 48% and 42% respectively. Appendix VII shows the detailed results of grass species germination assessment in the study while Table 4.7; Figs. 4.8/4.9 illustrate mean values of sprouts evaluated as number of seedlings enumerated at 1WAP.

Table 4.4: Initial Total Hydrocarbon concentrations (in mg/kg) of soils one week after Simulation (1WAS)

Crude oil/ Soil series	Control (0%) mg/kg	1%(24ml) mg/kg	2% (47) mg/kg	3%(83ml) mg/kg	5.5%(130ml) mg/kg	8%(189ml) mg/kg	10%(237ml) mg/kg
Alajo Series	649	5,674	6.757	16,551	28,077	40,466	48,881
Toje Series	2,688	11,516	15.295	16,088	33,270	51,110	68,338

Table 4.5: Ambient Temperature and Relative Humidity regimes in the green house [Note: Accra means =27^{O}C(T); 55%-65%(R/H)]

Phase 11	wk1	wk2	WK3	WK4	WK5	WK6	WK7	Total (mean)
Temperature ^{0}C	22.4– 37.6	23 – 38	23 – 38	19 – 37	19.6– 35.7	N/D	N/D	21 -37
Relative Humidity %	39.7 - 86	43.7 - 98	41 – 98	36 - 92	23.7– 86.5	N/D	N;D	38 - 92
Phase 111 Temperature ^{0}C	29 – 40>	31 – 40>	32– 40>	27 – 40>	26 – 40>	28-40>	31-40>	29 -40>
Relative Humidity %	44 - 97	49 - 100	55 – 100	50 - 96	46 – 96	59- 100	50-100	50 – 98

>: Indicating that weekly temperature peaks were above 40^{0}c.
Phase II spanned between March and May 2011.
Phase III spanned between November 2010 and January 2011.

Table 4.6: Sprouting of propagules of selected grasses and germination of seeds of selected legumes at one week after planting (1WAP)

	Plants species	Alajo series %	Toje series %
Vegetative parts of propagules of selected grasses	*P. maximum*	67	72
	P. vaginatum	83	61
	C. dactylon	63	79
Seeds of selected legumes			
	C. pubescence	38	67
	S. leptocarpa	43	48
	P. phaseoloides	64	42

4.2.9 Growth assessment for experimental plants

Figs 4.10 and 4.11 show growth indices for test plants examined in this study. Results indicated that mean values of plants heights were in the range of 38% and 83% on the Alajo soils series and 42% and 79% on the Toje soil series respectively

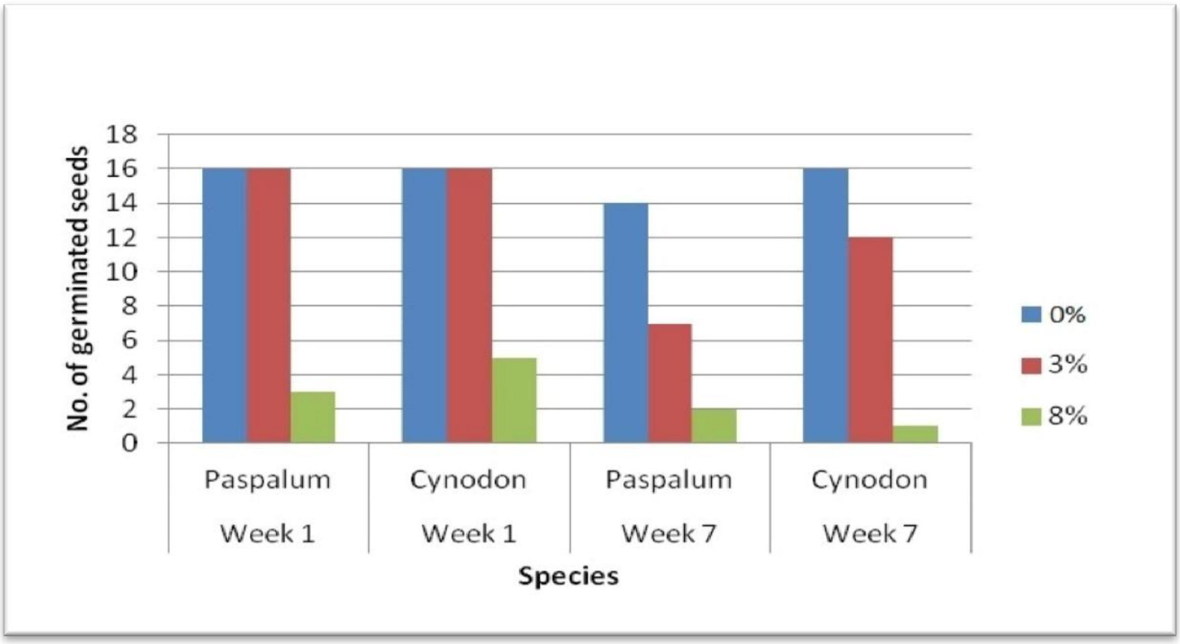


Fig 4.7: Number of propagules per plant of the selected grass species enumerated on the Toje soil series.

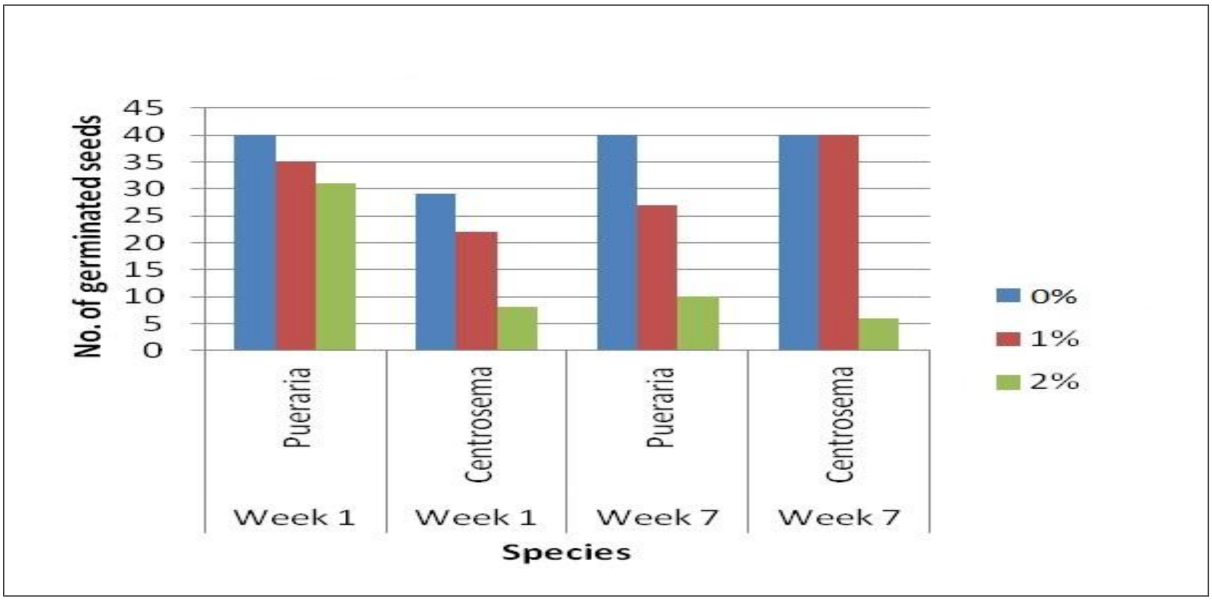


Fig 4.8: Number of seedlings per plant of the selected legumes enumerated on the Toje soil series.

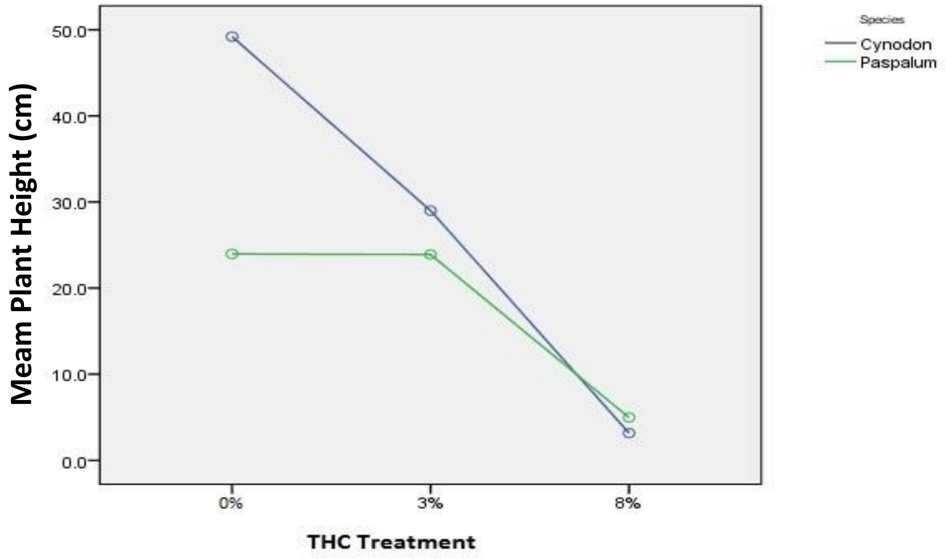


Fig 4.9: The effects of different concentrations of Hydrocarbon contamination on the growth and development of selected grasses grown on Toje soil series.

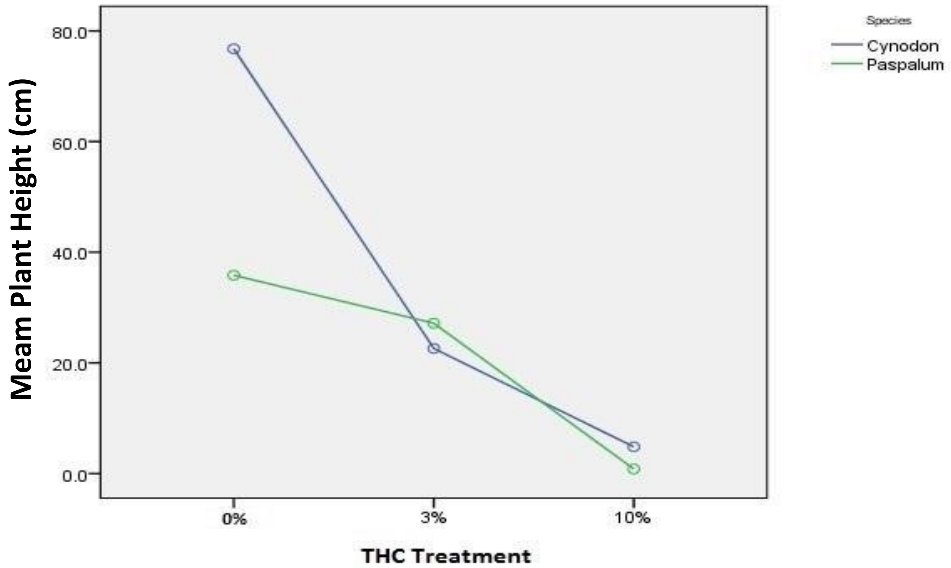


Fig 4.10: The effects of different concentrations of Hydrocarbon contamination on the growth and development of test grasses grown on Alajo soil series.

4.3 Phase III

4.3.1 General properties of the soils

Morphological characteristics of the soils from the catena of Accra Plains are described below while analytical data on selected physical and chemical properties are presented in Tables 4.7 and 4.8. Appendix IV (I and II) and V (I and II) show detailed descriptions of the individual pedons. Plates 8 and 9 shows profile photographs of Toje and Alajo soil series respectively.

Toje soil series was located at the upper to mid slope of the Legon hill and Alajo soil series could be found at the bottom slope of the catena at Asherley Botwe. Horizonation of the stratum along the the profiles were fairly uniform on both soils. Alajo series was characterized by cracks which varied in width from the surface down to the depth of about 10cm, and extended in depth beyond 20cm Alajo series had slickensides at greater depths because of its vertic properties. The depths of soils increase down the slope from the upper slope of the catena as Toje series had a depth of 0-155cm whereas Alajo series had a depth of 0-200cm (Plates 8 and 9). Soil colours were in the range of red for Toje and black for Alajo series respectively; and hues of between 10YR to 2.5 YR; Value scales ranged from 4 to 5, while chroma was between 4 and 6 for the Toje soil series. Alajo series had a dark grey to black colours, the hue of 10YR and value of between 2 and 3, while chroma intensified from 1 to 2.

The structure of Toje soil series varied from granular at the surface, sub-angular at mid profile and blocky at the base (Table 4.8). Structure was granular at the surface, blocky at mid profile and became massive at the base for the Alajo soil series (Table 4.9). Toje soilhad sandy clay loam in its surface texture, clay texture at mid profile and had clay- loam texture at the bottom-most horizon. Alajo series had sandy clay loam texture on the surface, clay mid profile and changed to sandy clay loam thereafter, and futher changed to clay texture at the base. Toje series had friable surface and firm mid to bottom moist consistence. Alajo series graded friable, firm and extremely firm in consistency from the surface to bottom horizons respectively. Carbonates were absent in all the horizons of Toje series while Alajo were slight, none and strong carbonate presence in all its horizons.

Rocks (gravels) were completely absent (none) in both soil series whereas roots distribution was abundant in all surface horizons examined in both soils. However, they (roots) decreased with profile depth with no trace of any in the last horizons of both soils.

Bulk densities were low on the surface (1.2g/cm^{-3}) but increased to (1.4g/cm^{-3}) from mid to bottom horizons of Toje soil series (Table 4.8); while Alajo soil series was equally low on its surface (1.2g/cm^{-3}) but increased fairly uniformly to 1.5/1.6g/cm^{-3} towards the bottom of the profile (Table 4.9).Porosity on both soils expectedly decreased with increase in profile depth from 54.7 to 39.6% on Toje and from 50.9 to 43.4% on Alajo series respectively.

The details of the grid analysis of Toje soil series are shown in Appendix IV (ii) whereas Plate 8 shows the picture of its profile pit. Results indicated that mean percentage (%) phosphorous was 0.105mg/kg with the range of between 0.03mg/kg and 0.36mg/kg. Mean percentage nitrogen of 0.064% with a range of between 0.04% and 0.1%: Mean organic carbon was 8% and a range of between 1 and 25%. pH read at the room temperature of 25.8^{0}C stood at 7 with a range between 6.7 and 7.7: while mean electric conductivity (EC) was 504.9µScm and a range between 220µScm and 900µScm. Surface cations exchange capacity (CEC) was 30.6meq/100g.

The detailed grid data on Alajo series are contained in Appendix IV (i) while Plate 9 shows picture of its profile. Alajo series was formed from fluvial deposits along a wide flat depression of approximately 0 – 1% in slope. The vegetation here consisted of Savanna regrowths comprising of Thickets and Neen as some of the common tree species. The pit dug here was also rock fragments free due to the origin of its parent material since the soil was alluvial. These descriptions are corroborated by the report of Eze (2009).

