RHIZO-REMEDIATION OF CRUDE OIL-CONTAMINATED AGRICULTURAL SOIL USING SELECTED CROP PLANTS

BY

UCHECHI AGNES UKAULOR (B.Sc. PLANT SCIENCE & BIOTECHNOLOGY)

20104772358

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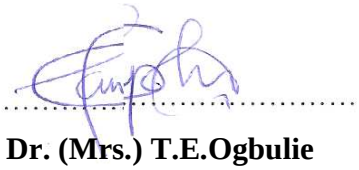
CERTIFICATION

This is to certify that this research work on **"RHIZO-REMEDIATION OF CRUDE OIL-CONTAMINATED AGRICULTURAL SOIL USING SELECTED CROP PLANTS"** is an authentic record of work carried out by **UCHECHI AGNES UKAULOR (20104772358)** of the Department of Biotechnology, School of Biological Sciences, Federal University of Technology Owerri.



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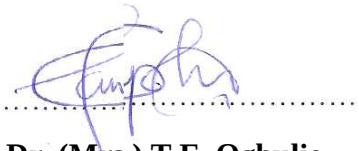
Dr. (Mrs.) T.E.Ogbulie Date

(Supervisor)



13/06/2017

Dr. (Mrs.) T.E. Ogbulie Date

(Head, Department of Biotechnology)

…… ……

Prof. J.N. Ogbulie Date

(Dean,School of Biological Sciences)

…… ……

Prof. (Mrs.) N.N. Oti Date

(Dean, Postgraduate School)

July 21, 2017

Prof. G.C. Okpokwasili Date

(External Examiner)

DEDICATION

In loving memory of my late identical twin sister, Ukaulor, Ugochi Agatha.

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GLOSSARIES

Zm= *Zea mays*

Mp = *Mucuna pruriens*

To = *Telfairia occidentalis*

Ps = Polluted soil

Us = Unpolluted soil

Pd = Poultry droppings

T = Treatment

T_1 = Polluted Soil before Planting

T_2= Polluted Soil One Month after Planting

T_3= Polluted Soil Stimulated with Poultry Droppings

T_4= Unpolluted Soil Stimulated with Poultry Droppings

ABSTRACT

Rhizo-remediation of crude oil-contaminated agricultural soils using *Zea mays, Mucuna pruriens* and *Telfairia occidentalis* was carried out to evaluate their effectiveness in environmental clean up. Soil samples polluted with different volumes (25 ml, 50 ml, 100 ml, 200 ml, 400ml) of 100 % Bonny light crude oil were used. Four parameters, crude oil polluted soil (polluted before planting), crude oil polluted soil (polluted one month after planting), crude oil polluted soil stimulated with poultry droppings and unpolluted soil stimulated with poultry droppings were used. The growth rate measurements showed increased growths from 8.5 ± 0.2cm to 106.0 ± 0.1cm by the stimulated treatment. Pre-and post microbial examinations of the polluted soil were carried out and the indigenous microbial flora present in the soil were identified to be *Proteus vulgaris, Pseudomonas* sp, *Staphylococcus* sp, *Bacillus* sp, *Acinetobacter* sp, *Escherichia coli, Micrococcus* sp, *Aspergillus* sp and *Penicillium* sp though *Escherichia coli* was absent in the latter. Molecular identification to confirm the presence of bacterial and fungal isolates persistent in the treated soil showed visible bands indicating the possibility of degradation by the acclaimed organisms. The total hydrocarbon content (THC) of treated soil was undertaken and the result revealed decrease from 16.05mg/kg to 0.08mg/kg. Statistical analysis showed significant difference in the degradation of crude oil amongst the treatment samples at 95% confidence level. This study revealed that *Telfairia occidentalis* had faster degradation than *Mucuna pruriens* and *Zea mays* hence this plant can be used more in remediation of polluted sites.

Keywords: Pollution, Rhizo-stimulation, Telfairia occidentalis, Petroleum hydrocarbon, Soil microbes.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Contamination of soil by crude oil during exploration, production and transportation significantly affects the environment and presents a high risk to the ecosystem. The exploration process has led to economic boom in oil producing countries thus providing raw materials for many petrochemical industries and serve as a source of energy. The availability and abundance of this product have led to increasing demand and prices for petroleum products. With the increasing demands of oil to meet the world's energy needs, more petroleum is transported to distant places and in the process it is spilled into the terrestrial and aquatic environment (Inoni *et al.,* 2008).Oil spillage into the terrestrial environment affect the physiochemical properties of the soil thus endangering the growth performance of plants due to prevention of water and oxygen from reaching the plant thereby resulting to either suffocation or loss of plant viability. Since commercial exploration of petroleum started in Nigeria in 1958(Okoh, 2008), petroleum has continuously grown to be mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and water ways. Oil exploration / exploitation is very lucrative and a major source of revenue in Nigeria. But like most industrial activities it produces environmental hazards that are "slow poison" in that they often take months and years to cause

diseases or death (WHO, 2013).The covert and slow action of the hazards created by oil exploration / exploitation make it difficult to fully appreciate their contribution to the disease burden in Nigeria, especially in the oil-bearing communities, even with the emergence of non-communicable disease as a major cause of ill health in Nigeria (WHO, 2013).

According to Odokuma and Ibor (2007), crude oil contamination on agricultural lands have made them become less productive. Several civil unrests due to environmental degradation caused by oil exploration have also been witnessed in the Niger Delta region of Nigeria (Inoni *et al.,* 2008). These oil producing communities such as the Niger Delta region of Nigeria have basically remained dependent and under developed, persistently disempowered, socio-culturally marginalized and psychologically alienated (Inoni *et al.,* 2008)and thus abandonment ofsuch land and emigration of the rural dwellers to seek ends meet in cities. Cleaning up of polluted sites is adopted in areas of oil spillage with toxic characteristics (Zverev *et al.,* 2008).

Recently,bioremediation like phytoremediation is being evaluated for the remediation of sites contaminated with petroleum(Lundstedt,2008).

Bioremediation is an option that can accelerate remediation kinetics at contaminated sites (Osam *et al.,* 2011).It is a relatively novel potential remediation technology that has been receiving increasing attention in recentyears

and has been shown to be effective for different kinds of pollutants (contaminants)and broad range of organic pollutants (Schnoor, 2007).According to Gianfreda and Rao (2004), bioremediation is the application of biological processes for cleanup of the hazardous chemicals present in the environment. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health. This can be used to clean up crude oil, solvents, and landfill leachates(Gerhard *et al.*, 2009).

Phytoremediation is the use of plants and/or associated microorganisms to remove or render harmful materials harmless (Merkl*et al.*,2005).According to Pivetz (2010), plants for phytoremediation should be appropriate for the climatic and soil conditions of the contaminated sites. Such plants should have the ability to tolerate conditions of stress (Siciliano and Germida, 2008).Phytoremediation has mostly involved the use of weeds (Banks *et al.*,2010). The use of food crops will improve the economic value of thetechnique. Although the conditions in the tropics favour phytoremediation, few researches have been carried on this technique in the tropics (Merkl*etal.*, 2005). However, it is necessary to develop simple, suitable accurate and sensitive methods to determine the extent of soil contamination by hydrocarbon mixture. The remediation efficiency of this technology can also be assessed rapidly by using the newly developed methods according to the removal of the contaminants after remediation (Mao *et al.*, 2009).There is the need therefore to evaluate the potentials of phytoremediation in

the tropics especially in Nigeria where pollution due to oil activitiesis high(Ayotamuno *et al.,* 2004). Nitrogen and phosphorus can be added to the soil, these can boost the population of naturally occurring microbes that will carry out the remediation process (Chaudhry *et al.,* 2005). Numerous soil bacteria including *Pseudomonas* sp have the ability to degrade organic contaminants and causing remediation. Different approaches like rhizoremediation and use of specific contaminant-degrading bacteria, plants and enzyme technology can be used to improve the efficiency of bioremediation (Nkwocha and Duru, 2010) and this is in accordance with Ogbulie *et al.* (2014).Plants may directly degrade the contaminants by root enzymes or in directly enhance rhizosphere microbial biodegradation by providing microorganisms with favourable microhabitats. Many studies have focused on screening feasible plants for successful phytoremediation whereas few have explored the mechanisms by which plants increase the remediation of contaminated soil. For crude oil contaminated soil, plants enhance the remediation efficiency mainly by directly removing the petroleum hydrocarbons (Ogbulie and Njoku, 2011).

Many aquatic and terrestrial environments are contaminated with various levels of toxicants. Among these, pesticides and total petroleum hydrocarbons (TPHs) from anthropogenic sources, pose a risk to human health. Government, industries and the public have already recognized the potential dangers of these contaminants to human health and natural environment (Lundstedt, 2008).

Nowadays, there is a considerable interest in developing cost-effective clean up mechanism based on microorganisms or plants. Researchers have shown that the removal of petroleum hydrocarbons is enhanced in vegetated soil compared with unvegetated soil. Plants may directly degrade the contaminants by roots enzymes or indirectly enhance rhizosphere microbial biodegradation by providing microorganisms with favourable microhabitats, for example by providing colonization surface and releasing root exudates to modify the soil environment (Ayotamuno *et al.,* 2004).

Soil and farmland contamination by crude oil contain petroleum substances or products that are poisonous thus making the land fouled and thus threatens the rich coastal habitat. Crude oil contamination of the soil is an environmental problem in Nigeria. It is common in oil producing areas. Oil is a very complex mixture of predominantly hydrocarbons; the light less dense portions of oil are more toxic but also more likely to evaporate. Soil contamination by crude oil makes it unsatisfactory for the plant growth. This is due to insufficient aeration of the soil because of air from the spaces between the soil particles by oil spillage. In Nigeria, the socio and environmental costs of oil spillage on soil farmland have been extensive. They include destruction of biodiversity, loss of fertile soil, pollution of land, degradation of farmland, damage to aquatic ecosystem and also cause serious health problems for the inhabitants of areas surrounding crude oil-contaminated or oil spillage areas.

1.1STATEMENT OF PROBLEMS

Crude oil spillage impacts negatively on the environment thereby making the soil unsatisfactory for plant growth. The problems are outlined as follows:

i.)Deterioration of the quality staple foods and food insecurity. Crude oil spillage has turned productive areas into wastelands and this significantly affects crops.

ii.)Reduced yield in economic trees and loss of food crops, thus endangering the growth performance of plants due to prevention of water from reaching the plants.

iii.)Reduced soil fertility and death of hundreds of plants and marine lives. Oil spillage renders the soil useless for farming.

1.3 AIM OF STUDY

The aim of this research work is to determine the rhizo-remediation of crude oil-contaminated agricultural soil using selected crop plants.

1.4OBJECTIVES OF STUDY

The objectives of this research work are as follows:

i.)To compare the growth performance of *Zea mays*, *Mucuna pruriens* and *Telfairia occidentalis* on stimulated and unstimulated treatments.

ii.) To determine total hydrocarbon content in the soil in order to evaluate the level of degradation.

iii.) To identify indigenous microorganisms in the remediation process.

iv.) To add to the existing phytoremediation model by incorporating the use of organic manure (poultry droppings) for remediation.

1.5 RESEARCH HYPOTHESIS

For this research work the following hypothesis were assessed:

i.)Null Hypothesis ($H_0 = 0$) = There is no significant difference in growth rate of plants on stimulated and unstimulated treatments.

Alternative Hypothesis ($H_1 \neq 0$) = There is significant difference in growth rateof plants on stimulated and unstimulated treatments.

ii.) Null Hypothesis ($H_0 = 0$) = There is no significant difference in total heterotrophic count on stimulated and unstimulated treatments.

Alternative Hypothesis ($H_1 \neq 0$) = There is significant difference in total heterotrophic count on stimulated and unstimulated treatments.

iii.)Null Hypothesis ($H_0 = 0$) = There is no significant difference in total hydrocarbon content degradation between stimulated and unstimulated.

Alternative Hypothesis ($H_1 \neq 0$) = There is significant difference in total hydrocarbon content degradation between stimulated and unstimulated.

1.6SIGNIFICANCE OF THE STUDY

The research will help in the understanding of crude oil contamination and appreciation of its effect on the environment. The significance of the research is as follows:

i.)Plant system solely is capable of remediation and uses its own natural processes to clean up the site with associated microorganisms and organic manure.

ii.) It is also an ecologically friendly driven clean-up technology based on the concept of using nature to cleanse nature.

iii.) The plants (vacuum cleansers) used to control spills can tolerate conditions of stress.

1.7 SCOPE OF STUDY

Contamination of soil by crude oil spill is a widespread environmental problem that often requires clean up of the contaminated sites. Phytoremediation which is a biological clean up was conducted using these three plants; *Zea mays, Mucunapruriens* and *Telfairia occidentalis.*Application of organic manure (poultry droppings) in phytoremediation was introduced in the soil. The growth characteristics of the plants were compared in the treatments. Also the microbial and biochemical analysis were carried out to identify the microorganisms involved in phytoremediation, and total hydrocarbon content degradation was carried out on the soil samples to know the rate of degradation. Molecular analysis was carried out to confirm the possible presence of bacteria and fungi present in the polluted soil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 EFFECTS OF CRUDE OIL ON PLANTS

Crude oils are chemicals which primarily include hydrogen and carbon (Glick, 2013).It is a fossil fuel that is made from decaying plant matter that has been under pressure in the earth crust for many years. Crude oil can affect the environment in many ways, one of which is by oil spillage thereby contaminating the soil as a result of agricultural and industrial activities. In Nigeria, crude oil contaminated soils have adversely affected the vegetation as well as the health of animals and humans in oil producing areas of the country (Ogbulie and Iwuala, 2006).The rate at which oil spill spreads will determine its effect on the environment. Thus, oil spill has done a tremendous damage to the environment affecting the overall inhabitants. What makes crude oil potentially even moredangerous is that it contains so many toxic chemicals that can all attack the organism. Though crude oil is predominantly composed of various hydrocarbons, certain nitrogen heterocyclic compounds, such as pyridine, picoline and quinoline are reported as contaminants associated with crude oil. Cunningham*et al.*(2010)stated that crude oil spills on agricultural land reduced soil fertility. This also smothered economic trees and food crops out rightly killing them or reducing their yield (Chung and Ka, 2012) causing a 60% reduction in household food security (Cole, 2013). The oil spills also reduced the quantity of food crops, thus studies carried out by Cunningham *et al.* (2010) shows that the ascorbic acid

content of water-leaf was reduced by 40%.The food insecurity and deterioration of the quality of the staple food led to 24% increase in the prevalence of childhood malnutrition in some communities. The crude oil spills also resulted in the bio-accumulation of heavy metals in the surviving food crops like Pumpkin (*Telfairia occidentalis)*and Cassava (*Manihot esculenta)*(Dean-Ross, 2007). Inoni *et al.*(2008) reported that crude oil pollution causes among other things low permeability and low infiltration of water into the soil. These conditions can lead to accumulation of water on the soil surface and an artificial drought in the subsurface layer of soil. This can lead to difficulty for the roots to absorb water and nutrients as such the water in the roots usually go deeper into the soil sub-surface layers.

2.2 REMEDIATION

Remediation is the clean up or abatement method used to remove a hazardous substance from an environment. It is used to remove a toxic spill or hazardous materials from a contaminated site. It is an action that involves the correction of a certain prevailing condition. Environmental remediation is the elimination of pollution and the action of remedying something, especially the reversal or stopping of damage to the environment. The goal of a remediation plan is to identify and treat the contamination so that the contaminated area will be usable again (Kaufmann and Cleveland, 2008). This deals with the removal of pollution

or contaminants from environmental media such as soil, groundwater, sediments or surface water. Remediation should be immediate due to the fact that it can impact negatively on human health and the environment. The treatment of environmental problems through biological means is known as bioremediationand the specific use of plants for treatment is called phytoremediation (Gregerand Landberg, 2009). Remediation is about safety and the ultimate goal for cleaning up any contaminated site is to eliminate any current or potential threatto plants which affect human health and the environment from the chemicals that have been released into the soil.

2.3 REMEDIATION TECHNOLOGIES

Remediation technologies are many and varied but can be categorized into *ex situ*and *in situ* method. *Ex situ*methods involves excavation of affected soil and subsequent treatment at the surface while *in situ* methods seek to treat the contamination without removing the soils (Fawole and Oso, 2007).

2.4 BIOREMEDIATION

Bioremediation is the application of biological processes for clean-up of the hazardous chemicals present in the environment (Gianfreda and Rao, 2014). It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health. Environmental biotechnologies through

decade of intensive study devised this technology that is used in combating the problems of environmental pollution (Agbor *et al.,* 2012). The process of bioremediation involves the transformation and breakdown of complex organic molecules through biostimulation and bioaugmentation into simpler substances such as fatty acid, carbon dioxide and water (Agbor *et al.,* 2012).In this technology,either naturally occurring or specially bred bacteriaare used to consume contaminants from extracted ground water or source(Burken *et al.,* 2011). According to Agbor *et al.*(2012), in some cases, bioremediation will use microbes already living in the soil at acontaminated site, in other cases, new microbes will be introduced into the soil. Timmis and Pieper (2009) stated that bioremediation has obvious advantage over physiochemical remediation methods due to several merits:cost-effective, convenient, complete degradation of organic pollutants and no collateral destruction of the site material or its indigenous flora and fauna. The aim of bioremediation is to accelerate remediation kinetics at contaminated sites (Alexander, 2009). This is inline with the work carried out by Njoku *et al.* (2009)and Bhatia and Malik (2011).Boudella *et al.*(2007)investigated the bioremediation of hydrocarbon contaminated soil using compositing process. Ebere *et al.*(2011)and Chikere and Okpokwasili (2009) reported on the effectiveness of cassava peels and poultry droppings in enhancing the degradation of crude oil-polluted soil in south-eastern Nigeria.Njoku *et al.*(2008)demonstrated that *Glycine max* germinates and grows in crude oil-

polluted soil serving as a remediation agent. Also Frick *et al.*(2009) included *G. max* in the list of plants that can grow and remediate petroleum hydrocarbon contaminated sites. Bioremediation is a technique that involves the use of natural occurrence or cultivated microorganisms that, through metabolic routes, promote physico-chemical reactions, transforming compounds of hard degradation into simple compounds, making degradation an easy process, being used in the removal of contaminants in surface water, groundwater and soils. Some studies report that most microorganisms used in this technique are bacteria and fungi, in reason of the ability to degrade a wide range of organic substances. Bioremediation is a viable and environmentally friendly alternative for the treatment of contaminated soils by organic compounds and metals that are potentially toxic. It is essentially the awakening of human consciousness to the need for development and application of these technologies in favor of the environment.

2.4.1 TYPES OF BIOREMEDIATION

There are two methods of bioremediation when referring to the location of work.

2.4.1.1*In situ*Bioremediation

This type of bioremediation is also known as natural remediation. In this technique, the contaminant remains in place and decontamination occurs through physical, chemical and biological processes. In general, it occurs lowly, requiring

monitoring of the site in long term, aiming to restore the environmental equilibrium.

2.4.1.2*Ex situ*Bioremediation

This is a type of bioremediation that requires the removal of the contaminated soil from the place so that it can be treated in another location. Removal may be required when there is possible contamination of people and of the environment near the soil to be bioremediated, or the presence of high concentrations of contaminants requires the use of techniques such as: composting, bioreactor, among others. Microorganisms can be used in the treatment of contaminated soils and are considered efficient biodegradation promoters, because of their abundance, diversity of species and catabolic and anabolic versatilities, as well as their capacity for adaptation to adverse environmental conditions. This makes it possible to understand the importance of using microorganisms in biotechnology for remediation of contaminated soils, considering that they use toxic substances as carbonsources, resulting in an effective and safe remediation.

2.5 BIOREMEDIATION PROCESSES

The bioremediation processes or methods have proven to be highly effective at reducing toxic organic contaminants to safe levels below regulatory thresholds at thousands of sites (Okoh, 2008).

2.5.1 Biostimulation

This is one of the common *in situ* methods of bioremediation. It involves the stimulation of existing naturally occurring micro-organisms beneath a site via the injection of nutrients such as fertilizers or food sweetners such as molassesinto soil. If done correctly, the injection will cause naturally occurring micro-organisms to multiply rapidly beneath the site, thus greatly enhancing the naturally occurring breakdown of toxic contaminant in the affected site. This process enhances the soil microbial activity for the contaminants to be degraded by organisms mostly associated with roots. Biostimulation can also involve aquatic organisms supporting active population of microbial degraders in its stimulation (Rupassara *et al.*, 2012).

2.5.2 Bioaugmentation

This is another *in situ*method of bioremediation. It involves the injection of a selection of engineered micro-organisms into ground waterbeneath a site, which are selected based on site conditions and the type of ground water contamination that is targeted for treatment. The injection introduces a large number of engineered micro-organisms into ground water beneath the site, which then ingest and breakdown the toxic contaminants in ground water. Biostimulation is sometimes used in conjunction with bioaugmentation to enhance the effectiveness of bioaugmentation. Bhatia and Malik (2011)

reported that bioaugmentation is a method to improve degradation and enhancethe transformation rate of xenobiotics by the seeding of specific microbes, able to degrade the xenobiotics of interest. Extensive degradation of petroleum pollutants generally is accomplished by mixed microbial populations, rather than single microbial species.

