KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF APPLIED AND THEORTICAL SCIENCE
DEPARTMENT OF ENVIRONMENTAL SCIENCE

POTENTIAL USE OF *ERAGROSTIS CURVULA* AND *CHROMOLAENA ODORATA* FOR PHYTOREMEDIATION ON HYDROCARBON CONTAMINATED SOIL: A CASE STUDY AT NEWMONT GHANA GOLD LIMITED – AHAFO KENYASI

BY

DESMOND ASARE

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ABSTRACT

Contaminated soil containing oil and grease and total petroleum hydrocarbon was phytoremediated by blending 3 Kg of the hydrocarbon contaminated soil with portions of compost, topsoil and fertilizer (urea). The soil was homogenized with the above mentioned nitrogen sources and monitored for a period of Twenty (20) weeks with seeds of *Chromolaena odorata* (Acheampong plant) and vegetative part of *Eragrostis curvula* (Love grass) nursed and planted respectively. The different treatment combinations used in this study were, Treatment A (Hydrocarbon contaminated soil (HCS) + Top soil), Treatment B (HCS + Inorganic fertilizer), Treatment C (HCS + Compost), Treatment D (HCS + Fertilizer + Topsoil), Treatment E (HCS + Compost + Topsoil), Treatment F (HCS + fertilizer + Compost) and the control treatment, Treatment G (HCS only). The different treatment combinations were augmented with different levels of inorganic nitrogen at 0.8, 1.0 and 1.2%. The 7 different treatments all reported significantly different rates of biodegradation of oil and grease and Total Petroleum Hydrocarbon (TPH), with most of the treatments resulting in significant reduction of oil and grease and TPH concentrations. The results of the phytoremediation experiment indicated measurable reduction of oil and grease as well as Total petroleum hydrocarbon (TPH) concentrations in the different treatment media as far as the two plants are concerned with Treatment E resulted in the best enhancement of oil and grease and TPH with over 90% reduction in contaminant levels after the 20-week period. Generally, the treatment combinations with the 0.8% nitrogen amendment recorded the lowest oil and grease and TPH phytoremediation rates using *Chromolaena odorata* and *Eragrostis curvula*. The residual Oil and Grease / TPH levels after the 20-week period were higher in 0.8% compared to the 1.0% and 1.2% Nitrogen levels. The phytoremediation experiment showed that, the higher the nitrogen amendment in the various treatments, the higher the plant growth and thus the higher the reduction of the petroleum contaminants. The addition of organic fertilizers and materials significantly ($p<0.05$) enhanced phytoremediation rates. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) yielded the best phytoremediation rates for the two plants probably because of the compost and topsoil combination as opposed to Treatment B (Hydrocarbon contaminated soil + fertilizer) which consistently produced the lowest phytoremediation rates in the different Nitrogen amendments.

Accumulation of oil and grease as well as Total petroleum was also higher in the root and shoot of the *Chromolaena odorata* as compared to the root and shoot of the *Eragrostis curvula* after the distractive sampling.

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DECLARATION

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made".

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

High use of hydrocarbons due to increased industrialization both in the mining and oil industry create a booming economy with these activities leading to negative socio-economic and environmental problems. The extraction of petroleum products to fuel for our industrial society inevitably results in spills, due to human and mechanical error (Robson, 2003).

Stroud *et al*. (2007) stated that aliphatic hydrocarbons (e.g. diesel fuel and engine oils) make up a substantial proportion of organic contamination in the terrestrial environment. There have been increasing international efforts to remediate contaminated sites using "green" technologies, either as a response to the risk of adverse health or environmental effects or to enable site redevelopment (Vidali, 2001).

The observation that some plants and microorganisms are capable of growing in hydrocarbon-contaminated soil prompted research into remediation using these organisms. Biological degradation of contaminants or pollutants in the environment has been described as a proven method of remediating petroleum– contaminated soils, and soils contaminated by many other organic chemicals (Jørgensen *et al*., 2000). Traditional engineering techniques to clean hydrocarbon-contaminated soils are often expensive, ranging from $20 to over $1,500 per ton of soil, and result in extensive disturbance of the site (Schnoor, 2002).

Phytoremediation is the use of plants and their associated rhizosphere microorganisms to degrade sequester or contain soil contaminants most commonly *in situ* (Cunningham

et al., 1996). Preliminary research on phytoremediation reveals that it may be more effective than using microorganisms alone (Robson, 2003).

Although phytoremediation is not a panacea that would be universally applicable, it is rapidly achieving pedagogical maturity and it has already earned an important place in the menu of alternatives from where we select solutions for our environmental pollution problems. In the last decade phytoremediation has gained increasing acceptance as an area of research and equally as a viable cleanup technology particularly for organic pollutants. A cost comparison by Frick *et al.* (1999) of phytoremediation to alternative remediation methods including physical/chemical, engineering and bioremediation revealed a clear overall advantage.

These promising results have prompted scientists to further investigate the potential of plant/microorganism combinations for remediation of contaminated soils.

There is therefore the need for this research work of using natural remediation approaches such as phytoremediation in the decontamination of hydrocarbon soil.

1.2 JUSTIFICATION OF STUDY

There is an increasing awareness of environmental issues and concerns throughout the world especially on hydrocarbon management. Contaminated land has generally resulted from past industrial activities where awareness of the environmental health effects was connected with the production, use, and disposal of these hazardous substances (Gaskin, 2008). Little has been done in its management in Ghana ranging from the vehicle repairs center's popularly called "Magazines" to the various established industries including manufacturing and mining companies. Mining as an

industry makes use of a variety of crude oil for running it operations from hauling of ore to its processing activities.

But in all this, spillage of hydrocarbons on site (eg. Newmont Ahafo Mine) is inevitable due to mechanical failure of fleet of heavy duty equipment or by accidental introduction into the environment. Stringent hydrocarbon management process has been instituted to manage such occurrences from having a negative toll on the environment. Popular among this management process is the volatilization of the contaminated soil in a pad which is left to photograde. However, this process is only not yielding the desired result and is also expensive and time consuming. In view of this that plants which are used in land reclamation activities at Newmont have been experimented to see how they can remediate this hydrocarbon contaminated soil.

This remediation option analysis for Newmont Ahafo Gold Limited (NGGL) site could be applied to other mining companies in Ghana and the local artisanal garages in the remediation of hydrocarbon contaminated lands which is now becoming an environmental menace.

1.3 Objectives of the Study

The objective of the study is to evaluate the efficiency of the *Chromolaena odorata* (locally known as Acheampong) and *Eragrostis curvula* (Love grass) to remediate hydrocarbon-contaminated soil at Newmont Ahafo Mine.

The Specific Objectives were to determine:

1. The baseline concentrations of the hydrocarbon contaminated soil with respect to Oil and Grease, Total Petroleum Hydrocarbons (TPH) and other physicochemical parameters in the soil before degradation.

2. The appropriate setup for the amended contaminated soil with the right nitrogen sources.
3. The concentrations TPH and oil and grease in the shoots and roots of the plants, and
4. The degradation process of the amended contaminated soil by measuring the oil and grease and TPH.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hydrocarbon Contamination

The problem is worldwide, and the estimated number of contaminated sites is significant and increasing (Gaskin, 2008). Hydrocarbon pollution is ubiquitous in the environment. For example, in the United Kingdom it accounts for over 15% of all pollution incidents (Stroud *et al.*, 2007). In the measurement of hydrocarbon contaminated soil TPH levels and the Oil and grease levels are considered.

Common fuels such as Petrol, Diesel and Kerosene and Lubricating Oils/Greases all fall within the TPH domain. The term total petroleum hydrocarbons (TPH) is used to describe a broad family of several hundred chemical compounds that originally come from crude oil. TPH is defined as the measurable amount of petroleum-based hydrocarbons in environmental media (Research Triangle Institute, 1999). In practice, TPH is defined by the method used to analyze it.

Oil and grease (O/G) contaminants are defined as any material recovered as a substance extracted in the form of organic solvent from a sample, and are composed primarily of fatty matter from animal and vegetable sources, hydrocarbons of petroleum origin, certain organic dyes, and chlorophyll. The hydrocarbon analyses can be used for environmental assessment of remediation (Douglas *et al.*, 1991) or soil bioremediation (Korda *et al.*, 1997; Jorgensen *et al.*, 2000). Different methods often give different results because they are designed to extract and measure slightly different subsets of petroleum hydrocarbons. No single method gives a precise and accurate measurement of TPH for any type of contamination. The four most commonly used TPH testing

methods include gas chromatography (GC), infrared spectrometry (IR), gravimetric analysis, and immunoassay.

2.2 Remediation Technologies for hydrocarbon contaminated soil

Numerous hydrocarbon remediation technologies have been developed in recent years. However most of these are only applicable in the temperate regions. Remediation technologies include both physical (mechanical) and biological methods (phytoremediation). Remediation is defined as an activity, process or action that leads to some correction, rectification or benefit. The remediation of contaminated soil is not aimed at the total clean-up of the contaminant, but rather at reducing or eliminating the undesirable effects of the contamination on human or environmental health.

Many different remediation options are currently available, with varying advantages and disadvantages, with the suitability of each option being dependent on the specifics of the site.

Generally, biological processes are one half to one third the cost of physical methods (Torma, 1994). Some of the biological methods are briefly discussed below with particular reference to the subject.

2.2.1 Bioremediation

The term bioremediation is sometimes thought to be synonymous with phytoremediation, but these terms describe two completely different methods. Bioremediation is defined as the action of microbes or other biological systems to degrade environmental pollutant. Bioremediation can be applied in situ without the removal and transport of polluted soils in order not to disturb the soil matrix (Caplan,

1993). Although both seek to exploit living organisms to alter contaminated environments, bioremediation involves the manipulation of microbial populations, and phytoremediation concerns the use of higher plants.

2.2.2 Rhizoremediation

Plant enzymes establish the degradation of pollutants during phytoremediation; whereas, during natural attenuation or bioamendment, the (indigenous) microbial population performs the degradation. In many of these studies, an important contribution to the degradation of pollutants is ascribed to microbes present in the rhizosphere of plants used during phytoremediation or of plants which are emerging as natural vegetation on a contaminated site. This contribution of the rhizomicrobial population is referred to as rhizoremediation (Anderson *et al.*, 1993). In some cases, rhizosphere microbes are even the main contributors to the degradation process. A plant can be considered to be a solar-driven biological pump and treatment system, attracting water with its root system, accumulating water-soluble pollutants in the rhizosphere, and concluding with the degradation or translocation of the pollutant (Erickson, 1997).

2.2.3 Phytoremediation

According to Barazani *et al*. (2004) for a plant to be considered for phytoremediation should have a few of the following traits to make its use feasible:

- Ability to extract, degrade or stabilize the contaminant
- Tolerance to high levels/concentrations of the contaminant
- Rapid growth rate and high biomass production
- Cosmopolitan growth and ease for harvesting.

Phytoremediation may be defined as an in situ remediation strategy that uses vegetation and associated microbiota, soil amendments, and agronomic techniques to remove, contain, or render environmental contaminants harmless. Phytoremediation is a word formed from the Greek prefix "phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore (Cunningham *et al*., 1996). Although plants are known to sequester and degrade some classes of organic contaminants from soils, in situ contaminant degradation by root-associated rhizosphere microorganisms (i.e., rhizodegradation) is likely the most important mechanism during the phytoremediation of hydrophobic compounds such as petroleum hydrocarbons (PHCs) (Siciliano and Germida, 1998).

2.2.3.1. Mechanisms for Phytoremediation

In general, phytoextraction and phytovolatization are considered as the main options for the removal of heavy metals and other elemental compounds, whereas phytodegradation and phytostabilisation are applied mostly to organic contaminants (Meagher, 2000). Phytoremediation can be accomplished by phytoextraction, phytodegradation, phytostabilization, phytovolatization and rhizofiltration. While stabilization or volatilization is acceptable in some situations, degradation of the contaminant into nontoxic compounds is the most desirable outcome.

1) **Phytoextraction**: the use of plants to remove contaminants from soils. Pollutant-accumulating plants are utilized to transport and concentrate contaminants (metal or organic) from the soil into harvestable parts of the roots and aerial parts of the plant; the term is mostly used to refer to metal removal from soils (Kumar *et al.*, 1995).

2) **Phytostabilization:** the use of plants to reduce the bioavailability of pollutants in the environment. In this process, the contaminant or its metabolite is released into the atmosphere (Pilon-Smits, 2005). This mechanism of contaminant removal may have implications regarding contamination of the atmosphere, and consequently, regulatory compliance issues with air quality guidelines (Schnoor, 2002).

3) **Phytovolatization:** the use of plants to volatilize pollutants. Plants extract volatile pollutants (e.g. selenium, mercury and arsenic) from the soil and biologically converts them to a gas which is released via transpiration from the foliage (Ghosh and Singh, 2005a; Ghosh and Singh, 2005b).

4) **Phytodegradation**: the use of plants to degrade organic pollutants. Plant roots are utilized to remediate contaminated soils by the breakdown of organic contaminants to simpler molecules which are stored in the plant tissue (Ghosh and Singh, 2005b).

5) **Rhizofiltration**: the approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants. This contaminated water is either collected from a waste site or brought to the plants, or the plants are planted in the contaminated area, where the roots then take up the water and the contaminants dissolved in it (Dushenkov *et al*., 1995).

Table 1: Summary of the uses and mechanisms for phytoextraction, phytovolatization, phytodegradation, phytostabilisation and rhizofiltration.

Technique	Plant mechanism	Surface medium
Phytoextraction	Uptake and concentration of metal via direct uptake into the plant tissue with subsequent removal of the plants	Soils
Phytodegradation	Enhances microbial degradation in Rhizosphere	Soils, groundwater within rhizosphere
Phytostabilisation	Root exudates cause metal to precipitate and become less available	Soils, groundwater, mine tailing
Phytovolatization	Plants transpire selenium, mercury, and volatile hydrocarbons	Soils and groundwater
Rhizofiltration	Uptake of metals into plant roots	Surface water

Mechanisms of phytoremediation happen on biochemical and ecological interactions between plants and bacteria. The most extensively characterized fibrous root systems belong to the grass family Poaceae. Grass root systems possess an extensive surface area compared to other plant types, and have been recognized in many studies for their potential for remediation of hydrocarbon contaminated sites (Xia, 2004).