Table 4.7: Morphological properties of Toje soil series (Legon hill catena)

Horizon (dept/cm)	Rocks	Roots	Structure	Colour (moist)	Consistency (moist)	Texture (Grade)	Carbonates	B/D g/cm^{-3}	Porosity %
0 – 11	None	Many	Granular	5YR4/4	Friable	scl	None	1.2	54
11 – 27	None	Many	Sub- ang	5YR4/5	Friable	scl	None	1.3	50
27 – 75	None	Few	Blocky	10 YR4/4	Firm	Clay	None	1.2	47
75 – 123	None	V.few	Blocky	2-5YR4/5	Firm	Clay	None	1.4	43
123 – 155	None	None	Blocky	2-5YR4/6	Firm	Clay loam	None	1.3	39.6

Sub-ang = Sub-angular; scl= sandy clay loam

Table 4.8: Morphological properties of Alajo soil series (Bottom hill catena-Asherley Botwe)

Horizon (depth/cm)	Rocks	Roots	Structure	Colour (moist)	Consistency (moist)	Texture (Grade)	Carbonates	B/D (mgm^{-3})	Porosity %
0 – 8	None	Many	Granular	10YR3/2	Friable	Scl	Slight	1.3	50.9
8 – 21	None	Many	Blocky	10YR3/1	Firm	Scl	Slight	1.5	43.4
21 – 45	None	Few	Blocky	10YR3/1	Firm	Clay	None	1.5	43.4
45 – 115	None	V. few	Blocky	10YR2/1	V. firm	Scl	Strong	1.6	39.6
115- 200	None	None	massive	10YR3/1	Ext. firm	Clay	Strong	1.5	43.4

S.c.l=Sandy-clay-loam;V=Very;Ext=Extremely

Plate 8: Profile pit of Toje soil series

Plate 9: Profile pit of Alajo soil series

4.3.2 Evapo-volatilization

Data on evapo-volatilization are presented in Table 4.10 and Figs 4.11- 4.14. The values were in the range of 1% to 3% at low contamination (1% & 2% treatment options) and between 4% and 8% at high contaminant concentrations (3% - 10%treatment options) for both soils. Correlation tests were R^2 = O.9329 and R^2= 0.9969 for legumes and grasses sown on Toje and R^2 = 0.8923 and R^2 = 0.6413 for grasses and legumes sown on Alajo soil at $p<0.05$.

Table 4.9: Effects of different concentrations of crude oil contamination on percentage evapovolatilization for the selected test plants.

Alajo Series

TO/Test Plants	1%	2%	3%	8%	10%
Puereria	0.9	2.7	-	-	-
Centrosema	0.9	2.9	-	-	-
Paspalum	-	-	4.3	-	7.4
Cynodon	-	-	4.6	-	7.5

Toje Series

T.O/Test Plants	1%	2%	3%	8%	10%
Puereria	0.9	2.7	-	-	-
Centrosema	0.9	2.9	-	-	-
Paspalum	-	-	5	7.4	-
Cynodon	-	-	5	7.5	-

All Evapovolatilization data represent Percentages of surface losses

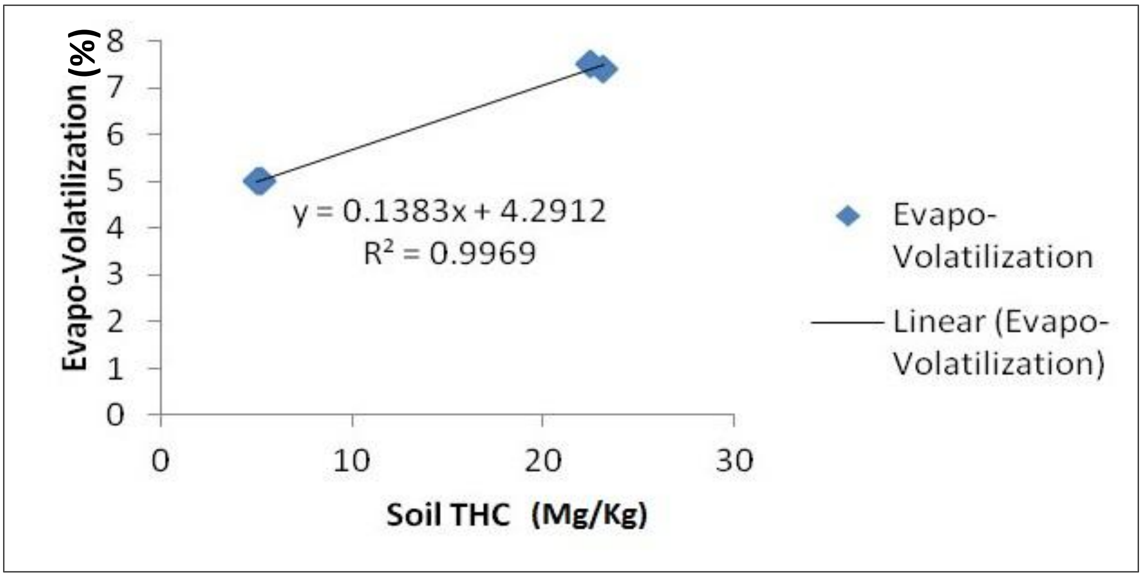


Fig 4.11: The correlation between different concentrations of crude oil concentrations and Evapovolatilization from the selected grass species and the Toje soil series

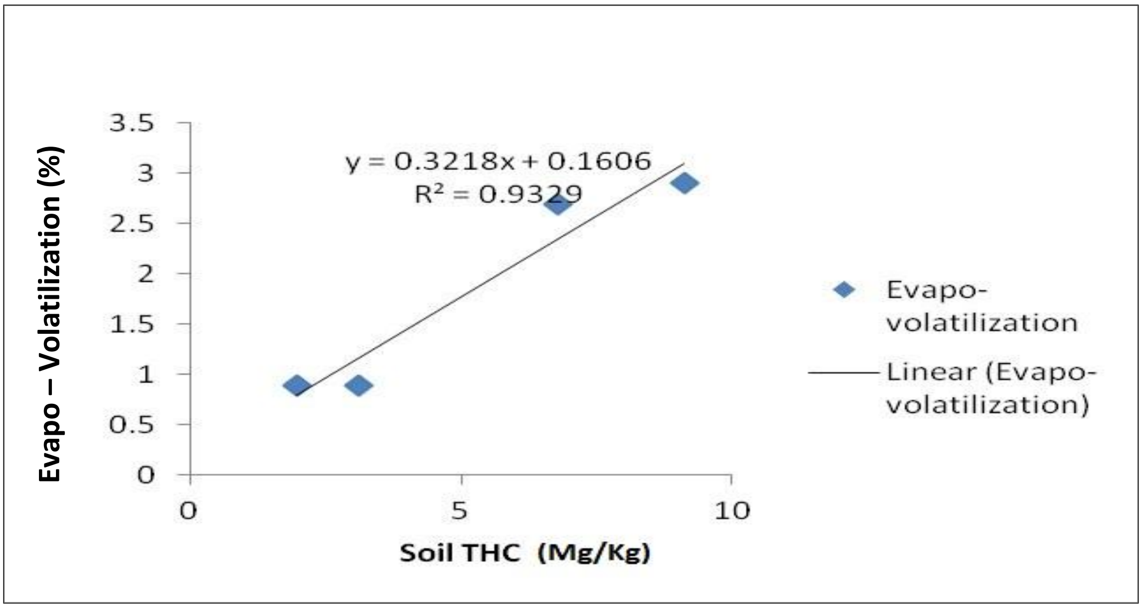


Fig 4.12.The correlation between different concentrations of crude oil concentrations and Evapovolatilization from the selected legumes and the Toje soil series.

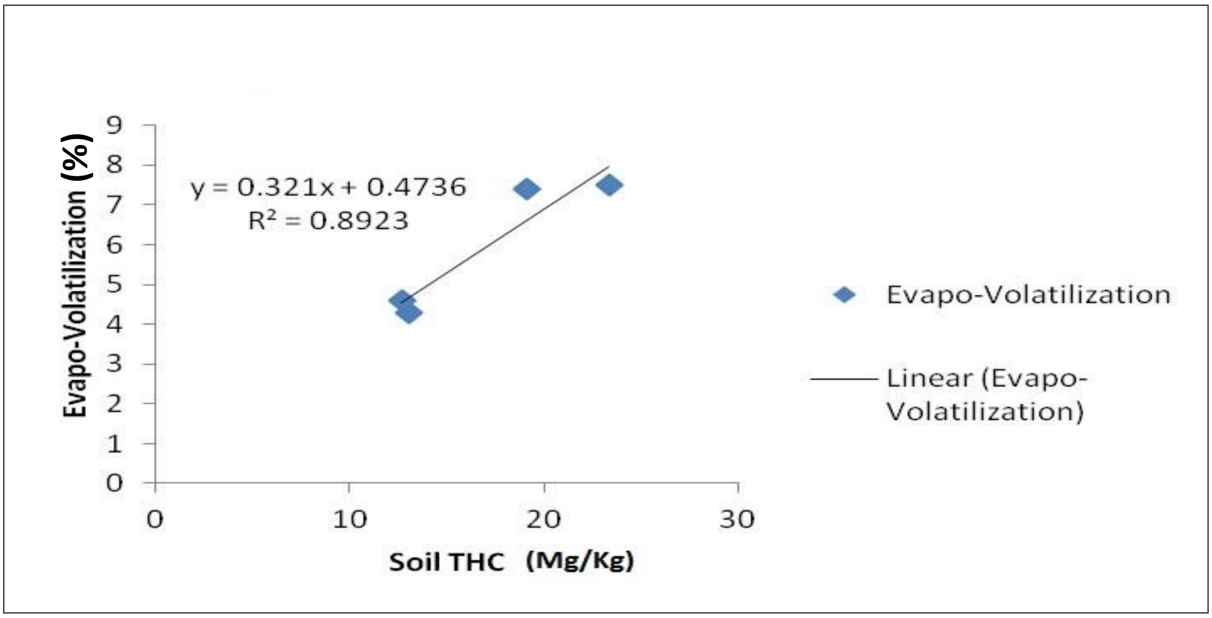


Fig 4.13: The correlation between different concentrations of crude oil and Evapovolatilization from selected grass species and the Alajo soil series.

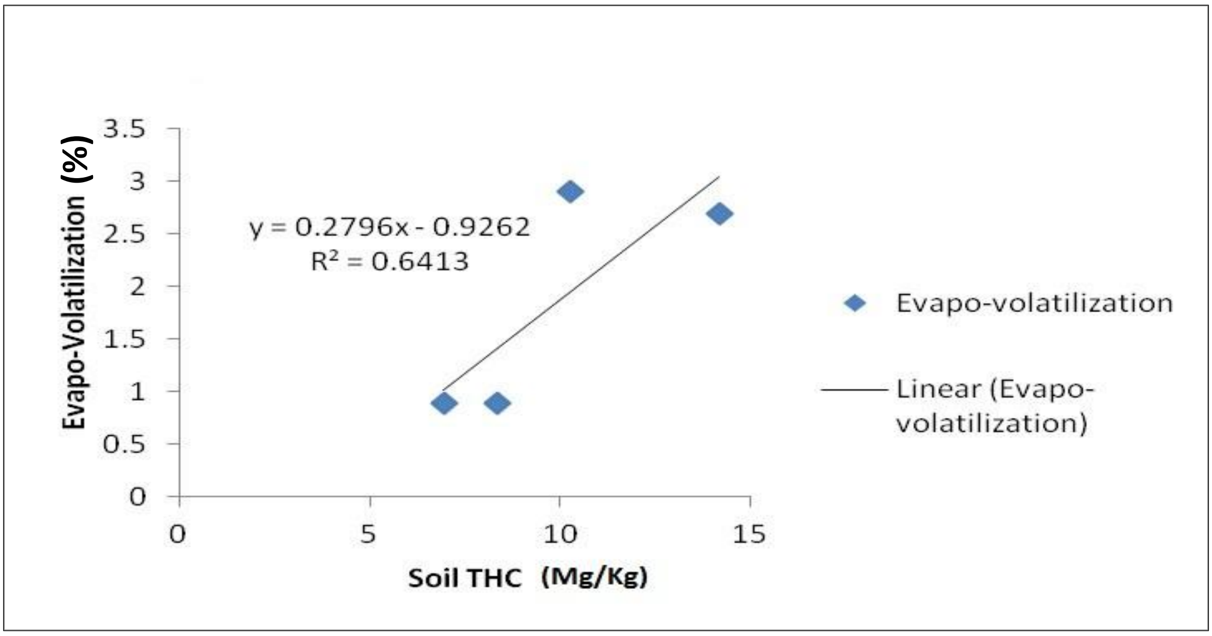


Fig 4.14: The correlation between different concentrations of crude oil and Evapovolatilization from legumes and the Alajo soil series.

4.3.3 Plant biomass

The data on plants biomass are presented in Figs 4.15 and 4.16. The best general performance of the test plants was at 0% treatments (control option) on both soils. The highest control value of 122g/fw was recorded on Alajo soil by *Paspalum vaginatum*, whereas*Centrosema pubescence* and *Pueraria phaseoloides* had the lowest joint value of 14g/ww. The leguminous plantshad 0.3g/fw (*Centrosema* sp.) at 2% treatment level on Toje soil and 20g/fw (*Pueraria* sp.) at 1% treatment level on the Alajo soil.

Furthermore, the grasses merely survived at 3% on both soils, but could not survive at 8% and 10% on both the Toje and Alajo soil series respectively. Highest biomass index of 72g/fw for this category was achieved by *Paspalum vaginatum* on Alajo soil, whereas 27g/fw was recorded by *Cynodon dactylon* on Toje soil.