2.6 PHYTOREMEDIATION

Phytoremediation is the use of plants and/or associated micro-organismsto remove or render harmful material harmless (Merkl, 2005). Phytoremediation can be understood as the use of plants (trees, shrubs, grasses and aquaticplants) and their associated microorganisms in order to remove, degrade or isolatetoxic substances from the environment (Chaney *et al.*, 1997).The word "phytoremediation" derives from the Greek "phyton", meaning "plant", and Latin "remedium", which means "to remedy" or "to correct". Substances that may be subjected to phytore mediation include petroleum hydrocarbons, metals and others.For the choosing of phytoremediator species, plants that show a set of specificcharacteristics must be considered for phytoremediation, as a potential to produce high amounts of biomass, fast growth rate, extensive root system, tolerance to the metal or contaminant. Phytoremediation technologies have been used to clean up crude oil, metals, pesticides, solvent, polyaromatic hydrocarbons and landfill leachates. This remediation mechanism has been used incombination

with other clean up approaches as a “finishing or polishing” step (Nkwocha and Duru, 2010).Njoku*et al.* (2009) reported that *Glycine max*can be usedfor control of crude oil contaminated soil. The ability of some plants like *G. max* to reduce the level of crude oil in oil-polluted soil can help to restore polluted soils back for agricultural use. The high acceptability and ease of propagation will make it an easy tool for remediation of soil contaminated with crude oil (Rupasara *et al.*, 2012). Njoku *etal.* (2008) demonstrated that *G.max*grows in crude oil polluted soil and Frick *et al.*(2009) included *G.max*in the list of plants that can grow and remediate petroleum hydrocarbon contaminated sites. In addition, the high nutritional value of plants used for remediation makes them acceptable by many. This is similar to the findings of Njoku*et al.*(2008)who noted that *G.max* has high nutritional value and has the potential of growing in sandy loam soil, a soil type found inthe Niger Delta region of Nigeria. According to reports of Ayotamuno *et al.*(2004)and Merkl*et al.*(2005), pH of contaminated soil decreases during remediation process as a result of degradation of crude oil. This decrease in the pH of soil with degradation of crude oil could be dueto accumulation of organic acid produced during degradation in the soil (Merkl *et al.*, 2005)or the production of acid radicals through nitrification (Tisdale and Nelson, 2010).In 2011, Bhatia and Maliks work on bioremediation and the use of plant associated bacteria, such as endophytic bacteria (non-pathogenic bacteria) that occur naturally in plants and rhizospheric bacteria (bacteria that live on and near the roots of plants) have

been shown to contribute to biodegradation of toxic organic compounds in contaminated soil and could have potential for improving phytoremediation. Cullie and Blanchet (2011) reported that phytotoxic effect of crude oil increases with the concentration of crude oil, the higher moisture content of the soil, the higher and the quantities of crude oil in plant. The inhibition of root growth can lead to low penetration of water and higher accumulation of water on the soil surface. Barker (2010) in his work reported that reduction of transpiration is one of the phytotoxic effects of crude oil. Bhatia and Malik (2011) reported that phytoremediation as an emerging technology that uses plants and associated bacteria for the treatment of soil and ground water contaminated by toxic pollutantshas several advantages such as; it preserves the natural properties of soil, it acquires energy mainly from sunlight, the high levels of microbial biomass in the rhizosphere can be activated, it is low in cost and has the potential to be rapid (Huana*et al.,* 2006b).

2.7 APPLICATION OF PHYTOREMEDIATION

Phytoremediation has been successfully applied in many locations but full scale applications are still limited. In Nigeria, this technology has been applied in different areas. Phytoremediation application has been successful as seen in the works of Agbor *et al.*(2012), Bhatia and Malik (2011), Njoku *et al.* (2009) and others. At theMilan Ammunition plant in Tennesse, USA, a constructed wetland

designed deployed submersed and emergent plants in surface/subsurface flow system is used to degrade contamination of soil and explosive contaminants on land and in ground water sites. In Canada, Environment Canada is working with a company that operates the world's largest smelter to treat land fill leachate using constructed wetlands.

2.8CHALLENGES FACED WITH PHYTOREMEDIATION

The application of phytoremediation as an environmentally sound technology involves a number of challenges including the development of local capacity to understand and apply phytoremediation technologies and the establishment of an effective regulatory frame work(Hannik *et al.*, 2010).In some countries, there is lack of experience in the use of phytoremediation. This is often coupled with a lack of data, performance standards and cost-benefit analysis regarding phytoremediation technologies. Hence, there is need for appropriate phytoremediation techniques application to different geographic regions with varied weather conditions. Also there is need for site characterization, clean up and technology selection criteria and assessment/evaluation methods that can be applied to determine the applicability and appropriateness of various phytoremediation techniques (Jadia and Fulekar, 2009).

However, to overcome these challenges there are also a number of areas where research is required. For example, the rate of biodegradation and mineralization

during phytoremediation is usually affected by the nature and concentrations of contaminants present, as well as surrounding oxygen levels, soil/air moisture pH, temperature soil element contents, their bioavailability and the supporting microbial media. Plant physiological and roots growth expansion studies are needed in optimizing plant uptake of contaminants and to maximize process output performance. Bhatia and Malik (2011) stated that even though phytoremediation has been shown to efficiently reduce chemical hazards associated with various classes of organic or inorganic pollutants, it also suffers serious limitations that prevent large-scale field applications. One of the main challenges that have so far prevented full-scale application of phytoremediation technologies is that contaminant-induced stress frequently leads to low rates of seed germination, slow rates of plant development and decrease in plant biomass. In many cases, this problem can be solved by using plant growth promoting rhizobacteria (Glick, 2013). Rhizobacteria that exert beneficial effects on the plant growth and development are called plant growth promoting rhizobacteria (PGPR). Examples include *Micrococcus, Pseudomonas, Flavobacterium* and others.

2.9MICROORGANISMS INVOLVED IN REMEDIATION

Microorganisms help in remediation and many researchers have reported that bacteria play good role in the degradation process of crude oil (Frick *et al.*, 2009).These microbes especially bacteria are capable of degrading certain kinds of organic pollutants such as hydrocarbons, polychlorinated biphenyls (PCBs) etc.

However, there are naturally occurring microorganisms in the soil. The bacterial species include: *Bacillus, Micrococcus, Escherichia coli, Proteus* spand *Streptococcus*sp.The fungal species include: *Rhizopus*sp, *Mucor*spand *Aspergillus* sp(Agbor *et al.,* 2012). The bacterial species associated with crude oil contaminated soil are *Pseudomonas* sp, *E. coli*, *Bacillus* sp, *Micrococcus* sp, *Staphylococcus* spand*Acinetobacter*sp while fungal species include *Mucor* sp, *Penicillium*spand*Aspergillus* sp(Agbor *et al.,* 2012).Okolo *etal.*(2010)stated that the presence of petroleum might cause an increase in microbial population in the soil. Guerin and Jones (2010) and Hamamura *et al.* (2006) in their worksreported that*Pseudomonas* spp possess more competent and active hydrocarbon degrading enzymes than other biodegraders.

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1Study Area

The field experiment was carried out in a screen house besides Federal University of Technology Owerri (FUTO) School of Science Extension building. The laboratory work which includes soil analysis, microbial identification/biochemical tests and DNA isolation were carried out at FUTO School of Agriculture and Agricultural Technology Soil Science laboratory, Department of Biotechnology laboratory and also at the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, respectively.

3.2Soil Sample

For the purpose of this experiment, the soil used was loamy, which is the best for cultivation. The loamy soil used for cultivation was collected from FUTO school farm behind School of Science Extension building within a depth of 15cmmeasured with a rule using surface sterilized hoe.

3.3Seed Sources and Selection

Three annual indigenous seeds used for this study include a cereal, maize (*Zea mays)*, a vegetative crop, fluted pumpkin*(Telfairia occidentalis)* of whichbothwere obtained from farmers in Owerri and a forage leguminous crop, velvet bean (*Mucuna pruriens)*which was collected from a bush/farm located at Ngor-Okpala in Imo state. These seeds were selected as described by Burton and Burton (2012) based on criteria as free from seed borne pest and viability.

3.4Source of Crude Oil

The crude oil used was Bonny light (100%)obtained from Agip oil well, Akin Oguta Local Government Area (LGA) in Imo State using a sterilized plastic container sterilized as described by Tee *et al.* (1998).

3.5Sterilization of Water

Water used for washing the seeds during this analysis was sterilized by filling clean conical flasks of various sizes with water, corking and sealing with masking tape and autoclaving at 121°C for 15minutes at 15psi (Cheesbrough, 2000).

3.6Seeds

The three seeds namely: *Zea mays*, *Mucuna pruriens and Telfairia occidentalis* used were washed with sterile water three times before use in order to maintain aseptic condition.

3.7 Sponge and Cotton Wool

They were washed with detergent and rinsed thoroughly with sterile water three times. They were surface sterilized using modified method of Tee *et al.* (1998) by soaking in sodium hypochloride for 30minutes and thereafter soaked with ethanol for 15 minutes. They were rinsed with sterile water three times for 10minutes. The water was squeezed off and they were sealed separately in foil, autoclaved for 15 minutes as described by Cheesbrough (2000) for 15 minutes.

3.8 Planting Pots and Hoe

The planting pots were perforated randomly at the base for drainage. The planting pots and hoe used for collection of soil were washed with detergent and rinsed thoroughly with sterile water and allowed to dry. They were soaked in sodium hypochloride and allowed to stand for 30 minutes. Thereafter, they were soaked with absolute ethanol and allowed to stand for 15 minutes and were rinsed with sterile water.

3.9 PROCESSING OF SEEDS

Pre-planting procedure is essential for the seeds in order to prevent problems and difficulties during the planting and growing periods. This maintains varietal purity, correct hygiene procedures and low risk of introducing weeds (Hemmati *et al.,* 2011).Floating method according to Anoliefo and Vwioko (2005) was used to determine the viability of three different seeds used. One hundred and twenty (120)of each of the three different seeds, giving a total of three hundred (360)seeds were soaked in a sterilized container with distilled water for a period of 30 minutes, the seeds that submerged were three hundred and thirty two (332) seeds and twenty eight (28) seeds that floated were discarded. One hundred five (105) seeds of each of the three different seeds giving a total of three hundred and fifteen (315) seeds were selected at random from the three hundred and thirty two (332) seeds that submerged for the study. The sterilized sponges were spread inside the sterile Petridishes and sterilized transparent bucket. The selected seeds were spread on the sponges and the sterilized cotton wool was used to cover them. The Petridishes and buckets were covered loosely and allowed to stand for 3 to 5 days to enhance the sprouting of shoot.

3.10 TREATMENT PROCESSES

For the purpose of this study, four different treatments were used, which include:

T_1: Polluted soil before planting

T_2: Polluted soil one month after planting

T_3: Polluted soil stimulated with poultry droppings

T_4: Unpolluted soil stimulated with poultry droppings and

controls

There were replications andthe 4treatments in the 5 different volumes of crude oil gave a total of 48 planting buckets withcontrols as shown in Table3.1.

Table 3.1: Treatment Design

Controls	Treatment1 (Polluted soil before planting)	Treatment 2 (Polluted soil/ soil polluted one month after planting)	Treatment 3 (Polluted soil stimulated with Poultry droppings)	Treatment4 (Unpolluted soil stimulated with Poultry droppings)
Us	Zm + T_1(25ml)	Zm + T_2(25ml)	Zm + T_3(25ml)	Zm + T_4
	Zm + T_1(50ml)	Zm + T_2(50ml)	Zm + T_3(50ml)	
Zm + Us	Zm + T_1(100ml)	Zm + T_2(100ml)	Zm + T_3(100ml)	
	Zm + T_1(200ml)	Zm + T_2(200ml)	Zm + T_3(200ml)	
	Zm + T_1(400ml)	Zm + T_2(400ml)	Zm + T_3(400ml)	
Mp + Us				Mp + T_4
	Mp + T_1 (25ml)	Mp + T_2(25ml)	Mp + T_3(25ml)	
	Mp + T_1(50ml)	Mp + T_2(50ml)	Mp + T_3(50ml)	
To + Us	Mp + T_1(100ml)	Mp + T_2(100ml)	Mp + T_3(100ml)	
	Mp + T_1(200ml)	Mp + T_2(200ml)	Mp + T_3(200ml)	
	Mp + T_1(400ml)	Mp + T_2(400ml)	Mp + T_3(400ml)	
Ps				To + T_4
	To + T_1 (25ml)	To + T_2(25ml)	To + T_3(25ml)	
Ps+ Pd	To + T_1(50ml)	To + T_2(50ml)	To + T_3(50ml)	
	To + T_1(100ml)	To + T_2(100ml)	To + T_3(100ml)	
Us + Pd	To + T_1(200ml)	To + T_2(200ml)	To + T_3(200ml)	
	To + T_1(400ml)	To + T_2 (400ml)	To + T_3(400ml)	

(Key: Us = Unpolluted soil, Ps = Polluted soil, Pd = Poultry droppings, Zm = *Zea mays*, Mp = *Mucuna pruriens*, To = *Telfairia occidentalis*).

3.11 SEED PLANTING

This exercise was carried out in the screen house. Polluted soil (polluted before seed planting), Polluted soil (polluted one month after seed planting), a mixture of Polluted soil and poultry dropping sand Unpolluted soil stimulated with poultry droppings were the four different treatments used. Prior to planting, one thousand three hundred and eighty grams (1380g) of loamy soil from aforementioned location was weighed into each of the sixty four (64) pots and two hundred and thirty grams (230g) of poultry droppings was weighed into the soils both polluted and unpolluted treatments that were stimulated. The ratio of the soil to the poultry dropping was 6:1.

Generally, fifteen (15)planting pots were used for each treatment set up grouped into three. Each group had five (5) separate planting pots for the three different test seeds. Crude oil volumes of 25ml, 50ml, 100ml, 200ml and 400ml were measured with a surface sterilized measuring cylinder and poured into the planting pots at the appropriate time. Five (5) seeds per each of the three (3) different seeds were planted in the various treatment soil. All pots were watered with 50ml of water before planting and subsequently every 48 hours during the treatment process.

3.12 VEGETATIVE PARAMETERS

The growth performance of the three different test seeds were monitored and observed in the various treatments. This include the growth height of the plants which was measured and recorded using a measuring tape, as described by De Rio (2003) and Ogbulie *et al.* (2012). Physical changes like colour changes in leaves, shading of leaves, senescence of plants observed and the time of occurrence were all recorded.

3.13DETERMINATION OF TOTAL HYDROCARBON CONTENT (THC)

The test soil sample was subjected to spectrophotometric method of Mao *et al.* (2009) to determine the THC of the soil. This was carried out by weighing 100g of test soil sample into 250ml of clean glass flask in which 200ml of hexane was added. The set up was placed in an ultrasonic bath for 1 hour. Thereafter, the organic suspension was incubated overnight at room temperature, the supernatants were transferred to another evaporation flasks. This separation process was repeated twice. The evaporating supernatants obtained were separated and read by the spectrophotometer after standardizing with hexane.

Secondly, the lambda max, λ_{max} (maximum wavelength) for the absorption of light by the crude oil was determined and recorded. A graded concentration of the crude oil was prepared using crude oil-hexane mixture as shown in Table 3.2.

Using the determined lambda max, the optical densities (OD) of the graded concentrations were determined and a linear plot of optical density against concentration was plotted. The graph was used to extrapolate the values of the initial sample figures.

Table 3.2: Protocol for Crude oil concentrations

Crude oil Concentration(mg/kg)	0	0.01	0.02	0.03	0.04	0.05	0.06
Crude oil volume (ml)	0	0.1	0.2	0.3	0.4	0.5	0.6
Hexane volume (ml)	10	9.9	9.8	9.7	9.6	9.5	9.4
Final volume(ml)	10	10	10	10	10	10	10

3.14MICROBIAL ANALYSIS

Aseptic condition was maintained in all work done. The soil sample collected were subjected to microbial analysis within 24h of collection. All microbial tests were carried out by methods of Cheesbrough(2000).

3.15Preparation of Media

These are basically an aqueous solution to which all necessary nutrients have been added. The four media used were: MacConkey agar, Nutrient agar, Salmonella-Shigella agar and Sabouraud dextrose agar. Each medium was prepared according to manufacturer's guide. After preparation they were aseptically poured into sterile Petridishes and allowed to solidify.

3.16 Serial Dilution

One gram of each of the soil sampleswas measured and dispersed in 9ml of sterile distilled water or peptones water and this was serially diluted.

3.17Inoculation of Media

A 0.1ml aliquot of nine folds serial dilution of the sample was inoculated ontothe media plates using spread plate method. The Petridishes were incubated for 18-72 hours depending on the medium.

3.18 Gram Staining

In Gram staining, a normal saline was dropped on a clean slide. Small amount of the test organism was smeared, allowed to air dry and the reverse side of the slide was quickly flamed in order to fix the bacteria. The smear was flooded with methyl violet stain for 30-60 seconds. Dye was drained quickly and washed with Lugol's iodine. Iodine solution was left for 60 seconds and drained off, slide was washed gently under a tap. Slide was washed with 95% ethanol until the slide appeared free of violet stain. Slide was rinsed under the tap and diluted with safranin for 30 seconds and washed off. This was air-dried and viewed under microscope.

3.19 Motility Test

Oil immersion was placed round the edge of the depression of the cavity slide. A loopful of the test organism/bacteria was transferred to the centre of a clean dry cover slip placed on a flat sheet of paper avoiding spreading the drop of culture. Cavity slide was inverted over the cover slip such that the culture drop was in the centre of the slide depression. Slide was pressed down firmly so that the oil seals the cover slip in position. Inverted slide appeared hanging and was examined for motility under the microscope reducing the light by lowering the sub-stage condenser. Low power objective was used to focus the edge of the drop and

turned to the higher power objective. This was raised gently with the fine adjustment and the motile cells were seen moving rapidly in the field.

3.20Sugar(Sucrose, Lactose and Glucose) Fermentation Test

Each sugar was put into the test tubes and an indicator phenol red (0.01%) was incorporated into the medium and the bacteria were inoculated. One tube of each uninoculated medium was left as control. Tubes were incubated at 35°C for 2-7 days, watched daily for observation. Colour change of the indicator from phenol red to yellow and growth in the inoculated tubes were observed.

3.21BIOCHEMICAL TESTS

Identification of test isolates was done based on morphological and biochemical tests. Catalase, Citrate, Indole and Coagulase tests were carried out for proper characterization of bacterial isolates as described by Cheesbrough (2000).

3.22Catalase Test

Two of thetest organisms/bacteria wereinoculated separately onto agar plates, leaving the third un-inoculated to serve as control and incubated for24-48hours. Three drops of 3% hydrogen peroxide were added. Effervescence, caused by the liberation of oxygen as gas bubbles indicated the production of catalase by the testbacterium.

3.23Citrate Test

This test showed the ability of organisms to use citrate as the sole source of carbon and energy. Simmon's citrate was inoculated using straight wire from 18-24 hour old colony. This was incubated at 35^{o}C for up to 7 days. Growth on some colony in the medium were observed, without color change from green (neutral) to blue (alkaline) indicated positive and negative no growth.

3.24Indole Test

Two tubes with the bacteria were inoculated leaving the 3rdtube uninoculated to serve as control. These tubes were incubated at 35^{O}C for 48 hours. After the incubation period, 2ml of chloroform were added to the culture and shaken gently. Two millilitres of Kovac's reagent was added, shakengently and returned to test tube rack. It was allowed to stand for 20minutes and this permitted the reagent to rise to the top. A red colour in some tube at the reagent layer indicated indole production.

3.25Coagulase Test

Coagulase was introduced into the test organism/bacterium. This was tightly bound to the surface of the bacterium. Microscopic clumping was found for positive result within 10seconds while negative result showed no clumping.

3.26Lactophenol Cotton Blue Test

A drop of 70% ethanol was placed on a clean microscopic glassslide. The test specimen was immersed in the drop of alcohol. Onedrop of the lactophenol cotton bluewas added before the alcohol dried. The coverslip was held between the index finger and thumb, one edge of the drop of mountant was touched with a coverslip edge andloweredgentlyavoiding airbubbles. This preparation was examinedusinglow power objective and higher power (40X) objective for more detailed examination of spores and other structures. The features of each fungus were used to differentiate one from another.

3.27MOLECULAR IDENTIFICATION

The analyses done include:

- DNA extraction
- PCR (Polymerase chain reaction)
- Gel electrophoresis

3.28 DNA Extraction

This was carried out using modified method of Sambrook and Michael (2012)by first adding 1ml of sterile water into pre-abelled Eppendorf tubes into which a loopful of cells were transferred. This was vortexed and centrifuged at10,000 rpm for 5minutes. Resulting supernatant was decanted and blotted on the paper towel.

This process was repeated and thereafter 200µl of sterilewater wasadded, vortexed to homogenize the pellets and boiled at 100^{O}Cfor10 minutes. After boiling, the cells were vortexed again and centrifuged at 10,000 rpm for5 minutes. Thesupernatantswere transferredinto another pre-labeled Eppendorf tube by gentle aspiration using micropipette and stored at -20°C until when needed for next analysis.