2.3 FACTORS AFFECTING PHYTOREMEDIATION

2.3.1 Environmental factors

Environmental factors that affect the success of phytoremediation include soil texture, organic matter content, pH, oxygen availability, moisture, fertility, temperature, solar radiation and weathering. These factors affect the properties and bioavailability of

hydrocarbons, germination and productivity of plants, and colonization and growth of rhizosphere microorganisms (Gaskin, 2008).

2.3.1.1 Soil pH

Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the acceptable range is pH 6 – 8 (US EPA, 2006).

2.3.1.2 Soil Moisture

All soil microorganisms require moisture for cell growth and function. Availability of water affects diffusion of water and soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. Anaerobic respiration produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation. Soil moisture content "between 45 and 85 percent of the water-holding capacity (field capacity) of the soil or about 12 percent to 30 percent by weight" is optimal for petroleum hydrocarbon degradation (US EPA, 2006). A soil water content of 60% is the ideal amount for degradation of hydrocarbons in loamy soil (Hutchinson *et al.,* 2001b).

2.3.1.3 Soil Composition and Quality

Soil quality is another important factor for determining successful germination, growth and health of plants. Heavily contaminated soils have a tendency towards poor physical conditioning which is unsuitable for vigorous growth of vegetation and rhizosphere bacteria. Common limitations are poor moisture-holding capacity, insufficient aeration,

low permeability and nutrient deficiencies. Organic amendments such as aged manure, sewage sludge, compost, straw, or mulch can be used to increase the water-holding capacity of a contaminated soil. Soil pH can be increased and decreased by the addition of lime and sulphur respectively (Kamath *et al.*, 2007). The addition of high carbon organic matter like sawdust improves plant germination by decreasing hydrocarbon bioavailability to plants, but decreases yield due to an increase in the C: N ratio (Amadi *et al*., 1993). Plants require different soil textures and organic matter contents for optimal germination and growth (Evans *et al*., 1977).

When screening plants for phytoremediation those species naturally adapted to the soil texture at the contaminated site will likely be more successful than those adapted to different soil textures. Clay and organic matter content also affect microbial populations via their ability to form soil aggregates (Paul and Clark, 1989).

2.3.1.4 Soil Oxygen

Soil contaminated with hydrocarbons may have low oxygen availability (Cunningham *et al*., 1996). Lack of oxygen reinforces seed dormancy of some plants, preventing growth in contaminated soil (Amadi *et al.*, 1993). As the most effective hydrocarbon degrading microorganisms are aerobic, lack of oxygen can negatively affect this process (Eweis *et al.*, 1998).

Animal manure increases plant yield more than inorganic fertilizers, likely due to the binding of hydrocarbons to organic matter (Amadi *et al*., 1993). Nitrogen and phosphorus are more limiting in freshly contaminated than in aged contaminated soils as

they tend to be immobilized by microorganisms shortly after contamination and mineralized when the C:N ratio decreases (Hutchinson *et al*., 2001a).

2.3.1.5 Temperature

Temperature affects the availability and toxicity of oil, and plant and microorganism growth. Indirectly, high temperatures lead to water stress, which decreases plant productivity (Larcher, 1980). Microorganisms benefit from heat; hydrocarbon degradation rates double for every 10 °C increase in temperature (Eweis *et al.*, 1998).

2.3.1.6 Fertilizer Requirements

Contaminated soils are usually deficient in macro- and micro-nutrients necessary for establishing healthy vigorously growing plants and stimulating microbial contaminant degradation. The source of nutrients also appeared to affect the germination and growth of plants. Organic sources of nitrogen are better than inorganic sources.

With respect to TPH degradation, nutrient addition during phytoremediation has yielded mixed results. Hutchinson *et al.* (2001b) observed better degradation of TPH using grasses with N/P amendments than without inorganic amendments. Microbial bioremediation of TPH contaminants with nutrient addition also produced widely varying results. However, Graham *et al.* (1999) assessed an array of N/P amendments for hexadecane biodegradation and suggested amendments above stoichiometric requirements can lead to diminished rates of degradation. This potentially occurs because addition of excessive nitrogen results in an increase in soil salinity and this increases the osmotic stress and suppresses the activity of hydrocarbon-degrading organisms. Walworth *et al.* (2003) showed that soil with initial low concentrations of N

or P is more likely to show decreased degradation with N/P addition. Many PAH-degrading organisms are adapted to low nutrient conditions and activity may decrease with the addition of soil amendments.

Table 2: Macro- and Micro-nutrients required for healthy plant growth.

Macronutrients a (~100 ppm)	Micronutrients b (~1 ppm)
Nitrogen (N)	Iron (Fe)
Phosphorus (P)	Boron (B)
Potassium (K)	Zinc (Zn)
Magnesium (Mg)	Copper (Cu)
Calcium (Ca)	Manganese (Mn)
Sulphur (S)	Molybdenum (Mo)

2.3.2 Biological Factors

Biological factors that may affect phytoremediation include degradation ability of associated microorganisms, and plant root architecture, growth rate, exudate production and productivity. Uncontaminated soils generally have lower numbers of hydrocarbon degrading species than soils that have been contaminated, because the microbial community adapts to the presence of hydrocarbons. Adaptation occurs via (i) induction and/or depression of enzymes, (ii) genetic changes resulting in new metabolic abilities, and (iii) selective enrichment of organisms (Leahy and Colwell, 1990).

Plants with extensive fibrous root systems, like grasses, are considered the most effective phytoremediators, as they explore larger volumes of soil than plants with taproots (Aprill and Sims, 1990). Plants with herringbone root morphology are more effective at soil exploration than plants with random or dichotomous morphologies

(Fitter *et al*., 1988). Studies on root architecture in mixed prairie show that while grasses form dense mats of roots in the top 0.5- to 1 m of soil, many tap rooted species typically reach soil depths greater than one meter, some up to four metres (Albertson, 1937). While grasses may be valuable for phytoremediation of soils with shallow contamination, certain tap rooted forbs may be more effective for remediation of deeper contamination.

Slow growing plants may have higher specific root lengths and relatively more fine roots than faster growing plants (Boot and Mensink, 1990). Since root exudates are hypothesized to improve degradation (Cunningham *et al.,* 1996), using species that produce more exudates may be advantageous.

Plants with high productivity have more root biomass and probably higher populations of rhizosphere microorganisms. Plants that are able to sustain their growth in contaminated soil would be more successful at phytoremediation than plants that cannot. However, plant productivity in uncontaminated soil is not indicative of productivity in hydrocarbon-contaminated soil (Kulakow *et al.,* 2000). Plant productivity is limited in hydrocarbon-contaminated soil largely due to low availability of nitrogen (Biederback *et al.*, 1993).

2.4. Characteristics of Plants for Degradation

These plants that have potential to phytoremediate petroleum hydrocarbon plant with a demonstrated potential to tolerate petroleum hydrocarbons. They are mostly grasses and legumes. The uniqueness of these grasses in phytoremediation stem from the fact that they have a fibrous root system which increases their contact with the pollutant because

of increase in surface area (Aprill and Sims, 1990). The legumes are also a good option for phytoremediation because of their ability to fix atmospheric nitrogen.

Generally, degradation occurs as result of these organisms using the organic contaminants for growth and reproduction. The organic contaminants provide the micro-organisms with the carbon and electron used by the organism to obtain energy (Frick *et al.*, 1999). Containment can be direct or indirect. Direct containment involves the accumulation of contaminants within the plants, adsorption of contaminants onto roots and binding of contaminants in the rhizosphere through enzymatic activities (Frick *et al*., 1999). Indirect containment involves plants supplying enzymes that bind contaminants into soil organic matter (or humus) in a process called humification and by increasing soil organic matter content, which allows for humification (Cunningham *et al.*, 1996).

Root exudates: Root exudates are the link between plants and microbes that leads to the rhizosphere effect (Frick *et al.*, 1999). The type and quantity of root exudate are dependent on plant species and the stage of plant development. The type of root exudate is also likely to be site and time specific (Siciliano and Germida, 1998).

2.5. Method for selecting plants for phytoremediation hydrocarbons

The use of native species always characterize the selection of plant in the rehabilitation at Newmont Ghana Gold Limited which involves higher plant like *Terminalia superba* (Ofram), *Ceiba pentandra* (Ceiba), Acacia among others and the use of dense cover grass like *Bracharia decumbens, Eragrostis curvula, Microlaena stipoides.*

Plant species belonging to the *Poaceae* and *Asteraceae* family was selected for the current study based on the following desirable criteria and its related benefit for this research;

1. Previous study by Baah (2011) on phytoremediation using *Chromolaena odorata* at the study site.
2. *Eragrostis curvula* is a predominant grass used for reclamation at Newmont Ahafo Mine.
3. Species selected are tolerant to environmental contaminants.
4. *Eragrostis curvula* has an extensive root system.
5. Both have rapid growth and dense coverage to provide good soil cover and prevent soil erosion.
6. Easy to establish and maintain except the *Chromolaena odorata.*
7. Aggressive root systems (common in grasses) which can disrupt soil aggregates and enhance access of trapped hydrophobic contaminants.
8. Suitable as site restoration species (long term stability).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area

Newmont Ghana Gold Ltd. (NGGL) has a Brong Ahafo Project in Ghana, West Africa. The Ahafo Project is located along a mineralized UTM Zone 30N {WSG84} (with location coordinates E00125958 (Easting), N00260919 (Northing) - that extends approximately 70 kilometers (km) in the central portion of Ghana. It is located some 300 km north west of the capital city of Accra and 40 km south east of the regional capital of Sunyani. An area noted for its semi deciduous vegetation with an average annual rainfall of 23000mm.The district lies within the wet semi- equatorial zone marked.

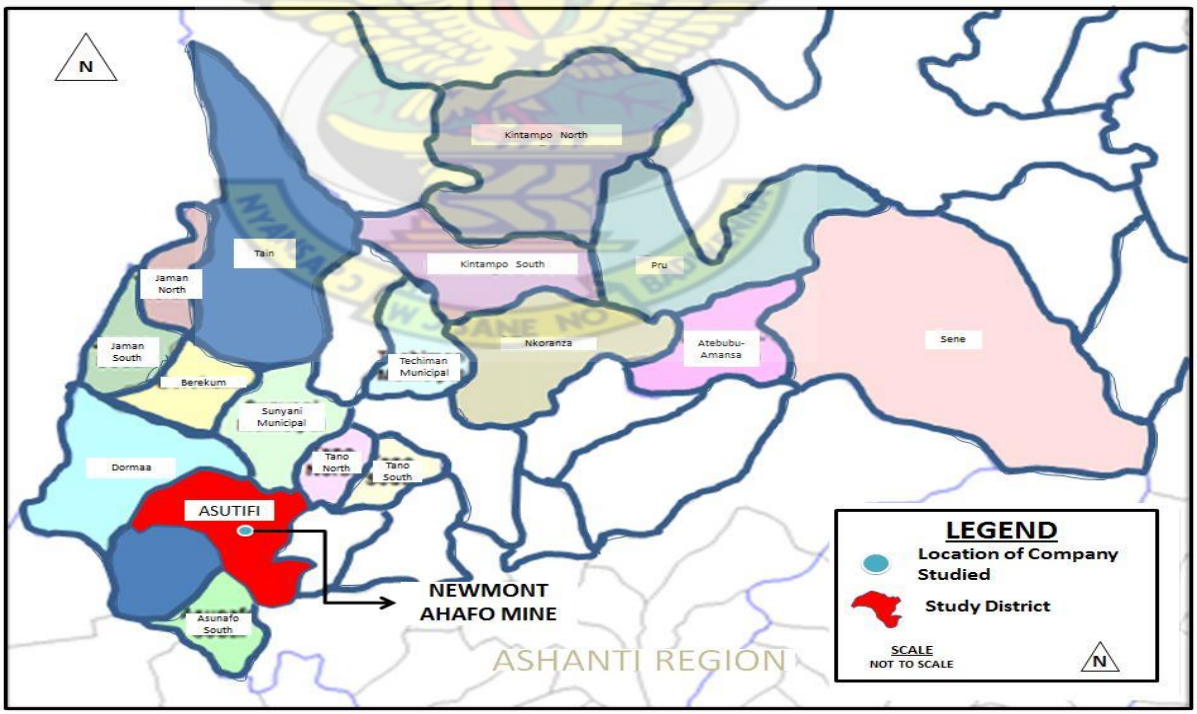


Fig 1: Map of Study area

3.2 Sample Preparation and Experimental Setup

Different sources of Nitrogen were used in this study namely; topsoil, compost and fertilizer (Urea). Topsoil without any hydrocarbon contamination history, hydrocarbon contaminated soil and compost made with sludge, wood shavings and food waste were collected from the Integrated Waste Management Facility (IWMF) at Newmont Ahafo Mine. Fertilizer (Urea) used was purchased from the open market.

Six (6) setups involving a mixture of 3 kg hydrocarbon contaminated soil with portions of compost/fertilizer/topsoil was used to boost the Nitrogen (N)-level to the suitable soil condition to support growth of the *Chromolaena odorata* and *Eragrostis curvula*. Seeds of *Chromolaena* and vegetative part of the *Eragrostis curvula* from a reclaimed site were then nursed and planted in buckets respectively (Plate 1 and 2).

The amendment was as a result of the Nitrogen (N)-level of the hydrocarbon contaminated soil. Baseline analysis (Appendix A1) indicated that the Nitrogen levels were very low. Nitrogen (N) levels were amended to 0.8%, 1.0% and 1.2% based on calculations considering the Nitrogen level of the contaminated soil and the other Nitrogen sources.

Laboratory assay of the levels of the Nitrogen was carried out to verify whether the levels were consistent with the calculated values. The experiment was replicated three times in a factorial design. Each block contained (21) different treatment. Codes from letters A to G were used to represent the various treatment combinations used for the experiment.

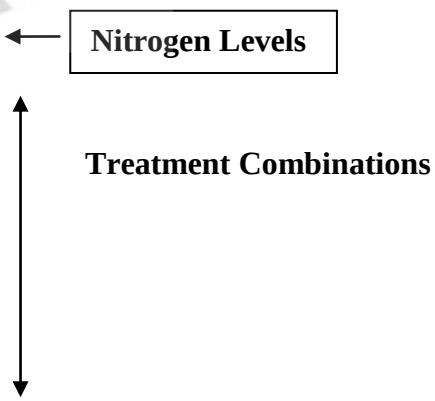
Table 3: The various soil treatments used in the experiments and their respective codes.