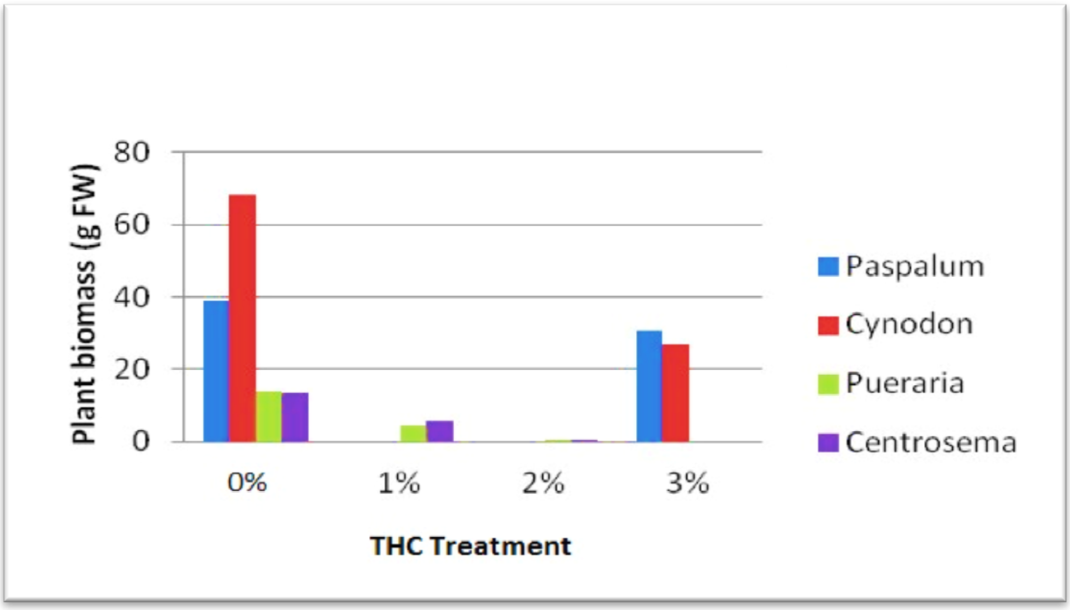


Fig 4.15: Fresh weights accumulation in selected experimental plants (g/fw) grown on the Toje soil series

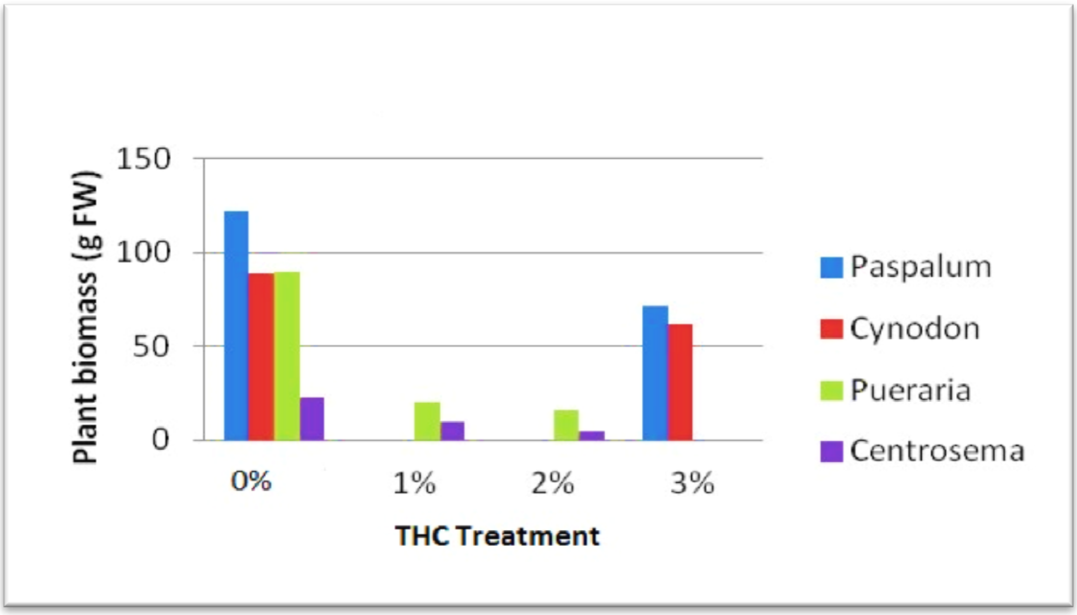


Fig 4.16:Fresh weight accumulation in selected experimental plants (g/fw) grown on the Alajo soil series

4.3.4 Laboratory determinations

4.3.5 pH

Data on pH determination are shown in Table 4.11. Results indicated that the pH values for the Alajo soil series ranged between 6.8 to 7.2 at the inception of field work and 7.7 to 8.1at the end of field study. Whereas the pH of Toje soil series was in the range of 4.5 to 5.7 and 6.4 to 6.8 at the beginning and the end of field study respectively.

Table 4.10: Physical and chemical properties of soils used in the study at 1 and 7 weeks after simulation.

Parameters	Alajo seris	Toje series
Ph@28^{0}C 0%	6.9 (7.7)	4.5 (6.8)
1%	7.0 (8)	5.5 (6.8)
2%	6.9 (8.1)	5.7 (6.4)
3.5%	6.8 (7.7)	5.1 (6.4)
5.5%	6.8 (7.8)	5.3 (-)
8%	7.2 (-)	5.3 (6.6)
10%	6.8 (7.8)	5.3 (-)
EC µs cm^{-1}		
0%	100 (126.8)	50 (31.4)
1%	600 (139.3)	70 (37.9)
2%	450 (128.9)	87 (35.6)
3.5%	150 (123)	100 (67.7)
5.5%	270 (-)	40 (-)
8%	120 (-)	60 (42.7)
10%	380.7 (115.5)	30 (-)
Total nitrogen(%)		
0%	6	4
1%	0.2	0.1
2%	0.2	0.1
3.5%	0.2	0.1
5.5%	0.2	0.1
8%	0.2	0.1
10%	0.2	0.2

Table 4.10 continued

Total organic carbon (%)		
0%	15.3	14.8
1%	15.7	14.5
2%	30.9	16.2
3.5%	31.6	30.8
5.5%	31	32
8%	31	32
10%	31.3	32.7
Total organic matter (%)	Alajo series	Toje series
0%	26.3	25.5
1%	27.1	24.9
2%	53.3	27.2
3.5%	53.4	27.9
5.5%	53.4	53.1
8%	53.4	55.2
10%	53.9	56.4
Carbon: Nitrogen ratio (%)		
0%	3:1	4:
1%	79:1	145:1
2%	134:1	148:1
3.5%	150:1	162:1
5.5%	158:1	308:1
8%	155:1	320:1
10%	157:1	164:1

4.3.6 Electrical conductivity (µs cm^{-})

Table 4.11 contains a summery of the data on EC. Results of Electrical Conductivity (EC) analysis indicated a range of mean values of 100-600 µs cm^{-1} at the inception of study and 115.5-139.3 µs cm^{-1} at the end of field study. Also, mean values were in the ranged of 50-100 µs cm^{-1}at the inception and 31.4-67.7 µs cm^{-1} for Toje soil series at the close of field study

4.3.7 Total nitrogen (%)

Table 4.11 and Figs 4.18a and b, contain data on total nitrogen. Results indicated that mean values of percentage nitrogen were in the range of 6% and 0.2% between control and treated options for the Alajo soil series and 4% and 0.1% for the Toje soil series respectively.

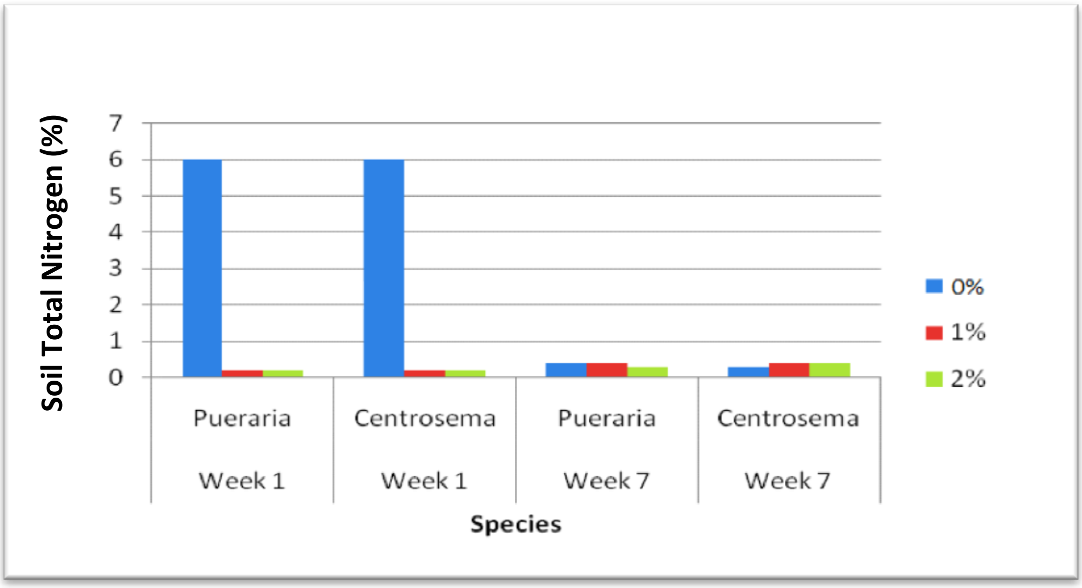


Fig 4.17a: Percentage nitrogen content of legumes grown on Alajo soil series at weeks 1 and 7.

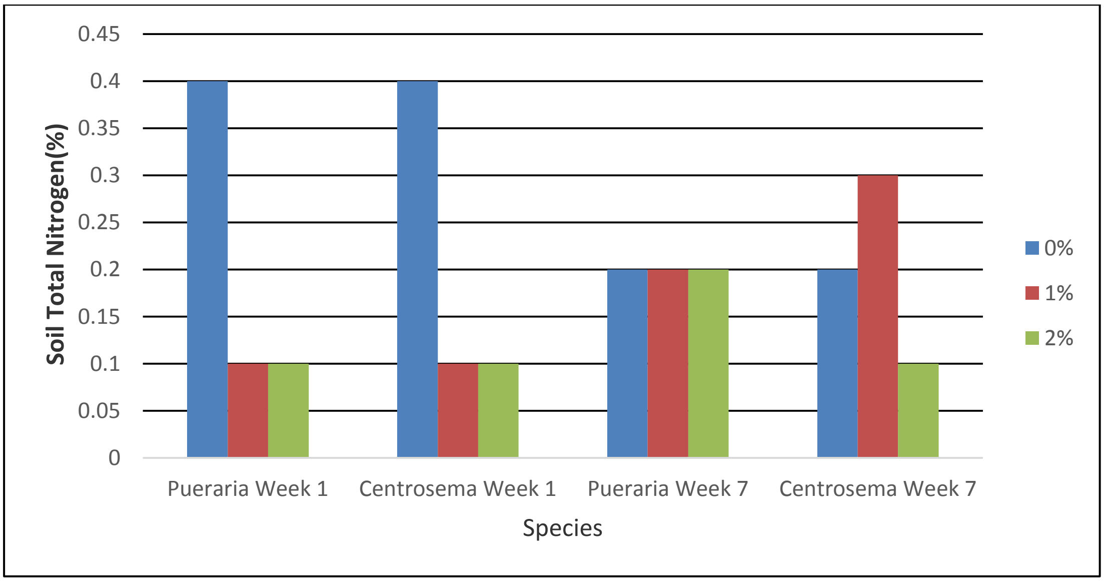


Fig 4.17b: Percentage nitrogen content of leguminous plants grown on the Toje soil series atweeks 1 & 7.

4.3.8 Total organic carbon/Organic matter content

Table 4.11 contains data on total organic carbon and organic carbon contents. Results of total organic carbon indicated that mean values were in the range of 31 % and 31.6 % for the Alajo soil series and between 14.5 and 32.7% for the Toje soil seriea respectively.

Organic matter contents also, recorded mean values in the range of 26.3 % and 53.9 % for the Alajo series and 24.9 % and 56.4 % for the Toje soil series respectively.

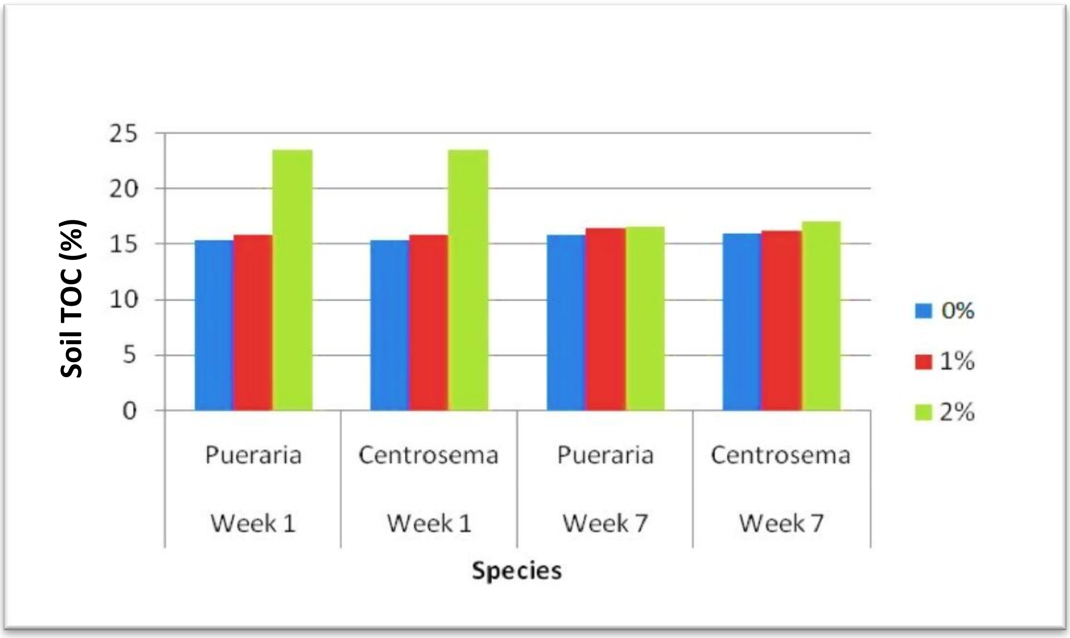


Fig 4.18: Percentage Total Organic Carbon (TOC) content of Toje soil series at weeks 1 & 7 occupied by Leguminous plants.

4.3.10 Total Hydrocarbons analyses (THC mg/kg) in soils

Details of soils THC are presented in Table 4.12. Results were in the ranges of 0.641mg/kg and 48.886mg/kg for the Alajo soil series and between 2.678mg/kg and 51.113mg/kg for Toje soil series for week one (1). They were however 0.365mg/kg % 25.505mg/kg and 0.293mg/g & 25.615mg/kg between weeks three (3) and seven (7) for the Toje and Alajo soil series respectively.

Table 4.11: Total hydrocarbons degradation trend evaluated in milligram per kilogram (mg/kg) of soils samples.