3.29Polymerase Chain Reaction (PCR)

During the PCR experiment carried out in this research, primers which are short pieces of DNA are designed to match the segment of DNA to be copied. The two primers (Forward and Reverse) used were DNA com1& com2 and NS7 & NS8.The volume required for the PCR component was calculated depending on the 47 samples and all reagents were completely dissolved,vortexed at low speed and centrifuged. These were placed on ice. Sample numbers(1 to 47) were labeled on top of the Eppendorf tubes and the codes forprimers written by the side of the tube. The reaction mixture comprised of 38.9µl of sterile de-ionized water, 5µl of PCR buffer,3µl of $MgCl_2$, 1 µlof DNTP, 0.2µl of each primer (primer 1/forward and primer 2/ reverse),0.2µl of Taq DNA polymerase, 0.2µl of the specific primer and 1.5µl of template DNA. These were added into 200µl PCR tubes per sample per number of primers used and the mixture wasvortexed at low speed andcentrifuged. These were placed in thermal cycler for 2 hours at

annealing temperature of 56°C, 72°C holding time and 95°C denaturation time for 30 cycles.

3.30Gel Electrophoresis

Analysis of PCR product was carried out using 1.5% agarose gel prepared by dissolving 1.5g of agarose powder in 100ml of 1xTBE buffer using a conical flask.The solution was dissolved by boiling using a microwave oven and allowed to cool to about 50°C. On cooling,Ethidium bromide (10µl)was added and mixed by swirling gently. This was poured into the electrophoresis tray to obtain a gel thickness of about 4-5mm and bubbles were avoided. Prior to gel casting, the comb was placed to create depression (well) into which the samples of PCR products were loaded.This was allowed to solidify for about 20 minutes, after which the comb was removed. The tray was placed in the electrophoresis tank.Tris Biric EDTA buffer (1x) was poured into the tank ensuring that the buffer covered the surface of the gel. During loading, 15µl of each sample was mixed with 2µl of the loading dye. Samples were loaded into the wells created by the combs, marker was loaded on lane 1 followed by the control. Electrodes were connected to the power pack in such a way that negative terminal was at the end where the sample has been loaded. The gel electrophoresis was ran at 60-100V until the loading dye has migrated about three quarters of the gel.The gel was observed on a UV- transilluminator (Philip, 2010).

3.31: Statistical Design

The experimental design was a four factor factorial experiment using Fishers probability where the means of the statistical data were separated using least significant difference.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

Three days after planting the seeds, germination was observed in unpolluted planting pots, control pots as well as in some polluted before planting pots of different treatments,that is polluted with different volumes of crude oil. Their growth rates were differently measured and values recorded. Appendix I a – I g shows the plants in treatments at different duration.

4.1.1Growth Characteristics for Treatment1(T_1)/Soil Polluted before Planting.

The T1, seeds planted on polluted soil /soil polluted before planting such as ZmT_1(25ml, 50ml), MpT_1(25ml, 50ml) and ToT_1(25ml, 50ml) all had growth while ZmT_1,MpT_1 and $ToT_1$of crude oil volumes of 100ml, 200ml and 400ml had no growth after two weeks of observation. Growth heights of ZmT_1(25ml, 50ml), MpT_1(25ml, 50ml) and ToT_1 (25ml, 50ml) were measured and values were recorded. It was also observed that these plants had slight slow growth rate possibly due to the presence of crude oil.ZmT_1(25ml, 50ml) growth rates were measured upto seven(7) weeks.MpT_1(25ml, 50ml) growth rates were measured for eight (8)weeks while ToT_1(25ml, 50ml) lasted for twelve (12) weeks. Colour changes in leaves were howeverobserved in the fifth(5th)week of plant growth.The mean height of growth rate measurement for each plant of different

crude oil volumes at fortnight intervals (4th week and 6th week) respectively were recorded as shown in Fig 4.1, with ZmT_1(25ml) depicting mean values of 15.2 ± 0.2cm and 22.5 ± 0.3cmwhich is higher than the mean heights of 11.0 ± 0.4cmand 16.3 ± 0.3 cmfor ZmT_1(50ml). Whereas MpT_1 (25ml) had 17.3 ± 0.2 cm and 24.6 ± 0.3cm which are higher thanthe mean heightsof 13.2 ± 0.4 cm and 18.7± 0.1cm for MpT_1(50ml) while ToT_1had 20.2± 0.4 cm and 31.3 ± 0.2cm which are also higher than the mean heights of16.4 ± 0.4 cm and 25.2 ± 0.3 cm for ToT_1 (50ml).

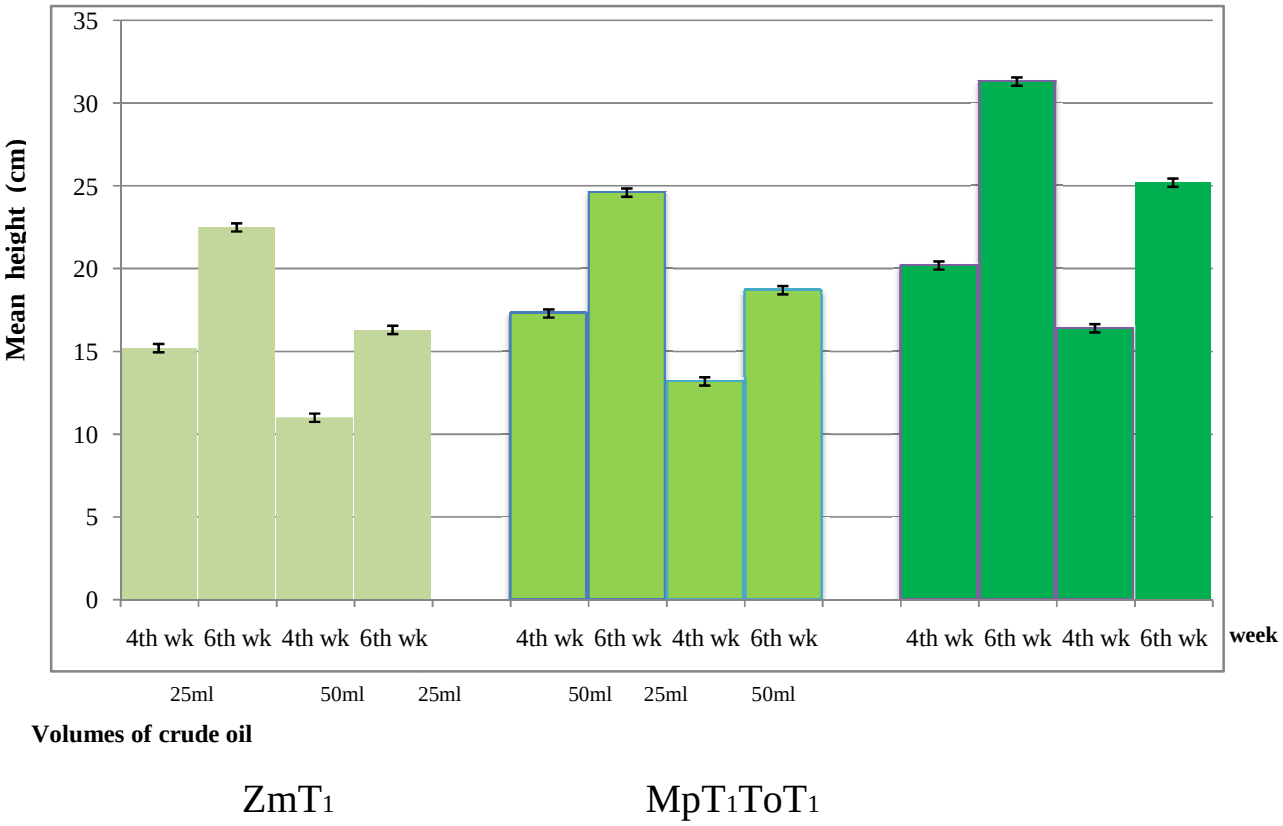


Fig 4.1: Mean height measurement (cm) of *Zea mays* (ZmT1), *Mucuna pruriens*(MpT1) and *Telfairia occidentalis* (ToT1) of 25ml and 50ml volumes each of fortnight interval

Key

- *Zea mays*+Treatment 1

- *Mucuna pruriens*+ Treatment 1

-*Telfairia occidentalis*+Treatment 1

4.1.2Growth Characteristics for Treatment 2 (T_2) / Soil Polluted one month after planting.

The $T_{2,}$ seedsplanted on polluted soil/soil polluted one month after planting such as ZmT_2, MpT_2 and ToT_2 all had rapid growth for one month before pollution.After pollution, it was observed that the plants (ZmT_2, MpT_2 and ToT_2) polluted with 25ml and 50ml volumes of crude oil respectively were still growing but at a reduced rate which lasted for seven weeks whereas those polluted with crude oil volumes of 100ml, 200ml and 400ml had very sluggish growth and afterward stopped growing.However, slight colour change in leaves was observed two weeks after pollution and shading of leaves was noticed.The mean height of measurementfor each plant of different crude oil volumesof fortnight interval (4th week and 6th week) respectively were recorded as shown in Fig 4.2, with $ZmT_2$of 25ml, 50ml, 100ml, 200ml and 400ml having theirfortnight mean as 36.4± 0.4 cm and 54.4± 0.3cm, 34.1± 0.4 cm and 50.6± 0.2 cm, 21.2± 0.2cm and 32.0± 0.1cm, 17.0± 0.4 cm and 23.5± 0.3 cm, 14.4± 0.3cm and 22.1± 0.4 cm.Whereas $MpT_2$of soil polluted with similar range of crude oil volumes had fortnight means as40.4± 0.1 cm and 60.5± 0.4cm, 35.5± 0.3 cm and 52.0± 0.2 cm, 20.1± 0.3cm and 30.2± 0.4 cm, 17.2± 0.2cm and 26.1± 0.3 cm, 15.3± 0.2 cm and 23.5± 0.4 cm while that of ToT_2had 42.3± 0.3cm and 63.0± 0.1cm, 40.3± 0.4 cm and 60.1± 0.4 cm, 30.1± 0.3cm and 45.0± 0.1 cm, 22.2± 0.3cmand 34.0± 0.4 cm,17.8± 0.3 cm and 26.4± 0.4 cm.

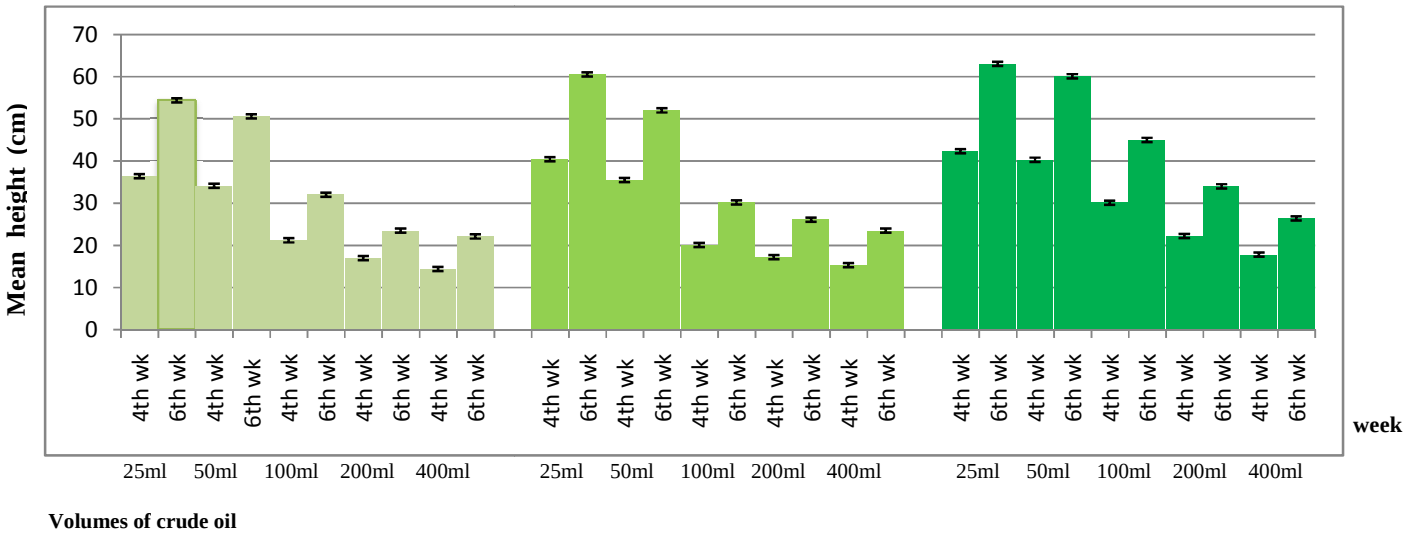


Fig 4.2: Mean height measurement (cm) of *Zea mays* (ZmT2), *Mucuna pruriens* (MpT2) and *Telfairia occidentalis*(ToT2) of 25ml, 50ml, 100ml, 200ml and 400 ml volumes each of fortnight interval

Key

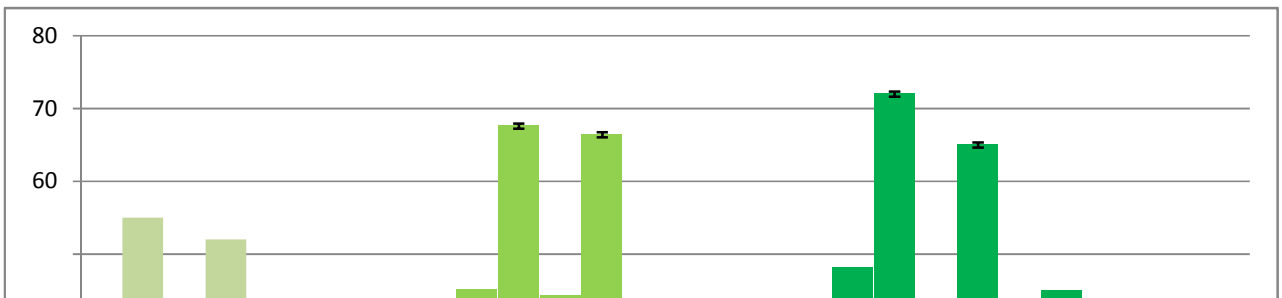
-*Zea mays* + Treatment 2

- *Mucuna pruriens* + Treatment 2

-*Telfairia occidentalis*+ Treatment 2

4.1.3 Growth Characteristics for Treatment 3/ (T_3) Polluted Soil stimulated with Poultry droppings

The $T_{3,}$ seeds planted on polluted soil stimulated with poultry droppings.ZmT_3 of (25ml, 50ml) grew till eight(8) weeks while ZmT_3(100ml, 200ml) grew for twelve (12) weeks, thereafter colourchange in leaves was observed. MpT_3(25ml, 50ml and 100ml) had rapid growth for eight (8) weeks, while MpT_3 (200ml)had late growth which was on the fourth (4th)week after planting. Generally no growth was discovered for ZmT_3(400ml)and MpT_3(400ml). Furthermore, ToT_3 (25ml, 50ml)had growth till ten (10) weeks whereas ToT_3(100ml, 200ml and 400ml) had growth which was later stunted, followed by decolouration of leaves. The mean height measurement for each plant of different crude oil volumes of fortnight interval (4th week and 6th week) respectively as shown in Fig 4.3,with ZmT_3 polluted with 25ml, 50ml, 100ml, 200ml volumes of crude oil having their means as 38.1± 0.4cm and 55.0± 0.3 cm, 35.0± 0.2cm and 52.0± 0.3cm, 25.2± 0.4 cm and 38.0± 0.1cm, 10.0± 0.4 cm and 16.0± 0.3cm. MpT_3 of 25ml, 50ml, 100ml and 200ml had 45.1± 0.3cm and 67.6± 0.2cm, 44.3± 0.1cm and 66.4± 0.3cm, 26.0± 0.2cm and 40.3± 0.2cm, 12.2± 0.3cm and 20.0± 0.1cm whileToT_3 polluted with 25ml, 50ml, 100ml, 200ml,400ml volumes of crude oil had 48.1± 0.2cm and 72.0± 0.2cm, 42.2± 0.2cm and 65.0± 0.4cm, 27.1± 0.3 cm and 45.0± 0.2cm, 13.3± 0.3cm and 20.0± 0.2cm, 8.5± 0.2 cm and 12.3± 0.4cm.



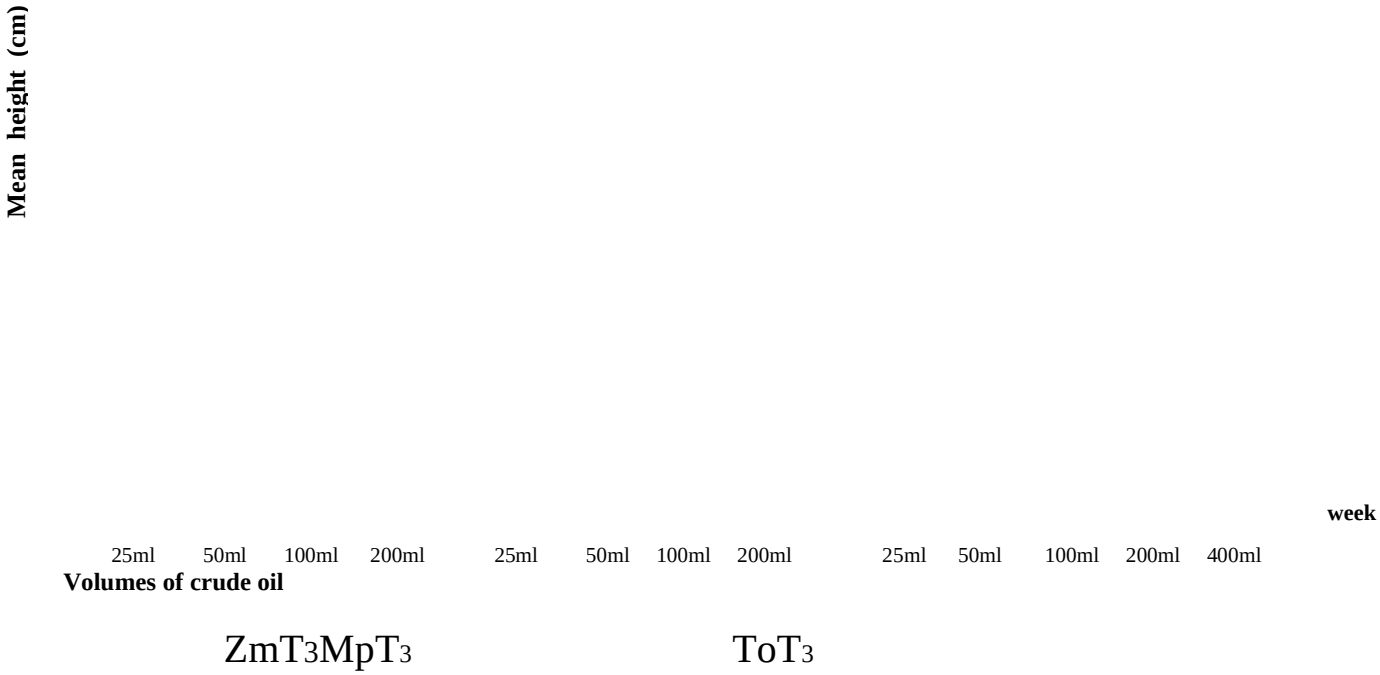


Fig 4.3:Mean height measurement (cm)of *Zea mays* (ZmT3), *Mucuna pruriens*(MpT3) and *Telfairia occidentalis*(ToT3) of 25ml, 50ml, 100ml, 200 ml volumes each 400 ml addition to ToT3 of fortnight interval

Key

- *Zea mays* + Treatment 3

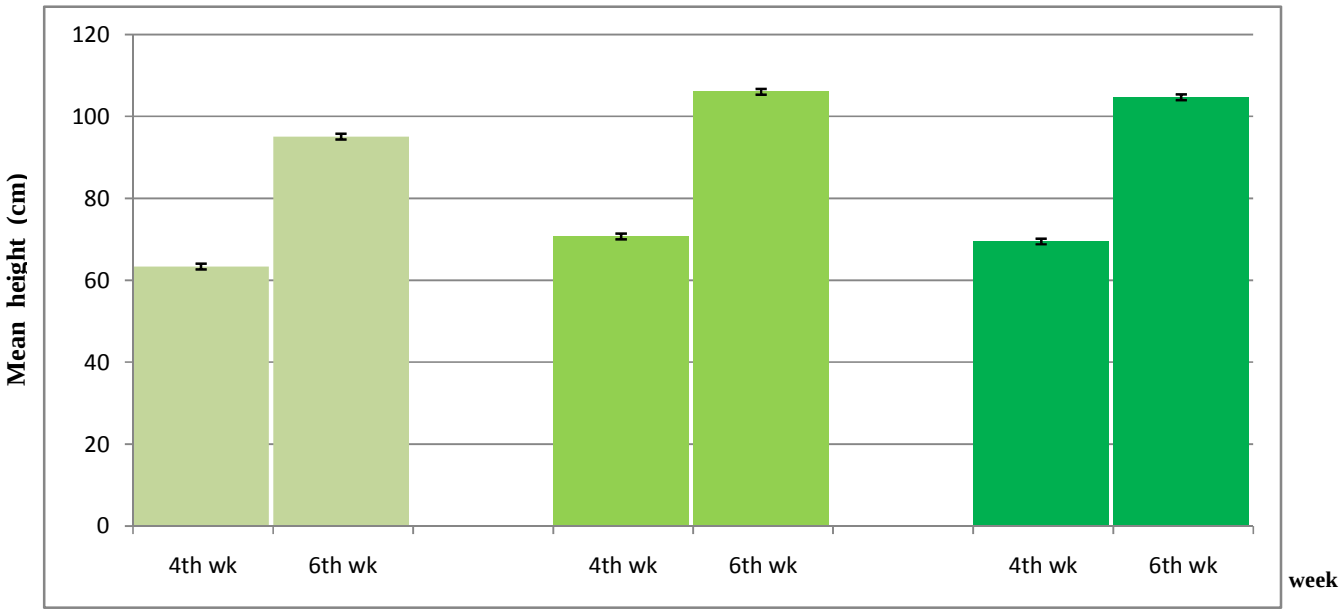
- *Mucuna pruriens* + Treatment 3

- *Telfairia occidentalis* + Treatment 3

4.1.4Growth Characteristics for Treatment 4 (T4)/Unpolluted soil

Stimulated with Poultrydroppings.