TREATMENTS	CODES
Hydrocarbon Contaminated Soil (HCS) (3kg) + Topsoil	A
Hydrocarbon Contaminated Soil (HCS) (3kg) + Fertilizer	B
Hydrocarbon Contaminated Soil (HCS) (3kg) + Compost	C
Hydrocarbon Contaminated Soil (HCS) (3kg) + Fertilizer + Topsoil	D
Hydrocarbon Contaminated Soil (HCS) (3kg) + Compost + Topsoil	E
Hydrocarbon Contaminated Soil (HCS) (3kg) + Fertilizer+ Compost	F
Hydrocarbon Contaminated Soil (HCS) Only (3kg) - Control	G

The block layout for the experiment of the two species using the factorial design is shown in Table 4 below;

Table 4: Block Layout Design for the Experiment

0.8%	1.0%	1.2%
A	C	F
B	D	G
C	E	A
D	F	B
E	G	C
F	A	D
G	B	E



3.3 Laboratory Analysis for the monitoring process

Soil analysis for Total Petroleum Hydrocarbon (TPH), and Oil and Grease (O&G) for the hydrocarbon contaminated soil was done weekly. Nitrogen, Moisture content, pH, phosphorous, Carbon - Nitrogen ratio (C/N) was also analyzed during the study. Distractive sampling was also done at the end of the experiment to determine the accumulated oil and grease and residual TPH in the shoots and roots for the two plants.

3.3.1 Moisture Content Determination

The container was cleaned, dried and weighed (W1). One Hundred (100) g of the soil sample was taken and weighed together with the container (W2). The sample was dried to constant temperature at 105 ^{o}C for a period of Twenty Four (24) hours. After drying the sample was removed from the oven and cooled in a desiccator for 30 minutes. The final constant weight (W3) of the container with dried soil sample was recorded. The percent moisture content in the soil is given by:

W (%) = [(W2-W1)-(W3-W1)/ (W2-W1)]*100

Water was added weekly to achieve the acceptable 40%-60% level range. (Standard methods book, 2005)

3.3.2 Determination of pH

The pH of the aqueous extract of all the contaminated soil, compost and topsoil were measured using the Orion-4-stra pH-conductivity meter. The meter was first calibrated with pH buffer 4.00, 7.00 and 10.00. 25 grams of the soil sample was weighed into a 1L beaker. It was then mixed with 125 ml of distilled water and stirred for a period of Thirty (30) minutes. The pH of the supernatant water was then measured.

3.3.3 Determination of Percent Total Nitrogen by Kjeldahls Method

Ten grams of air dry soil was weighed into a 500 ml long – necked Kjeldahl flask and followed by 10 ml distilled water. It was allowed to stand for 10 minutes to moisten. One spatula full of kjeldahl catalyst [mixture of l part Selenium + 10 parts $CuSO_4$ + 100 parts Na_2SO_4] and 20 ml conc. H_2SO_4 were added. It was digested for a period of two hours until it turned colourless or light greenish colour was observed. It was further allowed to cool. The fluid was decanted into a One Hundred (100) ml volumetric flask and made up to the mark with distilled water.

Distillation

An aliquot of Ten (10) ml of fluid by means of pipette was transferred into the kjeldahl distillation apparatus. Twenty (20) ml of 40% NaOH was dispensed. Distillate was collected over Ten (10) ml of 4% Boric acid and three (3) drops of mixed indicator in a Five Hundred (500) ml conical flask for Four (4) minutes. The presence of Nitrogen gave a light blue colour.

Titration

One Hundred (100) ml of collected distillate was titrated with 0.l N HCl till blue colour changes to grey and then suddenly flashes to pink. A blank determination was carried out without the soil sample.

Calculation

Thus, the percentage of Nitrogen in the soil sample is given by the equation:

$$\% N = \frac{14 \times (A - B) \times N \times 100}{1000 \times 1}$$

Where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

3.3.4 Oil and Grease Analysis

Exactly Thirty (30) g ± 0.1g soil sample was weighed into a 250 ml Schott bottle. 2 to 3 teaspoons of anhydrous Na_2S0_4 (more was added if the soil was very damp) followed by 30 mL Solvent and 2 ml concentrated HCl to the Schott bottle, The Schott bottle was boiled on a flame and shaken vigorously to break up any aggregates and it was sonicated for Ten (10) minutes.

The supernatant liquid was poured off into a phase separator filter set in a glass funnel with approximately Ten (10) g sodium sulphate and run into a pre-weighed beaker with 2 glass boiling chips added. Thirty (30) mL Solvent was further added to the Schott bottle. The sonication and filtering process was repeated three times. The extracts were combined and evaporated to dryness on a hotplate at or below 70 °C. Sample was cooled in a desiccator to constant weight and weight recorded. (Standard Methods Book, 2005)

Calculation

$$\text{Oil and Grease (mg/kg, dry weight)} = \frac{B\text{-}A \times 10^6 \times F}{M}$$

Where:

B = final weight of beaker and residue, corrected for blank (g)

A = initial weight of beaker, corrected for blank (g)

M= weight of sample taken (g)

F = moisture factor

3.3.5 Total Petroleum Hydrocarbon Determination

Procedure for TPH analysis of soil and the dried blended plant part by Infra-Red was carried out in accordance with standard methods for the examination of water and wastewater.

Approximately Twenty grams (20g) of soil/plant part was weighed into a 16 oz. French square bottle with minimum exposure, along with fifty millimeters of distilled water and adjusted to a pH of 3 with hydrochloric acid (HCl). The bottle was capped tightly using a Teflon line cap and shaken mildly to disperse the soil for 1 to 2 min. After shaking, Twenty Five (25) ml of Freon was pipetted into the bottle and shaken well again for 15 minutes using a paint or lateral shaker. Sample was allowed to stand to permit content of bottle to separate into distinct layers.

Ten millimeter (10ml) of Freon was pipetted from the appropriate layer and filtered through Five grams (5g) of activated silica gel and One gram (1g) of sodium sulphate into a reference cell. The TPH Analyzer was turned on and allowed to warm-up for 30 minutes. The instrument was calibrated with working standards prepared from reference oil. The analyzer was blanked with the extractant solvent and cell filled with sample inserted into the calibrated analyzer. The readings from the analyzer were then recorded.

3.4 Distractive Sampling

The length of experimental period was determined by the pertinent literature which suggested the bulk of aliphatics would be biodegraded within this stated frame (Pichtel and Liskanen 2001; Kaimi *et al.*, 2007b). After Twenty (20) weeks of growth, plants were harvested and separated into their various nitrogen levels. Plant roots were gently washed in distilled water to remove soil particles and separated according to the shoot and roots and placed in paper envelopes before oven drying. O&G and TPH were determined after blending.

Plate 1: Germination of *Chromolaena odorata* seeds (left) and vegetative growth of *Eragrostis curvula* (right) after Four (4) weeks of sowing and planting respectively.

Plate 2: Growth of *Chromolaena odorata* (left*)* and *Eragrostis curvula* (right*)* after Twelve (12) weeks.

3.5 Data Analysis

Differences among treatment means and the nitrogen amendment levels were tested by one-way analysis of variance (ANOVA), and all the possible treatment combinations compared using Turkey's Multiple Comparison Test to test for significance of variation between all the means. In all cases differences were considered significant at $p < 0.05$. The Student's *t*-test was used to test for differences in the phytoremediation rates of the two plant species.

Data analysis and the execution of graphs were carried out using the GraphPad Prism 5 Statistical Package for Windows.

CHAPTER FOUR

4.0 RESULTS

4.1 Initial Levels of TPH, Oil and Grease in Hydrocarbon contaminated soil

Contaminated soil, compost and topsoil collected were analyzed for TPH and oil and grease to establish the levels that already existed in these media. Compost and Topsoil recorded insignificant TPH and Oil and grease levels as compared to the Hydrocarbon contaminated soil which was 139.714mg/kg and 39,315.9mg/kg respectively. Below shows the Baseline TPH, Oil and grease levels.

Table 5: Baseline TPH, Oil and Grease levels in the Hydrocarbon Contaminated

Sample ID	**Oil and Grease (mg/kg)**	**TPH (mg/kg)**
Compost	<100	<10
Topsoil	< 100	<10
Hydrocarbon Contaminated Soil	139,714.5	39,315.9

4.2. Degradation of Oil and grease at 1.2% Nitrogen level for *E. curvula*

The trends in oil and grease breakdown in the treatments with the 1.2% Nitrogen amendment levels are shown in Table 6.0. Treatment A (Hydrocarbon-contaminated soil + topsoil) resulted in an 85.66% breakdown in oil and grease concentration in that media. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in just 9.48% reduction in the initial oil and grease concentration. Treatment C (Hydrocarbon

contaminated soil + Compost) resulted in an 84.08% reduction in oil and grease concentration recording a final concentration of 22000 $mgkg^{-1}$. Treatment D (Hydrocarbon contaminated soil + Fertilizer + Topsoil) resulted in a 92.03% reduction in oil and grease concentration from an initial concentration of 138000 $mgkg^{-1}$ to 11000 $mgkg^{-1}$. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement in oil and grease concentration as far as the 1.2% Nitrogen amendment level was concerned. The media resulted in a 96.37% reduction in oil and grease concentration. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) also resulted in a highly measurable reduction in oil and grease concentration from 136000 $mgkg^{-1}$ to 5700 $mgkg^{-1}$, representing a 95.80% reduction. Similar to Treatment B, the Control Treatment (Contaminated soil only) resulted in the lowest oil and grease remediation in the soil with a reduction of 7.77% over the 20-week period.

Table 6: Degradation rate of Oil and Grease at 1.2 % Nitrogen in the different treatment media by *Eragrostis curvula* in 20 weeks.

Treatments (Codes)	**Initial Concentration (mg/kg)**	**Final Concentration (mg/kg)**	**% Reduction**
A	139520±86.34	20000±23.33	85.66
B	139420±66.31	126200±44.57	9.48
C	138211±32.90	22000±21.34	84.08
D	138000±34.22	11000±12.76	92.03
E	135000±49.87	4900±11.10	96.37
F	136000±32.34	5700±9.95	95.80
Control	138620±32.12	128150±38.32	7.77

4.2.1 Degradation of Oil and grease at 1.0% Nitrogen level for *E. curvula*

Table 7 below shows the trends in oil and grease breakdown in the treatments with the 1.0% Nitrogen amendment levels over the 20-week period. The Hydrocarbon-contaminated soil + top soil (Treatment A) resulted in 82.72% breakdown in oil and grease concentration in that media. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in 8.00% reduction in the initial oil and grease concentration. Treatment C (Hydrocarbon contaminated soil + Compost) and Treatment D (Hydrocarbon contaminated soil + Fertilizer + Topsoil) resulted in a 70.29% and 89.15% reductions in oil and grease concentration respectively. Similar to the same treatment with the 1.2% Nitrogen amendment, Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement (95.81%) in oil and grease concentration as far as the 1.0% Nitrogen amendment level was concerned. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) produced a breakdown in oil and grease concentration of 64.23%.

Table 7: Degradation rate of Oil and Grease at 1.0 % Nitrogen in the different treatment media by *Eragrostis curvula* in 20weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	139042±66.34	24000±12.01	82.72
B	138920±11.44	127800±21.12	8.00
C	138021±12.02	41000±27.14	70.29
D	138200±21.00	15000±9.70	89.15
E	136000±11.02	5700±7.99	95.81
F	138620±13.64	49000±16.54	64.23
Control	138620±32.12	128150±38.32	7.77

4.2.2. Degradation of Oil and grease at 0.8% Nitrogen level for *E. curvula*

The treatments with the 0.8% Nitrogen amendment level showed reduced rates of phytoremediation as compared to their corresponding treatment media with the 1.0% and 1.2% Nitrogen amendment levels. Table 8 below summarizes the trends in oil and grease breakdown in the treatments with the 0.8% Nitrogen amendment levels. Treatment A (Hydrocarbon-contaminated soil + top soil) resulted in a 73.27% breakdown in oil and grease concentration in that media. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in lowest phytoremediation rate of just 6.42%. Treatment C (Hydrocarbon contaminated soil + Compost) resulted in a relatively higher rate of phytoremediation of 81.17%. The enhancement of oil and grease in Treatment D was 62.43%. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best phytoremediation of oil and grease concentration resulting in a 94.06% reduction. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) produced a final oil and grease concentration of 62.43%.

Table 8: Degradation rate of Oil and Grease at 0.8 % Nitrogen in the different treatment after 20 weeks

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	138420±71.00	37000±13.35	73.27
B	138920±31.31	126200±44.57	9.48
C	138100±12.09	26000±7.12	81.17
D	138400±33.22	52000±18.32	62.43
E	136400±62.27	8100±16.17	94.06
F	138400±21.05	52000±9.11	62.43
Control	138620±32.12	128150±38.32	7.77

4.2.3. Degradation of TPH at 1.2% Nitrogen level for *E. curvula*

Table 9 below shows the trends in TPH breakdown in the treatment blends with the 1.2% Nitrogen amendment levels. Treatment A (Hydrocarbon-contaminated soil + top soil) and Treatment B (The Hydrocarbon contaminated soil + fertilizer) resulted in just 48.65% and 38.13% breakdowns. The Hydrocarbon contaminated soil + Fertilizer + Topsoil blend (Treatment D) resulted in a 74.83% reduction in TPH concentration. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement in TPH concentration of 89.62%. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) also resulted in a highly quantifiable reduction in TPH concentration of 87.37% enhancement. The Control Treatment (Contaminated soil only) resulted in a 32.91% reduction in oil and grease concentration over the 20-week period.

Table 9: Degradation rate of TPH at 1.2 % Nitrogen in the different treatment media by *Eragrostis curvula* in 20 weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	37000±16.04	19000±13.00	48.65
B	38720±14.22	23978±9.21	38.13
C	38700±12.70	4780±6.41	87.65
D	36000±4.45	9058±12.76	74.83
E	37800±12.30	3920±5.14	89.62
F	39200±14.97	4950±9.95	87.37
Control	38200±32.12	25630±8.32	32.91

4.2.4. Degradation of TPH at 1.0% Nitrogen level for *E. curvula*

The Hydrocarbon-contaminated soil + Top soil (Treatment A) resulted in a 49.87% breakdown in TPH. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in just 23.08% reduction. Treatment C (Hydrocarbon contaminated soil + Compost) and Treatment D (Hydrocarbon contaminated soil + Fertilizer + Topsoil) resulted in a 72.71% and 68.08% reductions respectively. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement in TPH concentration of 87.27%. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) produced a breakdown in TPH concentration of 76.70%.