Soil series	Weeks	0% mg/kg	1% mg/kg	2% mg/kg	3% mg/kg	8% mg/kg
Toje series	**Wk 1**	2.698 ± 0.02	11.516 ± 0.02	15.295 ± 0.01	16.093 ± 0.01	51.113 ± 0.02
	Wk 3	0.978 ± 0.30	9.301 ± 0.94	14.484 ± 0.88	15.263 ± 0.12	25.505 ± 2.64
	Wk 5	1.830 ± 0.17	10.126 ± 1.48	13.429 ± 3.45	23.538 ± 4.95	18.247 ± 2.29
	Wk 7	0.365 ± 0.12	2.541 ± 0.63	7.957 ± 1.27	5.138 ± 0.16	22.826 ± 1.8
Alajo series						**10% mg/kg**
	Wk 1	0.641±0.002	5.782 ± 0.25	6.757 ± 0.26	16.537 ± 0.30	48.886 ± 0.01
	Wk 3	1.339 ± 1.52	4.895 ± 0.23	6.775 ± 0.24	6.352 ± 0.16	23.011 ± 2.36
	Wk 5	1.799 ± 0.23	6.358 ± 1.16	10.874 ± 3.25	11.042 ± 1.84	25.615 ± 7.35
	Wk 7	0.293 ± 0.01	7.634 ± 1.07	12.222 ± 2.16	12.892 ± 2.29	21.227 ± 2.59

± Represent Standard Error or deviations from mean values for allreplicate sets of data

4.3.11 Total Hydrocarbons analyses in plants (THC mg/kg of soil)

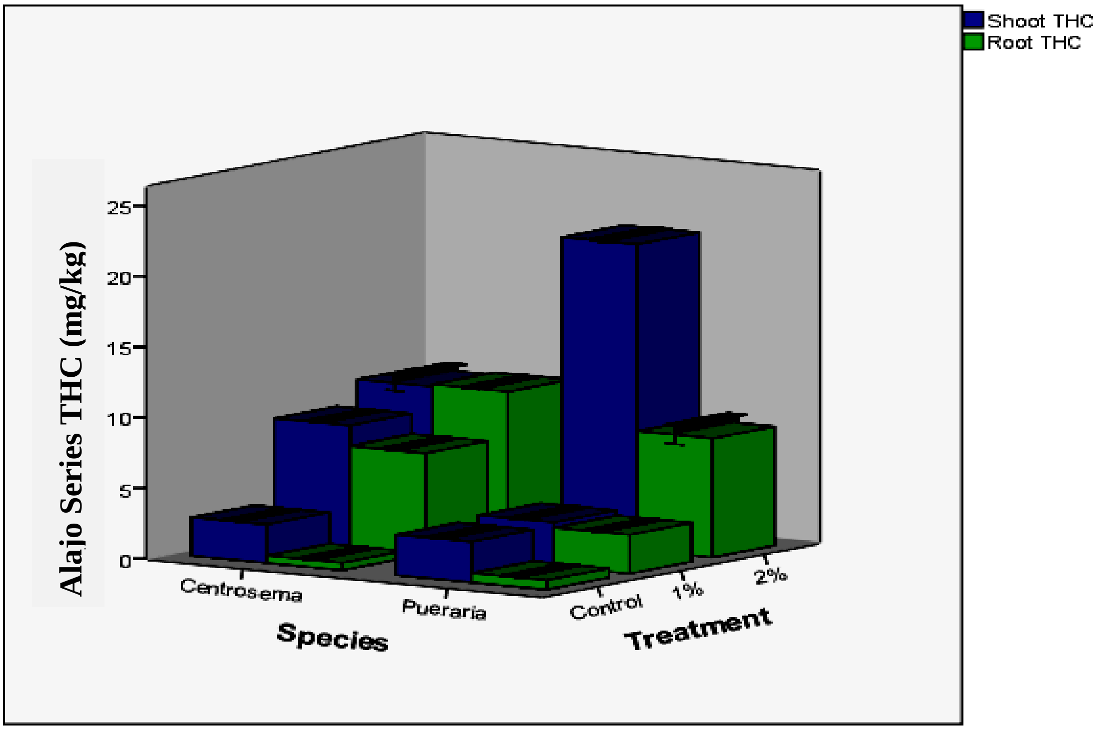
Data on plants' general phytoremediation characterizations (tissue THC content) are presented in Table 4.13 and Figs 4.22 to 4.25. Results of the plantsTHC enrichment indicated that *Cynodon dactylon*had 0.987mg/kg and 4.487mg/kg whereas *Paspalum vaginatum* had 1.179mg/kg and 3.123mg/kg at high contaminant concentration from the Alajo and Toje soil series respectively.

However, at low contaminant concentrations, results indicated that *Cynodon dactylon* had 1.29%, 2.83% and 0.14%, 1% whereas *Paspalum vaginatum* recorded 0.76%, 2.06% and 0.57, 1.27% at 1% and 2% respectively.

Furthermore, data on peak THC degradation/absorption are presented inand Figs 4.26 and 4.27. Results indicated that both *Cynodon dactylon* and Paspalum vaginatum achieved peak THC degradation/absorptionat 5.5% and 8% contamination level in the Toje and Alajo soil series. These results corresponded to total hydrocarbons concentrations of 14.13mg/kg and 8.38mg/kg respectively.

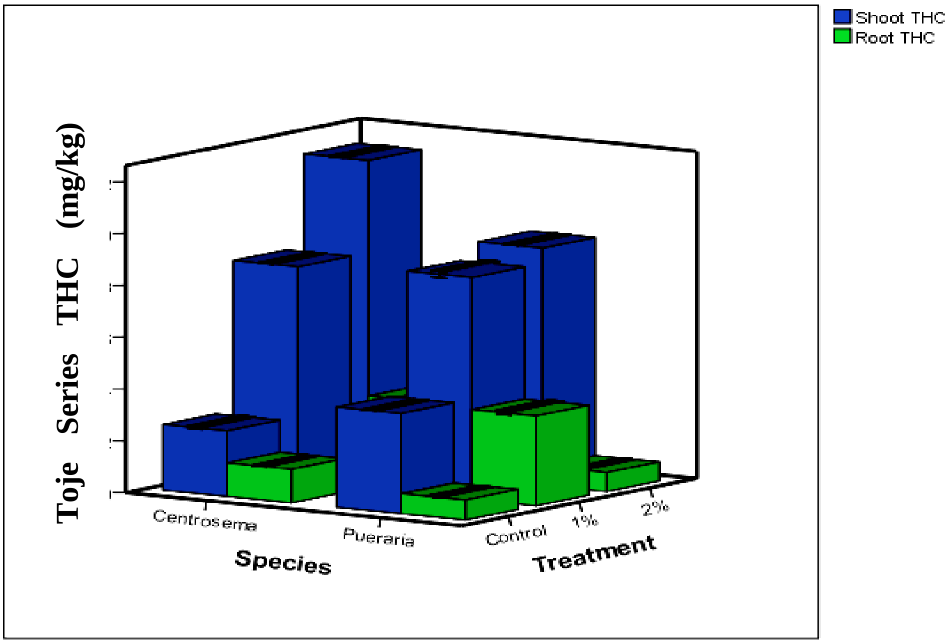
Table 4.12: Total Hydrocarbons content in plants grown on the Toje and Alajo soil series.

Species of High Conc.	Parameters (mg/kg)	Toje series 3%		Alajo series 3%	
C. dactylon	Rhizhofiltration (RF)	0.26		1.516	
	Translocation (TF)	2.734		1.951	
	Enrichment (EF)	0.987		4.487	
	SOIL THC @7WAP	12.62		5.027	
P. vaginatum	Rhizhofiltration (RF)	0.5		1.118	
	Translocation (TF)	1.188		1.793	
	Enrichment (EF)	1.179		3.123	
	SOIL THC @7WAP	13.09		5.249	
Species Low Conc.	Parameters	1%	2%	1%	2%
C. pubescens	Rhizhofiltration (RF)	0.25	0.95	0.1	0.75
	Translocation (TF)	3.75	1.96	1.0	2.73
	Enrichment (EF)	1.2	2.83	0.14	1.0
	SOIL THC @7WAP	2.54	7.96	7.63	12.2
P. phaseoloides	Rhizhofiltration (RF)	0.43	1.32	0.27	0.69
	Translocation (TF)	1.29	1.79	0.88	1.19
	Enrichment (EF)	0.76	2.06	0.57	1.27
	SOIL THC @7WAP	2.54	7.96	7.63	12.2



Error Bars: +/- 1 SE

Fig 4.24: Total Hydrocarbon content in roots and shoots of *Centrosema pubescens* and *Pueraria phaseoloides* grown in the Alajo soil series.



Error Bars: +/- 1 SE

Fig 4.25.Total Hydrocarbon content in roots and shoots of *Centrosema pubescens* and *Pueraria phaseoloides* grown in the Toje Soil series

4.3.12 Biological property

4.3.13 Enumeration of Total fungal and bacterial Populations

Details of total microbial population (TMP) are shown in composite charts in Figs 4.27 to 4.30; Table 4.14 and Plates 10 and11. The total mean microbial population count was 418 x10^5/g for the Toje soil series, and 183 x 10^5/g for the Alajo soil series. Total correlation coefficients of R^2 = 0.8988 & R^2 = 0.9614 at high contaminant concentrations and R^2= 0.9853 & R^2 = 0.4041 at low contaminant concentrations for the Toje and Alajo soil series respectively.

Table 4.13:Influence of varying concentrations of crude oil concentrations on mean bacterial and fungal populations in the Toje and Alajo soils series.

Microbe	Treatment options (%)	Population (x10^5/g) Toje series	Alajo series
Bacteria	0	67	3
	1	41	9
	2	67	4
	3.5	24	46
	8	69	-
	10	-	9
	Total	268	71
Fungi	0	125	44
	1	65	13
	2	83	33
	3.5	57	56
	8	88	-
	10	-	47
	Total	150	112
	Grand total	418	183

X 10^5/g = Hornby's dilution factor for mean total colony of microbial population

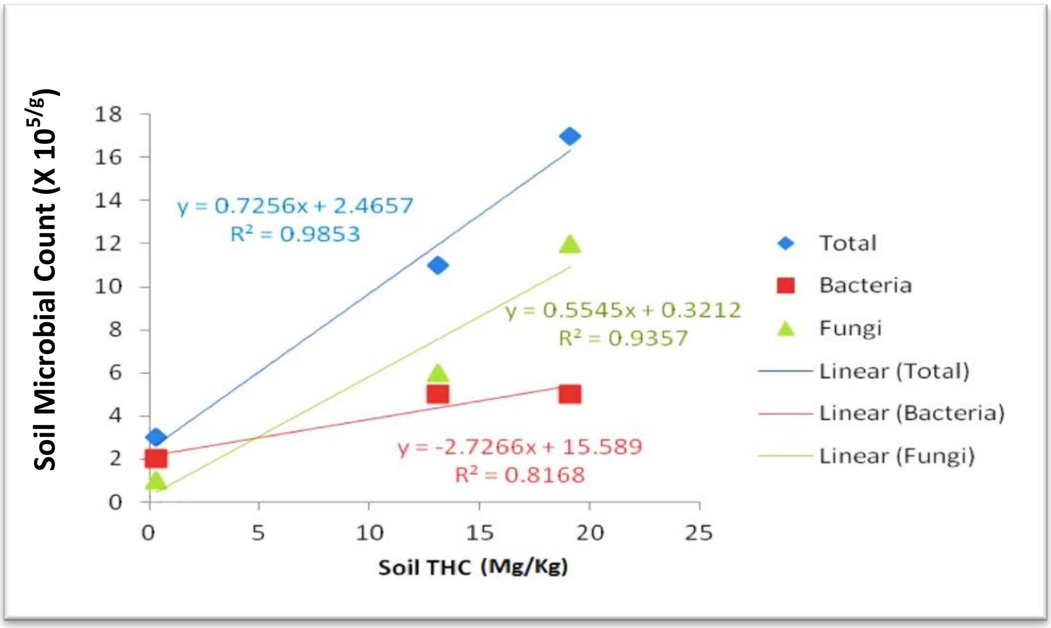


Fig 4.30: Correlation between soil THC and microbial population in the Alajo soil series under vegetative portion of *Paspalum vaginatum*

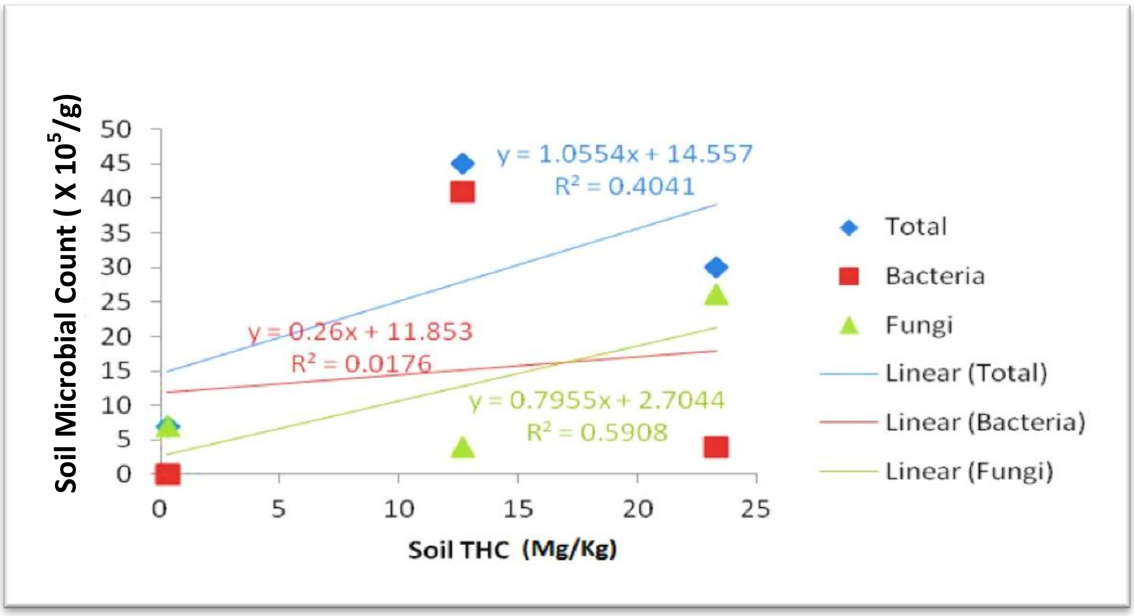


Fig 4.31: Correlation between soil THC and microbial population in the Alajo soil series under vegetative cover of *Cynodon dactylon.*

CHAPTER FIVE

DISCUSSION

5.1 Field work in Niger Delta

5.1.1 Particle Size Distribution, Textural Class determination and GPS locations of pilot survey Sites in the Niger Delta.

GPS locations taken at sites surveyed in the Niger Deltaindicated that the three locations belong to a common region in latitude (NE; 000^0; 5^0 42' 43" for Iriama in Delta State: NE: 000^0; 6^0 26' 33" for Kalaba in Bayelsa State and NE: 000^0;6^0 37' 21" for Ogbogu in Rivers State respectively) and corresponded with the actual locations of the sites in their respective States on the map of Nigeria.

Soil textural classesof sites were basically clay but laced with varying mixtures of silt and sand. This was suggestive of their deltaic origin.This inference is similar to an earlier report by Ayolagha and Dickson (1997), who described the texture of soils of the Niger Delta as a range between sand and clay, but predominantly loamy for agricultural soils; acidic and poorly drained.

5.2 Questionnaire Analysis

5.2.1 Biometric Data of Respondents

A critical analysis of respondents' data revealed as follows:

i) Sex and marital statuses of respondents had no significant influence on the quality of responses or inputs received. Although the influence of this parameter was yet to be reported in relevant literature, this outcome was expected as spills and their pollution effects would occur irrespective of the sex or marital statuses of inhabitants of host communities

ii) Farming and fishing are the major occupations of inhabitants of communities. Therefore, any incident of crude oil spill on farmlands and water bodies would have tremendous effects on the occupations or livelihood of the residents and consequently inflict economic hardship on the local population. These findings have been corroborated by Tanee and Kinako (2008) who reported that oil spill does not only cause immense ecological damage, but also incredible distortion of the livelihoods of inhabitants of the affected areas.

iii) On soil fertility status,educational background of respondents significantly influenced the quality of inputs as their input contradicted earlier reports by the Federal Department of Agriculture and Land Resources – Nigeria (FDALR, 1990) and Ayolagha and Dickson (1997) who reported that the soils of the study area are poorly drained and therefore, poor in nutrients.

iv) Majority of respondents (83%) have lived in the communities for 10 years and beyond suggesting that their viewscould be relied upon as credible; although there appear to be no information on this parameter as yet.