The T_4, seeds planted on unpolluted soil stimulated with poultry droppings, the three different kinds of plants had rapid growth and best growth so far.The plants whose soils were not polluted with crude oil were observed to be healthier than the other treatments. The slight colour change in leaves were seen after eight (8) weeks of plant growth.The mean height measurement for each plant of fortnight interval (4th week and 6th week) respectively were recorded as shown in Fig 4.4,with ZmT4 having its mean values as of 63.4± 0.4cm and 95.1± 0.3cm whereasMpT4 had 70.7± 0.2cm and 106.0± 0.1cmhence recorded the highest growth rate value amongst the test plant sampleswhile ToT4 had 69.5± 0.4cm and 104.7± 0.2 cm.



ZmT4MpT4ToT4

Fig 4.4:Mean height measurement (cm) of *Zea mays* (ZmT4), *Mucuna pruriens* (MpT4) and *Telfairia occidentalis* (ToT4) of 25mland 50 ml volumes each of fortnight interval

Key

- *Zea mays* + Treatment 4

- *Mucuna pruriens* + Treatment 4

- *Telfairia occidentalis* + Treatment 4

4.1.5 Growth Characteristics for Control

These areseeds planted in unpolluted soil which are the controls. The plants had rapid growth. The mean height measurement for each plant of fortnight interval (4th week and 6th week) respectively were recorded as shown in Fig 4.5, with ZmUS having a mean value of 60.5± 0.4cm and 90.8± 0.3cm, whereas MpUS had 68.2± 0.2 cm and 102.3± 0.1 cm while ToUS had 9.3± 0.4 cm and 103.5± 0.2cm. Generally all plants had slight leaf decolouration but for control samples, decolouration was observed after 8 weeks of growth.

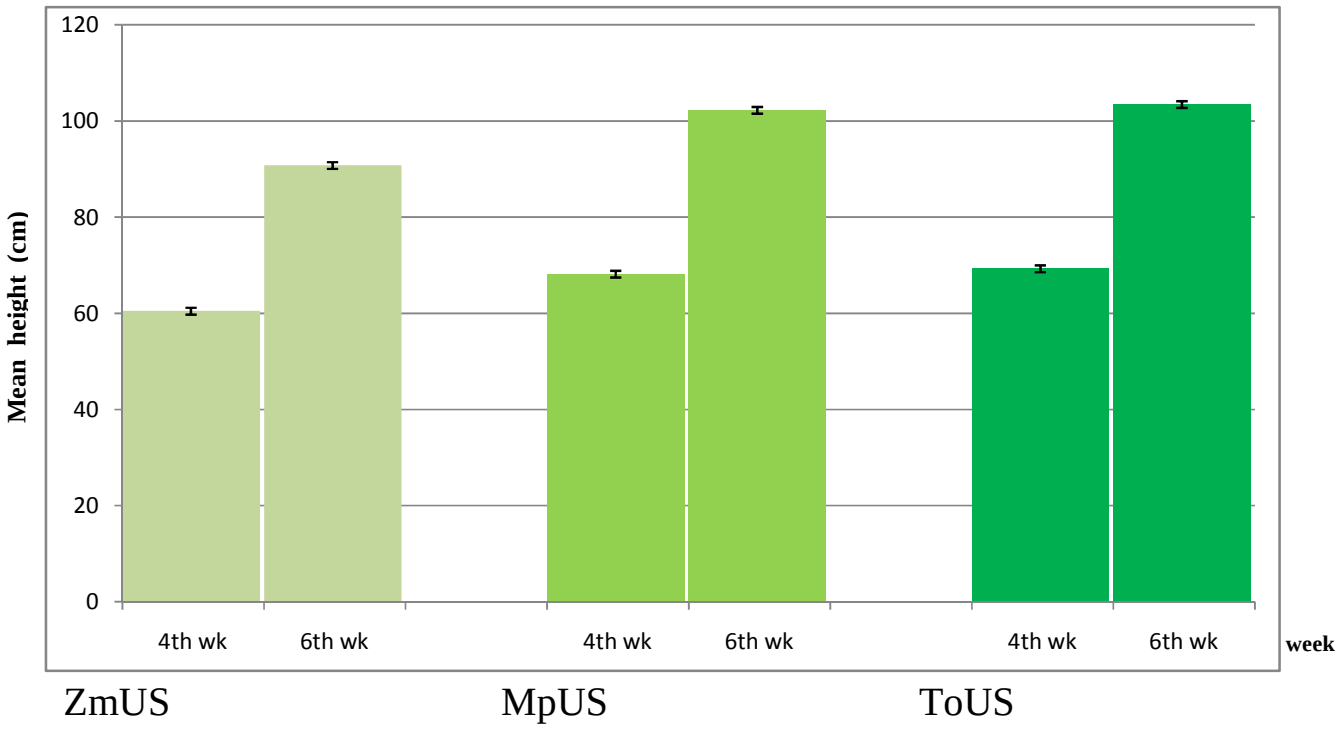


Fig 4.5: Mean heights measurement (cm)of Controls; *Zea mays* (ZmUS), *Mucuna pruriens* (MpUS) and *Telfairia occidentalis* (ToUS) each of fortnight interval

Key

- *Zea mays* + Unpolluted soil

- *Mucuna pruriens* + Unpolluted soil

-*Telfairia occidentalis* +Unpolluted soil

Table 4.1: ANALYSIS OF VARIANCE (ANOVA) SHOWING THE EFFECT OF PLANT TYPE ON PLANT HEIGHT
SINGLE

Plant Type	Mean height (cm)
Zea mays(Zm)	21.050± 0.1
Mucuna pruriens (Mp)	23.859 ± 0.3
Telfairia occidentalis (To)	27.589 ±0.2
Least significant difference LSD $_{0.05}$0.3403***	

*** = Highly significant at $p < 0.001$

Table 4.2: ANOVA SHOWING THE EFFECT OF CRUDE OIL POLLUTION VOLUME ON PLANT HEIGHT

Volume of polluting crude oil (ml)	Mean height (cm)
25	41.861± 0.2
50	37.683± 0.4
100	20.289± 0.2
200	13.204± 0.3
400	7.793± 0.1
Least significant difference LSD$_{0.05}$0.4393***	

*** = Highly significant at $p< 0.001$

Table 4.3: ANOVA SHOWING THE EFFECT OF TREATMENT ON PLANT HEIGHT

Treatment	Mean height (cm)
T1	7.727 ± 0.2
T2	31.478 ± 0.4
T3	33.293 ± 0.3
Least significant differenceLSD$_{0.05}$	0.3403***

*** = Highly significant at $p < 0.001$

Table 4.4: ANOVA SHOWING THE EFFECT OF FORTNIGHT INTERVAL ON PLANT HEIGHT

Fortnight interval	Mean height (cm)
4th week	19.531 ± 0.3
6th week	28.801 ± 0.2
Least significant differenceLSD $_{0.05}$	0.2779***

*** = Highly significant at $p < 0.001$

Table 4.5: ANOVA SHOWING THE COMBINED EFFECT OF PLANT TYPE AND CRUDE OIL POLLUTION VOLUME ON PLANT HEIGHT TWO COMBINATION

Plant Type	Volumes of polluting crude oil(ml)	Mean height(cm)
Zm	25	36.933± 0.2
Mp	25	42.517 ± 0.3
To	25	46.133 ± 0.2
Zm	50	33.167 ± 0.1
Mp	50	38.350 ± 0.4
To	50	41.533 ± 0.3
Zm	100	16.900 ± 0.2
Mp	100	19.433 ± 0.1
To	100	24.533 ± 0.2
Zm	200	12.167 ± 0.3
Mp	200	12.528 ± 0.1
To	200	14.917± 0.3
Zm	400	6.083 ± 0.4
Mp	400	6.467± 0.2
To	400	10.828± 0.3
Least significant difference $LSD_{0.05}$		0.7610***

*** = Highly significant at $p < 0.001$

Table 4.6: ANOVA SHOWING THE COMBINED EFFECT OF PLANT TYPE AND FORTNIGHT INTERVAL ON PLANT HEIGHT

Plant Type	Fortnight Interval	Mean height (cm)
Zm	4th week	17.607± 0.3
Mp	4th week	19.080 ± 0.2
To	4th week	21.907 ± 0.2
Zm	6th week	24.493 ± 0.1
Mp	6th week	28.638 ± 0.3
To	6th week	33.271 ± 0.4
Least significant difference $LSD_{0.05}$		0.4813***

*** = Highly significant at $p < 0.001$

Table 4.7: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT AND PLANT TYPE ON PLANT HEIGHT

Treatment	Plant Type	Mean height (cm)
T1	Zm	6.500 ± 0.1
T2	Mp	29.720 ± 0.2
T3	To	26.930 ± 0.2
T1	Zm	7.380 ± 0.3
T2	Mp	32.040 ± 0.4
T3	To	32.157 ± 0.2
T1	Zm	9.300 ± 0.4
T2	Mp	38.120 ± 0.1
T3	To	35.347 ± 0.3
Least significant difference LSD$_{0.05}$		0.5894***

*** = Highly significant at $p < 0.001$

Table 4.8: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT AND FORTNIGHT INTERVAL ON PLANT HEIGHT

Treatment	Fortnight Interval	Mean height (cm)
T1	4th week	6.229 ± 0.3
T2	4th week	27.360 ± 0.2
T3	4th week	25.004 ±0.4
T1	6th week	9.224 ± 0.2
T2	6th week	39.227± 0.2
T3	6th week	37.951±0.3
Least significant difference LSD $_{0.05}$		0.4813***

*** = Highly significant at $p < 0.001$

Table 4.9: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT AND CRUDE OIL POLLUTION VOLUME ON PLANT HEIGHT

Treatment	Volume of polluting crude oil (ml)	Mean height (cm)
T1	25	21.833 ± 0.3
T2	25	49.433 ± 0.2
T3	25	54.317 ± 0.3
T1	50	16.800 ± 0.1
T2	50	45.433 ± 0.2
T3	50	50.817 ± 0.4
T1	100	0.000 ± 0.2
T2	100	27.267 ± 0.3
T3	100	33.600 ± 0.2
T1	200	0.000± 0.1
T2	200	24.417± 0.3
T3	200	15.194 ± 0.2
T1	400	0.000 ± 0.3
T2	400	19.917 ± 0.1
T3	400	3.461± 0.2
Least significant difference LSD $_{0.05}$		0.7610***

*** = Highly significant at $p < 0.001$

Table 4.10: ANOVA SHOWING THE COMBINED EFFECT OF FORTNIGHT INTERVAL AND CRUDE OIL POLLUTION VOLUME ON PLANT HEIGHT

Fortnight Interval	Volume of polluting crude oil (ml)	Mean height (cm)
4th week	25	33.648 ± 0.2
6th week	25	50.074 ± 0.4
4th week	50	30.813 ± 0.2
6th week	50	45.144 ± 0.3
4th week	100	16.633 ± 0.1
6th week	100	23.944 ± 0.3
4th week	200	10.933 ± 0.3
6th week	200	15.474 ± 0.1
4th week	400	6.219 ± 0.4
6th week	400	9.367 ± 0.2
Least significant difference LSD $_{0.05}$		0.6213***

***= Highly significant at $p < 0.001$

Table 4.11: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT, PLANT TYPE AND FORTNIGHT INTERVAL ON PLANT HEIGHT THREE COMBINATION

Mean height (cm) for Fortnight Interval			
Treatment	**Plant Type**	**4th week**	**6th week**
T1	Zm	5.240 ± 0.3	7.760 ± 0.2
T2	Zm	21.660 ± 0.2	33.520 ± 0.3
T3	Zm	25.920 ± 0.4	32.200 ± 0.2
T1	Mp	6.100 ± 0.3	8.660 ± 0.4
T2	Mp	25.520 ± 0.3	38.793 ± 0.3
T3	Mp	25.620 ± 0.2	38.460 ± 0.3
T1	To	7. 347 ± 0.3	11.253 ± 0.1
T2	To	27.833 ± 0.4	42.860 ± 0.2
T3	To	30.540 ± 0.3	45.700 ± 0.4
Least significant difference LSD0.05		0.8336***	

*** = Highly significant at p< 0.001

Table 4.12: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT, PLANT TYPE AND CRUDE OIL POLLUTION VOLUME ON PLANT HEIGHT

Mean height(cm) Volume of pollutingcrude oil					
Treatment	**Plant Type**	**25ml**	**50ml**	**100ml**	**200ml**	**400ml**
T1	Zm	18.850 ± 0.2	13.650± 0.4	0.000 ± 0.0	0.000 ± 0.0	0.000± 0.0
T2	Zm	45.400 ± 0.3	42.350 ± 0.2	19.100± 0.4	23.500 ± 0.2	18.250 ± 0.2
T3	Zm	46.550 ± 0.2	43.500± 0.4	31.600 ± 0.2	13.000 ± 0.4	0.000 ± 0.0
T1	Mp	20.950± 0.4	15.950 ± 0.3	0.000 ± 0.0	0.000 ± 0.0	0.000 ± 0.0
T2	Mp	50.250± 0.3	43.750 ± 0.4	25.150 ± 0.4	21.650 ± 0.4	19.400 ± 0.4
T3	Mp	56.350 ± 0.3	55.350 ± 0.4	33.150 ± 0.4	15.933± 0.3	0.000 ± 0.0
T1	To	25.700 ± 0.4	20.800 ± 0.3	0.000 ± 0.0	0.000 ± 0.0	0.000± 0.0
T2	To	52.650± 0.4	50.200 ± 0.3	37.550 ± 0.3	28.100 ± 0.4	22.100 ± 0.4
T3	To	60.050± 0.3	53.600 ± 0.3	36.050 ± 0.4	16.650± 0.4	10.383 ± 0.4
Least significant difference LSD 0.05		1.3180***				

*** = Highly significant at p< 0.001

Table 4.13: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT, FORTNIGHT INTERVAL AND CRUDE OIL POLLUTION VOLUMEON PLANT HEIGHT

Mean height (cm) Volume of polluting crude oil Treatment	Fortnight Interval	25ml	50ml	100ml	200ml	400ml
T1	4th week	17.611± 0.4	13.533± 0.4	0.000± 0.0	0.000± 0.0	0.000± 0.0
T2	4th week	39.567 ± 0.2	36.633 ± 0.3	23.800 ± 0.1	20.967± 0.4	15.833± 0.1
T3	4th week	43.767 ± 0.4	40.500 ± 0.4	26.100 ± 0.4	11.833 ± 0.3	2.822± 0.4
T1	6th week	26.056 ± 0.3	20.067 ± 0.4	0.000± 0.0	0.000± 0.0	0.000± 0.0
T2	6th week	59.300 ± 0.4	54.233 ± 0.3	30.733 ± 0.4	27.867 ± 0.3	24.000± 0.3
T3	6th week	64.867 ± 0.4	61.133 ± 0.2	41.100 ± 0.3	18.556 ± 0.4	4.100 ± 0.4
Least significant difference LSD		0.05	1.0762***			

*** = Highly significant at p< 0.001

Table 4.14: ANOVA SHOWING THE COMBINED EFFECT OF PLANT TYPE, FORTNIGHT INTERVAL AND CRUDE OIL POLLUTION VOLUME ON PLANT HEIGHT

Mean height (cm) Volume of polluting crude oil Plant Type	Fortnight Interval	25ml	50ml	100ml	200ml	400ml
Zm	4th week	29.900± 0.3	26.700± 0.4	15.467± 0.2	11.167± 0.4	4.800± 0.4
Mp	4th week	34.133 ± 0.4	31.000± 0.3	15.367± 0.4	9.800± 0.2	5.100± 0.4
To	4th week	36.911± 0.4	32.967 ± 0.3	19.067 ± 0.3	11.833 ± 0.4	8.756± 0.3
Zm	6th week	43.967± 0.2	39.633± 0.4	18.333± 0.4	13.167 ± 0.3	7.367± 0.2
Mp	6th week	50.900± 0.4	45.700 ± 0.1	23.500 ± 0.4	15.256± 0.4	7.833± 0.4
To	6th week	55.356 ± 0.3	50.100 ± 0.4	30.000 ± 0.1	18.000 ± 0.4	12.900± 0.3
Least significant difference LSD		0.05	1.0762***			

*** = Highly significant at p< 0.001

Table 4.15: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT,PLANT TYPE, FORTNIGHT INTERVAL AND CRUDE OILPOLLUTION VOLUME ON PLANT HEIGHT

FOUR COMBINATION

Mean height (cm) Volume of polluting crude oil	Treatment	Plant Type	Fortnight Int.	25ml	50ml	100ml	200ml	400ml
	T1	Zm	4th week	15.200± 0.3	11.000± 0.4	0.000 ± 0.0	0.000 ± 0.0	0.000± 0.0
	T2	Zm	4th week	36.400 ± 0.4	34.100 ± 0.2	21.200± 0.4	23.500± 0.4	14.400± 0.4
	T3	Zm	4th week	38.100 ± 0.2	35.000 ± 0.4	25.200 ± 0.3	10.000 ± 0.1	0.000 ± 0.0
	T1	Mp	4th week	17.300 ± 0.4	13.200 ± 0.3	0.000± 0.0	0.000 ± 0.0	0.000± 0.0
	T2	Mp	4th week	40.000 ± 0.4	35.500 ± 0.4	20.100 ± 0.4	17.200 ± 0.3	15.300± 0.4
	T3	Mp	4th week	45.100 ± 0.1	44.300 ± 0.4	26.000 ± 0.3	12.200 ± 0.4	0.000± 0.0
	T1	To	4th week	20.333 ± 0.3	16.400 ± 0.4	0.000± 0.0	0.000± 0.0	0.000± 0.0
	T2	To	4th week	42.300 ± 0.4	40.300 ± 0.1	30.100± 0.4	22.200 ± 0.4	17.800 ± 0.2
	T3	To	4th week	48.100 ± 0.3	42.200 ± 0.4	27.100 ± 0.4	13.300 ± 0.2	8.467 ± 0.4
	T1	Zm	6th week	22.500 ± 0.4	16.300 ± 0.4	0.000± 0.0	0.000 ± 0.0	0.000± 0.0
	T2	Zm	6th week	54.400 ± 0.4	50.600 ± 0.2	17.000± 0.4	23.500 ± 0.3	22.100 ± 0.4
	T3	Zm	6th week	55.000 ± 0.2	52.000 ± 0.4	38.000 ± 0.3	16.000 ± 0.2	0.000± 0.3
	T1	Mp	6th week	24.600 ± 0.3	18.700 ± 0.4	0.000± 0.0	0.000± 0.0	0.000± 0.0
	T2	Mp	6th week	60.500 ± 0.4	52.000 ± 0.3	30.200± 0.4	26.100± 0.2	23.500± 0.3
	T3	Mp	6th week	67.600 ± 0.4	66.400 ± 0.4	40.300± 0.3	19.667 ± 0.4	0.000± 0.0
	T1	To	6th week	31.067 ± 0.4	25.200 ± 0.4	0.000± 0.0	0.000 ± 0.0	0.000± 0.0
	T2	To	6th week	63.000 ± 0.3	60.100 ± 0.2	45.000± 0.4	34.000 ± 0.4	26.400± 0.4
	T3	To	6th week	72.000 ± 0.4	65.000± 0.4	45.000±0.3	20.000 ± 0.2	12.300± 0.4
Least significant difference LSD 0.05				1.8640***				