Table 10: Degradation rate of TPH at 1.0 % Nitrogen in the different treatment media by *Eragrostis curvula* in 20weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	38900±14.39	19500±9.11	49.89
B	39000±8.36	30000±15.00	23.08
C	37564±13.11	10250±17.05	72.71
D	38531±22.38	12300±6.65	68.08
E	38500±10.08	4900±9.68	87.27
F	38560±10.02	9800±16.58	76.70
Control	38200±32.12	25630±8.32	32.91

4.2.5. Degradation of TPH at 0.8% Nitrogen level for *E. curvula*

Treatment A resulted in a 55.17% decrease in TPH concentration in that soil blend. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in just 20.56% reduction in TPH concentration. Treatment C (HCS + Compost) and D resulted in a relatively higher rate of phytoremediation of 69.57% and 62.15% reductions in TPH concentration. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil)

produced the best phytoremediation of TPH resulting in a 93.75% reduction in TPH concentration. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) in a decrease of 67.63%.

Table 11: Degradation rate of TPH at 0.8 % Nitrogen in the different treatment media by *Eragrostis curvula* in 20 weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	138420±71.00	37000±13.35	73.27
B	138920±31.31	126200±44.57	9.48
C	138100±12.09	26000±7.12	81.17
D	138400±33.22	52000±18.32	62.43
E	136400±62.27	8100±16.17	94.06
F	138400±21.05	52000±9.11	62.43
Control	138620±32.12	128150±38.32	7.77

4.2.6. Degradation of Oil and grease at 1.2% Nitrogen level for *C. odorata*

Table 8 below shows the phytoremediation rates of *Chromolaena odorata* on the different treatment combinations. Treatment A (Hydrocarbon-contaminated soil + top soil) resulted in a 54.53% reduction in the initial oil and grease concentration. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in the lowest phytoremediation of 5.85%. Treatment C (Hydrocarbon contaminated soil + Compost) resulted in a 70.29%. Treatment D resulted in an 82.56% reduction. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement in oil and grease concentration of 95.67%. Treatment F (Hydrocarbon contaminated soil

+ Compost + Fertilizer) resulted in a phytoremediation rate of 53.93%. The Control Treatment (Contaminated soil only) only resulted in a 7.24% reduction.

Table 12: Degradation rates of Oil and grease at 1.2 % Nitrogen in the different treatment media by *Chromolaena odorata* in 20 weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	139000±35.30	63200±13.99	54.66
B	138720±51.05	130600±31.22	9.48
C	138000±87.34	41000±11.62	70.29
D	138500±15.00	20000±11.02	85.56
E	138560±39.33	6000±8.16	95.67
F	139000±22.64	64000±18.44	53.93
Control	139500±44.09	129400±21.05	7.24

4.2.7. Degradation of Oil and Grease at 1.0% Nitrogen level for *C. odorata*

Treatment A resulted in a 47.41% breakdown in oil and grease concentration in that media. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in just 6.63% reduction in the initial oil and grease concentration. Treatments C and D resulted in a phytoremediation rate of 73.63% 63.07% respectively. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement in oil and grease concentration of 95.67% as far as the 1.0% Nitrogen amendment level was concerned. Treatment F (HCS + Fertilizer + Topsoil) produced a phytoremediation rate of 50.61%. Table 13 below shows the trends in oil and grease breakdown in the treatments with the 1.0% Nitrogen amendment levels over the 20-week period.

Table 13: Degradation rate of Oil and Grease at 1.0 % Nitrogen in the different treatment media by *Chromolaena odorata* in 20weeks

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	139000±46.05	73100±14.51	47.41
B	138700±28.42	129700±42.10	6.63
C	137300±66.73	36200±14.14	73.63
D	138100±26.77	51000±14.70	63.07
E	138560±10.82	6000±6.79	95.67
F	138700±32.53	68500±13.03	50.61
Control	139500±44.09	129400±21.05	7.24

4.2.8 Degradation of Oil and Grease at 0.8% Nitrogen level for *C. odorata*

Exactly 43.97% breakdown in oil and grease concentration was recorded for Treatment A. Treatment B (Hydrocarbon contaminated soil + fertilizer) produced the lowest phytoremediation rate of 6.13%. Treatment C (Hydrocarbon contaminated soil + Compost) resulted in a relatively higher rate of phytoremediation of 91.97% reduction in oil and grease concentration and ranking second only to Treatment E. The enhancement of oil and grease in Treatment D was 58.10%. Treatment E, similar to the same treatment with the 1.2% and 1.0% Nitrogen amendments, produced the best phytoremediation rate of 95.46%. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) produced a breakdown in oil and grease concentration of 46.21%.

Table 14: Degradation rate of Oil and Grease at 0.8 % Nitrogen in the different treatment media by *Chromolaena odorata* after 20weeks

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	139200 ± 51.00	78000 ± 23.40	43.47
B	138920 ± 31.31	130400 ± 32.53	6.13
C	137000± 30.79	11000 ± 13.17	91.97
D	138450 ± 27.28	58000 ± 15.77	58.10
E	138900 ± 42.10	6300± 9.10	95.46
F	138500± 65.23	74500 ± 11.18	46.21
Control	139500 ± 44.09	129400 ± 21.05	7.24

4.2.9 Degradation of TPH at 1.2% Nitrogen level for *C. odorata.*

Treatment A (Hydrocarbon-contaminated soil + top soil) recorded a 64.10% breakdown. The Hydrocarbon contaminated soil + fertilizer blend of Treatment B resulted in the lowest TPH breakdown of 16.54% with Treatment C (Hydrocarbon contaminated soil + Compost) recording an 83.35% reduction.(Treatment D) recorded an enhancement in the TPH concentration of 69.06%. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement in TPH concentration resulting in a 91.04% reduction. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) resulted in an 89.72% enhancement.

Table 15: Degradation rate of TPH at 1.2 % Nitrogen in the different treatment media by *Chromolaena odorata* after 20 weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	39000 ± 24.00	37000 ± 8.96	64.10
B	38340 ± 13.13	32000 ± 13.14	16.54
C	39023 ± 45.14	6500 ± 9.32	83.35
D	38789 ± 9.50	12000 ± 13.22	69.06
E	38796 ± 20.36	3362 ± 11.69	91.04
F	38920 ± 14.36	4000 ± 9.99	89.72
Control	39000 ± 20.04	32350 ± 18.85	32.91

4.3. Degradation of TPH at 1.0% Nitrogen level for *C. odorata.*

Treatment A resulted in a 47.78% breakdown in TPH concentration. The Treatment B substrate resulted in just 22.02% reduction in the initial TPH concentration. Treatment C (Hydrocarbon contaminated soil + Compost) resulted in a 69.61% reduction in TPH concentration. Treatment D recorded a reduction rate of 68.08%. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best phytoremediation of TPH resulting in an 89.61% reduction in TPH concentration. Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) produced a comparatively high breakdown in TPH concentration of 66.83%.

Table 16: Degradation rate of TPH at 1.0 % Nitrogen in the different treatment media by *Chromolaena odorata* after 20weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	38600 ± 20.00	20156 ± 4.84	47.78
B	39115 ± 8.63	30500 ± 10.10	22.02
C	38690 ± 21.21	11569 ± 9.64	69.61
D	38730 ± 16.72	15963 ± 13.32	58.78
E	38529 ± 14.09	4000 ± 4.53	89.61
F	38890 ± 12.73	12897 ± 5.70	66.83
Control	39000 ± 20.04	32350 ±18.85	32.91

4.3.1 Degradation of TPH at 0.8% Nitrogen level for *C. odorata.*

Treatment A resulted in a 51.61% decrease in TPH concentration. Treatment B (Hydrocarbon contaminated soil + fertilizer) resulted in just 18.52% reduction in TPH concentration, which was the lowest among the seven treatments. Treatment C resulted in a TPH phytoremediation of 67.52%. The improvement of TPH concentration in Treatment D was 59.65%. Like all the different Nitrogen amendments, Treatment E

(Hydrocarbon contaminated soil + Compost + Topsoil) produced the best phytoremediation of TPH resulting in an 87.85% reduction in TPH concentration. A total TPH phytoremediation rate of 60.62% was recorded by the Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) over the 20-week period.

Table 17: Degradation rate of TPH at 0.8 % Nitrogen in the different treatment media by *Chromoleana odorata* after 20 weeks.

Treatments (Codes)	Initial Concentration (mg/kg)	Final Concentration (mg/kg)	% Reduction
A	38765±11.34	18756±5.56	51.61
B	39282±21.32	32005±12.50	18.53
C	38954±20.59	12624±8.12	91.97
D	38900±20.43	15697±13.44	59.65
E	38892±8.10	4725±12.15	87.85
F	38100±35.21	15000±10.12	60.62
Control	39000±20.04	32350±18.85	32.91

The full breakdowns of oil and grease and TPH in the different media for the two plants are shown in Appendix B.

4.4 Comparative Assessment of the Phytoremediation Rates of the Different Treatment Media

The different treatment blends resulted in different phytoremediation rates over the 20-week period. Treatment E (Hydrocarbon contaminated soil + Topsoil+ Compost) and Treatment B(Hydrocarbon contaminated soil + Fertilizer) were highly significant ($P<0.05$). Appendix B1-12 presents the full ANOVA results as well as the Tukey's Multiple Comparison Tests for the different treatments at the different Nitrogen amendment levels. The summarized results are however presented in Table 13 below.

4.4.1 Assessment of the Phytoremediation Rates of the Treatment Media Planted with *E. curvula*

At the end of the 20-week period, there were significant differences ($p<0.05$) in the phytoremediation rates of oil and grease as far as Treatments A (Hydrocarbon Contaminated soil + Topsoil) and B (Hydrocarbon Contaminated soil + Fertilizer) as well as Treatment A (HCS +Topsoil) and the Control were concerned. This trend was observed in all the different Nitrogen amendment levels. There were however no significant differences ($p>0.05$) in the phytoremediation rates as far as Treatment A (Hydrocarbon Contaminated soil + Topsoil) and the other treatments were concerned. The phytoremediation rates of Treatment B (Hydrocarbon Contaminated soil + Fertilizer) varied significantly against all the other Treatments with the exception of the Control Treatment which recorded a fairly similar phytoremediation rate as Treatment B (Table 13). The phytoremediation rates of TPH with *Eragrostis curvula* in the different treatments did not show much variation with the only significant difference being recorded between Treatments B and E, and between the Control Treatment and Treatment E for the 1.2% N amendment. As far as the phytoremediation of TPH in the treatment blends with the 1.0% Nitrogen and 0.8% Nitrogen amendments were concerned, only Treatments B (Hydrocarbon Contaminated soil + Fertilizer) and E (Hydrocarbon Contaminated soil + Topsoil + Compost) exhibited significant differences ($p<0.05$).

Table 18: Tukey's Multiple Comparison Tests for the different indicates treatments at the different nitrogen amendment levels.

Treatment	N-Levels (%)	A	B	C	D	E	F	Control
Eragrostis curvula								
	1.2%	20000^{a}	126000^{b}	22000^{a}	11000^{a}	4900^{a}	5700^{a}	128200^{b}
O&G	1.0%	24000^{a}	127800^{b}	41000^{a}	15000^{a}	5700^{a}	49000^{a}	128200^{b}
	0.8%	37000^{a}	130000^{b}	26000^{a}	52000^{a}	8100^{a}	52000^{a}	128200^{b}
	1.2%	19000^{a}	23980^{ab}	4780^{a}	9058^{a}	3920^{ac}	4950^{a}	25630^{ab}
TPH	1.0%	19500^{a}	30000^{ab}	10250^{a}	12300^{a}	4900^{ac}	9800^{a}	25630^{a}
	0.8%	16980^{a}	30900^{ab}	11560^{a}	14570^{a}	2430^{ac}	12300^{a}	25630^{a}
Chromolaena								
	1.2%	63200^{a}	130600^{ab}	41000^{a}	20000^{a}	6000^{a}	64000^{a}	129400^{ab}
O&G	1.0%	73100^{a}	129700^{ab}	36200^{ac}	51000^{a}	6000^{ac}	68500^{a}	129400^{ab}
	0.8%	78000^{a}	130400^{ab}	11000^{ac}	58000^{a}	6300^{ac}	74500^{a}	129400^{ab}
	1.2%	14000^{a}	32000^{a}	6500^{a}	12000^{a}	3362^{a}	4000^{a}	32350^{a}
TPH	1.0%	20160^{a}	30500^{ab}	11570^{a}	15960^{a}	3500^{ac}	12900^{a}	32350^{ab}
	0.8%	18760^{a}	32010^{ab}	12650^{a}	15700^{a}	4725^{ac}	15000^{a}	32350^{ab}

Means (on same row) with different letters in superscript differ significantly ($p<0.05$)

4.4.2 Assessment of the Phytoremediation Rates of the Treatment Media Planted with *C. odorata*

With the exception of Treatments B and E, and Treatment E and the Control Treatment, there were no significant variations ($p>0.05$) in the phytoremediation rates of oil and grease with *Chromolaena* in all the other treatments pairings with 1.2% Nitrogen amendment. In the treatment combinations with the 1.0% Nitrogen amendment significant variations were found between Treatments B and C, between Treatments B and E, between Treatments D and E and between Treatment E and the Control Treatment (Table 4.4). In the 0.8% Nitrogen amendment levels, the phytoremediation rates of oil and grease at the end of the 20 weeks were fairly similar ($p>0.05$) between all the treatment pairings with the exception of Treatments B and C, between Treatments B and E, between Treatment C and the Control Treatment and between Treatment E and the Control Treatment, which exhibited significant variations in phytoremediation rates.

The Tukey's Multiple Comparison Tests showed no significant differences ($p>0.05$) in the phytoremediation rates of TPH by *Chromolaena odorata* in all the Treatment combinations with the 1.2% Nitrogen amendment. The treatment combinations with the 1.0% and 0.8% Nitrogen levels all exhibited significant differences ($p<0.05$) between the following treatments; B and E, and E and the Control.