5.2.2 The frequency of spills and impacts on the Environment

i) Spills were confirmed to have occurred severally in communities and impacted areas experienced losses of vegetation of herbaceous plants species, some trees and plants shed leaves while others lost their crowns and died (Plate 3). This observation was corroborated by Tanee and kinako (2008) who reported that despite stringent environmental regulations, the risk of an oil spill devastating ecosystems and biodiversity is still high and must be accepted as inevitable.

ii) Soils of none-remediate sites showed severe signs of cracks on their surfaces and did not look like recovery was imminent (Plate 4). This inference was corroborated by Plice (1948), Dobson and Odu (1972) who jointly reported complete breakdown of structure and dispersion of soil particles when oil was introduced to soil.

iii) Water body contamination was manifest on Mangrove trees as well as Water Hyacinth along the stream of Iriama (Plate 5).This observation finds credibility in the roport of NDC, (undated) which stated that immense tracts of mangrove forests become succeptible and were destroyed; also drinking water is also often contaminated when oil spilled into aquatic environments.

5.2.3 Evidence of fresh growths at sites of contamination

All the species which survived and therfore collected from sites of contamination in the Niger Delta where largely grasses and legumes confirming the submissions of Adam and Duncan (2002) and Merkl *et a.*, (2004 & 2005) that grasses and legumes are the best candidates for the phytoremediation process because of their multiple root ramifications which encourage microbial growth and activity in the rhishosphere.

5.2.4 Land restoration

Although awareness on the possible use of crops to cleanup spilled sites was lacking amongst majority of respondents (83%), none remediate sites (Plate 4) did not show any potential to support and or sustain organic life whereas remediate sites had growth of fresh species on them (Plate 6). This discovery was corroborated in the works of Dibble and Bartha (1979), Wiltse *et al*. (1998) and Ayotamuno *et al*. (2006) who reported that the carbon-hydrogen mixture of hydrocarbons causes its spill on soils to ultimately limit life of plants and animals.

5.3 Phase II: Morphological propertiesof experimental soils on the Accra Plains.

5.3.1 Soil characterization.

Soil characterization established Alajo series as clay and Toje series as sandy clay loam. These textural classifications conformed to the details in the Globe Soil Charaterization Protocol (2005) and finds support in the works of Dowuonna (1985) and Eze (2009).

5.3.2 Crude oil characteristics

As earlier stated, the confirmation of the Bonny Light grade of crude as oil used in study conforms to the American Petroleum Institute (API) and Nigerian National Petroleum Corporation (NNP 2010) specifications (Table 2.1). However, the absence of the appropriate equipment in Ghana and Nigeria was a major constraint to an independent determination/verification of its chemical composition.

5.3.3Crude oil application Rates

The range of crude oil applied in study indicated Total Hydrocarbons (THC) concentrations to have increased in accordance with increasing simulation levels on both soil, but varied significantly between soils at $p \leq 0.05$ even when equal volumes of THC applied. Although it may seem that there is no information as yet on the influence of soils ambient THC levels on contaminant toxicity, this outcome could be attributable to the influence of soil's ambient THC levels which upon determination revealed significant variance in accordance with the soil type.

5.4 Green house determinations

5.4.1 Ambient environmental conditions

Temperature and Relative Humidity regimes in the greenhouse were higher than the Accra mean weather conditions as corroborated by Akutam (2012).

Irrigation rates were inversely proportional to soil's clay status as higher application volume applied to the soil with lower clay status (Toje soil seriea) whereas it (irrigation rate) decreased as clay content increased (Alajo soil series). This observation conforms to the report by Spectrum Analytic Incorporated (2005) where it was stated that the higher the clay content of a soil the higher its water/nutrient retention quotient and the slower the release of same and vice versa.

Ambient Total Hydrocarbons (THC) contents of soils at 0% or control option varied in accordance with the soil series, indicating that the absence of pollution does not suggest the absence of hydrocarbons in the environment. Again, the influence of this parameter is yet to be reported, but the observation was implied as far back as in the year 1981 when Boehm when reported that natural sources of ambient THC included biogenic, petrogenic and pyrogenic means.

5.4.2 Germination of propagules and sprout of seeds

A negative correlation between test plants and crude oil contamination levels was observed in study as mean counts of germination/sprout decreased as contaminant concentrations increased. This observation was normal as plants were expected to perform maximally under ambient growth conditions than under conditions of stress and conforms to the reports of Agbogidi and Ofuoku (2005), and Agbogidi *et al*. (2006), who reported that plants general growth performances were significantly affected at higher levels of oil contamination than at lower concentrations.

One important result of interest in this study was that legumes attained very poor establishment rates within the study period but weeks after, they where observed to have continued sprouting from sown seeds even after the demobilization of the greenhouse segment of studythereby mystifying their choice criterion. Although literature is yet to capture this observation, it may be attributable to delayed germination of un-scarified seeds more than tolerance and extraction potentials. The argument therefore, is that prolonged dormancy may have sustained hard seeds of these leguminous plants on polluted soils long enough for toxicity effects of contaminant to havereduced considerably so that when seeds eventually sprouted, they were able to tolerate contaminant in its current state of low toxicity as it were, and were therefore able to sustain their growth at the sites of contamination.

5.4.3 Growth assessment of test plants

Test plants lost height significantly ($p \leq 0.05$) as the serial crude oil contamination of the Toje and Alajo series of soils increased. This inference was evident in the works of Chaineau *et al.* (1997), Molina-Barahona *et al*. (2005), Ogbo *et al.* (2009), who observed growth rate reduction of beans and wheat by 80%, and of stems, roots and also in shoot height, leaf area and total chlorophyll content of test plants respectively due to immobilization of nutrients by contamination.

5.4.4 Determination of Plant Survival Difference (PSD)

Plant survival difference between weeks one (1) and five (5) had a direct negative correlation as performances of experimental plants decreased with increase in contaminant concentration.*Cynodon dactylon* and *Paspallum vaginatum* species were more tolerant of hydrocarbon contamination than were *Centrosema pubescens* and *Pueraria phaseoloides* on both soils. Although, available literature was yet to report on the plant suevival difference (PSD), this outcome was understandable as experimental soils had tended towards neutrality (Toje soil series) and alkaline (Alajo soil series) towards the close of field study thereby confirming the work of Wallace and Nelson (as cited in Kuperberge 1999) who reported both *Centrosema pubescens* and *Pueraria phaseoloides* (legumes) to betolerant of soil acidity.

5.5 Phase 111

5.5.1 Evapo-volatilization

Correlation coefficient indicated a very positive relationship between surface losses (from soils and plants) and soil crude oil contents on both soils at $p \leq 0.05$, as evapo-volatilization rates increased in accoedance with increasing simulation levels of THC. This observation/inference implies that the more crude oil there is in soil, the higher the loss factor. Although no information exists as yeton evapovolatilization rates as a parameter, the reported correlation was expected as contaminant loss/absorption from any growth matrix under normal environmental condition, should depend on available concentrations within the matrix in question. This inference finds credence in the works of Baker and Brooks (1989), and Zhou *et al.* (2006), who stated that the bioavailability of contaminants depends on the contaminant's concentration in the habitat. Isirimah (2001) also confirmed that plants reaction to soil contamination is genetic and depends on the physical distribution and chemical characteristics of the contaminant.

5.5.2 The effects of serial crude oil contamination on the biomass (g/ww) of experimental plants

A negative correlation between plant biomass and contaminant concentrations was observed as plants wet/fresh weights decreased with increase in contaminant concentration on both soil series. Consequently, the high contaminant concentration plant species (grasses) could only tolerate contaminant concentration at 3% on both soils since they could not yield sufficient critical mass for the biomass determination at 8% and 10% on Toje and Alajo soil series respectively- a development that was attributable to the THC toxicity effect. This observation is similar to those reported in the works of Baker and Brooks (1989), Zhou *et al.* (2006), Chaineau *et al*. (1997), Brandt *et al*. (2006) and Ogbo *et al*. (2009) who reported that biomass, plant height and specific root surface were significantly reduced under the influence of petroleum oil.

5.6.1 The effects of crude oil on the chemical properties of experimental soils

i) Soil THC

Mean values of weekly THC degradation increased significantly (at $p \leq 0.05$) as serial crude concentrations increased between weeks one (1) and seven (7) indicating a strong positive relationship between crude oil degradation and time. This observation is corroborated in the work of Kogbara (2007) who reported that degradation rate tends to increase as the treatment period increases especially after some weeks of remediation treatment.

However, there was a sudden upsurge in themean values of THC concentrations in week 5 across board and week 7 at 1% & 3% on Alajo soil. This was not expected as crude oil was applied just once throughout the duration of the study. This observation may have been induced by poor development of test plantsand high activity clay (HAC) content which could also have induced low oxygen (anoxic) supply within the soil structureenough to impede contaminant oxidation and degradation. This inference was corroborated by Vance (2002), and Ayotamuno and Kogbara (2006) who suggested that the sudden increase in contaminant concentration was probably an effect induced by the high levels of bacterial activity stimulated in the hydrocarbon rich soil. They argued further that the bacteria active in the degradation of the contaminant excrete extra surfactant-like polymers during their metabolic cycle which in turn mobilized the hydrocarbon concentration to levels physically higher than those in the soils from which the samples were collected.

The general increase in hydrocarbon concentration levels observed at week five on both soils could also be attributable to increased biological oxygen demand (BOD) caused by the activity of bacteria. This position was corroborated by Kogbara (2007) who reported that at the initial stage of his study, soils contained adequate oxygen but as remediation progressed affected options were no longer receiving regular tillage thus anoxic conditions resulted.

ii) Soil pH

pH values on treated soils were higher than those of the control for both the Alajo and Toje soils series. The pH range of between 6.7 and 7.2 for Alajo soil series was good for hydrocarbon mineralization since most bacterial capable of metabolizing hydrocarbon develop best at pH condition close to neutral (6-9). This observation was corroborated by the worksof Atlas and Bartha (1992), Manuel *et al*. (1993), Aman (2004), Andrade (2004), Ayotamuno and Kogbgara (2006), and Njoku *et al.* (2009) who reported positive correlations between soil pH and THC, as pH was reported to have increased in soil with increase in crude oil concentrations.

pH in the range of 4.5 – 5.3 was the values forthe Toje series before soil was simulated with crude oil. This pH range has implications for nutrient availability in the oil-polluted pots as it could have inhibitive effect on the solubility of minerals. This result/observation was corroborated by Manahan (1994) who reported that strongly acidsoils with the pH range of 4–5 usually have high concentrations of soluble aluminum and manganese which are toxic to many plants; Nitrogen fixation and decomposition activities were also reported to have been inhibited. Also, crude oil degradation time-line could have influenced final pH values because mean pH values for all treated options in both the Alajo and Toje soil series increased as degradation time increased. This observation finds support in the reports of Andrade *et al*. (2004), Ayotamuno and Kogbara (2006) and Njoku *et al.* (2009).

iii) Electric conductivity (EC).

Although Electrical Conductivity values declined as degradation time increased on both soils, contamination of soils with crude petroleum oil may have been directly responsible for the observed changes. This finding was similar to that in the work of Xiao-yu *et al.* (2009) who reported that soil petroleum contamination resulted in the increase of the EC. Also, Osuji & Nwoye (2007) reported that organic compounds like crude oil do not conduct electrical current very well. Again, Aman (2004) reported that application of crude oil to soil did not show any effect on soil EC. Although the time between sampling andcontamination was not indicated in their reports, it is possible from finding that if degradation period had prolonged beyond seven weeks in this work, EC could have been reduced to insignificant levels in contaminated soils when compared to control options.

iv) Cation exchange capacity (CEC)

CEC had a positive correlation with the Alajo soil series as cation exchange capacity (CEC, 31meg/100g) increased with increase in clay contents and vice versa on Toje soil series (24meg/100g). System Analytic Inc. (2005) corroborated finding when it reported that a significant percentage of the CEC of most soils are due to clay and organic matter; that these soil fractions have large numbers of negative charges on their surfaces thus, they attract cations and contribute to a higher CEC, whereas they also repel anionic nutrients. Therefore, the loss of organic matter and acidification in (low claycontent) the Toje soil series was also corroborated by Kang and Juo (as cited in Spectrum Analytic, 2005) to result to decrease in the effective cations exchange capacity (ECEC).

v) Total organic carbon (TOC)

Total organic carbon content had a positiverelationship between soil and contaminant concentrations for both Alajo and Toje soil series (at $p< 0.05$) as organic carbon concentrations increased with increase in crude oil concentrations. This observation was healthy and surported by April and Simms (1990) and Njoku *et al*. (2009) who concluded that organic carbon contents improved the binding processes and water retention ability of soils, as well as a proven dependable source of energy necessary for microbial growth and development. It conforms to reports by Wikipedia (2010) and Canadian Center for Energy Information (2011) which seperately reported that crude oil is made up of carbon and hydrogen in proportions of about 83-87% for carbon and 10-14% for hydrogen.

vi) Carbon nitrogen ratio (C: N)

C: N ratio varied significantly throughout the study period from initial control values of 3:1 to as high as 158:1 for Alajo series at 3% and from 4: 1 to 320: 1 for Toje soil series at 8% contamination levels, respectively. The observed variations in C: N ratio may be attributable to the increase in microbial activity of the carbon utilizing class- since microbes are known to be heavy carbon utilizers. This position was corroborated by April and Simms (1990), Njoku *et al*. (2009) and Jobson *et al*. (1974) who reported changes in C: N ratio when large volume of hydrocarbon spilled unto soils causing depletion of soil nitrogen. Carbon utilizer (Azobacter) was reported by the same researchers to have increased at the expense of nitrifying bacteria –Nitrosomonas consequently. Dibble and Bartha (1979) also corroborated finding by repoting decreases in available nitrogen as crude oil concentration levels increased in test soils.

5.7 Biological property

5.7.1 The effects of THC on microbial population.

Enumeration of microbial population indicated both positive and negative correlations at high and low contaminant concentrations. This seems to suggest that results were influenced in no particularly order but probably by the interaction of the individual plant with the contaminant on one hand and the plant with the growth matrixes on the other hand within the rhizosphere. These inferencesalso confirm the widely held opinion that plants react differently to contaminant stress which was corroborated by Gallego *et al*. (2000) and Kogbara (2007). Furthermore, they argued thatthere was a general increase in bacterial numbers as remediation treatments progressed. Besides, the mere presence of microbes in polluted soils would no doubt have significantly influenced contaminant degradation. This assumption was reinforced by the report of Frick *et al*. (1999) where it was stated that the stimulation of the growth and activity of degrading organisms in the rhozhosphere remains the single but very important factor responsible for most contaminant degradation at polluted sites.