*** = Highly significant at p = 0.001

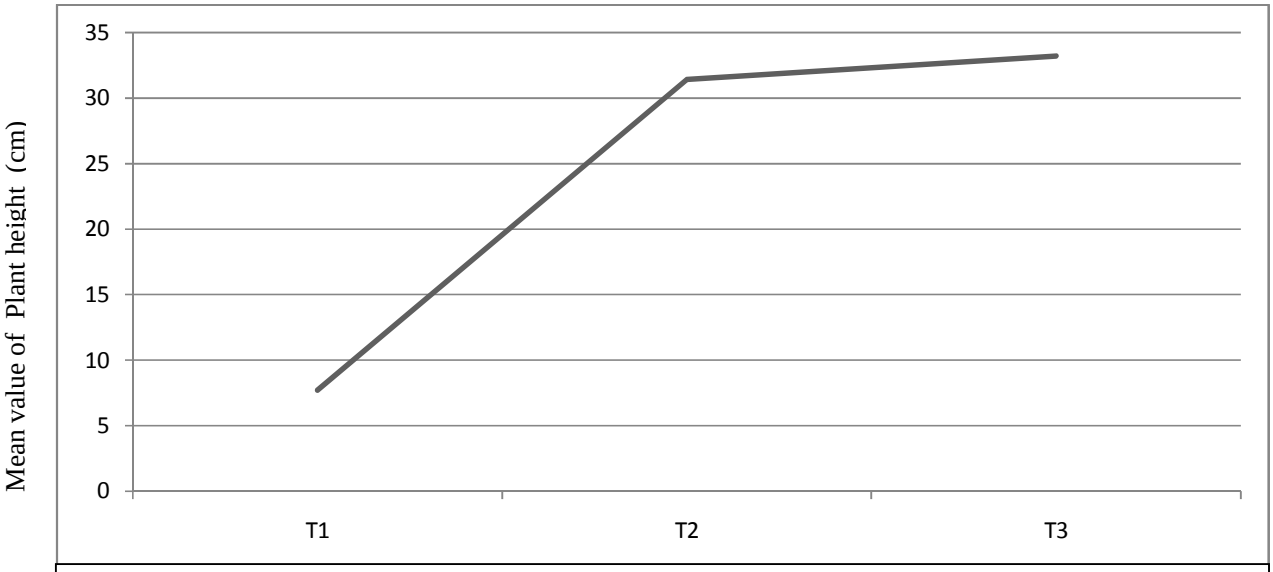


Fig 4.6: Effect of Treatment on Plant height

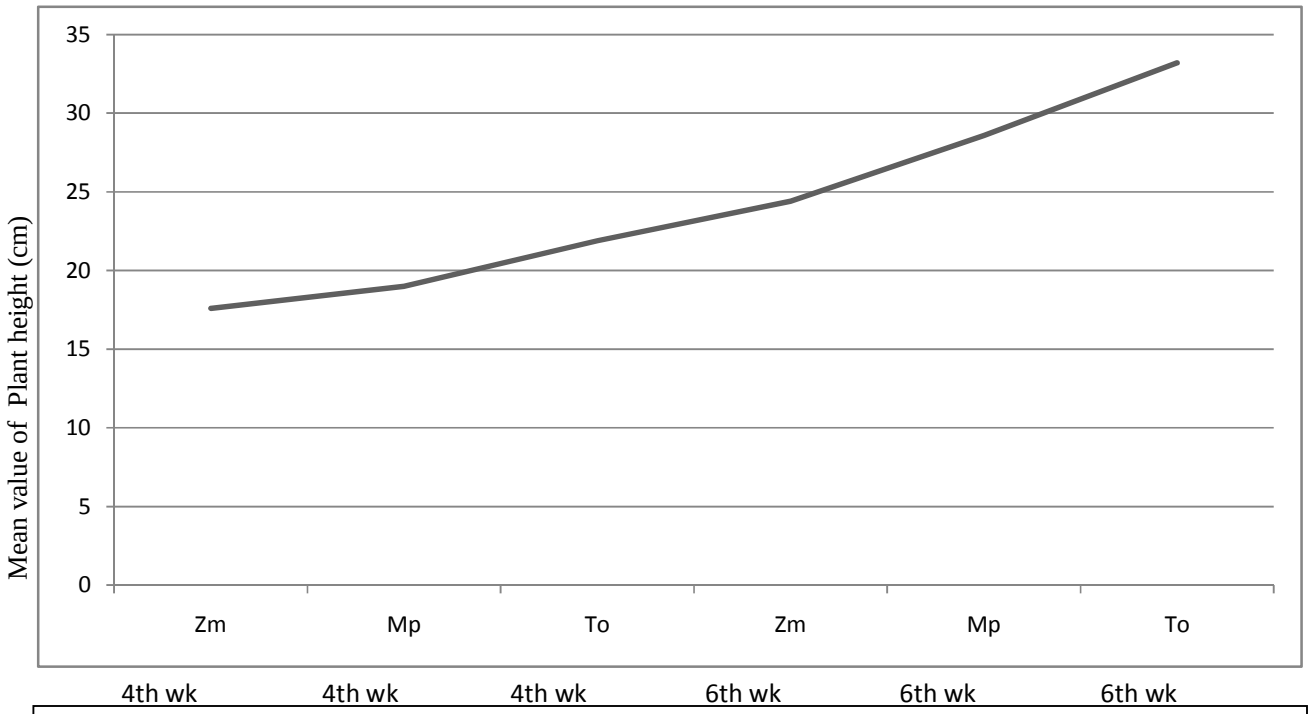


Fig 4.7: Combined effect of Plant type and Fortnight interval on Plant height

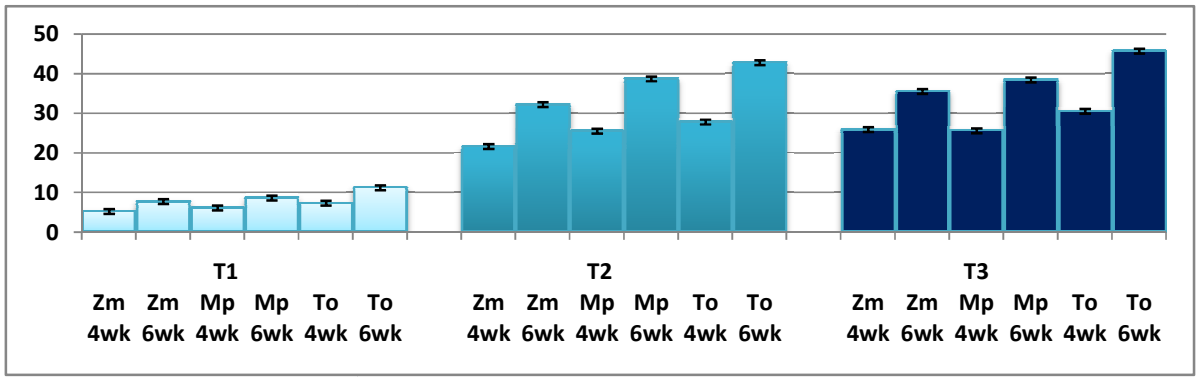


Fig 4.8: Combined **effect of Treatment, Plant type and Fortnight interval on Plant height (cm)**

Key

- Zm, Mp & To in Treatment 1 (Polluted soil before planting) of Fortnight interval

- Zm, Mp & To in Treatment 2 (Polluted soil one month after planting) of Fortnight interval

- Zm, Mp & To in Treatment 3 (Polluted soil stimulated with poultry droppings) of Fortnight interval

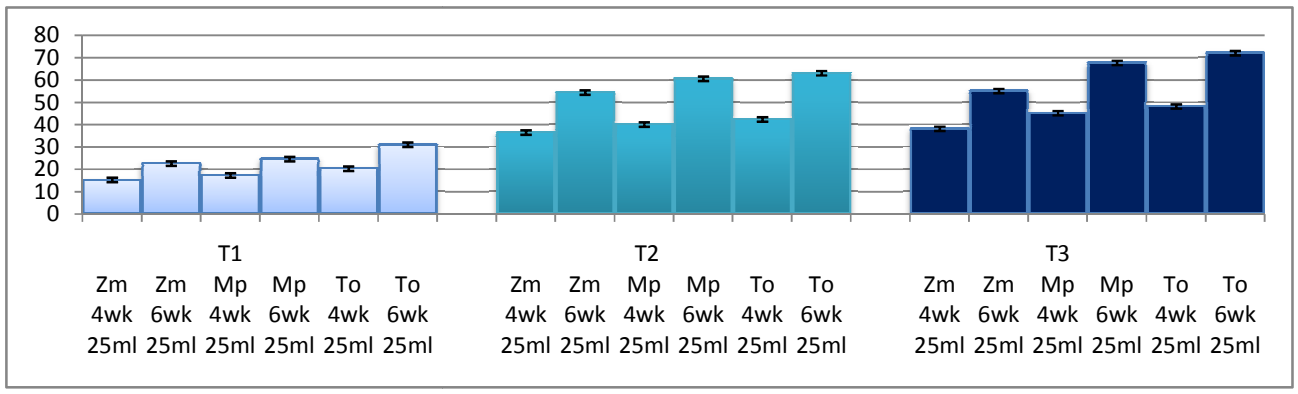


Fig 4.9: Combined effect of Treatment, Plant type, Fortnight interval and Crude oil pollution volume on Plant height (cm)

Key

- Zm, Mp & To in Treatment 1 (Polluted soil before planting) of Fortnight interval

- Zm, Mp & To in Treatment 2 (Polluted soil one month after planting) of Fortnight interval

- Zm, Mp & To in Treatment 3 (Polluted soil stimulated with poultry droppings) of Fortnight interval

4.1.6Total Hydrocarbon Contents of Soil

Total hydrocarbon content in the treatments of fortnight interval was determined in the varying volumes from the graph.

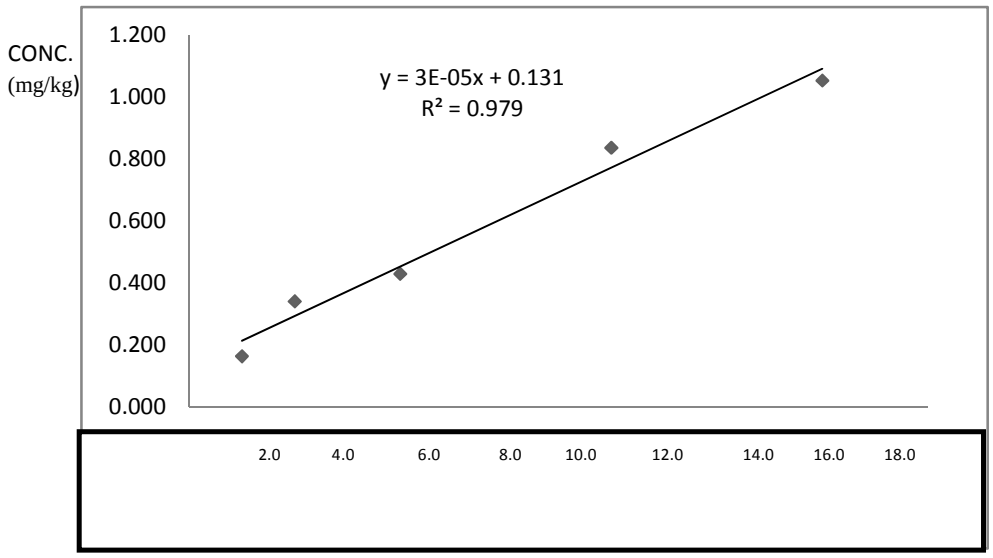


Fig 4.10: Standard curve from which the values were extrapolated.

Table 4.16: Total Hydrocarbon content of Soil samples

Control	Treatment1(Polluted soil before planting)	Treatment 2 (Polluted soil/ soil polluted one month after planting)	Treatment 3 (Polluted soil stimulated with Poultry droppings)	Treatment 4(Unpolluted soil stimulated with Poultry droppings)
Us **4 weeks = 0.00** **6 weeks = 0.00**	Zm + T_1 (25ml) **4 weeks = 6.65(mg/kg)** **6 weeks = 3.07**	Zm + T_2 (25ml) **4 weeks = 6.46 (mg/kg)** **6 weeks = 3.05**	Zm + T_3(25ml) **4 weeks = 5.25 (mg/kg)** **6 weeks = 2.03**	
	Zm + T_1 (50ml) **4 weeks = 7.37** **6 weeks = 3.13**	Zm + T_2 (50ml) **4 weeks = 6.90** **6 weeks = 2.12**	Zm + T_3 (50ml) **4 weeks = 6.40** **6 weeks = 1.05**	Zm + T_4 **4 weeks = 0.00** **6 weeks = 0.00**
Zm + Us **4 weeks = 0.00** **6 weeks = 0.00**	Zm + T_1(100ml) **4 weeks = 8.65** **6 weeks = 1.22**	Zm + T_2 (100ml) **4 weeks = 7.84** **6 weeks = 2.20**	Zm + T_3(100ml) **4 weeks = 7.00** **6 weeks = 3.12**	

	Zm + T 1 (200ml) **4 weeks = 11.22** **6 weeks = 6.58**	Zm + T2 (200ml) **4 weeks = 9.44** **6 weeks = 3.38**	Zm + T3(200ml) **4 weeks = 8.05** **6 weeks = 2.25**	
	Zm + T 1 (400ml) **4 weeks = 16.01** **6 weeks = 1.15**	Zm + T2(400ml) **4 weeks = 7.36** **6 weeks = 0.79**	Zm + T3 (400ml) **4 weeks = 4.20** **6 weeks = 1.48**	
Mp + Us **4 weeks = 0.00** **6 weeks = 0.00**				Mp + T4 **4 weeks = 0.00** **6 weeks = 0.00**
	Mp + T 1 (25ml) **4 weeks = 6.66** **6 weeks = 2.05**	Mp + T2 (25ml) **4 weeks = 6.42** **6 weeks = 2.04**	Mp + T3(25ml) **4 weeks = 6.21** **6 weeks = 1.02**	
	Mp + T 1 (50ml) **4 weeks = 6.32** **6 weeks = 2.11**	Mp + T2 (50ml) **4 weeks = 6.80** **6 weeks = 3.08**	Mp + T3(50ml) **4 weeks = 6.44** **6 weeks = 1.04**	
To + Us **4 weeks = 0.00** **6 weeks = 0.00**	Mp + T 1(100ml) **4 weeks = 8.64** **6 weeks = 3.25**	Mp + T 2 (100ml) **4 weeks = 7.65** **6 weeks = 3.14**	Mp + T 3 (100ml) **4 weeks = 6.88** **6 weeks = 1.07**	
Ps **4 weeks = 11.42** **6 weeks = 6.82**	Mp + T 1 (200ml) **4 weeks = 11.20** **6 weeks = 3.33**	Mp + T2(200ml) **4 weeks = 7.32** **6 weeks = 3.30**	Mp + T 3 (200ml) **4 weeks = 6.76** **6 weeks = 2.16**	
	Mp + T 1(400ml) **4 weeks = 15.18** **6 weeks = 2.98**	Mp + T2 (400ml) **4 weeks = 12.57** **6 weeks = 2.61**	Mp + T3 (400ml) **4 weeks = 8.42** **6 weeks = 1.30**	To + T4 **4 weeks = 0.00** **6 weeks = 0.00**
	To + T 1 (25ml) **4 weeks = 6.52** **6 weeks = 1.03**	To + T2 (25ml) **4 weeks = 6.38** **6 weeks = 1.03**	To + T3(25ml) **4 weeks = 5.18** **6 weeks = 1.00**	

Ps + Pd **4 weeks = 3.37** **6 weeks = 0.60**	To + T 1 (50ml) **4 weeks = 7.04** **6 weeks = 2.06**	To + T2 (50ml) **4 weeks = 7.75** **6 weeks = 2.05**	To + T3(50ml) **4 weeks = 7.30** **6 weeks = 1.02**	
	To + T 1 (100ml) **4 weeks = 8.06** **6 weeks = 3.14**	To + T2 (100ml) **4 weeks = 7.51** **6 weeks = 2.13**	To + T3 (100ml) **4 weeks = 6.50** **6 weeks = 1.04**	
Us + Pd **4 weeks = 0.00** **6 weeks = 0.00**	To + T 1 (200ml) **4 weeks = 10.16** **6 weeks = 1.28**	To + T2 (200ml) **4 weeks = 3.03** **6 weeks = 1.19**	To + T3(200ml) **4 weeks = 5.11** **6 weeks = 0.08**	
	To + T 1 (400ml) **4 weeks = 13.12** **6 weeks = 2.55**	To + T2(400ml) **4 weeks = 11.02** **6 weeks = 1.39**	To + T3(400ml) **4 weeks = 8.20** **6 weeks = 1.16**	

(Key: Us = Unpolluted soil, Ps = Polluted soil, Pd = Poultry droppings, Zm = *Zea mays*, Mp = *Mucuna pruriens*, To = *Telfairia occidentalis*).

From the above results, the hydrocarbon content in the soil polluted with 25ml of crude oil had least quantities of total hydrocarbon content.

Comparatively, the controls had the highest hydrocarbon content than the treatments, with control of polluted soil without plant having its fortnight hydrocarbon means as 11.42mg/kg and 7.82mg/kg while polluted soil stimulated with poultry droppings (T3) had 9.37mg/kg and 3.06mg/kg.

Generally for the treatments, all polluted soil samples stimulated with poultry droppings (T3) had lesser hydrocarbon content, followed by samples polluted one month after planting (T2)while the samples with polluted soil before planting (T1) had the highest hydrocarbon content (Table 4.19). Appendix I h shows the test soil samples.

However, from the experiment, it was observed for the fortnight interval (additional two weeks to the time the first record was taken), the hydrocarbon content was gradually reducing for the treatment samples.

TABLE 4.17: ANALYSIS OF VARIANCE (ANOVA) SHOWING THE EFFECT OF PLANT TYPE ON HYDROCARBON CONTENT
SINGLE

Plant Type	Mean hydrocarbon content (mg/kg)
Zea mays (Zm)	1.550 ± 0.2
Mucuna pruriens (Mp)	1.398 ±0.4
Telfairia occidentalis (To)	1.135 ±0.4
Least significant difference LSD $_{0.05}$	0.0528***

*** = Highly significant at $p < 0.001$

Table4.18: ANOVA SHOWING THE EFFECT OF CRUDE OIL POLLUTION VOLUME ON HYDROCARBON CONTENT

Volume of polluting crude oil (ml)	Mean hydrocarbon content (mg/kg)
25	0.226± 0.4
50	0.443± 0.3
100	0.893± 0.4
200	1.713± 0.2
400	3.529± 0.4
Least significant difference LSD$_{0.05}$	0.0681***

*** = Highly significant at $p< 0.001$

TABLE 4.19: ANOVASHOWING THE EFFECT OF TREATMENT ON HYDROCARBON CONTENT

Treatment	Mean hydrocarbon content (mg/kg)
T1	1.992 ± 0.2
T2	1.400 ± 0.3
T3	0.691 ± 0.3
Least significant difference $LSD_{0.05}$	0.0528***

*** = Highly significant at $p < 0.001$

Table 4.20: ANOVA SHOWING THE EFFECT OF FORTNIGHT INTERVAL ON PLANT HEIGHT

Fortnight interval	Mean hydrocarbon content (mg/kg)
4th week	2.494 ± 0.4
6th week	0.228 ± 0.3
Least significant difference $LSD_{0.05}$	0.0431***

*** = Highly significant at $p < 0.001$

Table 4.21: ANOVA SHOWING THE COMBINED EFFECT OF PLANT TYPE AND CRUDE OIL POLLUTION VOLUME ON HYDROCARBON CONTENT
TWO COMBINATION

Plant Type	Volumes of polluting crude oil (ml)	Mean hydrocarbon content(mg/kg)
Zm	25	0.252 ± 0.3
Mp	25	0.233 ± 0.2
To	25	0.192 ± 0.4
Zm	50	0.495 ± 0.4
Mp	50	0.465 ± 0.1
To	50	0.370 ± 0.3
Zm	100	0.938 ± 0.2
Mp	100	0.730 ± 0.4
To	100	1.987 ± 0.3
Zm	200	1.845 ± 0.4
Mp	200	12.528± 0.1
To	200	1.308 ± 0.3
Zm	400	4.005 ± 0.4
Mp	400	3.510 ± 0.1
To	400	3.073 ± 0.4
Least significant difference $LSD_{0.05}$		0.1180***

*** = Highly significant at $p < 0.001$

Table 4.22: ANOVA SHOWING THE COMBINED EFFECT OF PLANT TYPE AND FORTNIGHT INTERVAL ON HYDROCARBON CONTENT

Plant Type	Fortnight Interval	Mean hydrocarbon content (mg/kg)
Zm	4th week	2.792± 0.3
Mp	4th week	2.565 ± 0.4
To	4th week	2.125± 0.3
Zm	6th week	0.308± 0.1
Mp	6th week	0.232 ± 0.2
To	6th week	0.144± 0.4
Least significant difference $LSD_{0.05}$		0.0746***

*** = Highly significant at $p < 0.001$

TABLE 4.23: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT AND PLANT TYPE ON HYDROCARBON CONTENT

Treatment	Plant Type	Mean hydrocarbon content (mg/kg)
T1	Zm	2.209± 0.3
T2 Mp	1.558 ± 0.4	
T3	To	0.883± 0.2
T1Zm	2.072± 0.1	
T2Mp	1.393± 0.4	
T3	To	0.730± 0.3
T1 Zm	1.696± 0.4	
T2	Mp	1.248± 0.3
T3	To	0.460± 0.4
Least significant difference LSD$_{0.05}$		0.0914**

** = Highly significant at p = 0.006

Table 4.24: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT ANDFORTNIGHT INTERVAL ON HYDROCARBON CONTENT

Treatment	Fortnight Interval	Mean hydrocarbon content (mg/kg)
T1	4th week	3.656± 0.3
T2	4th week	2.566 ± 0.4
T3	4th week	1.260 ± 0.2
T1	6th week	0.329 ± 0.4
T2	6th week	0.233 ± 0.1
T3	6th week	0.122± 0.4
Least significant difference LSD $_{0.05}$		0.0746***

*** = Highly significant at p< 0.001

TABLE 4.25: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT AND CRUDE OIL POLLUTION VOLUME ON HYDROCARBON CONTENT

Treatment	Volume of polluting crude oil (ml)	Mean hydrocarbon content (mg/kg)
T1	25	0.330± 0.2
T2	25	0.230± 0.4
T3	25	0.117± 0.3
T1	50	0.672± 0.4
T2	50	0.450± 0.2
T3	50	0.208± 0.3
T1	100	1.327± 0.4
T2	100	0.918± 0.4
T3	100	0.435± 0.3
T1	200	2.628± 0.3
T2	200	1.777± 0.4
T3	200	0.735 ± 0.2
T1	400	5.005± 0.4
T2	400	3.623± 0.1
T3	400	1.960± 0.4