4.5 Comparative Assessment of the Different Nitrogen Amendments in the Treatment Blends

Overall, the treatments with the 0.8% nitrogen amendment recorded the lowest oil and grease and TPH phytoremediation rates using *Chromolaena odorata* and *Eragrostis curvula* plants. The residual Oil and Grease / TPH levels after the 20-week period were

thus higher in 0.8% compared to the 1.0% and 1.2% Nitrogen levels. As far as the phytoremediation of oil and grease by *Chromolaena odorata* was concerned, only Treatment A exhibited significant differences ($p<0.05$) among the different Nitrogen amendments after the 20-weeks period.

The Tukey's Multiple Comparison Test revealed that the actual differences were between the 1.2% and 0.8% Nitrogen levels and between the 1.0% and 0.8% Nitrogen levels. Table 19 below summarises the percentage reduction in oil and grease as well as TPH in the various treatments at the different Nitrogen amendment levels.

Table 19a: Comparative Assessment of phytoremediation rates of oil and grease of *Eragrostis curvula* Amendments in the Treatment Blends

% Nitrogen	A	B	C	D	E	F	Control
Eragrostis curvula							
1.2% Nitrogen							
Initial Conc	139520	139420	138211	138000	135000	13600	138620
Final Conc	20000	126200	22000	11000	4900	5700	128150
% Reduction	85.66	9.48	84.08	92.03	96.37	95.80	7.77
1.0% Nitrogen							
Initial Conc	139042	138920	138021	138200	136000	138620	138620
Final Conc	24000	127800	41000	15000	5700	49000	128150
% Reduction	82.72	8.00	70.29	89.15	95.81	64.23	7.77
0.8% Nitrogen							
Initial Conc	138420	138920	138100	138400	136400	138400	138620
Final Conc	37000	130000	26000	52000	8100	52000	128150
% Reduction	73.27	6.42	81.17	62.43	94.06	62.43	7.77

Table 19b: Comparative Assessment of phytoremediation rates of oil and grease of *Chromolaena odorata* in the Treatment Blends

% Nitrogen	A	B	C	D	E	F	Control
Chromolaena odorata							
1.2% Nitrogen							
Initial Conc	139000	138720	138000	138500	138560	139000	139500
Final Conc	63200	130600	41000	20000	6000	64000	129400
% Reduction	54.53	5.85	70.29	85.56	95.67	53.93	7.24
1.0% Nitrogen							
Initial Conc	139000	138920	137300	138100	138560	138700	139500
Final Conc	73100	129700	36200	51000	6000	68500	129400
% Reduction	47.41	6.63	73.63	63.07	95.67	50.61	7.24
0.8% Nitrogen							
Initial Conc	139200	138920	137000	138450	13890	138500	139500
Final Conc	78000	130400	11000	58000	6300	74500	129500
% Reduction	43.97	6.13	91.97	58.10	95.46	46.21	7.24

Table 20a: Comparative Assessment of phytoremediation rates of TPH of the *Eragrostis curvula* in the Treatment Blends

% Nitrogen	A	B	C	D	E	F	Control
Eragrostis curvula							
1.2% Nitrogen							
Initial Conc	37000	38720	38700	36000	37800	39200	38200
Final Conc	19000	23978	4780	9058	3920	4950	25630
% Reduction	48.65	38.13	87.65	74.83	93.75	87.37	32.91
1.0% Nitrogen							
Initial Conc	38900	39000	37564	38531	38500	38560	38200
Final Conc	19500	30000	10250	12300	4900	9800	25630
% Reduction	49.87	23.08	72.71	68.08	89.62	76.70	32.91
0.8% Nitrogen							
Initial Conc	37927	38900	38000	38486	38900	38000	38200
Final Conc	16978	30900	11564	14568	2430	12300	25630
% Reduction	55.17	20.56	69.57	62.15	87.27	67.63	32.91

Table 20b: Comparative Assessment of phytoremediation rates of TPH of the *Chromolaena odorata* Nitrogen Amendments in the Treatment Blends

% Nitrogen	A	B	C	D	E	F	Control
Chromolaena curvula							
1.2% Nitrogen							
Initial Conc	39000	38340	39023	38789	38796	38920	39000
Final Conc	37000	32000	6500	12000	3362	4000	32350
% Reduction	64.10	16.54	83.35	69.06	91.04	89.72	17.05
1.0% Nitrogen							
Initial Conc	38600	39115	38690	38730	38529	38890	39000
Final Conc	20156	30500	11569	15963	4000	12897	32350
0.8% Nitrogen							
% Reduction	47.78	22.02	69.61	58.78	89.61	66.83	17.05
Initial Conc	38765	39285	38954	38900	38892	38100	39000
Final Conc	18756	32005	12654	15697	4725	15000	32350
% Reduction	51.61	18.53	67.52	59.65	87.85	60.62	17.05

4.6 Uptake and Accumulation of Hydrocarbons by the Two Plant Species

Root and shoot tissue were separately assessed for TPH accumulation at the end of the 20 weeks study, for the two plant species. The harvested plant tissue were washed thoroughly in distilled water and separated into roots and shoots. The assessment of roots and shoots of the two plant species for TPH accumulation showed varying concentrations in the two plants.

4.6.1 Uptake of Oil and Grease

The highest mean oil and grease accumulated in the *Chromolaena odorata* shoot was 30750±535.55 $mgkg^{-1}$ in the 1.2% Nitrogen amendment. The shoots of the *Eragrostis curvula* on the other hand recorded a highest mean concentration of 26400±565 $mgkg^{-1}$, significantly lower than the highest mean oil and grease in the shoots of the *Chromolaena*. The roots generally recorded lower accumulation of oil and grease levels compared to the shoots for both plants. Similar to the shoots, the roots of the *Chromolaena* generally recorded higher concentrations of oil and grease concentrations with a highest concentration of 18600±707.71 $mgkg^{-1}$ recorded in the 1.0% Nitrogen amendment levels. The highest mean oil and grease concentration in the roots of the *Eragrostis curvula* was 17650±494.98 $mgkg^{-1.}$

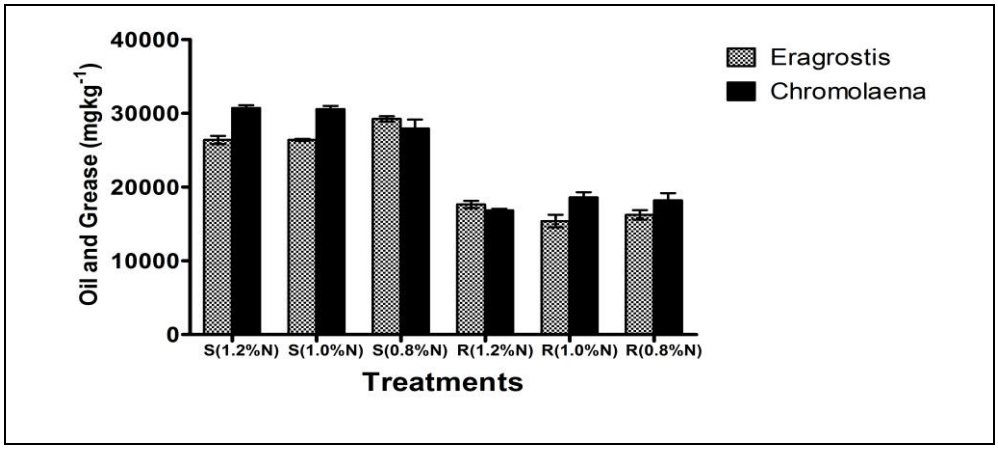


Fig. 2 Mean oil and grease concentrations accumulated in the tissues of the two plants at the end of 20 weeks

4.6.2 Uptake of Total Petroleum Hydrocarbon

The mean TPH concentrations accumulated in the shoots of the *Eragrostis curvula* were slightly higher by Thirty Five percent (35%) than the concentrations in the *Chromolaena odorata*. The highest mean TPH concentrations of 22450±777.19 and 21750±777.19 $mgkg^{-1}$ were recorded in the shoots of *Eragrostis curvula* and *Chromolaena odorata* respectively. The roots of the *Chromolaena* generally recorded slightly higher TPH concentrations than the *Eragrostis curvula*, recording a highest mean concentration of 6800 $mgkg^{-1}$. *Chromolaena* on the other hand recorded a highest mean concentration of 5950 $mgkg^{-1.}$ Similar to the oil and grease, the TPH concentrations accumulated in the roots were significantly lower ($p<0.05$) than the concentrations recorded in the shoots. There were, however, no significant differences ($p>0.05$) in the concentrations of the TPH accumulated in the roots of the plants as shown in Figure 2.

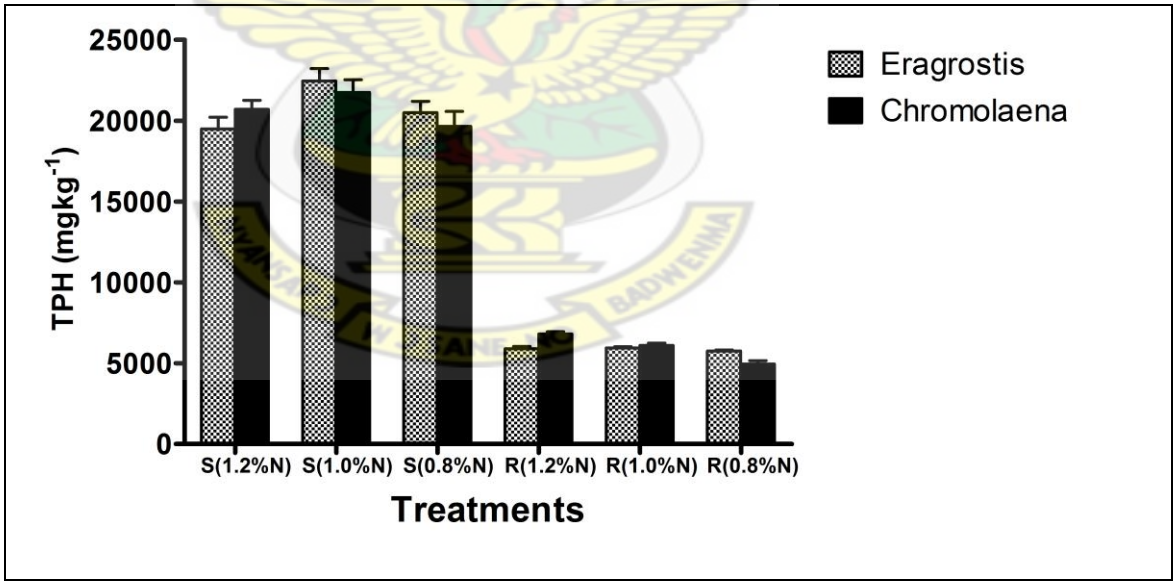


Fig. 3 Mean TPH concentrations accumulated in the tissues of the two plants at the end of 20 weeks

CHAPTER FIVE

5.0 DISCUSSION

5.1. Oil and grease and TPH degradation at different nitrogen levels for *Chromolaena odorata*

Phytoremediation has proven to be a good method for cleaning up soils that have low or intermediate contamination of petroleum hydrocarbons. It is cheap in comparison with many in situ methods. Nitrogen is essential for plant growth and the higher the concentration the better it is for the plants to grow. At the Naval Air Station Reserve Base in the US, phytoremediation with particular emphasis on nitrogen amendment of the soil gave a similar confirmation of the need for higher nitrogen levels (Betts, 1997). The results of this study demonstrate that growth of *C. odorata* on treatment E (Hydrocarbon + topsoil + compost) for the different nitrogen amendment groups (1.2%, 1.0% and 0.8%) enhances the degradation of the oil and grease concentration in the contaminated soil (Table 6).

Nitrogen level amendment at 1.2% recorded the highest amount of Oil and Grease / TPH reduction for the treatment (E) combination of HCS + Topsoil + Compost (Table 8). Growth of *C. odorata* was observed to be better in all the treatments blends with Topsoil and/Compost at the various nitrogen amendment and a higher remediation of oil and grease reduction as well as TPH was also recorded as shown in Tables 7 and Table 8. The poor growth of *C. odorata* and the higher containments of hydrocarbons in the contaminated soils after the 20^{th} week for the treatment B and the control could be attributed to the fact that the oil and grease concentration in the soil was too high for the plant to mobilize (Table 6), since no growth media was added to help neutralize the hydrocarbon concentration. According to U.S. EPA, (2000), extremely high contaminant

concentrations may not allow plants to grow or survive; phytoremediation is likely to be more effective or reasonable for lower concentrations of contaminants. The 0.8% nitrogen level (mg/kg) in all the experiment with *C. odorata* recorded the lowest oil and grease and TPH percentage reduction for treatment E. The residual Oil and Grease / TPH levels were thus higher in 0.8% compared to the 1.0%, and 1.2% as indicated in Table 6 and 7. The higher the nitrogen amendment in the various treatments, the better the plants grew and thus the higher the reduction of the contaminants. Tables 14, 15 and 16 show the different nitrogen levels and their residual oil & grease concentrations by the 20th week of sampling in the various treatment blends.

The rapid degradation of hydrocarbons in the topsoil and/compost system was expected since topsoil and/compost blend improve the soil quality by improving soil structure and increasing porosity, leading to better water infiltration, providing nutrient and increasing soil organic carbon (Schnoor, 1997), resulting in the better growth and higher percentage reduction of hydrocarbons of C. *odorata* in the treatment E (Tables 4.2.6, 4.2.7 and 4.2.8).

In general the addition of Topsoil and/ compost resulted in a better growth performance of *C. odorata* in treatment E, D, C, A and F in all the nitrogen amendment levels. Degradation of Oil and Grease in the topsoil and/ compost blends was highly significant (p 0.05) as compared to those of fertilizer. This may be attributed to the fact that, the topsoil and/compost could be rich in microorganisms and also had well stratified layers with air spaces. These synergistically helped in the development of sufficient root and shoot mass of the plant and the subsequent bioaccumulation of the hydrocarbon contaminants. Other studies have indicated that plants can enhance hydrocarbon containment only when they have established sufficient root and shoot mass (Frick *et al*., 1999).

The present results also showed that the poor performance in growth of the *C. odorata* in the Treatment B (Hydrocarbon contaminated soil + fertilizer) and the control

(Contaminated soil only) as shown in Tables 4, 5 and 6 can be attributed to oil-concentration dependent growth of plant decreasing with increasing in oil and grease level in soil. Oil and grease contamination of soil has been reported to cause reduction in the germination, growth and their performance and even yield of plants (Anoliefo *et al.*, 2006; Vwioko *et al.*, 2006; Agbogidi and Dolor, 2007).