5.8 Evaluation of test plants for phytoremediation potential

5.8.1 Total Hydrocarbons (THC-mg/kg) content in plants species

Plant THC analysis indicated thata positive correlation existed between tissue THC and soils contaminant concentrations as accumulation trends in plants increased with increase in simulation levels in soils. Also, at high contaminant concentration, plants exhibited poor tolerance to contaminant stress andfailed to yield the needed critical biomass for THC analysis beyond 5% and 8% for theToje and Alajo soil series respectively. This inference was corroborated by Merkl *et al*. (2004) who suggested that the understanding of plants reaction to contaminant stress was key to the successful application of the phytoremediation technology.

5.8.2 Phytoremediation Classification of plants species: assessment of THC accumulation potential in the tissues of grasses and Legumes.

Test of Multiple analyses of variance (MANOVA) ran on plant THC data indicated that accumulation trends increased with increase in contaminant concentration levels in the soil as test plants succeeded in absorbing contaminant from the substrates into their root cells and also trans-located part of it to their above ground tissues (stems and leaves). To this end, the results of the THC concentration assessed in the below (roots) and above ground portions of the plants as well as soils indicated that test plants were able to store more contaminant concentrations in their above ground tissues than in their below ground tissue; which made them better phyto-extractors than rhizo-filtrators. *Cynodon dactylon* posted the higher shoot THC value of 14.13mg/kg on Toje soil, than *Paspalum vaginatum* which recorded the lower shoot THC value of 8.38mg/kg on Alajo soil.

THC values of 21.67mg/kg and 11.89mg/kg were accumulated in the shoots of *Centrosema pubescens* at 2% on Alajo and Toje soils respectively for leguminous plants at low contaminant concentrations. These findings may have resulted from the bioavailability of contaminant to plants' uptake andinfluenced by ambient THC levels as earlier stated. Findings have been corroborated by Isirimah (2001), who reported that in a normal plant translocation process, water, mineral and contaminants taken up by plants from growth matrixes move upward through the xylem tissues in a normal standard and acceptable plant production process; and by Agunbiade *et al*. (2009) who also reported that the success of phytoremediation of a contaminant depends ultimately on the bioavailability of contaminant to plants uptake in a complex interface interaction influenced by climatic conditions prevalent at the site of contamination. Also, by Ernst (1996), Lasat (2002), Harun *et al*. (2008), and Wei *et al*. (2009), who argued that these findings make phytoremediation more site specific than generic.

5.8.3 The determination of THC tolerance and extraction limits for test plants.

THC contamination tolerance and degradation (extraction) at 7WAP revealed that plants absorption and accumulation of contaminants in their tissues increased as simulation percentages/levels increased. Peak degradation was at 5.5% on the Toje soil series and 8% on the the Alajo soil series. Beyond this contaminant concentration levels, degradation decreased at rates particular to the plant and soil type as well. This observation is attributable to plants exposure to THC contamination as *Paspalum vaginatum* failed to produce the critical mass necessary for tolerance/extraction determination beyond the 5.5% mark on the Toje soil series, but was able to tolerate contaminant concentration upto 8% on the Alajo soil series where toxicity was lower.

Cynodon dactylon could tolerate contaminant concentrations at 5.5% and 8% on the Toje and Alajo soil series respectively. This result implied that *Cynodon dactylon* and *Paspalum vaginatum* could tolerate and degrade contaminant (hydrocarbons) concentrations from growth matrixes contaminated up to 33.270mg/kg of low clay activity soil and 40.466mg/kg of a high clay activity soil. This finding was corroborated by Isirimah (2001), as proof of appropriate biological condition to potentially aid plants in the remediation of crude oil contamination of similar concentration levels in a phytoremediation trial.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summaryof findings

Emanating from the results obtained from the study and the subsequent discussions that followed, the following are presented as summary of findings:

i) Inferences from inputs of respondents through oral interview and answers to questionnaire during the site tours in the pilot study indicated that majority of the respondents were married /adults (51%/65%) who have lived in host communities for 10 or more years and above and as such their imputs were highly credible.

ii) Farming and fishing are the primary occupations of residents in the study areas; although these occupations (ie, their activities) are threatened by frequent crude oil spillage through operations of the oil companies.

iii) Educational qualification of respondents influenced the quality of their inputs

iv) The prominent texture of soils sampled from the sites visited was clay laced with varying content of silt and sand.

v) All the respondents (-i.e 100% of them) indicated that oil exploration and exploitation activities have had negative impacts on the socio-econiomic and cultural norms in all the study sites; and these include:

a) High levels of unemployment.

b) Disrespect for elders, customs and institutions of study area by the youth.

c) Sex trade/Prostitution and teenage pregnancy among girls; seduction and harassment of women by security operatives and high rates of divorce.

vi) Biophysical features that were negatively affected include:

a) Vegetative distortion and loss

b) *Land degradation with cracks on soil surface at unremediated sites*

c) *Contamination of water bodies*

d) *Loss of human lives and other organisms.*

vii) Physical observations made during the field tour and inputs from respondents indicated that scoop and burn or dig and bury were the choice cleanup measures adopted by conssessionaires (-i.e companies); and these measures merely/basically left oil spilled soil surfaces temporarily free of crude oil because each and every inundation arising from/after any precipitation released absorbed crude oil to the surface of the soil.

Furthermore, unremediated soils did not only show/ exhibit serious surface cracks and deformation but were incapable of supporting any form of life.

ix) Remediated soils were tilled and treated *in-situ* with "Dust exorbents" which practically left the contaminant within the same environment and could support growth of fresh vegetation.

x) Plant samples collected from sites visited during the pilot phase were dominated by grasses and legumes. A total of fifteen plants were collected and identified.

xi) Aftermath of impacts of crude oil spillage include the loss of farmlands with the attendant waiting for meager handouts paid as compensation which always took very long times in coming or to recieve.

xii) The effects of frequent crude oil spillage and impoverishment of residents in the Niger Delta have led to the taking of foreign staff of multinational companies as "hostages" in addition to militancy among the youth from the region.

These developments have attracted global attention and also forced the Federal Government of Nigeria to put together an armnesty programme of reformation, rehabilitation and reintegration of restive youth back into societies.

xiii) Characteristic features of plants such as: leaf burn, wilting, stunted plant growth and even outright loss of vegetation after crude oil spillage were common features observed in the toured sites.

xiv) Local people lacked information on the use of plants in clean-up operation after crude oil spillage (-ie, phytoremediation); although they are/were excited about its potential value and were looking forward to it.

xv) Six (6) plants consisting of three (3) grasses (*Paspalum vaginatum*, *Panicum maximum* and *Cynodon dactylon*) and three (3) legumes (*Centrosema pubescens*, *Pureria phaseoloides* and *Schrankia leptocarpa*) were selected for phase 11 of the research project.

xvi) Based on the outcome of an initial screening study which used "Plant Survival Difference/Differential" (PSD) as a criterion, indicated (after treatment with varying concentrations of crude oil) that *Paspalum vaginatum* ranked overall best, followed by *Cynodon dactylon.*

Both *Centrosema pubescens* and *Pureria phaseoloides* performedpoorly in the initial screening but were included in the next stage of the project on the assumption that because they are leguminous plants, they should potentially correct for nitrogen deficiency in soils caused be crude oil spillage and contamination if they are able to survive contamination stress.

Xvii) *Paspalum vaginatum* and *Cynodon dactylon* are highly tolerant of hydrocarbons contamination and could therefore be good plants for further phytoremediation trials at the 1% and 10% treatment option adopted for Alajo soil series and 1% and 8% for Toje soil series.

xviii) Legumes did not thrive well on clay (Alajo) soil series compared to their performance on the sandy clay loam (Toje) soil series.

Centrosema pubescens and *Pueraria phaseoloides* are acid soil-loving plants.

Clay soils have been reported to impede root growth and establishment.

xix) Remarkable variations in physical and chemical properties exist between the two (2) soils used in the study (Toje soil series and Alajo soil series).

Alajo soil series had higher surface bulk density of 1.3mg/m^{-3}; porosity of 50.9% and a Cation Exchange Capacity (CEC) of 30.6 meq/100g compared to 1.2mg/m-3 for soil bulk density; 54.0% for porosity and 2.3meq/100g for CEC recorded by Toje soil series.

xx) Textural classes also varied from clay on Alajo soil to sandy-clay-loam on Toje soil series.

Alajo soil series is a High Activity Clay soil (HAC).

HAC soils are those with good nutrient retention and release potential, poor leach ability and very good effective cation exchange capacity (ECEC) and these properties were reinforced by the relatively higher soil bulk density, reduced porosity and relatively higher CEC values of the Alajo soil series

Toje soil series is a Low Activity Clay soil (LAC).

LAC soils are those with poor nutrient retention and release potential, higher leach ability and very poor ECEC. These qualities were further evident in the relatively lower values of surface bulk density, porosity and CEC recorded in the Toje soil series.

Overall, these qualities influenced THC retention, bioavailability and consequent uptake by the experimental plants because final THC values were higher for Alajo (mean serial THC 13.5mg/kg) than Toje soil series (mean serial THC 9.6mg/kg).

xxi) Similarly, THC enrichment factor was higher for Toje soils (4.49% for *Cynodon dactylon* and 3.12% for *Paspalum vaginatum*) than for Alajo soils with corresponding values of 0.99% and 1.98% for both plant species.

Low porosity medium would naturally experience higher surface gradient flow from logging in the event of heavy crude oil spillage and a possibility of contamination for adjoining land masses; whereas high porosity medium holds possibilities for crust and ground water contamination.

xxii) Plant Survival Difference (PSD) parameter used as an analytical index in evaluating contaminant tolerance indicated that there was a systematic decline/decrease or negative relationship between PSD and concentration of crude oil contamination - i.e., the observed decline/decrease in PSD was correlated with contaminant concentration; and was highest at peak contaminant concentrations of 10% and 8% for Alajo and Toje soil series, respectively.

xxiii) On Alajo soil series, *Centrosema* and *Pueraria* Recorded 6% and 16% survival count respectively at the 10% concentration treatment; whereas on Toje soil series, both plant species had 14% and 6% as lowest PSD values at the 8% concentration treatment level followed by *Pueraria* and *Centrosema* spp with 25% and 15%, respectively.

xxiv) Generally, legumes had very poor establishment records within the study period. However, these plant species (*Pueraria* and *Centrosema* species)were observed to have continued sprouting and their seeds germinating even after the formal demobilization/completion of the experimental phase seven (7) and eight (8) weeks after planting respectively – i.e,7WAP and 8 WAP. However, germination data of *Pueraria phaseoloides* and *Centrosema pubescens* on both soil series (41% and 23% respectively) and (25% and 40%), respectively for Alajo soil series and Toje soil series clearly indicated that delayed germination of unscarified seeds and sprouting of their seedlings rather than their tolerance and extraction potentials may have been responsible for the survival and presence of these species on soils of sites that were visited during the pilot survey phase of this research project.

In other words, prolonged dormancy may have sustained hard seeds of these leguminous species on crude oil-polluted soils long enough for the toxicity effects of crude oil-contaminated soils to have waned considerably so that when the seeds germinated eventually, they were able to tolerate the contaminant at its relatively low toxicity; and thus be able to sustain their groth.

xxv) There was a strong positive correlation between Total Hydrocarbons content (THC) of the experimental or test soils and surface loss of contaminant/crude oil (R^2 = 0.997 for legumes and grasses grown on Toje soil series and R^2 = 0.892 and R^2 = 0.641 for grasses and legumes grown on Alajo soil series; implying that evapovolatilization rates increased with increase in serial crude oil simulation levels.

xxvi) Large counts of fungi and bacteria were observed in all soils used for study indicating that crude oil contaminant concentration applied in the different treatment options in this study were not sufficiently toxic as to hinder their growth: considering that phytoremediation of crude oil contaminated soils has been defined as a synergy between micro-organisms and plants.

The total mean microbial population was 418 x 10^5/g for Toje soil series and 183 x 10^5/g for Alajo soil series; and could have influenced contaminant degradation positively. This difference in mean microbial population index was attributable to the loose and compact nature of the Toje and Alajo soils, respectively.

xxvii) Results of PSD and THC degradation were inversely proportional. Whereas *Paspalum vaginatum* had a higher PSD index, *Cynodon dactylon* posted a better phytoremediation potential.

6.5 Conclusion

Results emanating from the study and the discussions which followed indicated that germination and sprouting of selected grasses and seeds of leguminous plants were affected by serial crude oil concentrations. Consequently, a positive correlation was established between sequential crude oil contamination and species' absorption/uptake of contaminant (THC enrichment factor). This conclusion was arrived at because plants were able to absorbed and accumulate up to 33,270mg/kg at 5% and 40,466mg/kg at 8%, of total hydrocarbons (THC) in the Toje and Alajo soil series respectively. Also, experimental plants established their potential as better phyto-extractors than they were rhizho-filtrators and by so doing confirmed the efficacy of the phytoremediation technology and validated the study's *Alternate Hypothesis* (H_1) which stated that "All or some of the selected plants species for this study have the potential to tolerate and extract petroleum hydrocarbons from soil contaminated with crude oil".

6.6 Recommendations

Emanating also from the results obtained from experimental studies and the subsequent discussions, the following recommendations are made;

i) That the social vices observed to be associated with the oil and gas industry in Nigeria should serve as a useful precautionary lesson to the emerging oil and gas industry in Ghana and elsewhere.

These vices include:

- *Prostitution (sex trade)*
- *Teenage pregnancy*
- *Disrespect of elders, social institutions and cultural norms by the youth.*

Some parliatives to the above vices would include:

- *Skills acquisition awareness and enlightenment campaigns on the dangers inherent in indulgence in such vices*
- *Investments in the proper education (circular) of the youthfull population and*
- *The creation of employment opportunities.*

ii) Improvement of company-community relations such as

a) *Facilitating reportage of spills and reduced industrial reaction time*

b) *Timely payment of appropriate compensation to oil-spilled and pollution-affected communities.*

c) *Making geuine and concerted efforts at remediating crude oil polluted soils* *back to pre-spilled conditions early enough.*

iii) All phytoremediation field studies and petroleum oil industry production processes should be preceded by site characterization in a pre-commencement Environmental Impact Assessment.

iv) Further germination tests/evaluations of unscarified seeds of leguminous plant species should be carried out under varying conditions of crude oil contamination to verify and establish their tolerance potential to crude oil contamination.

v) Adherence to standard plant selection criteria for phytoremediation should be observed in order to prevent possible introduction of new pests and diseases into effected crude oil-polluted areas/sites.

vi) More site-specific research and development efforts should be carried out in order to obtain more reliable data for successful application of the phytoremediation process.

vii) A soil clay activity content as a novel parameter that was used in this study in searching for appropriate site specific phytoremediation methods/technologies always should be considered.

viii) The Percentage Plant Survival Difference/Differential (% PSD) parameter that was introduced in the execution of the experiments in this study should be considered in the determination of appropriate phytoremediation tolerant- plant- species for potential use in post crude-oil spill clean-up efforts

ix) The possible role of leguminous plants (such as those used in this study) should be further investigated in order to establish their use in the restoration of the Carbon: Nitrogen (C: N) ratio etc during phytoremediation.

x) Because ambient Total Hydrocarbons content (THC) of soils vary among soil types, it is essential that this parameter should be determined in order to effectively assess the extent of ecosystem damage in the search and development of site-specific phytoremediation methods/technologies.

xi) Both *Cynodon dactylon* and *Paspalum vaginatum* found in this study to be able to tolerate and degrade crude oil contamination up to 33.3mg/kg on a low clay activity soil and 48.466mg/kg on a high clay activity soil in tropical climates should be subjected to further field trials in phytoremediation studies in tropical climates.