Least significant difference LSD $_{0.05}$0.1180***

*** = Highly significant at p< 0.001

TABLE 4.26: ANOVA SHOWING THE COMBINED EFFECT OF FORTNIGHT INTERVAL AND CRUDE OIL POLLUTION VOLUME ON HYDROCARBON CONTENT

Fortnight Interval	Volume of polluting crude oil (ml)	Mean hydrocarbon content (mg/kg)
4th week	25	0.414± 0.4
6th week	25	0.037± 0.3
4th week	50	0.813± 0.4
6th week	50	0.073± 0.2
4th week	100	1.641± 0.4
6th week	100	0.146 ± 0.4
4th week	200	3.143± 0.1
6th week	200	0.283 ± 0.4
4th week	400	6.458± 0.4
6th week	400	0.601± 0.4

Least significant difference LSD$_{0.05}$0.0963***

*** = Highly significant at p< 0.001

Table 4.27: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT, PLANT TYPE AND FORTNIGHT INTERVAL ON HYDROCARBON CONTENT THREE COMBINATION

Mean hydrocarbon content (mg/kg) for Fortnight Interval			
Treatment	**Plant Type**	**4th week**	**6th week**
T1	Zm	3.988 ± 0.4	0.430 ± 0.4
T2	Zm	2.808 ± 0.4	0.308 ± 0.3
T3	Zm	1.550 ± 0.4	0.816 ± 0.4
T1	Mp	3.800 ± 0.4	0.344 ± 0.3
T2	Mp	2.552± 0.4	0.234 ± 0.4
T3	Mp	1.342 ± 0.4	0.118 ± 0.2
T1	To	3.180 ± 0.4	0.212 ± 0.4
T2	To	2.338 ± 0.4	0.158 ± 0.3
T3	To	0.858 ± 0.4	0.100 ± 0.4

Least significant difference LSD 0.050.1292

Not significant at p = 0.051

Table4.28: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT, PLANT TYPE AND CRUDE OIL POLLUTION VOLUME ON HYDROCARBON CONTENT

Mean hydrocarbon content (mg/kg) Volume of pollutingcrude oil					
Treatment	**Plant Type**	**25ml**	**50ml**	**100ml**	**200ml**	**400ml**
T1	Zm	0.360± 0.4	0.750± 0.3	1.435 ± 0.2	2.900± 0.3	5.600± 0.4
T2	Zm	0.255± 0.3	0.510± 0.4	1.040± 0.3	1.910± 0.2	4.075± 0.4
T3	Zm	0.140± 0.4	0.225± 0.2	0.560 ± 0.3	1.150± 0.4	2.340± 0.2
T1	Mp	0.355 ± 0.2	0.715 ± 0.4	1.445± 0.4	2.765± 0.4	5.080± 0.4
T2	Mp	0.230 ± 0.1	0.440± 0.3	0.895 ± 0.3	1.180± 0.2	3.590± 0.3
T3	Mp	0.115 ± 0.3	0.240 ± 0.4	0.475± 0.2	0.960± 0.4	1.860± 0.4
T1	To	0.275 ± 0.4	0.550± 0.3	1.100 ± 0.4	2.220 ± 0.3	4.335 ± 0.1
T2	To	0.205 ± 0.2	0.440 ± 0.4	0.820 ± 0.4	1.610± 0.4	3.205± 0.4
T3	To	0.095 ± 0.4	0.160 ± 0.1	0.270 ± 0.3	0.095± 0.2	1.680± 0.4

Least significant difference LSD 0.05 0.2044***

*** = Highly significant at p< 0.001

Table 4.29: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT,FORTNIGHT INTERVAL AND CRUDE OIL POLLUTION VOLUMEON HYDROCARBON CONTENT

Mean hydrocarbon content (mg/kg)						
Volume of pollutingcrude oil						
Treatment	Fortnight Interval	25ml	50ml	100ml	200ml	400ml
T1	4th week	0.610± 0.3	1.234± 0.4	2.450± 0.3	4.860± 0.2	9.117± 0.4
T2	4th week	0.420± 0.4	0.817 ± 0.1	1.680± 0.4	3.263± 0.4	6.650± 0.3
T3	4th week	0.213± 0.2	0.380± 0.4	0.793± 0.1	1.307± 0.3	3.607± 0.4
T1	6th week	0.050± 0.4	0.100± 0.3	0.203± 0.4	0.397± 0.4	0.893± 0.3
T2	6th week	0.040± 0.1	0.083± 0.4	0.157± 0.4	0.290± 0.1	0.597± 0.4
T3	6th week	0.020± 0.4	0.037± 0.4	0.077± 0.2	0.163± 0.3	0.313± 0.2
Least significant difference LSD 0.05 0.1669***						

*** = Highly significant at p< 0.001

TABLE 4.30: ANOVA SHOWING THE COMBINED EFFECT OF PLANT TYPE, FORTNIGHT INTERVAL AND CRUDE OIL POLLUTION VOLUME ON HYDROCARBON CONTENT

Mean hydrocarbon content (mg/kg)						
Volume of polluting crude oil						
Plant Type	Fortnight Interval	25ml	50ml	100ml	200ml	400ml
Zm	4th week	0.453 ± 0.3	0.890± 0.4	1.843± 0.3	3.570± 0.4	7.203± 0.4
Mp	4th week	0.430± 0.4	0.853± 0.2	1.723± 0.2	3.427± 0.3	6.390± 0.2
To	4th week	0.360 ± 0.4	0.697 ± 0.4	1.357 ± 0.4	2.433± 0.4	5.780± 0.3
Zm	6th week	0.050± 0.2	0.100 ± 0.4	0.180± 0.3	0.403± 0.3	0.807± 0.4
Mp	6th week	0.037± 0.4	0.077 ± 0.1	0.153± 0.4	0.263 ± 0.4	0.630± 0.4
To	6th week	0.023± 0.3	0.043 ± 0.4	0.103± 0.4	0.183± 0.4	0.367 ± 0.3
Least significant difference LSD 0.05 0.1669***						

*** = Highly significant at p< 0.001

Table 4.31: ANOVA SHOWING THE COMBINED EFFECT OF TREATMENT,PLANT TYPE, FORTNIGHT INTERVAL AND CRUDE OIL POLLUTION VOLUME ON HYDROCARBON CONTENT
FOUR COMBINATION

Mean hydrocarbon content (mg/kg)						
Volume of polluting crude oil						
Treatment	**Plant Type**	**Fortnight Interval**	**25ml**	**50ml**	**100ml**	**200ml**	**400ml**
T1	Zm	4th week	0.650± 0.4	1.370± 0.3	2.650± 0.3	5.220± 0.4	10.050± 0.4
T2	Zm	4th week	0.460± 0.2	0.900 ± 0.4	1.880± 0.4	3.440± 0.2	7.360 ± 0.4
T3	Zm	4th week	0.250± 0.4	0.400± 0.4	1.000 ± 0.3	2.050 ± 0.4	0.400± 0.3
T1	Mp	4th week	0.660± 0.3	1.320± 0.3	2.640± 0.3	5.200 ± 0.3	9.180 ± 0.4
T2	Mp	4th week	0.420± 0.1	0.880± 0.4	1.650 ± 0.4	3.320± 0.2	6.570 ± 0.2
T3	Mp	4th week	0.210± 0.4	0.440 ± 0.2	0.880± 0.2	1.760± 0.4	3.420 ± 0.4
T1	To	4th week	0.520± 0.3	1.040± 0.4	2.060± 0.4	4.160± 0.4	8.120 ± 0.3
T2	To	4th week	0.380± 0.4	0.750± 0.2	1.510± 0.1	3.030± 0.3	6.020 ± 0.4
T3	To	4th week	0.180± 0.4	0.300± 0.1	0.500± 0.3	0.110± 0.4	3.200 ± 0.1
T1	Zm	6th week	0.070 ± 0.3	0.130 ± 0.4	0.220 ± 0.4	0.580± 0.3	1.150± 0.2
T2	Zm	6th week	0.050± 0.4	0.120± 0.3	0.220 ± 0.3	0.380± 0.4	0.790± 0.4
T3	Zm	6th week	0.030± 0.4	0.050 ± 0.4	0.120± 0.4	0.250± 0.3	0.050± 0.2
T1	Mp	6th week	0.050± 0.2	0.110± 0.3	0.250± 0.2	0.330 ± 0.3	0.980± 0.4
T2	Mp	6th week	0.040± 0.4	0.080± 0.4	0.140 ± 0.4	0.300± 0.4	0.610± 0.3
T3	Mp	6th week	0.020± 0.4	0.040± 0.4	0.070± 0.4	0.160± 0.3	0.300± 0.4
T1	To	6th week	0.030± 0.1	0.060 ± 0.3	0.140± 0.3	0.280± 0.3	0.550± 0.3
T2	To	6th week	0.030± 0.4	0.050± 0.4	0.130± 0.4	0.190± 0.4	0.390± 0.4
T3	To	6th week	0.010± 0.3	0.020± 0.4	0.040± 0.3	0.080 ± 0.4	0.160± 0.4
Least significant difference LSD 0.05			0.2890**				

** = Highly significant at p = 0.008

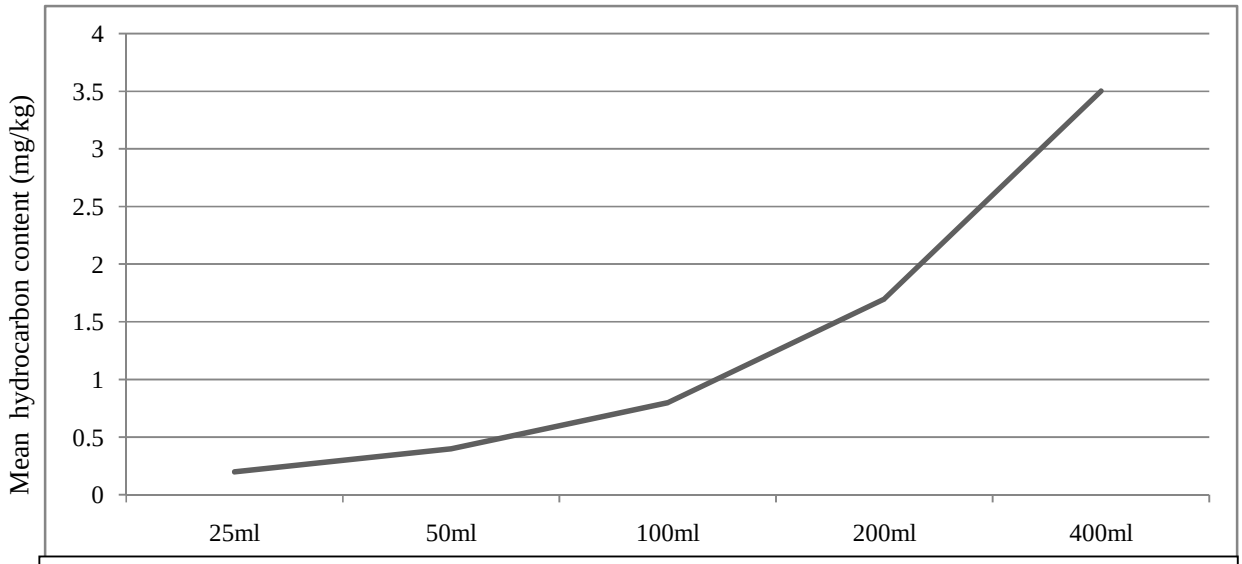


Fig 4.11: Effect of Crude oil pollution volumes of Plant samples in reducing the Hydrocarbon content

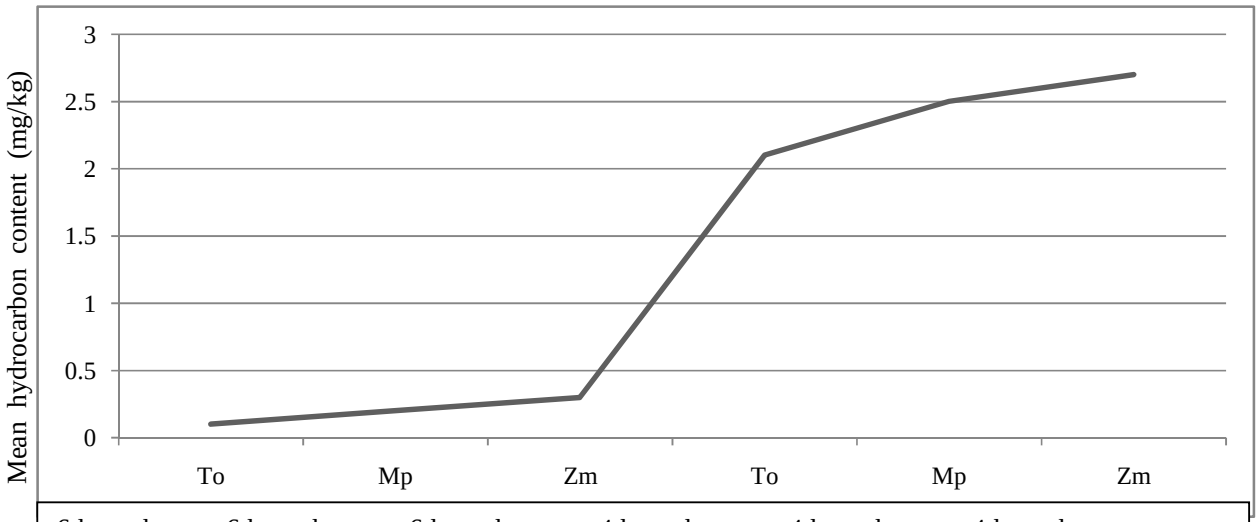


Fig 4.12: Combined effect of Plant type and Fortnight interval in reducing the Hydrocarbon content

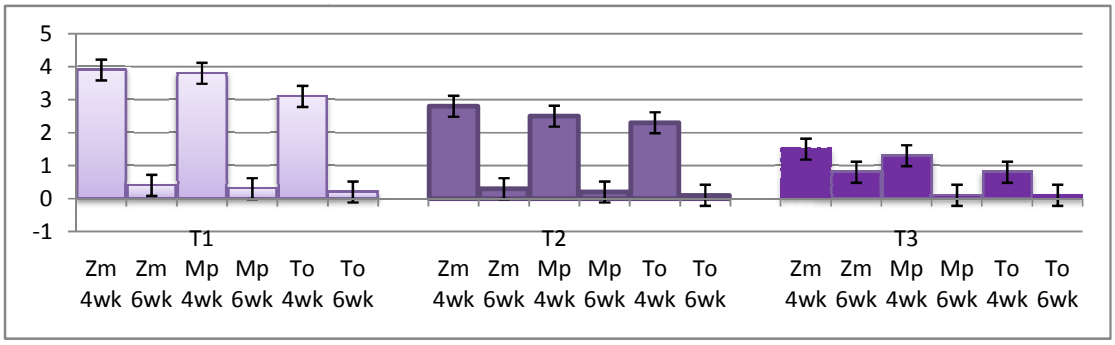


Fig 4.13: Combined effect of Treatment, Plant type and Fortnight interval in reducing hydrocarbon content (mg/kg)

Key

- Zm, Mp & To in Treatment 1 (Polluted soil before planting) of Fortnight interval
- Zm, Mp & To in Treatment 2 (Polluted soil one month after planting) of Fortnight interval
- Zm, Mp & To in Treatment 3 (Polluted soil stimulated with poultry droppings) of Fortnight interval

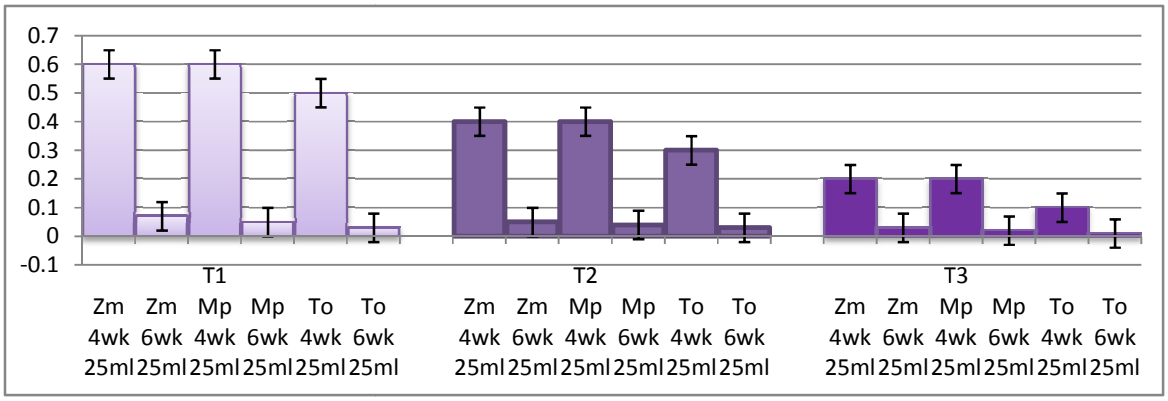


Fig 4.14: Combined effect of Treatment, Plant type, Fortnight interval and Crude oil pollution volume in reducing hydrocarbon content (mg/kg)

Key

- Zm, Mp & To in Treatment 1 (Polluted soil before planting) of Fortnight interval
- Zm, Mp & To in Treatment 2 (Polluted soil one month after planting) of Fortnight interval
- Zm, Mp & To in Treatment 3 (Polluted soil stimulated with poultry droppings) of Fortnight interval

4.1.7 Microbial Characteristics

The result in Table 4.32 shows the probable crude oil utilizing bacteria and fungi present in the unpolluted and polluted soils. Appendix I i – I lshows the microbial result.

Table 4.32: Crude oil utilizing microorganisms

Crude oil utilizing microorganisms	Unpolluted soil	Polluted soil
Proteus vulgaris	+	+
Pseudomonas sp	+	+
Staphylococcus sp+	+	
Bacillus sp+	+	
Acinetobacter sp+	+	
Escherichia coli	+	-
Micrococcus sp	+	+
Aspergillus sp+	+	
Penicillium sp+	+	

Key:

+, positive

-, negative

sp, species

4.1.8 Biochemical Characteristics

The biochemical tests undertaken are as shown in Table 4.33 and Appendix I i

Table 4.33: Morphological and biochemical characteristics of the isolates from polluted soilsamples.

Morphological Characterization		Gram staining	Motility test	Catalase test	Citrate test	Indole test	Coagulase test	Sugar fermentation test			Isolates
Colour	Consistency/Texture							Glucose	Lactose	Sucrose	
Creamy Rod	Flat colonies	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	*Proteus vulgaris*
LightSlightly yellowRod	raised	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	*Pseudomonas* sp
Green Rod	Rough surface	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	*Escherichia coli*
Creamy edge cluster	Raised/smooth cocci	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	*Staphylococcus* sp
White mucoidRod	Flat colonies	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	*Bacillus* sp
Creamy colony	Small circular	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve *Acinetobacter* sp
Light yellowRod	Flat colonies	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	*Micrococcus*sp

Legend:

+, positive

-, negative

sp, species

4.1.9: Molecular Characteristics

Molecular analysis was carried out to confirmthe possible presence of bacteria and fungi present in the polluted soil.The presence of band in the test samples in Plates 4.1 - 4.8 shows that there are possible bacteria and fungi involved in the degradation of hydrocarbons within the treated soil. Although Plate 4.8 had no visible band.

From the DNA com1 primer, specifically used for detecting the presence of bacteria, visible bands were seenin lanes 1, 2, 3,4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 17, 18, 19, 21, 22, 23, 24 and 37.While no visible band was seen in lanes12, 16, 20, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46 and 47(Plate 4.1 – 4.4) .

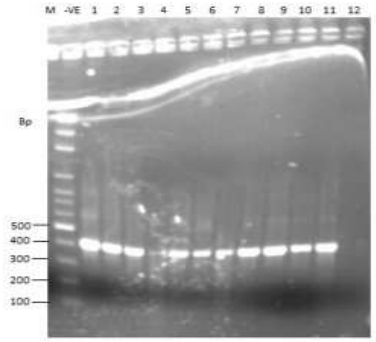


Plate 4.1: DNA bands depicting the presence of bacteria intest soil samples.

Legend:

M = Marker, -VE = Negative, 1 = ZmT1(25ml) , 2 = ZmT2(25ml), 3 = ZmT3(25ml),
4 = MpT1(25ml), 5 = MpT2(25ml), 6 = MpT3(25ml), 7 = ToT1(25ml),8 = ToT2(25ml),
9 = ToT3(25ml), 10 = ZmT1(50ml), 11 = ZmT2(50ml), 12 = ZmT3(50ml)

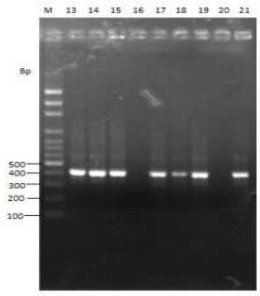


Plate 4.2:DNA bands depicting the presence of bacteria in test soil samples.

