**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**DEPARTMENT OF ENVIRONMENTAL SCIENCE**

**COLLEGE OF SCIENCE**

# RHIZOREMEDIATION OF HYDROCARBON CONTAMINATED SOILS:

## A CASE STUDY AT NEWMONT GHANA GOLD LIMITED (NGGL)

## AHAFO- KENYASI

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

BY

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**DECLARATION**

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.



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## ABSTRACT

This study investigated the rate of degradation of Total Petroleum Hydrocarbon (TPH) and Oil and Grease in Hydrocarbon contaminated soil samples obtained from a mine site (Newmont Ghana Gold Limited) using Rhizoremediation technology with different levels of nutrient amendments for a native species. The levels of N (nitrogen) used were; in Compost (0.2%, 0.5% and 0.8%), Urea (0.2%, 0.5% and 0.8%) and Topsoil (0.2%, 0.5% and 0.8%).

A Ghanaian native grass species *Paspalum* Spp from the (*Poaceae*) family was selected following the development of essential and desirable growth criteria. Vegetative parts of the species were subjected to ten (10) treatments in a Randomized Complete Block Design (RCBD) in three replicates.

The plant-associated microbial community was examined in *Paspalum* Spp. The assessment of the influence of grass on the abundance and activity of microorganisms in the rhizosphere revealed a buildup of microbial communities over the period. This was assessed using the Freidman's test which showed that at p value of 5% rhizospheric samples from the different treatments were significantly different.

Multiple comparisons showed how microbial populations built up in the rhizosphere for the different treatments.Treatments G(0.2% compost+ 3kg HCS) , H(0.5% compost+3kg HCS) and I( 0.8% compost+3kg HCS) showed no significant difference with regards to rhizospheric TPH and Oil and grease degradation levels whiles treatments E( 0.5% topsoil+ 3kg HCS) and F(0.8% topsoil+ 3kg HCS) also showed no significant difference in performance. Furthermore treatment A (0.2% urea+ 3kg HCS), B (0.5% urea+ 3kg HCS), C (0.8% urea+ 3kg HCS) and D (0.2% topsoil+ 3kg HCS) also showed no significant difference in terms of rhizospheric TPH and oil and grease degradation patterns.

The presence of a single species successfully enhanced the removal of hydrocarbons from soil.

*Paspalum* Spp subjected to compost levels( 0.5% and 0.8%) and topsoil levels (0.5% and 0.8%) showed significantly lower residual hydrocarbon concentrations than those treated with Urea after 80days ($p < 0.00$).

A strong relationship between abundance of hydrocarbon degrading microorganisms in the rhizosphere and hydrocarbon biodegradation was demonstrated for rhizospheric samples with treatment G (0.2% compost+3kg HCS), H (0.5% compost+ 3kg HCS) and I (0.8% compost+ 3kg HCS) ($p < 0.001$).

This study showed that *Paspalum* a native Ghanaian grass species could be a possible candidate for in situ rhizoremediation potential at field scale and that some level of amendment with 0.8% compost (N-level) can improve the effectiveness of the application.

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**ACRONYMS**

| | |
|---|---|
| NGGL | Newmont Ghana Gold Limited |
| Vol. Pad | Volatilization Pad |
| TPH | Total Petroleum Hydrocarbon |
| mg/kg | milligrams per kilogram |
| mg/L | milligrams per liter |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PCB | Polychlorinated biphenyl |
| TPH | Total Petroleum Hydrocarbons |
| MPN | Most Probable Number |
| BTEX | Benzene, Toluene, Ethylbenzene, and Xylenes |
| DRO | diesel-range organics |
| HCS | Hydrocarbon Contaminated Soil |
| VOC | Volatile Organic Compound |

# CHAPTER ONE

## 1.0 INTRODUCTION

Contamination of the environment with persistent organic and inorganic pollutants is a common occurrence in today's industrialized world. Aliphatic hydrocarbons (e.g. diesel fuel and engine oils) make up a substantial proportion of organic contamination in the terrestrial environment (Stroud *et al*., 2007). This class of contaminant is characterized by saturated and unsaturated linear or branched open-chain structures and is subject to physicochemical processes which can affect their fate and behaviour in soil such as degree of removal, sequestration and interaction with micro flora (Germida *et al.,* 2002). The physical properties of aliphatic hydrocarbons determine their susceptibility to microbial attack and the potential for degradation in soils (Mougin, 2002). There have been increasing international efforts to remediate hydrocarbon contaminated sites using "green" technologies, either to mitigate risks of adverse health or environmental effects or to enable site redevelopment (Vidali, 2001).