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APPENDICES

Appendix I

I: The Questionnaire.

UNIVERSITY OF GHANA, LEGON.
ENVIRONMENTAL SCIENCE PROGRAMME

A QUESTIONNAIRE ON SOCIO-CULTURAL VIEWS ON CRUDE OIL POLLUTION; IMPACT AND CLEANUP MEASURES IN THE NIGER DELTA AREA OF NIGERIA.

Dear Respondent,

The administrator of this questionnaire is a research student at the University of Ghana, Legon, seeking general information on the socio-economic and ecological impact of Crude Oil exploration and exploitation in the Niger Delta Area of Nigeria. Therefore, whatever information you provide shall be treated with confidentiality and used only for academic purposes.

Thank you.

BACKGROUND INFORMATION

TOWN:…….L.G.A:……………………… STATE

Date: ☐☐ ☐☐ ☐☐☐☐

PERSONAL INFORMATION

1.0 NAME: ……………………………………….. SEX: ……. AGE: …… MARITAL STATUS: (a) married (b) single (c) separated (d) divorced

2.0 EDUCATIONAL QUALIFICATION: (a) None (b) Primary (c) Mid/JSS (d) SSS (e) Poly/ University (f) Others-Specify ……………..

3.0 OCCUPATION: (a) Farmer (b) Fisher (c) Trader (d) Civil/ Public Servant (e) Student (f) Self Employed (g) Others-Specify ………………

4.0 How long have you lived in this locality? a) Below 5 years b) 5-10 yrs c) above 10 yrs.

5.0 What is the general occupation of your people (a) farming (b) fishing (c) trading (d) Civil/Public Servants (e) Others-specify ………………

6.0 What soil type do you have in your area? (a) Is it productive? YES/NO (b) Is it stony? YES/NO (c) What is its colour? ………… (d) Other qualities please specify …………

7.0 How do you rate the fertility status of the soils of your area? (a) Very fertile (b) fairly fertile (c) not fertile

8.0 What farming system(s) do you practice in your area? (a) Crop rotation (b) bush fallow (c) continuous cropping (d) mixed farming/cropping (e) others specify…. …

9.0 Has your choice of farming system improved soil status/fertility in any way? (a) Yes (b) No

9.1 If yes, in what way?..

10.0 Do people in your area use fertilizers? (a) Yes (b) No
10.1 Why?..

11.0 Do they use Manure? (a) Yes (b) No

11.1 Why?...

12.0 Does your community host crude oil exploration/ exploitation operations (a) Yes (b) No

13.0 Has your community experienced crude oil spillage before? (a)Yes (b) NO

14.0 If yes, how many times? (a) Once (b) twice (c) thrice (d) others – specify….

15.0How long did it last (a) Days (b) Weeks (c) Months (d) Others specify ………

16.0What was the impact on socio-cultural life of the community (occupation, taboos, customs etc)? a) Positive b) negative c) indifferent d) others- specify…..

17.0 Any effect on (a) Soil? Yes/ NO (b) Vegetation? Yes/ NO (c) Water? Yes/ No d) Animal life? Yes/No

18.0 What cleanup measure(s) was/were adopted? (a) Dig & Burn (B) Detergents (c) Mechanical (d)Others - specify ………………..

19.0Were you involved in the clean-up as a: (a) community (b) group (c) individual (c) others ….

20.0a) Did any plant(s) regrow/regenerate naturally on the clean-up site? Yes / No

b) If yes, can you name any of such plants:……………………………….

c) Is any of them edible? Yes / No

d) If yes, can you please name it / them………………………………….

21.0 Was your Land restored back to its original or close to its original state? (a)Yes (b) No

22.0 Are you aware of the possible use of local crops to cleanup oil contaminated sites? (a) Yes (b) No

23.0 If no, how would you welcome the news of the use of crops to restore oil contaminated sites? (a) Very well (b) well (c) badly (d) indifferent

24.0 How would you feel being part of the process of site restoration using crops? (a) Very well (b) well (c) badly (d) indifferent

25.0 Any other relevant information you may want to provide?................................

Thank you.

…………………………………………………………

Name/Signature of Administrator

II: Questionnaire analysis

INFO/QUESTIONS	OPTIONS	DELTA	BAYELSA	RIVERS	TOTAL	%
SEX	Male/Female	10/6	32/nil	32/3	74/9	89/10
AGE	a. 1-19	-	-	6	6	7
	b. 20-39	10	6	13	29	34
	c. 40 ->	6	26	16	48	57
MARITAL STATUS	Married/Single	7/9	26/6	21/14	54/29	65/34
EDUCATIONAL QUALIFICATION	a. None	-	-	-	-	-
	b. Primary	-	-	6	6	7
	c. MID/JSS	6	5	8	19	22
	d. SSS	6	9	10	25	30
	e. Tertiary	4	18	11	23	27
OCCUPATION	a. Farmer	4	13	11	28	33
	b. Fisher	4	13	-	17	20
	c. Trader	-	-	8	8	9
	d. Civil/public	-	3	-	3	3
	e. Student	6	3	9	18	21
	f. Self Employ	2	-	7	9	10
DURATION IN LOCALITY	a.<5yrs	2	6	-	8	9
	b.5-10yrs	2	-	5	7	8
	c.>10yrs	12	26	30	68	81
GENERAL OCCUPATION OF PEOPLE	a. Farming	12	30	19	61	73
	b. Fishing	4	2	8	14	16
	c. Trading	-	-	6	6	7
	d. Civil/public	-	-	2	2	2
	e. Others	-	-	-		-

Analysis continued

SOIL TYPE IN LOCALITY	a. Productive? Yes/No b. Stony? Yes/No c. Colour?	10-Yes 6-No 16-No 14-Dark grey/brown/black	32 - 32 9 Brown, red, white	21 14 35 35 Brown, red,white	63 20 83 58	75 24 100 69
SOIL FERTILITY RATING	a. Very fertile b. Fairly fertile c. Not fertile	12 4 -	21 6 5	15 12 8	48 22 13	57 26 15
FARMING SYSTEM IN PRACTICE	a. Crop rotation b. Bush fallow c. Continuous cropping d.Mix farming	6 10 - - 10	5 8 19 -	8 9 - 18	19 27 19 - 28	22 32 22 - 33
ANY CONSEQUENT IMPROVEMENT ON SOIL STATUS?	a. Yes b.No Why?	14-Soil enrichment 2-No reason	28 enrichment 6 No reason	20-Soil enrichment 16- No reason was given	62 24	74 28
DO LOCALS USE FERTILIZER?	a. Yes b. No	2-No fertile soil	- 32- Soil is	5- Soil not fertile	7 76	8 91

	Why?	14 Fertile Soil	fertile/fund / awareness	30-Soil is fertile		

Analysis continued

DO THEY USE MANURE?	a. Yes	2	-	5-Soil not fertile	7	8
	b, No Why?	14-Soil is fertile	32-good soil/awareness	30-Soil is fertile	76	91
DOES YOUR COMMUNITY HOST CRUDE OIL OPERATIONS?	a. Yes	16	32	35	83	100
	b. No	-		-	-	
ANY SPILL EXPERIENCE?	a. Yes	16	32	35	83	100
	b. No					
IF YES, HOW MANY TIMES?	a. Once	-	-	2	2	2
	b. Twice	14	-	2	16	19
	c. Thrice	2	-	-	2	2
	d. Others	-	32 severally	31 severally	63	75
DURATION OF SPILL	a. Days	3	6	-	9	10
	b. Weeks	10	-	10	20	24
	c. Months	3	28	25	56	67
	d. Others	-	-	-	-	-
IMPACT ON SOCIO-CULTURAL/GEO-PHYSICAL FACTORS	a. Positive	1	-	4	5	6
	b. Negative	14	32	29	75	90
	c. Indifferent	1	-	2	3	3
WHAT CLEANUP MEASURES WERE ADOPTED?	a. Dig & Burn	12	28	26	66	79
	b. Detergents	-	-	-	-	-
	c. Mechanical	4	4	6	14	16
	d. Others		-	3	3	3

				unspecif ied		

Analysis continued

HOW WERE YOU INVOLVED?	a. Community b. Youth group c. Individual	12 - 4	8 20 6	3 18 14	23 38 24	27 45 28
WHAT CROPS REGENERATED, ANY, EDIBLE?	Names	Cassava, Palm, Rubber, Centroce ma, Cotton.	Raffia, cassava,rub ber	Cassava, Elephant grass	Cassava Palm/raf fia Rubber Centroc ema Cotton Elephant grass	
LAND RESTORATION	a. Yes b. Not yet c. No	4 4 8	8 - 24		18 13 52	21 15 62
AWARENESS ON USE OF LOCAL CROPS	a. YES b. No	2 14	- 32	12 23	14 69	16 83
ACCEPTANCE OF USE OF LOCAL CROPS	a. Very well b. Well c. Badly d. Indifferent	2 5 9	17 10 - 5	16 3 6 10	35 18 15 15	42 21 18 18

APPENDIX III

I: Some merits and drawbacks of phytoremediation processes

Advantages	Disadvantages / Limitation
Amendable to a broad range of organic and inorganic contaminants including many metals with limited alternative options.	Restricted to sites with shallow contamination within rooting zone of premeditative plants; ground surface at the site may have to be modifies to prevent flooding or erosion.
In Situ I Ex Situ application possible with effluent/soil substrate respectively; soil can be left at site after contaminants are removed, rather than having to be disposed or isolated.	A long time is often required for remediation; may take up the treatment is generally limited to soils at a meter from the surface; soil amendments may be required.
In Situ application decrease the amount of soil disturbance compared to conventional methods; It can be performed with minimal environmental disturbance; topsoil is left in usable condition and may be reclaimed for agricultural use; organic pollutants may be d e graded to CO_2 and H_2O, removing environmental toxicity.	Restricted to sites with low contaminant concentration; the treatment is generally limited to soils at a meter from the surface and groundwater within a few meters of the surface; soil amendments may be required.
Reduces the amount of waste to be land filled (up to 95%), can be further utilized as bio-ore of heavy metals	Harvested plant biomass from phytoextraction may be classified as a hazardous waste hence disposal should be proper.
In Situ applications decrease spread of contaminant via air and water; possibly less secondary air/or water wastes generated than with traditional methods.	Climate conditions are a limiting factor; climatic or hydrologic conditions may restrict the rate of growth of plants that can be utilized.
Does not require expensive equipment r highly specialized personnel; it is cost-effective for large volumes of water having low concentrations of contaminants; it is cost effective for large areas having low to moderately contaminated low moderately contaminated surface soils.	Introduction of non-native species may affect biodiversity.
In large scale applications the potential energy can be utilized to generate thermal energy; plant uptake of contaminated groundwater can prevent off-site migration.	Consumption/utilization of contaminated plant biomass is a cause of concern; contaminants may still enter the food chain through animals/insects that eat plant material containing contaminants.

Source: Schwitzguébel (2000)

APPENDIX III

II: A Summary of some phytoremediation processes.

Mechanism	Process Goal	Media	Contaminants	Plants	Status of research
Phytoextraction	Hyper-accumulation, contaminant extraction and capture	Soil, sediment, sludges	Inorganics: Metals: Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Zn, Radionuclides: Sr, Cs, Pb, U	Indian mustard, pennycrees, alyssum, sunflower, hybrid, poplars	Laboratory, pilot, and field applications.
Rhizofiltration	Rhizosphere accumulation, contaminant extraction and capture	Groundwater, surface water.	Organics/Inorganics:Metals, radionuclides	Sunflowers, Indian mustard, water hyacinth	Laboratory, pilot scale
Phytostabilization	Complexation, contaminant destruction	Soil, sediment, sludges	Inorganics: As, Cd, Cr, Cu, Hs, Pb, Zn	Indian mustard, hybrid poplar, grasses,	Field application
Rhizodegradation	Contaminant destruction	Soil,sediment, sludge, groundwater	Organics, compounds, (TPH, PAHs, pesticides, chlorinated solvents, PCBs)	Red mulberry, grasses, hybrid poplar,cattails, rice	Field application
Phytodegradation	Contaminant destruction	Soil, sediment, sludge, groundwater, surface water	Organic compounds, chlorinated solvents, phenols, herbicides, munitions	Algae, stonewort, hybrid poplar, black willow, bald cypress	Field demonstration
Phytovolatilization	Volatilization by leaves, Contaminants extraction from media & release into air	Groundwater, soil, sediment, sludge	Organics/Inorganics: Chlorinated solvents, some organics & inorganics	Poplar, alfalfa, black locust, Indian mustard	Laboratory & field application
Hydraulic Control (plume control)	Contaminant degradation/containment	Groundwater, surface water	Water soluble organics/inorganics	Hybrid poplar. Cottonwood, willow	Field demonstration
Vegetative cover (evapotranspiration cover)	Containment erosion control	Soil, sediment, sludge	Organics & inorganic compounds	Poplar, grasses	Field application Field application
Riparian c	Contaminant destruction	Surface water, groundwater	Water-solubleorganics &inorganics	Poplars	Field application

Source: Adapted from Kania et al, (2002); Ghosh and Singh (2005)

APPENDIX III

III: Examples of sites that demonstrated phytoremediation mechanisms.

Location	Application	Containments	Medium	Plants
Edgewood, MD	Phytovolitilization Rhizofiltration Hydraulic control Phytodegradation	Chlorinated solvents	Ground water	Hybrid poplar
Forth Worth	Phytovolatilization Rhizodegradation Hydraulic control	Chlorinated solvents	Ground water	Eastern cottonwood
Ogdon, UT	Phytoextraction Rhizodegradation	Petroleum hydrocarbon	Soil, Groundwater	Alfalfa, poplar, juniper, fescue
Porthsmouth, VA	Phytodegradation Rhizodegradation	Petroleum	Soil	Grasses, Clover
Trenton, NJ	Phytoextraction	Heavy metals, Radionuclides	Soil	Hybrid poplar, grasses
Chernobyl, Ukraine	Rhizofiltration	Radionuclides	Ground water	Sunflower

Source: Adapted from EPA (Gosh and Singh, 2005).