Legend:

M = Marker, 13 = MpT1(50ml),14 = MpT2(50ml), 15 = MpT3(50ml), 16 = ToT1(50ml),

17 = ToT2(50ml), 18 = ToT3(50ml), 19 = ZmT1(100ml), 20 = ZmT2(100ml),

21 = ZmT3(100ml)

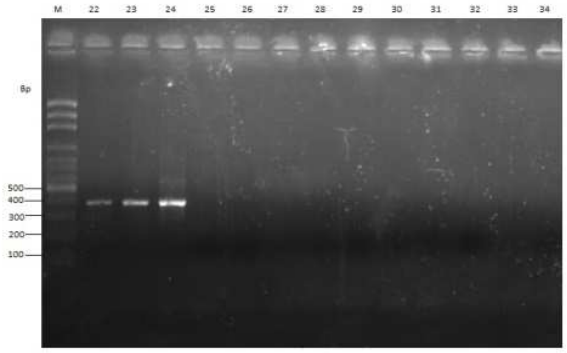


Plate 4.3:DNA bands depicting the presence of bacteria in test soil samples.

Legend:

M= Marker, 22 = MpT1(100ml), 23 = MpT2(100ml), 24 = MpT3(100ml),

25 = ToT1(100ml)**,** 26 = ToT2(100ml), 27= ToT3(100ml), 28 = ZmT1(200ml),

29 = ZmT2(200ml),30 = ZmT3(200ml), 31 = MpT1(200ml), 32 = MpT2(200ml), 33 = MpT3(200ml), 34 = ToT1 (200ml)

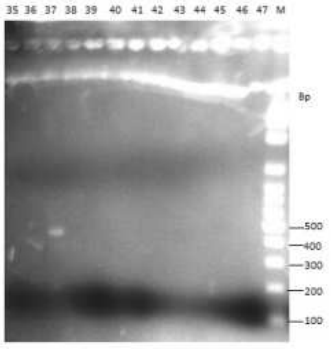


Plate 4.4: DNA bands depicting the presence of bacteria in a test soil sample.

Legend:

35 = ToT2(200ml), 36 = ToT3(200ml), 37 = ZmT1(400ml), 38 = ZmT2(400ml),

39 = ZmT3(400ml), 40 = MpT1(400ml), 41 = MpT2(400ml), 42 = MpT3(400ml),

43 = ToT1(400ml), 44 = ToT2(400ml), 45 = ToT3(400ml), 46 = Unpolluted soil + Zm

47 = Control (soil), M = Marker.

From the NS7F primer, specifically for detecting the presence of Fungi, visible bands were seen in lanes 1, 2, 6, 8, 9, 11, 14, 15, 16, 18, 20, 21, 22, 25, 30, 37 and 38. Whereas no visible band was seenin lanes 3, 4, 5, 7, 10, 12, 13, 17, 19, 23, 24, 26, 27, 28, 29, 31,32, 33, 34, 35, 36, 39, 40, 41, 42, 43, 44, 45, 46 and 47 (Plate4.5 – 4.8) .

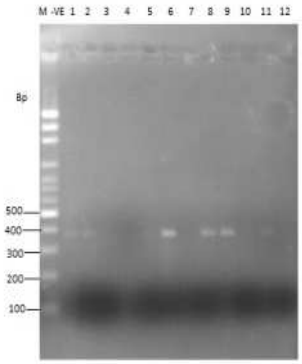


Plate 4.5: DNA bands depicting the presence of fungi in test soil samples.

Legend:

M = Marker, -VE = Negative, 1 = ZmT1(25ml), 2 = ZmT2(25ml), 3 = ZmT3(25ml),

4 = MpT1(25ml), 5 = MpT2(25ml), 6 = MpT3(25ml), 7 = ToT1(25ml), 8 = ToT2(25ml),

9 = ToT3(25ml), 10 = ZmT1(50ml), 11 = ZmT2(50ml), 12 = ZmT3(50ml)

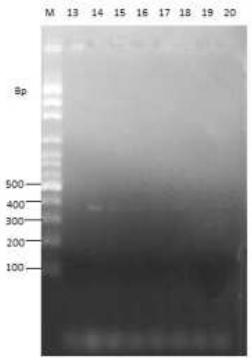


Plate 4.6:DNA bands depicting the presence of fungi in test soil sample.

Legend:

M = Marker, 13 = MpT1(50ml),14 = MpT2(50ml), 15 = MpT3(50ml), 16 = ToT1(50ml),

17 = ToT2(50ml), 18 = ToT3(50ml), 19 = ZmT1(100ml), 20 = ZmT2(100ml)

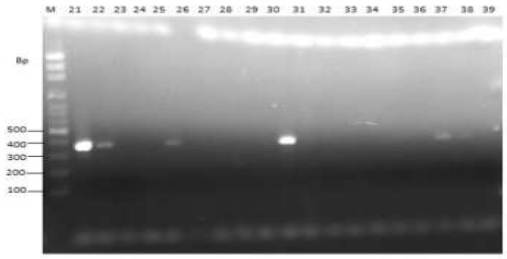


Plate 4.7:DNA bands depicting the presence of fungi intest soil samples.

Legend:

M= Marker, 21 = ZmT3(100ml), 22 = MpT1(100ml), 23 = MpT2(100ml), 24 = MpT3(100ml),

25= ToT1(100ml), 2 = ToT2(100ml), 27= ToT3(100ml), 28 = ZmT1(200ml), 29 = ZmT2(200ml),

30 = ZmT3 (200ml), 31 = MpT1(200ml), 32 = MpT2(200ml), 33 = MpT3(200ml), 34 = ToT1 (200ml),

35 = ToT2(200ml), 36 = ToT3(200ml), 37 = ZmT1 (40 ml), 38 = ZmT2(400ml), 39 = ZmT3(400ml)

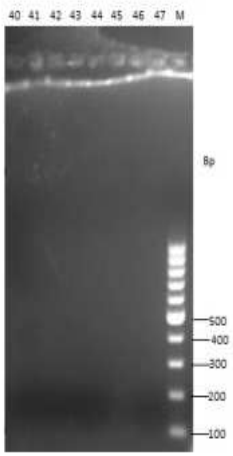


Plate 4.8: No visible DNA bands in testsoil samples.

Legend:

40 = MpT1(400ml), 41 = MpT2(400ml), 42 = MpT3(400ml), 43 = ToT1(400ml),

44 = ToT2(400ml), 45 = ToT3(400ml), 46 = Unpolluted soil + Zm, 47 = Control (soil),

M = Marker.

4.2Discussion

4.2.1 GrowthMeasurement

In this study,growth measurement result obtained from the treatments used indicated that plants on unpolluted soil but stimulated with poultry droppings (positive control) had the best growth, followed by those on polluted soil and stimulated with poultry droppings.Plants whose soils were polluted one month after planting also had good growths while others planted on soil that was polluted before planting had the least growthperformance. Growths of controls were also measured (Fig 4.1 - 4.5). From the plant growths, Towith 27.589cm had the highest growth followed by Mp which had23.859cm while Zm had 21.050cm and this was very highly significant ($p < 0.001$).For the crude oil volumes, 25ml growth was 41.861cm the highest, 50ml had 37.683cm growth, 100ml had 20.289cm, 200ml had 13.204 cm and 400ml had the least 7.793cm growth, and the difference was very highly significant($p < 0.001$)(Tables 4.1 - 4.15).It was observed from this study that crude oil pollution ofagricultural soil affects agricultural productivity. This is line with the observationsreported by several researchers (Adenipekun *et al.,* 2009; Okoh, 2008; Ogbulie *et al.,* 2011; Ogbulie *et al.,*2014).

Generally,oil contamination in whatever form is toxic to plant and soil micro-environments. Several researchers (Adenipekun and Kassim, 2006; Anoliefo and Vwioko, 2005)have reported that crude oil affects the physiological, ecological and anatomical development of plants grown on such soils. This is in consonance with the findings of the present study. Treatments where soils were polluted with crude oil depict that germination of seeds were delayed in crude oil polluted soil.This was possibly due to the effect of higher volumes of crude oil in the polluted soil since all treatment was done with the same quantity of soil, hence it is volume dependent. This is in agreement with the result obtained by Adenipekun *et al.*(2009) that oil in soil above 2% concentration affects the growth of seeds adversely and severely. Crude oil spill into agricultural soil therefore affects the germination, plant physiology and ecology, and ultimately the growth and anatomical development of plants. The presence of crude oil in the plant soil micro-environment appears to affect normal soil chemistry, nutrient release and uptake and as well the amount of water available to the plants are reduced (Nwoko *et al.*, 2007).The decrease in height/retarded growth of plants in soils polluted withhigher volumes of crude oil is due to thelarge quantities of crude oil which may have made the soil unable to retain water, which possibly affected the nutrient uptake and mobility.However, the value obtained in treatment three (T_3) samples is an indication that stimulation enhanced remediation during interaction with plant root.

4.2.2 Total Hydrocarbon Content

The analytical method for total hydrocarbon was successfully applied to determine the level of hydrocarbon mixture in the soil. The method was based on the extraction and separation of total hydrocarbons from the indigenous contaminated soil using spectrophotometer. Generally, this analysis carried out showed that the total hydrocarbon content (THC) of the treatments of lesser crude oil volumes were lower thanthe control samples. It was observed that the polluted soil stimulated with poultry droppings had the least hydrocarbon content; this is possibly as a result of more crude oil utilizing microorganisms present in the poultry droppings. This was followed by polluted soil one month after planting while samples of polluted soil before planting had the highest total hydrocarbon contents (Table 4.16). UsingSPSS software, comparisons were made with analysis of variance (ANOVA) based on the volumes used and the resultant total hydrocarbon contents were obtained (Tables 4.17–4.31). To showed a much better remediation effect followed by MP and lastly ZM. Crude oil volumesof 25ml had hydrocarbon content of0.226mg/kg, 50ml had0.443mg/kg, 100ml had 0.893mg/kg, 200ml had 1.713mg/kgwhile 400ml had 3.52mg/kg and the difference was highly significant ($p<0.001$).Hydrocarbon values obtained from the statistical analyses were observed to be increasing with increase in the crude oil pollution volume but decreasing within the fortnight interval (Figs 4.11-4.14).This study showed that the interaction between the plants and

microorganisms encouraged crude oil reduction from stimulated treatment faster than other treatments. This corroborates the reports made by Kuiper *et al* . (2004), Motoyama *et al.* (2005), Jussila (2006) and Ogbulie and Njoku (2011) who attributed the success to the ability of the legume to harbor large numbers of bacterial and fungal species on their highly branched root systems.Thedecrease in hydrocarbon content of the polluted soil in the treatments is due to the crude oil degradation action of microorganisms in interaction with plant roots (rhizosphere).This is similar to the work of Frick *et al.* (2009) who reported that bacteria play good role in the degradation of crude oil.It also supports the findings of Ogbulie and Njoku(2011) and Ogbulie *et al.* (2012) who stated that the roots release exudates for microorganisms to utilize and degrade hydrocarbons. Soils polluted with 25ml crude oil concentration majorly had least hydrocarbon content followed by 50ml.The amount of crude oil lost from the polluted soil was enhanced by the growths of the plant. Similar works have been reported by Njoku *et al.* (2009)who stated that the amount of crude oil lost from the soil contaminated with 25ml crude oil was enhanced by the growth of plant. Soils polluted with 100ml of crude oil had higher hydrocarbon contents.The trend was observed in soils with 200ml and 400ml of crude oil pollution recorded in the hydrocarbon content, evidently the rate of reduction depends on volume as well as number of plants per soil present during remediation.Among the three treatments, plants with a combination of polluted soil and poultry droppings had

lower and reducing hydrocarbon contentspossibly due to the higher microbial activities.This is an indication that the higher the concentration of agro-wastes in the soil, the higher the microbial population and activity, the lowerthe hydrocarbon content (Agbor *et al.*, 2012). This is however in line with this study.Okpokwasili (1994) observed that the use of NPK fertilizer, urea fertilizer and poultry droppings effectively stimulated bacterial growth into utilization ofcrude oil.

4.2.3 Microbial Characteristics

Microbiological analysis showed that crude oil utilizing microorganisms were *Aspergillus* sp, *Penicillium* sp,*Proteus vulgaris, Pseudomonas* sp, *Staphylococcus* sp,*Bacillus* sp,*Acinetobacter* sp,*Escherichia coli*and *Micrococcus* sp as shown in Table 4.362.However, after pollution there was absence of *Escherichia coli*. This is in accordance with the findings of Nkwelang *et al.* (2008) whose report showed that *Pseudomonas* sp, *Bacillus* sp,*Aspergillus* sp and *Penicillium* spwere present in the polluted soil throughout the experimental period. Similarly, Okoh *et al.* (2010) isolated*Aspergillus* sp, *Penicillium* spand *Candida albicans* from sampleswhich persisted even after treatments throughoutthe experimental process.The number of microorganisms were more in the polluted soil than in the natural soil, this is similar to the works of Ogbulie*et al.* (2013) which stated that the presence of petroleum might cause an increase in microbial population in

the soil. Plant-associated bacteria such as endophytic bacteria (non-pathogenic bacteria that occur naturally in plants) and rhizospheric bacteria (bacteria that live on and near the roots of plants) contribute to biodegradation of toxic organic compounds in contaminated soil and have potential for improving phytoremediation. Poultry droppings used as stimulant has the potential of increasing the microbial population in crude oil-contaminated soil due to the high nitrogen and phosphorus contents of the wastes.

Agbor *et al.*(2012)observed that poultry droppings effectively stimulated bacterial growth into utilization of crude oil. The use of NPK fertilizer, Urea fertilizer and poultry droppings effectively stimulated bacterial growth into utilization of crude oil. This is an indication that the higher the concentration of these agro-wastes, the higher the microbial population in the soil. This shows that for a speedy degradation rate of crude oil (clean-up) in the soil, the microorganisms must be high in population.These microorganisms especially bacteria can degrade certain kinds of organic pollutants such as hydrocarbons. This therefore is in consonance with the findings of this study.

Furthermore, the bacterial and fungal species associated with crude oil degradation obtained in this studyare in line with the findings of Jussila (2006) andOgbulie *et al.* (2010).Zajic and Suplison (1992) reported that *Pseudomonas* sp had a more competent and active hydrocarbon degrading enzymes than other biodegraders. Some bacteria exhibit a spreading growth pattern in the plate

suppressing the growth of other bacterial colonies from developing. This group of bacteriaareknown for antibiotic production.The high microbial counts of crude oil utilizing bacteria and fungi during the bioremediation study can thus be said to be responsible for the high degradation rate of total petroleum hydrocarbon content in the soil. Among the four treatments studied, soil polluted with crude oil and mixed with poultry droppings had increased microbial population and activity. This can be used in oil producing regions to enhance the degradation of crude oil spill in the environment.

4.2.4 Biochemical Characteristics

The biochemical tests showed the morphological features of isolates from polluted soil samples.Proper characterization was done and the bacterial isolates were identified hence they are oil-degrading microorganisms.The bacterial isolates identified are mostly Gram negative rods. The resultsof the characterization and identification showed that *Aspergillus* sp, *Penicillium* sp, *Proteus vulgaris*, *Pseudomonas* sp, *Staphylococcus* sp, *Bacillus* sp,*Acinetobacter* sp,*Escherichia coli* and *Micrococcus* sp were involved in crude oil degradation as shown in Table 4.33. The stimulated treatment had more microorganisms that aided in faster degradation; this could be as a result of the poultry droppings added. Ayotamuno *et al*. (2004) stated that addition of an organic manure could increase the rate of oil degradation.

4.2.5 Molecular Features

Molecular analysis carried out in this study to identify thebacterial and fungal species thatwere in crude oil-polluted soil proved their preferential presence in the polluted soil. This was depicted by the visible bands indicating their presence.From the DNA Com 1 primer, specifically used for detecting the presence of bacteria, bands were seen mostly in soil treated with lesser crude oil volumes of 25 ml, 50 ml, 100 ml whereas absence of bands was observed in 200 ml and 400 ml treatments. This could possibly be as a result of high concentration of crude oil volumes in the test soil samples. Few fungal species were seen from the bands mostly in lower crude oil pollution volumes of 25 ml, 50 ml, 100 ml while one band was seen in 200 ml and two bands in 400 ml. From this study, it was observed that more bacterial species were involved in the degradation process than fungal species as shown in Plates 4.1 - 4.8. This is line with the work carried out by Ensley (2000) who stated that bacterial species are the main microorganisms involved in crude oil degradation. This supported the findings of Yeates *et al.* (1998)who studied the methods for microbial DNA extraction from soil for PCR amplification and obtained results supporting the findings of this study.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1Conclusion

This study has revealed that introduction of crude oil into agricultural soil inhibits agronomic growth and developments of*Zea mays*, *Mucuna pruriens and Telfairia occidentalis*(for the treatments that were crude oil polluted)and also reduced the height. Therefore agricultural soil contamination by crude oil should be avoided and public awareness should be created on the detrimental effects of crude oil pollution in our terrestrial ecosystem. Phytoremediation process is promising approach to restore contaminated sites using plant associated endophytic and rhizospheric bacteria to degrade a wide range of contaminants of concern in environmental soil and the use of plant-associated bacteria to degrade contaminants in the soil may provide an efficient, economic and sustainable green remediation technology for future environments. It can be stated that since the treatment with crude oil polluted soil and poultry droppings mixture increased the soils microbial population that aided degradation, it should be used for clean-up of crude oil contamination in the environment.

5.2 Recommendation

Based on the findings of this work, the following recommendations are hereby made:

1.) Environmentally friendly remediation like phytoremediation should be carried out on our agricultural soils that have been grossly contaminated by crude oil exploitation.

2.) Further research is recommended to sequence the extracted DNA of persistent bacterial and fungal isolates to:

i.) determine and map the dehydrogenase gene responsible for degradation, and

ii.) to determine possible presence of unculturable isolates.

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APPENDIX I

Appendix I a: Samples of Sterilized seeds of *Zea mays, Mucuna pruriens* and*Telfairia occidentalis* used for the research work.

Appendix I b: The seeds within 3 days of planting.

Appendix I c: The seeds sprouting after one week of planting.

Appendix I d: The seeds sprouting after one week of planting.

Appendix I e: Different treatments in the screen house after two weeks of planting.

Appendix I f: Plants in different treatments in the screen house after three weeks of planting.

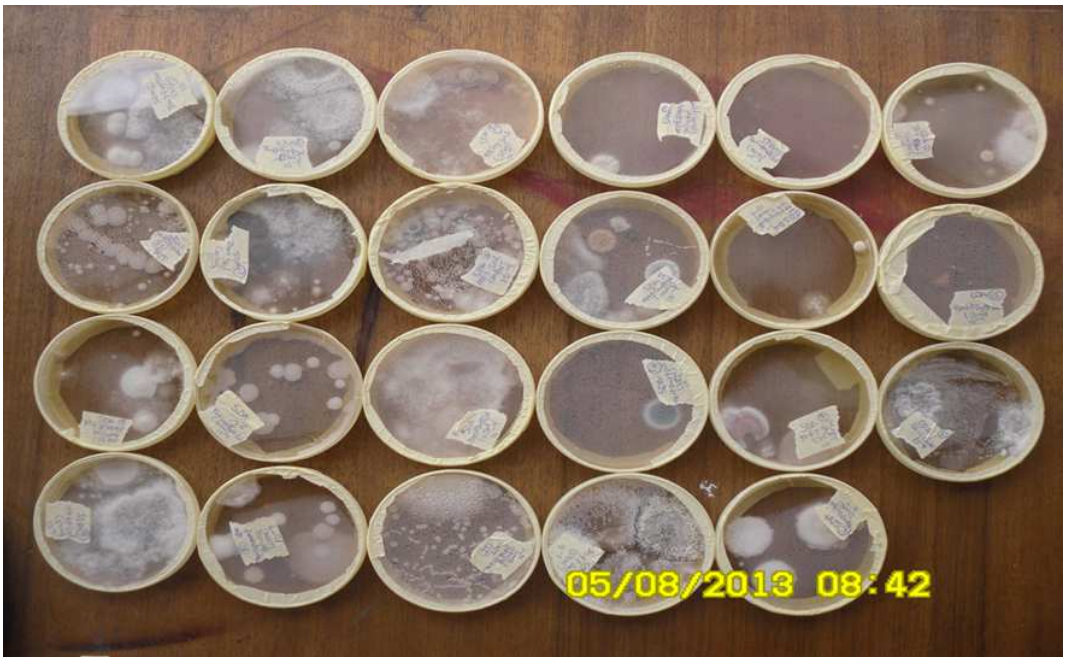
Appendix I g: The plants in polluted soils.

Appendix I h: The soil samples under soil analysis.

Appendix I i: The Microbial and Biochemical tests.

Appendix I j: The isolates on MacConkey Agar.

Appendix I k: The isolates on Nutrient Agar.



Appendix I l: The isolates on Sabouraud Dextrose Agar.