The use of plants and their associated microorganisms to detoxify hydrocarbon pollutants for the treatment of contaminated soils has gained increasing acceptance as a viable cleanup technology (Alkorta and Garbisu, 2001; van der Lelie *et al*., 2001; Arthur *et al*., 2005). Such technology is referred to as rhizoremediation and is suggested to be the primary mechanism responsible for hydrocarbon degradation in plant-assisted remediation efforts (Frick *et al*., 1999; Hutchinson *et al.,* 2003; Yateem *et al*., 2007).

Although the extent of soil contamination in Ghana is uncertain; rhizoremediation technology that has been developed overseas cannot be readily transferred to Ghana due to significant differences in climate, soil types, endemic plant species and environmental regulation performance criteria (Michael *et al*., 2007).

With the development of the economy and petroleum exploration, contamination of soil with petroleum compounds is of concern (Banks *et al*., 2003). The estimated costs for the cleanup of contaminated sites with conventional techniques such as incineration and land filling are enormous.

However recent works done has identified some grass species and legumes to be most suitable candidates for further investigation of their rhizoremediation potential (Gaskin *et al*., 2005).

Bioremediation of contaminated soil is supposed to be low cost, have less interference with soil structure and higher public acceptability as compared to other approaches such as soil thermal desorption and soil leaching treatment. There are two different approaches for bioremediation of petroleum contaminated soil: microbial remediation and phytoremediation.

Phytoremediation is a strategy that uses plants to degrade, stabilize and/or remove soil contaminants. Phytoremediation of TPH (Total Petroleum Hydrocarbon) has the potential to be a sustainable waste management technology if it can be proven to be effective in the field (Gurska *et al*., 2009).

Whiles microbial remediation strategy uses the action of microbes or other biological systems to degrade environmental pollutant (Caplan, 1993).

Recently, a combination of microbial remediation and phytoremediation has become a general practice in the field treatment of petroleum contaminated soil. This technique can be defined as rhizoremediation, which is a specific type of phytoremediation that involves both plants and their associated rhizosphere (volume of soil adjacent to and influenced by plant roots) microbes, and can occur naturally or can be actuated by deliberately introducing specific microbes (Gerhardta *et al*., 2009).

The petroleum industry is anticipated to create economic boom for Ghana and at the same time their activities can lead to environmental and socio-economic problems.

There is therefore the need for research work into various natural remediation techniques.

## 1.1 Justification

As part of Newmont Ghana Gold Limited's best environmental management practices, soils contaminated with oil (diesel fuel and engine oils) are supposed to be treated and later incorporated into the natural environment. To this end a volatilization facility (Vol. Pad) has been established where Hydrocarbon contaminated soils are exposed to sunlight for the breakdown of hydrocarbons.

However data on total petroleum hydrocarbon collected so far from 2009 has not shown any significant break down levels with values ranging between 35000 pm and 50000 ppm which is far above 5000 ppm Australian Standard (standard adopted by Newmont Ghana Gold Ltd).

This research therefore seeks to explore the potential of microbial communities and plant root associations in breaking down these hydrocarbons compared with breakdown rates by sunlight (photo remediation) at the Volatalization Pad facility.

The findings will be useful in cleaning contaminated soils on Newmont Ghana Gold Limited's mining site at Ahafo- Kenyase and will also be useful in the management of Ghana's oil in the near future since oil spill are inevitable.