APPENDIX IV

I :SITE CHARACTERIZATION: Toje series grid analysis

TOJE ERIES	Pmgkg^{-1}	N(%)	OC (%)	PH@25.8oC	EC µs cm $^{-1}$
1	0.036	0.07	5	5.4	125
2	0.035	0.035	4	5.8	80
3	0.035	0.105	1	5.7	65
4	0.052	0.07	2	5.8	60
5	N/D	N/D	N/D	N/D	N/D
6	0.04	0.07	2	5.6	55
7	0.033	0.07	2	5.6	190
8	0.121	0.07	2	5.7	126
9	0.061	. 0.035	2	5.6	100
10	0.048	0.07	2	61	105
11	N/D	N/D	N/D	N/D	N/D
12	0.05	0.07	2	5.7	70
13	0.041	0.07	2	5.2	135
14	0.042	0.14	2	5.7	140
15	0.046	0.105	5	5.5	130
16	0.052	0.105	1	5.4	75
17	0.084	0.105	3	5.5	110
18	N/D	N/D	N/D	N/D	N/D
19	0.056	0.07	2	5.6	40
20	0.045	0.07	2	5.6	125
21	0.039	0.07	2	5.5	60
22	0.048	0.07	3	5.4	41
23	0.06	0.105	1	5.5	65
24	0.04	0.105	2	5.5	100
25	0.039	0.07	2	5.6	105
26	0.047	0.07	2	5.6	110
27	0.049	0.07	2	5.9	85
28	0.051	0.105	11	5.5	65
29	0.788	0.07	3	5.4	70
30	0.041	0.07	2	5.4	95
31	0.041	0.07	2	5.4	130
32	0.049	0.07	2	5.5	75
33	0.059	0.07	3	5.6	50
34	0.051	0.07	2	5.8	65
35	0.04	0.07	2	5.9	155
36	0.044	0.07	3	5.5	155
mean	0.071606	0.07875	2.575758	7.254545	95.66667
range	0.033-0.788	.035-.14	1 - 11%	5.2-5.9	40-155

APPENDIX IV
II: Toje exchangeable cations complex

			pH			Cations Meq/100g	exchange	complex	
Pedon	Horizon	Depth(cm)		dsm^{-1}	K	Mg+	Ca+	Na+	CEC
Tojeseries									
	A	O – 11	5.2	30	0.21	0.833	0.879	0.003	23.5
	B	11 – 27	5.1	30	0.11	0.55	0.735	0.001	24.2
	C	27 – 75	5.7	45	0.072	1.09	1.32	0.002	18.1
	D	75 – 123	5.4	40	0.064	1.15	1.57	0.002	22
	F	123 – 200	5.3	20	0.069	1.131	1.09	0.002	22.7

APPENDIX V

I: SITE CHARACTERIZATION: Alajo series grid analysis

ALAJO SERIES	Pngkg^{-1}	N(%)	OC (%)	PH	EC µs cm $^{-1}$
1	0.012	0.035	12	7.7	310
2	0.09	0.035	9	7.1	590
3	0.146	0.035	1	7.3	610
4	0.092	0.035	9	7.2	550
5	0.112	0.035	6	7.1	420
6	0.069	0.035	12	7.5	560
7	0.123	0.14	14	7.3	335
8	0.147	0.07	9	6.8	653
9	0.062	0.35	1	7.1	760
10	0.069	0.035	9	7.2	380
11	0.133	0.07	1	7.6	450
12	0.094	0.07	12	7.2	275
13	0.126	0.07	7	7.2	400
14	0.109	0.105	11	7.2	460
15	0.204	0.105	13	7.3	350
16	0.109	0.035	8	7	535
17	0.108	0.07	1	7.2	550
18	0.127	0.035	9	7.4	750
19	0.014	0.035	5	7.2	500
20	0.099	0.035	1	7.1	640
21	0.359	0.035	17	7.4	440
22	0.102	0.035	25	7.3	545
23	0.029	0.07	1	7.3	610
24	0.017	0.035	7	7.4	220
25	0.154	0.07	7	6.9	390
26	0.207	0.035	9	7	610
27	0.064	0.035	6	7.2	900
28	0.106	0.07	9	7.3	410
29	N/D	N/D	N/D	N/D	N/D
30	0.081	0.07	8	7	460
31	0.042	0.07	5	7.1	520
32	0.117	0.07	9	7.7	420
33	N/D	N/D	N/D	N/D	N/D
34		0.07	8	7	540
35	0.077	0.035	7	7.1	460
36	0.063	0.035	6	7.4	565
Mean	0.105294	0.063824	8.058824	7.229412	504.9412
Range	.033- .359	.035 - .14	1 - 25%	6.8 - 7.7	220-900

						Cations Meq/100 g	exchange	complex	
Pedon	Horizon	Depth(cm)	pH	ECdsm^{-1}	K	Mg+	Ca+	Na+	CEC
Alajo series									
	A	O – 8	7.1	650	0.795	2.63	13.6	0.002	30.6
	B	8 – 21	7	620	0.308	2.23	16.6	0.014	36.4
	C	21 – 45	6.7	620	0.169	3.14	13.9	0.025	21
	D	45 – 115	7.2	400	0.628	2.11	15.7	0.023	19.4
	F	115 - 150	7.5	454	0.103	4.38	12.3	0.053	16.8

III: *Pueraria phaseoloides*

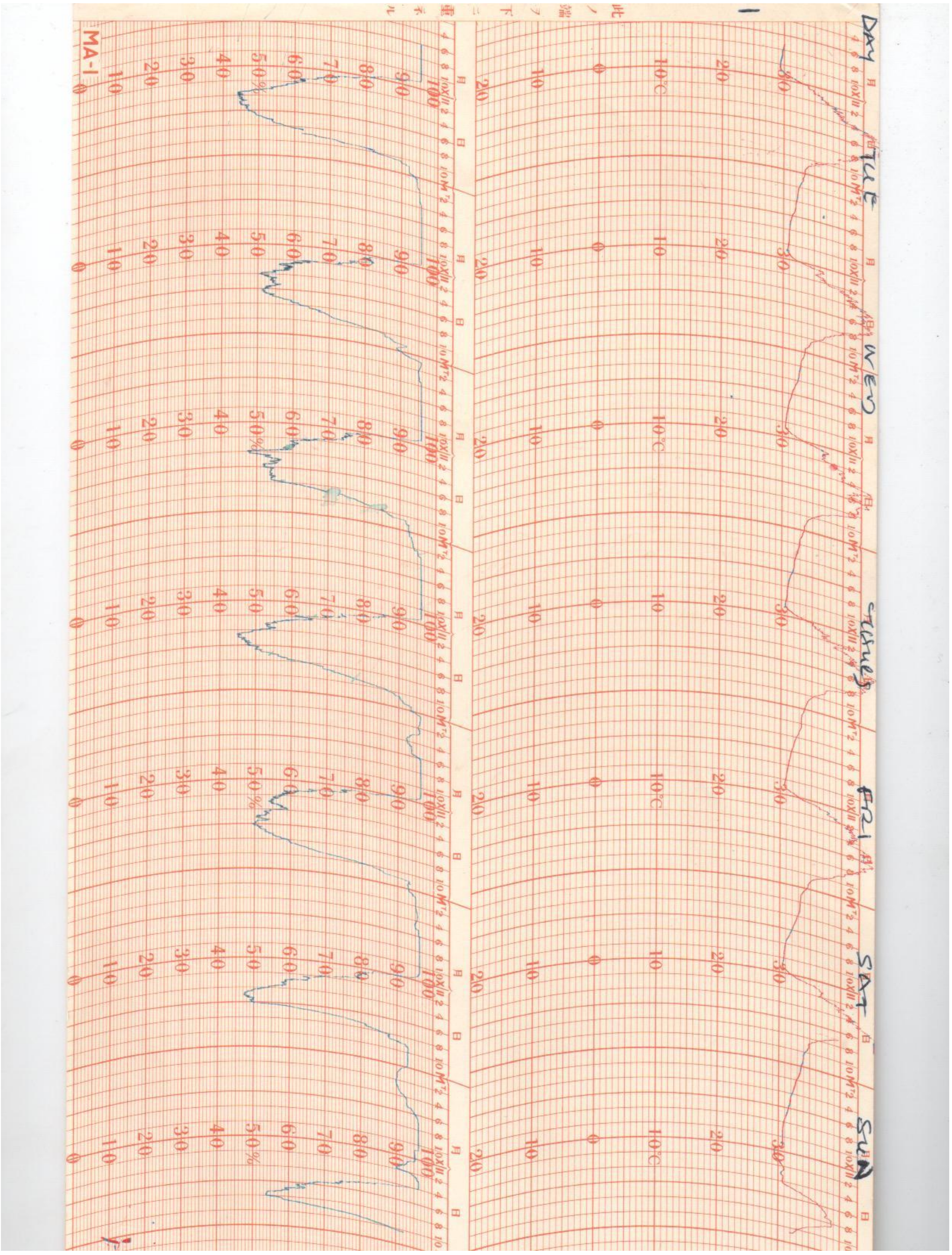
APPENDIX VII

Assessment of percentage Germination and sprouts of propagules and seeds

SOIL TYPE	ALAJO SOIL SERIES						TOJE SOIL SERIES					
Parameter measured / Name of selected plant	No of seeds sown	no of vegetative Propagules planted	No of seeds germinated	% germinate	no of vegetative Propagules planted	% Sprouts	No of seeds sown	no of vegetative Propagules planted	No of seeds germinate	% germinate	no of vegetative Propagules planted	% Sprouted
a) Grass												
P. maximum	-	18	-	-	12	12/18=67	-	18	-	-	13	13/18=72
P. vaginatum	-	18	-	-	15	15/18=83	-	18	-	-	11	11/18=61
C. dactylon	-	24	-	-	15	15/24=63	-	24	-	-	19	12/24=79
b)**Legumes**												
C. pubescens	60	-	23	23/60=38	-	-	60	-	40	40/60=67	-	-
S. leptocarpa	60	-	26	26/60=43	-	-	60	-	29	29/60=48	-	-
P. phaseoloides	60	-	41	41/60=64	-	-	60	-	25	25/60=42	-	-

Appendix IX

A sample of hygrogragh weekly sheet showing temperature (left of sheet) and Relative Humidity records(right)



GLOSSARY OF KEY WORDS AND ABBREVIATIONS USED IN THIS STUDY

AMBIENT: Prevailing natural environmental conditions

AMNESTY: To be granted a general pardon for an offence committed.

ANOVA: Analysis of variance (a statistical tool for data analysis).

ANOXIC: Condition of low oxygen supply.

API: American Petroleum Institute

BIOAVAILABILITY: The extent to which contaminant concentration is available in aqueous phase for uptake by plants.

BIOCENE: The sum of, or total biological population.

BIOGEOPHYSICAL: A phrase that refers to the living and non living things in an environment.

BIOMASS: The unit quantity or weight of a living material.

BIOMETRIC: The quantitative data of man/respondents in his/their environment.

BOD: Biological Oxygen Demand.

BONNY LIGHT: A brand of Nigerian light crude oil with premium commercial Value.

CEC: Cation Exchange Capacity.

C:N: The Carbon Nitrogen ration.

CONCESIONNAIRE: A person or organization with the license to explore and exploit for natural resources within an area.

CRAMP: An iron contrivance with a moveable part which can be used to screw things together- a clamp.

DEGRADATION: Reduction in hydrocarbons concentration.

DUST EXHORBENT: Fine particles or powdery matter used in the petrochemical industry to treat hydrocarbon contaminated soil matrixes.

EC: Electric Conductivity.

ECEC: Effective Cation Exchange Capacity.

EF: Enrichment factor.

EPA: (US) Environmental Protection Agency.

EVAPOVOLATILIZATION: A phrase that refers to the sum or total loss of hydrocarbons from unit surface.

EQUIPMENT FAILURE: Hydrocarbons contamination induced by faulty or dis-functional facilities.

FC: Field Capacity (ability of soil's to retain moisture).

FMANR: (Nig.) Federal ministry of agriculture and natural resources.

GNP: Gross national product.

GROWTH MATRIX: Medium of growth for experimental plants.

GTZ: (GER.) Geseksechaff for Techeische zussame Narbeit.

HAC: High Activity Clay content soil.

HORIZON: Soil visible layers or strata in a profile.

HYDROCARBONS: A phrase that refers to the alternate name for crude petroleum oil and allied products.

HYDROPHOBIC: Allergy to water or inability to dissolve in water (also lipophilic).

HYPERACCUMMULATORS: Plants with very high tissues contaminant accumulation potentials.

INFERNO: A condition of horror and confusion induced by serious fire outbreak.

IN-SITU: On the spot in the original condition.

JSS: Junior Secondary School.

LAC: Low activity clay content soil.

LSD: Less Significant Difference (statistical tool for data analysis).

LIPOPHILIC: Ability to dissolve in non- polar solvents (benzene, toluene etc).

MANOVA: Multiple Analyses of Variance (statistical tool for data analysis).

MEQ: Milligram Equivalent (in CEC determination).

NDDC: Niger Delta Development Commission.

NNPC: The Nigerian National Petroleum Corporation.

OC: Organic Carbon (also TOC-Total organic carbon).Osuji

OM: Organic matter.

PHYTOEXTRACTION: The use of plants to absorb and accumulate contaminants into their tissues.

PHYTOREMEDIATION: The use of plants to clean-up contaminants from the environment.

PHYTOVOLATILIZATIO: The use of plants to absorb and volatilize contaminants into and from their tissues.

PLANTS BLOCKS: This refers to homogenous units of experimental plants blocked to ensure equal access and treatments.

POCEAE: Family name for grass species.

PS: Particle Size.

PSD: Plants Survival Difference - evaluated in percentage of tolerance to contaminant concentration.

RF: Rhizhofiltration Factor; The use of plants roots to extract and store contaminants from the rhizosphere or root zone.

SSS: Senior Secondary School.

SCARIFICATION: Treatment given to hard seeds with long dormancy periods to

open up or break up their surfaces to induce germination.

SIMULATION: To recreate a condition of spill.

SOCIOMETRIC: A survey of the interrelationships of respondents within their communities.

SPDC: Shell Petroleum Development Corporation.

SPECIFIC GRAVITY: The relative density of experimental crude oil.

TERATOGENIC: Ability to distort the growth of fetus/embryo.

THC: Total Hydrocarbons- measurement index for crude oil and allied products (see also TPH – total petroleum hydrocarbon).

THERMOHYGROGRAGH: Instrument that measures temperature and relative humidity.

TN: Total Nitrogen.

UNDP: United Nations Development Programme.

USDA: United State Department of Agriculture.

VISCOSITY: Not free flowing or highly resistant to flow.

VOLATILIZATION: The loss of the volatile constituent of hydrocarbons.

WAP: Week(s) After Planting.

WAS: Week(s) After Simulation.

WELL HEAD: Or Christmas tree; an oil rig (flow station) valve facility.

WW: Wet weight of sample.