Present results confirms to the report of Agbogidi and Eshegbeyi (2006), and Agbogidi and Dolor, (2007), who noted that as hydrocarbons from oil polluted soil accumulate in the chloroplasts of leaves, photosynthetic ability of the leaves becomes reduced affecting translocation in affected plants probably due to obstruction of the xylem and phloem vessels hence reduction in growth and matter content resulting in a low remediation of hydrocarbons.

5.2. Oil and grease and TPH degradation at different levels of nitrogen for *Eragrostis curvula*.

The trends in oil and grease breakdown in the treatments with the 1.2%, 1.0% and 0.8% Nitrogen amendment levels are shown in Table 14 and 15. Treatment E (Hydrocarbon contaminated soil + Compost + Topsoil) produced the best enhancement in oil and grease concentration as far as the 1.2% Nitrogen amendment level was concerned with a reduction percentage of 96.37% in oil and grease concentration (Table 4.6). Treatment F (Hydrocarbon contaminated soil + Compost + Fertilizer) also resulted in a highly measurable reduction in oil and grease concentration from 136000 $mgkg^{-1}$ to 5700 $mgkg^{-1}$, representing a 95.80% enhancement.

According to Tang *et al.* (2010), contaminated soils are usually deficient in macro- and micro-nutrients necessary for establishing healthy vigorously growing plants and stimulating microbial contaminant degradation. In view of this Organic source of nitrogen

was observed to be better than inorganic sources. This is probably because organic nitrogen sources provide a low release source of nitrogen, and also help to improve soil structure and soil water relationships for plant growth.

Significantly, with the amendment of nitrogen, all the treatments blended with topsoil and or compost recorded a higher reduction in the oil and grease and/TPH concentration in the contaminated soil Tables 7 and 8.

Extremely high contaminant concentrations may not allow plants to grow or survive; phytoremediation is likely to be more effective or reasonable for lower concentrations of contaminants (U.S. EPA, 2000b), as in the case of Treatment B and the control, the percentage reduction recorded in the 1.2% nitrogen level was 9.48% and 7.77% respectively (Table 2). Relatively very low percentages of reduction for these two treatments were recorded in tables 4.5 and 4.6 as the nitrogen level decreases. This is in agreement with the above statement of the U.S. EPA (2000), and could be attributed to the fact that, the concentration of the contaminant was too high for the plant, such that the addition of fertilizer did not improve a good growth for *E. curvula* either at the higher nitrogen level or the lower nitrogen levels over the 20 week period.

5.3. Uptake and Accumulation of Hydrocarbons by the Two Plant Species

The assessment of roots and shoots of the two plant species for TPH accumulation showed varying concentrations in the roots or shoots of two plants. Uptake and transport as well as microbial metabolism could be the primary mechanism for the removal of oil and grease and TPH from the contaminated soils in this study. Increasing Nitrogen levels appears to result in increased uptake and accumulation of oil and grease by both plants. *Chromolaena odorata* accumulated higher levels of oil and grease than the *Eragrostis curvula* in ascending levels of nitrogen amendment (refer to Fig 1).

According to Crowley and Bicnnewr, (1996); Haby and Crowley, (1996), the process of plant degradation of hydrocarbon involve the root of the living plants which function as solar driven pumps that extract and concentrate compound and elements from the soil. From physiological view, the different plants species, *C. odorata* and *E. curvula* differ in their root orientation and subsequent penetration into the soil, a necessary ingredient for mineral absorption and plant growth. It was therefore expected that plants with higher root penetration would have a higher rate of modifying the soil by affecting a wide spectrum of biological activities capable of speeding up oil and grease degradation and accelerating plant growth. *C. odorata* which has a higher root penetration than *E. curvula* and recorded the highest mean of oil and grease accumulation in the shoot (30750±535.55 $mgkg^{-1)}$ and was measured in the 1.2% Nitrogen amendment. The shoot of the *E. curvula* on the other hand recorded a mean concentration of 26400±565 $mgkg^{-1}$, significantly lower than the mean accumulation of oil and grease in the shoot of the *C. odorata* (Fig.2). According to a study by Palmroth *et al*., (2002), grass roots accumulated 10,000 mg diesel-range compounds per kg dry plant tissue. The findings were similar to the concentrations of TPH recorded in the roots of the *Chromolaena* and *Eragrostis curvula* in this study although the accumulated levels recorded in this study were relatively lower.

The roots generally recorded lower accumulation of oil and grease levels compared to the shoots for both plants. It was shown during the study that uptake of hydrocarbons into aerial parts was possible (Fig. 2) despite the relatively high molecular weight of the studied hydrocarbons which would usually be adsorbed to roots rather than uptake into the shoot. The roots of the *C. odorata* generally recorded higher concentrations of oil and grease containment of 18600±707.71 $mgkg^{-1}$ recorded in the 1.0% Nitrogen amendment levels. The highest mean of oil and grease concentration in the roots of the *E. curvula* was 17650±494.98 $mgkg^{-1}$ (Fig. 2).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The contaminated soil containing high levels of oil and grease and total petroleum hydrocarbon levels were reduced at the end of the treatment. Amendment of the topsoil, compost and inorganic fertilizer to hydrocarbon contaminated soils was beneficial in creating the optimum conditions for the plants to grow. The residual Oil and Grease / TPH levels after the 20-week period were thus higher in 0.8% compared to the 1.0% and 1.2% Nitrogen levels.

The different treatment combinations at different levels of inorganic nitrogen (0.8, 1.0 and 1.2%) all reported significantly different rates of biodegradation of oil and grease and TPH, with most of the treatments resulting in significant reductions of oil and grease and TPH concentrations. The results of the phytoremediation experiment indicated measurable reduction of oil and grease as well as Total petroleum hydrocarbon (TPH) concentrations in the different treatment media using the plants studied.

Treatment E (Hydrocarbon Contaminated soil+ Topsoil+ Compost) resulted in the best enhancements of oil and grease and TPH with over 90% reduction in contaminant levels after the 20-week period in the various nitrogen levels. The Control Treatment on the other hand, consistently recorded less than 10% reductions in oil and grease concentrations.

Overall, the treatment combinations with the 0.8% nitrogen amendment recorded the lowest oil and grease and TPH phytoremediation rates as far as both *Chromolaena odorata* and *Eragrostis curvula* were concerned. The residual Oil and Grease / TPH levels in the contaminated soil after the 20-week period were thus higher in 0.8% compared to the 1.0% and 1.2% Nitrogen levels. The phytoremediation experiment revealed that, the higher the nitrogen amendment in the various treatments, the better the plant growth and thus the

higher the reduction of the petroleum contaminants. The general trend in oil and grease and TPH remediation over the 20-week period was 1.2% Nitrogen>1.0 Nitrogen>0.8 Nitrogen.

Overall reductions in oil and grease and TPH levels appeared to be as a result of uptake, accumulation and transpiration as well as microbial activity in the rhizosphere regions of the two plants. *Chromolaena odorata* could be the best plant for phytoremediation as it reduces drastically the hydrocarbon concentration in the soil better than *Eragrostis curvula.*

6.2 Recommendations

This study recommends the following:

- That treatment E (Compost, topsoil and hydrocarbon contaminated soil) at any 1.2% nitrogen should be used in remediating hydrocarbon contaminated soil.
- As nitrogen is the most frequently deficient nutrient in most hydrocarbon contaminated soils, legume-plants could be a good candidate in future phytoremediation research.
- Similar study could be done on contaminated soils from vehicle repairs centers popularly called "Magazines" using same or other established plants.
- Fertilizer as a nitrogen source is expensive and did not perform well in this study. It is highly recommended that in future studies the performance of other organic sources such as organic manure (animal droppings) could be investigated.

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APPENDICES

Appendix A1 Baseline Physiochemical analyses of various soil types used in this study

SAMPLE TYPE	NITR-OGEN	PHOSPHORUS		POTASSIU-M		ORGANIC MATTER	CA-RBON	N:C	pH	MOIST-URE
	% Total	% Total	Mg/g	% Total	Mg/g	% Total	%			%
COMPOST	0.95	0.331	3.307	0.131	1.304	10.19	5.91	1:4	7.96	43.00
CONTAMINATED SOIL	0.042	0.124	1.243	0.083	0.826	2.77	1.60	1:4	7.62	23.00
TOP SOIL	0.69	0.157	0.571	0.100	1.004	1.90	1.10	1:1	7.22	41.00

Appendix A2 Baseline levels of Oil/grease, TPH in Compost, HC and Topsoil

Sample ID	Oil and Grease (mg/kg)	TPH (mg/kg)
Compost	<100	<100
Topsoil	< 100	<100
Hydrocarbon Contaminated Soil	139714.5	39315.9

Appendix A3: Weight of compost /topsoil/fertilizer added to 3kg contaminated soil

Nitrogen levels **Treatment**	**0.8**	**1.0**	**1.2**
Compost(g) added to HC	2393g	3025g	3656g
Topsoil(g) added to HC)	3295g	4165g	5304g
Fertilizer(g) HC added)	49g	62g	75g

Appendix A4: Calculations for the Quantities of Topsoil, Compost and Fertilizer added to the Contaminated Soil for Amendment

Nitrogen levels	**(0.8%, 1.0%, and 1.2%)**
Nitrogen level in topsoil (%)	0.69
Weight of HC cont. soil used/g	3000
Nitrogen level in HC (%)	0.042
Weight of N in contaminated soil/g	1.26
Level of nitrogen (%)	0.80
Expected weight of 0.8% nitrogen in HC	24.00
Nitrogen deficit(g)	22.74g
Amount of compost that contains 24g of N	**2393g**
Level of nitrogen (%)	0.8
Expected weight of 0.8% nitrogen in HC	36
Nitrogen deficit	24.0
Amount of compost that contains 24g of N	**3295g**
Level of nitrogen (%)	0.8

Expected weight of 0.8% nitrogen in HC	36
Nitrogen deficit	24.0
Amount of fertilizer that contains 24g of N	49**g**
Level of nitrogen (%)	1.0
Expected weight of 1.0% nitrogen in HC	36g
Nitrogen deficit	28.74
Amount of compost that contains 28.74g of N	**3025g**
Level of nitrogen (%)	1.0
Expected weight of 1.0% nitrogen in HC	36
Nitrogen deficit	28.74
Amount of topsoil that contains 28.74g of N	**4165g**
Level of nitrogen (%)	1.0
Expected weight of 1.0% nitrogen in HC	36g
Nitrogen deficit	28.74
Amount of fertilizer that contains 28.74g of N	**62g**
Level of nitrogen (%)	1.2
Expected weight of 1.2% nitrogen in HC	36
Nitrogen deficit	34.74
Amount of compost that contains 34.74g of N	**3656g**
Level of nitrogen (%)	1.2
Expected weight of 1.2% nitrogen in HC	36
Nitrogen deficit	34.74
Amount of topsoil that contains 38.4g of N	**5304g**
Level of nitrogen (%)	1.2
Expected weight of 1.2% nitrogen in HC	36

Nitrogen deficit	34.74
Amount of fertilizer that contains 38.4g of N	**75g**

APPENDIX B1: Phytoremediation of Oil and Grease by *Eragrostis curvula* [a=1.2%N, b=1.0%N and c=0.8%N]

a

	A	B	C	D	E	F	Control
Week 2	139520	139420	138211	138000	135000	136000	138620
Week 4	137520	138046	133460	131600	121000	120000	135000
Week 6	110624	133962	116462	109200	102000	105500	131000
Week 8	90000	129000	91000	96000	85000	90000	130400
Week 10	81000	128000	76400	85000	70000	76000	129424
Week 12	50000	127000	50000	71000	50000	40000	129000
Week 14	42000	126300	41000	42000	10000	15000	128900
Week 16	30000	126000	35000	32000	7800	11000	128700
Week 18	25000	126800	27000	21100	6100	8500	128400
Week 20	20000	126200	22000	11000	4900	5700	128150

b

	A	B	C	D	E	F	Control
Week 2	139042	138920	138021	138200	136000	137000	138620
Week 4	137000	137900	133460	131900	120000	131425	135000
Week 6	109900	136500	116462	108800	105500	115800	131000
Week 8	87000	133000	91000	101600	90000	104900	130400
Week 10	79500	132100	78400	98000	76000	95000	129424
Week 12	59000	131700	76000	87000	40000	87000	129000
Week 14	44000	130000	69000	75000	15000	73000	128900
Week 16	40000	129100	60000	43000	11000	62000	128700
Week 18	31000	128600	49000	19000	8500	55000	128400
Week 20	24000	127800	41000	15000	5700	49000	128150

c

	A	B	C	D	E	F	Control
Week 2	138420	138920	138100	138400	136400	138400	138620
Week 4	136980	137900	133900	132900	120800	132900	135000
Week 6	110900	136500	115000	116200	103000	116200	131000
Week 8	79000	135000	97000	105400	87000	105400	130400
Week 10	60000	134100	78000	96000	69000	96000	129424
Week 12	54000	133700	60000	87000	45000	87000	129000
Week 14	49000	133400	55000	74000	25000	74000	128900
Week 16	45000	131600	49000	68000	18000	68000	128700
Week 18	41000	130300	36000	61000	11000	61000	128400
Week 20	37000	130000	26000	52000	8100	52000	128150