## 1.2 Objective

The main objective of this study is to investigate the rate of breakdown of diesel fuel, oil and grease in contaminated soil samples using Rhizoremediation technology.

The specific objectives of this study are to:

- design a rhizoremediation set up and determine TPH and Oil/grease levels.
- establish the rate of microbial population build up in the rhizosphere of selected plant species in set ups.
- Identify which of the media combinations is most suitable for the effective breakdown of Hydrocarbon contaminated soil in the rhizosphere.
- determine the rate of breakdown of diesel fuel, oil and grease in contaminated soil

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

The increased use of hydrocarbons has led to the contamination of the environment. In most industries where these hydrocarbons are used; there is lack of proper storage and disposal of spills causing contamination of the environment**.** The release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Holliger *and Gaspard*, 2000). Soil contamination with hydrocarbons causes extensive damage of local systems since accumulation of pollutants in animals and plant tissues may cause death or mutations (Alvarez *et al*., 1991) The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants.

## 2.1 Total petroleum Hydrocarbons

Total petroleum hydrocarbons (TPH) comprise a diverse mixture of hydrocarbons that occur at petrochemical sites and storage areas, waste disposal pits, refineries and oil spill sites. TPH's are considered persistent hazardous pollutants, and include compounds that can bioconcentrate and bioaccumulate in food chains (McElroy *et al.*, 1989). They are acutely toxic, and some such as benzene and benzopyrene are recognized mutagens and carcinogens (Mortelmans *et al.*, 1986).

Since this group includes chemicals that have physical and chemical characteristics that vary over orders of magnitude, TPHs are divided into two categories.

- Gasoline range organics (GRO) corresponds to small chain alkanes (C6-C10) with low boiling point (60-170 °C) such as isopentane, 2,3-dimethyl butane, *n*-butane and *n*-pentane, and volatile aromatic compounds such as the mono aromatic hydrocarbon benzene, toluene, ethylbenzene, and xylenes (BTEX).

- Diesel range organics (DRO) includes longer chain alkanes (C10–C40) and hydrophobic chemicals such as polycyclic aromatic hydrocarbons (PAH) (Mortelmans *et al.*, 1986).

Whereas most of these contaminants do have natural sources, concentration and release of contaminants through anthropogenic activities has led to significant contamination of soil and groundwater. Current treatment techniques usually involve excavation and *ex situ* treatment of the source material and the contaminated soils.

The large volume of soil affected precludes *ex-situ* treatment due to economic constraints and requires the use of relatively inexpensive remediation schemes, such as rhizoremediation.

The different mechanisms mentioned above could be utilized for the remediation of a wide variety of contaminants. Rhizoremediation could therefore be applied for the remediation of numerous contaminated sites. However, not much is known about contaminant fate and transformation pathways, including the identity of metabolites (Mortelmans *et al.*, 1986).

**2.2 Technologies for the remediation of Hydrocarbon (HC) contaminated soi1s**

Numerous hydrocarbon remediation technologies have been developed in recent years. However most of these are on1y applicable in the temperate regions. Remediation technologies include both physical (mechanical) and biological methods (phytoremediation). Physical methods include i) soi1washing, ii) excavation and land filling, iii) incineration and thermal desorption and iv) Vacuum extraction while biological methods include i) infiltration galleries and ii) biopiles and Iandfarming. Generally, biological processes are one half to one third the cost of physical methods (Torma, 1994). Some of the physical and biological methods are briefly discussed below with particular reference to the strengths and weaknesses.

2.3 **Physical Methods**

2.3.1 **Soil Washing**

Soil washing involves an on-site set-up to scrub soil and remove HCs. which are then treated separately.

Soil washing can be carried out with the aid of surfactants, emulsifiers and other additives to increase hydrocarbon solubility (Kosaric, 1993). The major drawback with this technology is that abrasive additives can harm the natural microbial flora and damage the soil environment (Loss of mineral cycling capacities) (Atlas and Bartha, 1993).

### 2.3.2 **Incineration and Thermal Desorption**

Thermal desorption and incineration use heat to volatilize and destroy hydrocarbon contaminants.