APPENDIX II

***** ANALYSIS OF VARIANCE *****

VARIATE: HYDROCARBON

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Singles					
1.) Treatment	2	76.40792	38.20396	1187.36	<.001
2.) Plant_type		27.95074	3.97537	123.55	<.001
3.) Fortnight_interval		1346.59603	346.596	031.077E+04	<.001
4.) Crude_oil_pollution vl	4	387.52281	96.88070	3011.00	<.001
Double					
5.) Treatment.Plant_type	4	0.47828	0.11957	3.72	<.006
6.) Treatment.Fortnightint2		54.07328	27.03664	840.29	<.001
7.) Treatment.Crudeoil p_vl	8	49.16495	6.14562	191.00	<.001
8.) Plant_type.Fortnightint2		2.99258	1.49629	46.50	<.001
9.) Plant_type.Crudeoil_pvl	8	5.43683	0.67960	21.12	<.001
10.)Fortnight_interval.Crude_oil_pollution_volume	4	266.40045	66.60011	2069.90	<.001
Triple					
11.) Treatment.Plant_type.Fortnight_interval	4	0.30968	0.07742	2.41	<0.051
12.) Treatment.Plant_type.Crude_oil_pollution_volume	16	1.40915	0.08807	2.74	<.001
13.) Treatment.Fortnight_interval.Crude_oil_pollution_volume	8	32.77599	4.09700	127.33	<.001
14.) Plant_type.Fortnight_interval.Crude_oil_pollution_volume	8	2.23619	0.27952	8.69	<.001
Quadriple					
15.)Treatment.Plant_type.Fortnight_interval.Crude_oil_pollution_volume	16	1.10575	0.06911	2.15	<0.008
Residual	180	5.79160	0.03218		
Total	269	1240.65223			

* MESSAGE: the following units have large residuals.

units 5	-0.970	s.e. 0.146
units 15	0.880	s.e. 0.146
units 95	1.020	s.e. 0.146
units 105	-0.860	s.e. 0.146
units 124	-0.440	s.e. 0.146
units 224	-0.460	s.e. 0.146

***** Tables of means *****
Variate: **Hydrocarbon**

Grand mean 1.361

Single

1.) Treatment T1 T2 T3
1.992 1.400 0.691

2.) Plant_type Zm Mp To
1.550 1.398 1.135

3.) Fortnight_interval 4th week 6th week
2.494 0.228

4.) Crude_oil_pollution_vol 25ml50ml 100ml200ml 400ml
0.226 0.4430.893 1.713 3.529

Two combination

5.) Treatment . Plant_type
Zm Mp To
T12.2092.072 1.696
T2 1.5581.393 1.248
T3 0.8830.730 0.460

6.) Treatment . Fortnight_interval
4th week6th week
T13.656 0.329
T2 2.566 0.233
T3 1.260 0.122

7.) Treatment . Crude_oil_pollution_volume
25ml 50ml 100ml 200ml 400ml
T10.330 0.672 1.327 2.628 5.005
T20.230 0.450 0.918 1.777 3.623
T30.117 0.208 0.435 0.735 1.960

8.) Plant_type . Fortnight_interval
4th week 6th week
Zm 2.792 0.308
Mp 2.565 0.232
To 2.125 0.144

9.) Plant_type .Crude_oil_pollution_volume
25ml 50ml 100ml 200ml 400ml
Zm 0.252 0.495 1.012 1.987 4.005
Mp 0.233 0.465 0.938 1.845 3.510
To 0.1920.370 0.730 1.308 3.073

10.) Fortnight_interval . Crude_oil_pollution_volume

		25ml	50ml	100ml	200ml	400ml
	4th week	0.414	0.813	1.641	3.143	6.458
	6th week	0.037	0.073	0.146	0.283	0.601

Three Combination

11.) Treatment . Plant_type . Fortnight_interval

		4th week	6th week
T1	Zm	3.988	0.430
T1	Mp	3.800	0.344
T1	To	3.180	0.212
T2	Zm	2.808	0.308
T2	Mp	2.552	0.234
T2	To	2.338	0.158
T3	Zm	1.580	0.186
T3	Mp	1.342	0.118
T3	To	0.858	0.062

12.) Treatment . Plant_type . Crude_oil_pollution_vpolume

		25ml	50ml	100ml	200ml	400ml
T1	Zm	0.360	0.750	1.435	2.900	5.600
T1	Mp	0.355	0.715	1.445	2.765	5.080
T1	To	0.275	0.550	1.100	2.220	4.355
T2	Zm	0.255	0.510	1.040	1.910	4.075
T2	Mp	0.230	0.440	0.895	1.810	3.590
T2	To	0.205	0.400	0.820	1.610	3.205
T3	Zm	0.140	0.225	0.560	1.150	2.340
T3	Mp	0.115	0.240	0.475	0.960	1.860
T3	To	0.095	0.160	0.270	0.095	1.680

13.) Treatment.Fortnight_interval.Crude_oil_pollution_volume

		25ml	50ml	100ml	200ml	400ml
T1	4th week	0.610	1.243	2.450	4.860	9.117
T1	6th week	0.050	0.100	0.203	0.397	0.893
T2	4th week	0.420	0.817	1.680	3.263	6.650
T2	6th week	0.040	0.083	0.157	0.290	0.597
T3	4th week	0.213	0.380	0.793	1.307	3.607
T3	6th week	0.020	0.037	0.077	0.163	0.313

14.) Plant_type.Fortnight_interval.Crude_oil_pollution_volume

		25ml	50ml	100ml	200ml	400ml
Zm	4th week	0.453	0.890	1.843	3.570	7.203
Zm	6th week	0.050	0.100	0.180	0.403	0.807
Mp	4th week	0.430	0.853	1.723	3.427	6.390
Mp	6th week	0.037	0.077	0.153	0.263	0.630
To	4th week	0.360	0.697	1.357	2.433	5.780
To	6th week	0.023	0.043	0.103	0.183	0.367

Four Combination

15.)Treatment.Plant_type.Fortnight_interval.Crude_oil_pollution_volume

25ml50ml 100ml 200ml 400ml						
T1Zm	4th week	0.650	1.370	2.650	5.220	10.050
6thweek	0.070	0.130	0.220	0.580	1.150	
Mp 4th week	0.6601.320	2.640	5.200	9.180		
6th week	0.0500.110	0.250	0.330	0.980		
To	4th week	0.5201.040	2.060	4.160	8.120	
6th week	0.030	0.060	0.140	0.280	0.550	
T2	Zm	4th week	0.460	0.900	1.880	3.440 7.360
		6th week	0.040	0.120	0.200	0.380 0.790
Mp	4th week	0.4200.800	1.650	3.320	6.570	
6th week	0.0400.080	0.140	0.300	0.610		
To	4th week	0.3800.750	1.510	3.030	6.020	
6th week	0.0300.050	0.130	0.190	0.390		
T3	Zm	4th week	0.250	0.400	1.000	2.050 0.400
		6th week	0.030	0.050	0.120	0.250 0.050
Mp	4th week	0.2100.440	0.880	1.760	3.420	
6th week	0.0200.040	0.070	0.160	0.300		
To 4th week	0.1800.300	0.500	0.110	3.200		
6th week	0.0100.020	0.040	0.080	0.160		

*** Standard errors of means ***

Single1. 2. 3. 4.

Table	Treatment	Plant_type	Fortnight_interval	Crude_oil_pol_vol
rep.	90	90	135	54
d.f.	180	180	180	180
e.s.e.	0.0189	0.0189	0.0154	0.0244

Two combination5. 6. 7. 8.

Table	Treatment.	Treatment.	Treatment.	Plant_type.
Plant_type	Fortnight_interval	Crude_oil_pol_vol		Fortnight_int
rep.	30	45	18	45
d.f.	180	180	180	180
e.s.e.	0.0327	0.0267	0.04230.0267	

9. 10.**Three combination**11. 12.

Table	Plant_type.	Fortnight_int.	Treatment.	Treatment.
Crudeoil_p_vol	Crudeoil_p_vol	Plant_type.	Plant_type.	
Fortnight_int	Crudeoil_p_vol			
rep.	18	27	15	6
d.f.	180	180	180	180
e.s.e.	0.0423	0.0345	0.0463	0.0732

13. 14. **Four combination** 15.
Table Treatment.Plant_type.Treatment.
Fortnight_int.Fortnight_int.Plant_type.
Crude_oil_p_vol Crude_oil_p_vol Fortnight_int.
Crude_oil_p_vol
rep. 9 9 3
d.f. 180 180 180
e.s.e. 0.0598 0.0598 0.1036

*** Standard errors of differences of means ***

Single 1. 2. 3. 4.
Table Treatment Plant_type Fortnight_interval Crude_oil_pollution_vol
rep. 90 90 135 54
d.f. 180 180 180 180
s.e.d. 0.0267 0.0267 0.0218 0.0345

Two combination 5. 6. 7. 8.
Table Treatment.Treatment.Treatment. Plant_type.
Plant_typeFortnight_intervalCrude_oil_pol_vol Fortnight_int
rep. 30 45 45 18
d.f. 180 180 180 180
s.e.d. 0.0463 0.0378 0.0378 0.0598

9. 10. **Three combination** 11. 12.
TablePlant_type.Fortnight_int.Treatment. Treatment.
Crudeoil_p_volCrudeoil_p_volPlant_type. Plant_type.
Fortnight_intCrudeoil_p_vol
rep. 18 27 15 6
d.f. 180 180 180 180
s.e.d. 0.0598 0.0488 0.0655 0.1036

13. 14. **Four combination** 15.
Table Treatment.Plant_type.Treatment.
Fortnight_int.Fortnight_int.Plant_type.
Crude_oil_p_vol Crude_oil_p_vol Fortnight_int.
Crude_oil_p_vol
rep. 9 9 3
d.f. 180 180 180
s.e.d. 0.0846 0.0846 0.1465

***** Least significant differences of means (5%level)*****

Single	1.	2.	3.	4.
Table	Treatment	Plant_type	Fortnight_interval	Crude_oil_pol_vol
rep.	90	90	135	54
d.f.	180	180	180	180
l.s.d.	0.0528	0.0528	0.0431	0.0681

Two combiation	5.	6.	7.	8.
Table	Treatment.Treatment.	Treatment.	Plant_type.	
Plant_type	Fortnight_interval	Crude_oil_pol_vol	Fortnight_int	
rep.	30	45	45	18
d.f.	180	180	180	180
l.s.d.	0.0914	0.0746	0.0746	0.1180

9.	10. **Three combination**	11.	12.
Table	Plant_type.Fortnight_int.Treatment.	Treatment.	
Crudeoil_p_vol	Crudeoil_p_vol	Plant_type.	Plant_type.
Fortnight_int	Crudeoil_p_vol		
rep.	18	27	15	6
d.f.	180	180	180	180
l.s.d.	0.1180	0.0963	0.1292	0.2044

13.	14.	**Four combination** 15.
Table	Treatment.Plant_type.Treatment.	
	Fortnight_int.Fortnight_int.Plant_type.	
	Crude_oil_p_vol	Crude_oil_p_volFortnight_int.
Crude_oil_p_vol		
rep.	9	9	3
d.f.	180	180	180
l.s.d.	0.1669	0.1669	0.2890

*****Stratum standard errors and coefficients of variation*****

Variate: **Hydrocarbon**

d.f.	s.e.	cv%
180	0.1794	13.2

APPENDIX III

ANALYSIS OF VARIANCE

VARIATE: PLANT_HEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Single					
1.) Treatment	2	36631.979	18315.989	1.368E+04	<.001
2.) Plant_type	2	1936.795	968.397	723.50	<.001
3.) Fortnight_interval	1	5800.007	5800.007	4333.27	<.001
4.) Crude_oil_pollution_vl	4	48552.894	12138.223	9068.65	<.001
Two combination					
5.) Treatment.Plant_type	4	398.645	99.661	74.46	<.001
6.) Treatment.Fortnight_int	2	1341.657	670.829	501.19	<.001
7.) Plant_type.Fortnight_int	2	228.370	114.185	85.31	<.001
8.) Treatment.Crudeoil_polvol	8	7473.002	934.125	697.90	<.001
9.) Planttype.Crudeoil_polvol	8	353.206	44.151	32.99	<.001
10.)Fortnightint.Crudeoil_p.vl	4	1982.274	495.568	370.25	<.001
Three combination					
11.) Treatment.Plant_type.Fortnight_interval	4	82.647	20.662	15.44	<.001
12.) Treatment.Plant_type.Crude_oil_pollution_volume	16	846.159	52.885	39.51	<.001
13.) Treatment.Fortnight_interval.Crude_oil_pollution_volume	8	408.405	51.051	38.14	<.001
14.) Plant_type.Fortnight_interval.Crude_oil_pollution_volume	8	58.005	7.251	5.42	<.001
Four combination					
15.)Treatment.Plant_type.Fortnight_interval.Crude_oil_pollution_volume	16	190.394	11.900	8.89	<.001
Residual	180	240.927	1.338		
Total	269	106525.367			

* MESSAGE: the following units have large residuals.

units 171 -2.800 s.e. 0.945

***** Tables of means *****
Variate: **Plant_height**

Grand mean 24.166
Single
1.) Treatment T1 T2 T3
7.727 33.293 31.478

2.)Plant_type Mp To Zm
23.859 27.589 21.050

3.) Fortnight_interval 4th week 6th week
19.531 28.801

4.) Crude_oil_pollution_vol 25ml 50ml 100ml 200ml 400ml
41.861 37.683 20.289 13.204 7.793

Two combination
5.) Treatment . Plant_type
Zm Mp To
T16.5007.380 9.300
T2 29.72032.040 38.120
T326.93032.157 35.347

6.) Treatment . Fortnight_interval
4th week 6th week
T1 6.229 9.224
T227.360 39.227
T3 25.004 37.951

7.) Treatment . Crude_oil_pol_volume
25ml 50ml 100ml200ml 400ml
T121.83316.8000.000 0.000 0.000
T2 49.43345.43327.267 24.41719.917
T354.31750.817 33.600 15.194 3.461

8.) Plant_type . Fortnight_interval
4th week 6th week
Zm 17.607 24.493
Mp 19.080 28.638
To 21.907 33.271

9.) Plant_type . Crude_oil_pollution_volume
25ml 50ml 100ml200ml 400ml
Zm 36.93333.167 16.900 12.1676.083
Mp 42.51738.35019.433 12.5286.467
To 46.13341.53324.533 14.917 10.828

10.) Fortnight_interval . Crude_oil_pollution_volume

	25ml	50ml	100ml	200ml	400ml
4th week	33.648	30.222	16.633	10.933	6.219
6th week	50.074	45.144	23.944	15.474	9.367

Three combination

11.) Treatment . Plant_type . Fortnight_interval

	4th week	6th week
T1	Zm	5.240	7.760
	Mp	6.100	8.660
	To	7.347	11.253
T2	Zm	25.920	33.520
	Mp	25.620	38.460
	To	30.540	45.700
T3	Zm	21.660	32.200
	Mp	25.520	38.793
	To	27.833	42.860

12.) Treatment . Plant_type . Crude_oil_pollution_volume

		25ml	50ml	100ml	200ml	400ml
T1	Zm	18.850	13.650	0.000	0.000	0.000
	Mp	20.950	15.950	0.000	0.000	0.000
	To	25.700	20.800	0.000	0.000	0.000
T2	Zm	45.400	42.350	19.100	23.500	18.250
	Mp	50.250	43.750	25.150	21.650	19.400
	To	52.650	50.200	37.550	28.100	22.100
T3	Zm	46.550	43.500	31.600	13.000	0.000
	Mp	56.350	55.350	33.150	15.933	0.000
	To	60.050	53.600	36.050	16.650	10.383

13.) Treatment.Fortnight_interval.Crude_oil_pollution_volume

		25ml	50ml	100ml	200ml	400ml
T1	4th week	17.611	13.533	0.000	0.000	0.000
	6th week	26.056	20.067	0.000	0.000	0.000
T2	4th week	39.567	36.633	23.800	20.967	15.833
	6th week	59.300	54.233	30.733	27.867	24.000
T3	4th week	43.767	40.500	26.100	11.833	2.822
	6th week	64.867	61.133	41.100	18.556	4.100

14.) Plant_type.Fortnight_interval.Crude_oil_pollution_volume

		25ml	50ml	100ml	200ml	400ml
Zm	4th week	29.900	26.700	15.467	11.167	4.800
	6th week	43.967	39.633	18.333	13.167	7.367
Mp	4th week	34.133	31.000	15.367	9.800	5.100
	6th week	50.900	45.700	23.500	15.256	7.833
To	4th week	36.911	32.967	19.067	11.833	8.756
	6th week	55.356	50.100	30.000	18.000	12.900

Four combination

15.) Treatment.Plant_type.Fortnight_interval.Crude_oil_pollution_volume

25ml 50ml100ml 200ml400ml
T1 Zm 4th week 15.200 11.000 0.000 0.000 0.000
6th week 22.500 16.300 0.000 0.000 0.000
Mp4th week 17.30013.200 0.0000.000 0.000
6th week 24.60018.700 0.0000.000 0.000
To 4th week 20.33316.400 0.0000.000 0.000
6th week 31.06725.200 0.0000.000 0.000
T2 Zm 4th week 36.400 34.100 21.200 23.500 14.400
6th week 54.400 50.600 17.000 23.500 22.100
Mp 4th week 40.00035.500 20.100 17.200 15.300
6th week 60.500 52.000 30.200 26.100 23.500
To 4th week 42.30040.300 30.100 22.200 17.800
6th week 63.00060.100 45.000 34.000 26.400
T3 Zm4th week 38.10035.000 26.000 10.000 0.000
6th week 55.00052.000 40.300 16.000 0.000
Mp4th week 45.10044.300 27.100 12.000 0.000
6th week 67.60066.400 45.000 19.667 0.000
To4th week 48.10042.200 25.200 13.300 8.467
6th week 72.00065.000 38.000 20.000 12.300

*** Standard errors of means ***

Single	1.	2.	3.	4.
Table	Treatment	Plant_type	Fortnight_interval	Crude_oil_pol_vol
rep.	90	90	135	54
d.f.	180	180	180	180
e.s.e.	0.1220	0.1220	0.0996	0.1574

Two combination	5.	6.	7.	8.
Table	Treatment.Treatment.	Treatment.	Plant_type.	
	Plant_type	Fortnight_interval	Crude_oil_pol_vol	Fortnight_int
rep.	30	45	45	18
d.f.	180	180	180	180
e.s.e.	0.2112	0.1725	0.1725	0.2727

9.	10.	**Three combination**	11.	12.
Table	Plant_type.Fortnight_int.		Treatment.	Treatment.
	Crudeoil_p_vol	Crudeoil_p_vol	Plant_type.	Plant_type.
			Fortnight_int	Crudeoil_p_vol
rep.	18	27	15	6
d.f.	180	180	180	180
e.s.e.	0.2727	0.2227	0.2987	0.4723

13. 14.**Four combination** 15.
Table Treatment.Plant_type.Treatment.
Fortnight_int.Fortnight_int.Plant_type.
Crude_oil_p_vol Crude_oil_p_volFortnight_int.
Crude_oil_p_vol
rep. 9 9 3
d.f. 180 180 180
e.s.e. 0.3856 0.3856 0.6680

*** Standard errors of differences of means ***

Single1. 2. 3. 4.
Table TreatmentPlant_typeFortnight_intervalCrude_oil_pol_vol
rep. 90 90 135 54
d.f. 180 180 180 180
s.e.d. 0.1725 0.1725 0.1408 0.2227

Two combination5. 6. 7. 8.
Table Treatment.Treatment.Treatment. Plant_type.
Plant_typeFortnight_interval Crude_oil_pol_vol Fortnight_int
rep. 18 27 15 6
rep.30 45 45 18
d.f.180 180 180 180
s.e.d. 0.2987 0.2439 0.2439 0.3856

9. 10.**Three combination** 11. 12.
TablePlant_type.Fortnight_int.Treatment. Treatment.
Crudeoil_p_volCrudeoil_p_volPlant_type. Plant_type.
Fortnight_int Crudeoil_p_vol
rep. 18 27 15 6
d.f. 180 180 180 180
s.e.d. 0.3856 0.3149 0.4225 0.6680

Triple13. 14. **Quadriple** 15.
Table Treatment.Plant_type.Treatment.
Fortnight_int.Fortnight_int.Plant_type.
Crude_oil_p_vol Crude_oil_p_volFortnight_int.
Crude_oil_p_vol
rep. 9 9 3
d.f. 180 180 180
s.e.d. 0.5454 0.5454 0.9446

***** Least significant differences of means (5% level)*****

Single Table

	1. Treatment	2. Plant_type	3. Fortnight_interval	4. Crude_oil_pol_vol
rep.	90	90	135	54
d.f.	180	180	180	180
l.s.d.	0.3403	0.3403	0.2779	0.4393

Two combination Table

	5. Treatment.Plant_type	6. Treatment.Fortnight_interval	7. Treatment. Crude_oil_pol_vol	8. Plant_type. Fortnight_int
rep.	30	45	45	18
d.f.	180	180	180	180
l.s.d.	0.5894	0.4813	0.4813	0.7610

Three combination Table

	9. Plant_type.Crudeoil_p_vol	10. Fortnight_int.Crudeoil_p_vol	11. Treatment. Plant_type. Fortnight_int	12. Treatment. Crudeoil_p_vol
rep.	18	27	15	6
d.f.	180	180	180	180
l.s.d.	0.7610	0.6213	0.8336	1.3180

Four combination Table

	13. Treatment.Plant_type.Fortnight_int. Crude_oil_p_vol	14. Treatment.Fortnight_int.Plant_type. Crude_oil_p_vol	15. Fortnight_int. Crude_oil_p_vol
rep.	9	9	3
d.f.	180	180	180
l.s.d.	1.0762	1.0762	1.8640

*****Stratum standard errors and coefficients of variation *****

Variate: **Plant_height**

d.f.	s.e.	cv%
180	1.1569	4.8