APPENDIX B2: Phytoremediation of TPH by *Eragrostis* (Love grass) [a=1.2%N, b=1.0%N and c=0.8%N]

a

	A	B	C	D	E	F	Control
Week 2	37000	38760.0	38700.0	36000	37800	39200	38200.00
Week 4	36700	37800.0	38280.0	35890	30250	37000	37800.00
Week 6	36000	37000.0	37450.0	33500	25500	36000	36025.00
Week 8	35000	35991.0	35000.0	30000	34000	34000	36512.00
Week 10	29000	35712.0	20000.0	24000	28000	29100	36238.72
Week 12	27500	35560.0	16700.0	20000	12500	28000	36120.00
Week 14	24500	34227.3	15000.0	19152	7957	20000	34803.00
Week 16	21000	33768.0	13000.0	16562	5733	10000	25740.00
Week 18	20000	31700.0	8500.0	13485	4453	6000	28248.00
Week 20	19000	23978.0	4780.0	9058	3920	4950	25630.00

b

	A	B	C	D	E	F	Control
Week 2	38900	39000	37564.0	38531	38500	38360.00	38200.00
Week 4	38400	38600	36100.0	36489	37000	38113.25	37800.00
Week 6	37200	38000	32000.0	34561	37900	37056.00	36025.00
Week 8	37900	37300	26785.0	32540	32000	30421.00	36512.00
Week 10	32601	36900	24590.0	29754	29500	26600.00	36238.72
Week 12	26555	36200	21579.0	21000	13000	26100.00	36120.00
Week 14	25000	36000	19540.0	19543	10000	21170.00	34803.00
Week 16	23000	35300	18900.0	15000	7000	18600.00	25740.00
Week 18	20000	34800	15553.0	14124	6000	11000.00	28248.00
Week 20	19500	30000	10250.0	12300	4900	9800.00	25630.00

c

	A	B	C	D	E	F	Control
Week 2	37927.08	38900	38000	38468	38900	38000	38200.00
Week 4	34245.00	38100	37872	36231	36240	37800	37800.00
Week 6	28834.00	37450	35214	33457	37080	35000	36025.00
Week 8	24490.00	36890	33590	31540	36540	28000	36512.00
Week 10	24000.00	35754	26451	28988	30000	24000	36238.72
Week 12	21600.00	34345	21000	21569	11089	23500	36120.00
Week 14	20874.00	34000	19000	20789	10900	21000	34803.00
Week 16	20655.00	32900	15600	17956	7000	20000	25740.00
Week 18	17220.00	32190	13500	16879	5000	19000	28248.00
Week 20	16978.00	30900	11564	14568	2430	12300	25630.00

APPENDIX B3: Phytoremediation of Oil and Grease by *Chromolaena* [a=1.2%N, b=1.0%N and c=0.8%N]

a

	A	B	C	D	E	F	Control
Week 2	139000	138720	138000	138500	138560	139000	139500
Week 4	131000	137900	134000	134000	132000	130450	139300
Week 6	119000	136300	120000	126000	120000	123000	138200
Week 8	109000	135600	111000	119000	103000	115000	136000
Week 10	101000	134100	103500	113000	90000	103000	133450
Week 12	85000	133700	96000	97000	75600	97000	132500
Week 14	79000	132800	77000	78000	12500	86000	131500
Week 16	71200	131600	54300	57000	10350	79000	130000
Week 18	65000	130700	44900	35000	8200	67000	129650
Week 20	63200	130600	41000	20000	6000	64000	129400

b

	A	B	C	D	E	F	Control
Week 2	139000	138920	137300	138100	138560	138700	139500
Week 4	135000	137700	133000	133600	132000	130000	139300
Week 6	131000	136500	115000	129000	120000	121500	138200
Week 8	123000	135000	97000	123000	103000	111000	136000
Week 10	115000	134100	79000	114000	90000	99000	133450
Week 12	105200	133700	64000	101000	75600	94500	132500
Week 14	90000	132600	57000	89000	12500	87000	131500
Week 16	81000	131600	50000	70000	10350	80000	130000
Week 18	75000	130500	43000	64000	8200	76000	129650
Week 20	73100	129700	36200	51000	6000	68500	129400

c

	A	B	C	D	E	F	Control
Week 2	139200	138920	137000	138450	138900	138500	139500
Week 4	137900	137900	131000	133400	133500	136000	139300
Week 6	132000	136800	124000	129400	126000	129000	138200
Week 8	125000	135500	116000	120000	110000	121000	136000
Week 10	113000	134900	100000	111000	85000	111000	133450
Week 12	107000	133400	74000	101000	40000	104000	132500
Week 14	94000	132000	52000	91300	11000	98500	131500
Week 16	87000	131500	39000	80000	9800	81000	130000
Week 18	80000	130790	24700	65000	7700	76000	129650
Week 20	78000	130400	11000	58000	6300	74500	129400

APPENDIX B4: Phytoremediation of TPH by *Chromolaena* [a=1.2%N, b=1.0%N and c=0.8%N]

a

	A	B	C	D	E	F	Control
Week 2	39000	38340	39023	38789	38796.8	38920.0	39000.00
Week 4	37900	37289	38580	36987	38280.0	38000.0	38100.00
Week 6	35000	36896	38100	34123	37000.0	37500.0	37500.00
Week 8	33000	36300	35000	32145	36500.0	35000.0	37000.00
Week 10	31000	35555	31000	29631	27000.0	33000.0	36031.50
Week 12	28000	35300	24230	26451	22680.0	30000.0	35775.00
Week 14	27400	34000	20000	22354	11250.0	21000.0	34190.00
Week 16	23300	33800	15000	18564	7600.0	9000.0	33800.00
Week 18	22467	33100	11000	15687	3362.0	5400.0	33709.00
Week 20	14000	32000	6500	12000	4650.0	4000.0	32350.00

b

	A	B	C	D	E	F	Control
Week 2	38600	39115	38690	38730	38529.9	38890	39000.00
Week 4	38500	38900	37100	37956	38135.0	38587	38100.00
Week 6	38000	38200	34000	36489	36540.0	38000	37500.00
Week 8	37000	37580	30000	33541	29900.0	34589	37000.00
Week 10	34000	37100	27825	31564	28028.0	32888	36031.50
Week 12	29000	36800	25655	26789	17000.0	29875	35775.00
Week 14	27000	34000	22530	23798	14000.0	26000	34190.00
Week 16	24700	32000	19566	21659	9900.0	24100	33800.00
Week 18	21500	31000	12845	19874	3500.0	20456	33709.00
Week 20	20156	30500	11569	15963	4000.0	12897	32350.00

c

	A	B	C	D	E	F	Control
Week 2	38765	39285.0	38954	38900.0	38892	38100	39000.00
Week 4	38100	38000.0	37895	37421.0	37380	37850	38100.00
Week 6	32876	37578.0	36552	35741.0	36540	36000	37500.00
Week 8	29990	37000.0	33896	34024.0	30800	31000	37000.00
Week 10	27345	36390.0	28907	32963.0	24650	27000	36031.50
Week 12	23000	35671.0	24568	30489.0	12000	25000	35775.00
Week 14	22876	35056.0	19800	28986.0	8250	22000	34190.00
Week 16	21800	33235.0	17952	26325.0	7350	21500	33800.00
Week 18	19800	32689.0	15000	21589.0	5775	18000	33709.00
Week 20	18756	32005.0	12654	15697.0	4725	15000	32350.00

APPENDIX C: Full ANOVA Statistical annalysis for the Various Treatment Combinations at the Different Nitrogen Amendments

Appendix C1: ANOVA Results for the Phytoremediation of Oil and Grease by *Eragrostis curvula* in the Different Treatment Media at 1.2%N amendment

Table Analyzed	LG 1.2% O&G				
One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	5.975				
R squared	0.3627				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	64.02				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	58010000000	6	9668000000		
Residual (within columns)	101900000000	63	1618000000		
Total	159900000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-57510	4.521	Yes	*	-112300 to -2679
A vs C	-486.9	0.03828	No	ns	-55310 to 54340
A vs D	-1124	0.08833	No	ns	-55950 to 53700
A vs E	13390	1.052	No	ns	-41440 to 68210
A vs F	11800	0.9274	No	ns	-43030 to 66620
A vs Control	-58190	4.575	Yes	*	-113000 to -3365
B vs C	57020	4.483	Yes	*	2192 to 111800
B vs D	56380	4.433	Yes	*	1555 to 111200
B vs E	70890	5.573	Yes	**	16060 to 125700
B vs F	69300	5.448	Yes	**	14470 to 124100
B vs Control	-686.6	0.05398	No	ns	-55510 to 54140
C vs D	-636.7	0.05006	No	ns	-55460 to 54190
C vs E	13870	1.091	No	ns	-40950 to 68700
C vs F	12280	0.9657	No	ns	-42540 to 67110
C vs Control	-57710	4.537	Yes	*	-112500 to -2878
D vs E	14510	1.141	No	ns	-40320 to 69340
D vs F	12920	1.016	No	ns	-41910 to 67750
D vs Control	-57070	4.487	Yes	*	-111900 to -2242
E vs F	-1590	0.1250	No	ns	-56420 to 53240
E vs Control	-71580	5.627	Yes	**	-126400 to -16750
F vs Control	-69990	5.502	Yes	**	-124800 to -15160

Appendix C2: ANOVA statistical analysis of Phytoremediation of Oil and Grease by *Eragrostis curvula* in the Different Treatment Media at 1.0%N amendment

Table Analyzed	LG 1.0				
One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	6.279				
R squared	0.3742				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	65.62				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	45380000000	6	7563000000		
Residual (within columns)	75870000000	63	1204000000		
Total	121200000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-57520	5.241	Yes	**	-104800 to -10210
A vs C	-10190	0.9285	No	ns	-57490 to 37110
A vs D	-6706	0.6110	No	ns	-54010 to 40600
A vs E	14270	1.301	No	ns	-33030 to 61580
A vs F	-15970	1.455	No	ns	-63270 to 31330
A vs Control	-55720	5.077	Yes	*	-103000 to -8412
B vs C	47330	4.313	Yes	*	24.63 to 94630
B vs D	50810	4.630	Yes	*	3509 to 98120
B vs E	71790	6.542	Yes	***	24490 to 119100
B vs F	41550	3.786	No	ns	-5754 to 88850
B vs Control	1803	0.1643	No	ns	-45500 to 49110
C vs D	3484	0.3175	No	ns	-43820 to 50790
C vs E	24460	2.229	No	ns	-22840 to 71770
C vs F	-5778	0.5265	No	ns	-53080 to 41520
C vs Control	-45530	4.148	No	ns	-92830 to 1778
D vs E	20980	1.912	No	ns	-26320 to 68280
D vs F	-9263	0.8440	No	ns	-56570 to 38040
D vs Control	-49010	4.466	Yes	*	-96310 to -1706
E vs F	-30240	2.756	No	ns	-77550 to 17060
E vs Control	-69990	6.378	Yes	***	-117300 to -22690
F vs Control	-39750	3.622	No	ns	-87050 to 7556

Appendix C3: ANOVA Statistical analysis of the Phytoremediation of Oil and Grease by *Eragrostis curvula* in the Different Treatment Media at 0.8%N amendment

Table Analyzed	LG 0.8%				
One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	7.289				
R squared	0.4097				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	68.02				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	45420000000	6	7570000000		
Residual (within columns)	65430000000	63	1039000000		
Total	110800000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-59010	5.791	Yes	**	-102900 to -15090
A vs C	-3670	0.3601	No	ns	-47600 to 40260
A vs D	-17960	1.762	No	ns	-61890 to 25970
A vs E	12800	1.256	No	ns	-31130 to 56730
A vs F	-17960	1.762	No	ns	-61890 to 25970
A vs Control	-55630	5.459	Yes	**	-99560 to -11700
B vs C	55340	5.431	Yes	**	11420 to 99270
B vs D	41050	4.028	No	ns	-2875 to 84980
B vs E	71810	7.047	Yes	***	27890 to 115700
B vs F	41050	4.028	No	ns	-2875 to 84980
B vs Control	3383	0.3319	No	ns	-40540 to 47310
C vs D	-14290	1.402	No	ns	-58220 to 29640
C vs E	16470	1.616	No	ns	-27460 to 60400
C vs F	-14290	1.402	No	ns	-58220 to 29640
C vs Control	-51960	5.099	Yes	*	-95890 to -8033
D vs E	30760	3.018	No	ns	-13170 to 74690
D vs F	0.0000	0.0000	No	ns	-43930 to 43930
D vs Control	-37670	3.696	No	ns	-81600 to 6257
E vs F	-30760	3.018	No	ns	-74690 to 13170
E vs Control	-68430	6.715	Yes	***	-112400 to -24500
F vs Control	-37670	3.696	No	ns	-81600 to 6257

Appendix C4: ANOVA Statistical analysis of the Phytoremediation of TPH by *Eragrostis curvula* in the Different Treatment Media at 1.2%N amendment

Table Analyzed	TPH [1.2% Nitrogen]				
One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	6.948				
R squared	0.3982				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	23.30				
P value	0.0007				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	48780000000	6	8129000000		
Residual (within columns)	73710000000	63	1170000000		
Total	122500000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-24300	2.246	No	ns	-70920 to 22330
A vs C	13250	1.225	No	ns	-33380 to 59870
A vs D	21320	1.971	No	ns	-25310 to 67940
A vs E	19730	1.824	No	ns	-26900 to 66350
A vs F	14680	1.357	No	ns	-31950 to 61300
A vs Control	-55400	5.122	Yes	*	-102000 to -8776
B vs C	37550	3.471	No	ns	-9079 to 84170
B vs D	45620	4.217	No	ns	-1006 to 92240
B vs E	44020	4.070	No	ns	-2600 to 90650
B vs F	38980	3.603	No	ns	-7647 to 85600
B vs Control	-31100	2.875	No	ns	-77730 to 15520
C vs D	8073	0.7463	No	ns	-38550 to 54700
C vs E	6479	0.5990	No	ns	-40150 to 53100
C vs F	1432	0.1323	No	ns	-45190 to 48060
C vs Control	-68650	6.346	Yes	***	-115300 to -22020
D vs E	-1594	0.1473	No	ns	-48220 to 45030
D vs F	-6641	0.6140	No	ns	-53270 to 39980
D vs Control	-76720	7.093	Yes	***	-123300 to -30090
E vs F	-5048	0.4666	No	ns	-51670 to 41580
E vs Control	-75130	6.945	Yes	***	-121700 to -28500
F vs Control	-70080	6.479	Yes	***	-116700 to -23450

Appendix C5: ANOVA Statistical analysis of the Phytoremediation of TPH by *Eragrostis curvula* in the Different Treatment Media at 1.0%N amendment

Table Analyzed	TPH [1.0% Nitrogen]				
One-way analysis of variance					
P value	0.0070				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	3.290				
R squared	0.2386				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	24.45				
P value	0.0004				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1686000000	6	281000000		
Residual (within columns)	5381000000	63	85420000		
Total	7068000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-6304	2.157	No	ns	-18900 to 6293
A vs C	5620	1.923	No	ns	-6978 to 18220
A vs D	4521	1.547	No	ns	-8076 to 17120
A vs E	8326	2.849	No	ns	-4272 to 20920
A vs F	4184	1.431	No	ns	-8414 to 16780
A vs Control	-3626	1.241	No	ns	-16220 to 8972
B vs C	11920	4.080	No	ns	-673.8 to 24520
B vs D	10830	3.704	No	ns	-1772 to 23420
B vs E	14630	5.006	Yes	*	2032 to 27230
B vs F	10490	3.588	No	ns	-2110 to 23090
B vs Control	2678	0.9164	No	ns	-9919 to 15280
C vs D	-1098	0.3757	No	ns	-13700 to 11500
C vs E	2706	0.9259	No	ns	-9892 to 15300
C vs F	-1436	0.4913	No	ns	-14030 to 11160
C vs Control	-9246	3.163	No	ns	-21840 to 3352
D vs E	3804	1.302	No	ns	-8794 to 16400
D vs F	-337.8	0.1156	No	ns	-12940 to 12260
D vs Control	-8147	2.788	No	ns	-20750 to 4450
E vs F	-4142	1.417	No	ns	-16740 to 8456
E vs Control	-11950	4.089	No	ns	-24550 to 646.1
F vs Control	-7810	2.672	No	ns	-20410 to 4788

Appendix C6: ANOVA Statistical analysis of the Phytoremediation of TPH by *Eragrostis curvula* in the Different Treatment Media at 0.8%N amendment

Table Analyzed	TPH [0.8% Nitrogen]				
One-way analysis of variance					
P value	0.0118				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	3.014				
R squared	0.2230				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	25.91				
P value	0.0002				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1487000000	6	247800000		
Residual (within columns)	5180000000	63	82210000		
Total	6666000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-10460	3.648	No	ns	-22820 to 1899
A vs C	-496.8	0.1733	No	ns	-12860 to 11860
A vs D	-1362	0.4751	No	ns	-13720 to 11000
A vs E	3164	1.104	No	ns	-9195 to 15520
A vs F	-1178	0.4107	No	ns	-13540 to 11180
A vs Control	-8849	3.086	No	ns	-21210 to 3510
B vs C	9964	3.475	No	ns	-2395 to 22320
B vs D	9098	3.173	No	ns	-3261 to 21460
B vs E	13630	4.752	Yes	*	1266 to 25980
B vs F	9283	3.237	No	ns	-3076 to 21640
B vs Control	1611	0.5619	No	ns	-10750 to 13970
C vs D	-865.4	0.3018	No	ns	-13220 to 11490
C vs E	3661	1.277	No	ns	-8698 to 16020
C vs F	-680.9	0.2375	No	ns	-13040 to 11680
C vs Control	-8353	2.913	No	ns	-20710 to 4007
D vs E	4527	1.579	No	ns	-7833 to 16890
D vs F	184.5	0.06435	No	ns	-12170 to 12540
D vs Control	-7487	2.611	No	ns	-19850 to 4872
E vs F	-4342	1.514	No	ns	-16700 to 8017
E vs Control	-12010	4.190	No	ns	-24370 to 345.4
F vs Control	-7672	2.676	No	ns	-20030 to 4687

Appendix C7: ANOVA Statistical analysis the Phytoremediation of Oil and Grease by *Chromolaena odorata* in the Different Treatment Media at 1.2%N amendment

Table Analyzed	Chrom O&G 1.2% Nitrogen				
One-way analysis of variance					
P value	0.0002				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	5.133				
R squared	0.3283				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	70.68				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	33450000000	6	5576000000		
Residual (within columns)	68430000000	63	1086000000		
Total	101900000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-37960	3.642	No	ns	-82890 to 6961
A vs C	4270	0.4097	No	ns	-40650 to 49190
A vs D	4490	0.4308	No	ns	-40430 to 49410
A vs E	26620	2.554	No	ns	-18300 to 71540
A vs F	-4105	0.3939	No	ns	-49030 to 40820
A vs Control	-37710	3.618	No	ns	-82630 to 7213
B vs C	42230	4.052	No	ns	-2691 to 87160
B vs D	42450	4.073	No	ns	-2471 to 87380
B vs E	64580	6.197	Yes	***	19660 to 109500
B vs F	33860	3.249	No	ns	-11070 to 78780
B vs Control	252.0	0.02418	No	ns	-44670 to 45180
C vs D	220.0	0.02111	No	ns	-44700 to 45140
C vs E	22350	2.144	No	ns	-22570 to 67270
C vs F	-8375	0.8036	No	ns	-53300 to 36550
C vs Control	-41980	4.028	No	ns	-86900 to 2943
D vs E	22130	2.123	No	ns	-22790 to 67050
D vs F	-8595	0.8247	No	ns	-53520 to 36330
D vs Control	-42200	4.049	No	ns	-87120 to 2723
E vs F	-30720	2.948	No	ns	-75650 to 14200
E vs Control	-64330	6.172	Yes	***	-109300 to -19410
F vs Control	-33610	3.224	No	ns	-78530 to 11320

Appendix C8: ANOVA Statistical analysis of the Phytoremediation of Oil and Grease by *Chromolaena odorata* in the Different Treatment Media at 1.0%N amendment

Table Analyzed	Chrom O&G 1.0% Nitrogen				
One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	6.178				
R squared	0.3704				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	69.30				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	35290000000	6	5882000000		
Residual (within columns)	59980000000	63	952000000		
Total	95270000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-27300	2.798	No	ns	-69360 to 14750
A vs C	25580	2.622	No	ns	-16480 to 67640
A vs D	5460	0.5596	No	ns	-36600 to 47520
A vs E	37110	3.803	No	ns	-4948 to 79170
A vs F	6110	0.6262	No	ns	-35950 to 48170
A vs Control	-27220	2.790	No	ns	-69280 to 14840
B vs C	52880	5.420	Yes	**	10830 to 94940
B vs D	32760	3.358	No	ns	-9295 to 74820
B vs E	64410	6.601	Yes	***	22350 to 106500
B vs F	33410	3.424	No	ns	-8645 to 75470
B vs Control	82.00	0.008404	No	ns	-41970 to 42140
C vs D	-20120	2.062	No	ns	-62180 to 21940
C vs E	11530	1.182	No	ns	-30530 to 53590
C vs F	-19470	1.995	No	ns	-61530 to 22590
C vs Control	-52800	5.411	Yes	**	-94860 to -10740
D vs E	31650	3.244	No	ns	-10410 to 73710
D vs F	650.0	0.06662	No	ns	-41410 to 42710
D vs Control	-32680	3.349	No	ns	-74740 to 9377
E vs F	-31000	3.177	No	ns	-73060 to 11060
E vs Control	-64330	6.593	Yes	***	-106400 to -22270
F vs Control	-33330	3.416	No	ns	-75390 to 8727

Appendix C9: ANOVA Statistical analysis of the Phytoremediation of Oil and Grease by *Chromolaena* in the Different Treatment Media at 0.8%N amendment

Table Analyzed	Chrom O&G 0.8% Nitrogen				
One-way analysis of variance					
P value	P<0.0001				
P value summary	***				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	5.861				
R squared	0.3582				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	74.25				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	37590000000	6	6265000000		
Residual (within columns)	67340000000	63	1069000000		
Total	104900000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Chrom vs O&G	-24900	2.408	No	ns	-69460 to 19660
Chrom vs 0.8%	28440	2.751	No	ns	-16120 to 73000
Chrom vs Nitrogen	6555	0.6340	No	ns	-38010 to 51120
Chrom vs E	42490	4.110	No	ns	-2074 to 87050
Chrom vs F	2360	0.2283	No	ns	-42200 to 46920
Chrom vs Control	-24640	2.383	No	ns	-69200 to 19920
O&G vs 0.8%	53340	5.159	Yes	**	8777 to 97900
O&G vs Nitrogen	31460	3.043	No	ns	-13110 to 76020
O&G vs E	67390	6.518	Yes	***	22830 to 112000
O&G vs F	27260	2.637	No	ns	-17300 to 71820
O&G vs Control	261.0	0.02524	No	ns	-44300 to 44820
0.8% vs Nitrogen	-21890	2.117	No	ns	-66450 to 22680
0.8% vs E	14050	1.359	No	ns	-30510 to 58610
0.8% vs F	-26080	2.523	No	ns	-70640 to 18480
0.8% vs Control	-53080	5.134	Yes	**	-97640 to -8516
Nitrogen vs E	35940	3.476	No	ns	-8629 to 80500
Nitrogen vs F	-4195	0.4058	No	ns	-48760 to 40370
Nitrogen vs Control	-31200	3.017	No	ns	-75760 to 13370
E vs F	-40130	3.881	No	ns	-84690 to 4434
E vs Control	-67130	6.493	Yes	***	-111700 to -22570
F vs Control	-27000	2.612	No	ns	-71560 to 17560

Appendix C10: ANOVA Statistical analysis of the Phytoremediation of TPH by *Chromolaena* in the Different Treatment Media at 1.2%N amendment

Table Analyzed	Chrom TPH 1.2% Nitrogen				
One-way analysis of variance					
P value	0.0328				
P value summary	*				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	2.472				
R squared	0.1906				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	45.52				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1533000000	6	255500000		
Residual (within columns)	6510000000	63	103300000		
Total	8043000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-6151	1.914	No	ns	-20010 to 7705
A vs C	3263	1.015	No	ns	-10590 to 17120
A vs D	2434	0.7570	No	ns	-11420 to 16290
A vs E	6395	1.989	No	ns	-7461 to 20250
A vs F	3925	1.221	No	ns	-9931 to 17780
A vs Control	-6639	2.065	No	ns	-20490 to 7217
B vs C	9415	2.929	No	ns	-4441 to 23270
B vs D	8585	2.671	No	ns	-5271 to 22440
B vs E	12550	3.903	No	ns	-1310 to 26400
B vs F	10080	3.134	No	ns	-3780 to 23930
B vs Control	-487.6	0.1517	No	ns	-14340 to 13370
C vs D	-829.8	0.2581	No	ns	-14690 to 13030
C vs E	3131	0.9741	No	ns	-10720 to 16990
C vs F	661.3	0.2057	No	ns	-13190 to 14520
C vs Control	-9902	3.080	No	ns	-23760 to 3954
D vs E	3961	1.232	No	ns	-9895 to 17820
D vs F	1491	0.4639	No	ns	-12370 to 15350
D vs Control	-9072	2.822	No	ns	-22930 to 4784
E vs F	-2470	0.7684	No	ns	-16330 to 11390
E vs Control	-13030	4.055	No	ns	-26890 to 822.4
F vs Control	-10560	3.286	No	ns	-24420 to 3293

Appendix C11: ANOVA Statistical analysis the Phytoremediation of TPH by *Chromolaena* in the Different Treatment Media at 1.0%N amendment

Table Analyzed	Chrom TPH 1.0% Nitrogen				
One-way analysis of variance					
P value	0.0050				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	3.468				
R squared	0.2483				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	30.90				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1467000000	6	244500000		
Residual (within columns)	4441000000	63	70500000		
Total	5908000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-4674	1.760	No	ns	-16120 to 6771
A vs C	4868	1.833	No	ns	-6577 to 16310
A vs D	2209	0.8321	No	ns	-9235 to 13650
A vs E	8892	3.349	No	ns	-2552 to 20340
A vs F	1217	0.4585	No	ns	-10230 to 12660
A vs Control	-4900	1.845	No	ns	-16340 to 6544
B vs C	9542	3.594	No	ns	-1903 to 20990
B vs D	6883	2.592	No	ns	-4561 to 18330
B vs E	13570	5.109	Yes	*	2122 to 25010
B vs F	5891	2.219	No	ns	-5553 to 17340
B vs Control	-226.1	0.08514	No	ns	-11670 to 11220
C vs D	-2658	1.001	No	ns	-14100 to 8786
C vs E	4025	1.516	No	ns	-7420 to 15470
C vs F	-3650	1.375	No	ns	-15090 to 7794
C vs Control	-9768	3.679	No	ns	-21210 to 1677
D vs E	6683	2.517	No	ns	-4761 to 18130
D vs F	-991.9	0.3736	No	ns	-12440 to 10450
D vs Control	-7109	2.678	No	ns	-18550 to 4335
E vs F	-7675	2.891	No	ns	-19120 to 3770
E vs Control	-13790	5.195	Yes	**	-25240 to -2348
F vs Control	-6117	2.304	No	ns	-17560 to 5327

Appendix C12: ANOVA Statistical annalysis of the Phytoremediation of TPH by *Chromolaena odorata* in the Different Treatment Media at 0.8%N amendment

Table Analyzed	Chrom TPH 0.8% Nitrogen				
One-way analysis of variance					
P value	0.0015				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	7				
F	4.105				
R squared	0.2811				
Bartlett's test for equal variances					
Bartlett's statistic (corrected)	37.34				
P value	P<0.0001				
P value summary	***				
Do the variances differ signif. (P < 0.05)	Yes				
ANOVA Table	SS	df	MS		
Treatment (between columns)	1736000000	6	289300000		
Residual (within columns)	4440000000	63	70480000		
Total	6176000000	69			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
A vs B	-8360	3.149	No	ns	-19800 to 3083
A vs C	713.0	0.2686	No	ns	-10730 to 12160
A vs D	-2883	1.086	No	ns	-14330 to 8560
A vs E	6695	2.522	No	ns	-4748 to 18140
A vs F	185.8	0.06999	No	ns	-11260 to 11630
A vs Control	-8415	3.170	No	ns	-19860 to 3028
B vs C	9073	3.418	No	ns	-2370 to 20520
B vs D	5477	2.063	No	ns	-5966 to 16920
B vs E	15050	5.671	Yes	**	3612 to 26500
B vs F	8546	3.219	No	ns	-2897 to 19990
B vs Control	-54.65	0.02059	No	ns	-11500 to 11390
C vs D	-3596	1.354	No	ns	-15040 to 7847
C vs E	5982	2.253	No	ns	-5461 to 17420
C vs F	-527.2	0.1986	No	ns	-11970 to 10920
C vs Control	-9128	3.438	No	ns	-20570 to 2315
D vs E	9577	3.608	No	ns	-1866 to 21020
D vs F	3069	1.156	No	ns	-8374 to 14510
D vs Control	-5532	2.084	No	ns	-16980 to 5911
E vs F	-6509	2.452	No	ns	-17950 to 4934
E vs Control	-15110	5.691	Yes	**	-26550 to -3666
F vs Control	-8601	3.240	No	ns	-20040 to 2842