Incineration uses a closed-vesse1 combustion unit to completely destroy hydrocarbon components at high temperature, whereas thermal desorption can be carried out in or ex situ and uses lower temperature ranges to volatilize Hydrocarbon components from the soil.Volatilized components are then captured and or treated. (Kostecki *et al.,* 1990). Low treatment volumes, reduced efficiency and increase costs for large-scale treatment, makes incineration and/or thermal desorption inappropriate.

### 2.3.3 **Vacuum Extraction**

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

## 2.4 **Biological Methods**

### 2.4.1 **Infiltration Galleries**

An infiltration gallery is an in situ soil treatment technology that seeks to minimize microbial activity for hydrocarbon degradation.

It is accomplished with down-gradient groundwater pumping wells that recirculate nutrient and oxygen-amended water through the petroleum-impacted soils (Reynolds and Wolf, 1999).

Equipment requirements are extensive and include pumping wells, recirculating pumps and holding mixing tanks. In addition, monitoring the extent of Hydrocarbon degradation within the impacted zone may prove difficult (Reynolds and Wolf, 1999).as there is the need for free-flowing sub-surface water. Finally, the need for complex amendment mixing and circulation systems can make this technology economically infeasible for treating large volumes of contaminated soil.

### 2.4.2 **Biopiles and Landfarming**

Biopiles and landfarmings are aboveground treatment cells for the bioremediation of contaminated soil. They can be coupled with biostimulation (addition of nutrients) and or bioaugmentation (inoculation with microbes). Biopiles involve placing soil in mounds or windrows to promote higher temperatures. For land farming, soil is excavated, spread thinly (1 5-30 cm) over a large area to ensure adequate aeration and is periodically tilled.

The amount of equipment required depends on the degree of process control required.

Regulatory guidelines for Volatile Organic Compound (VOC) emissions may require that Off-gases from the treatment cells be captured and treated. Biopiles and/or landfarms can be used for al1 soil types and can treat large volumes of soi1 efficiently and economically (Reynolds and Wolf, 1999).

There are other biological means or technologies that can be employed in the remediation of contaminated soils these are:

- Bioremediation
- Phytoremediation
- Rhizoremediation

## 2.5 **Bioremediation**

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).

## 2.6 **Phytoremediation**

Phytoremediation on the other hand is defined as the use of plants to extract, sequester, or detoxify pollutants (Meagher and Rugh, 1996).

This remediation method is environmental friendly and visually attractive, and the structure of the soil is highly maintained (Khan *et al.,* 2000). Pollutants which can be a target for phytoremediation can be divided into two groups; the elemental pollutants and the organic pollutants (Meagher and Rugh,1996).

### 2.6.1 **Elemental pollutants**

The group referred to as elemental pollutants contains contaminants such as toxic heavy metals and radionuclides. Compared with the organic pollutants, only a few remediation techniques are available for this kind of pollutant, and the use of plants to strip heavy metals from soil is an emerging tool (Clemens *et al.,* 2002; Khan *et al*., 2000). In these cases, the major principles of phytoremediation are

i) Extraction of the pollutant from soil and translocation to aboveground tissues,

ii) Sequestering of the pollutant in the root system to prevent further spreading and leaching into soil or ground water

iii) Conversion into less toxic chemicals. For this kind of phytoremediation, various plant species, such as tobacco, sunflower, mustard, maize, and sand rocket, are used, based on the ability to adsorb or hyper-accumulate the pollutant (Meagher and Rugh, 1996).

The natural ability to hyper accumulate metals was found in plants grown on soils which were naturally enriched with heavy metals, and this accumulation was thought to be a defense mechanism against herbivores (Gleba *et al.,* 1999).

However, the distribution of such plants is limited and the hyper accumulating ability is contaminant specific. Genetic alterations of plants can improve the success of phytoremediation. For example, phytoextraction can be improved by an increase in the transpiration rate of plants.

An Indian mustard cell line with a transpiration rate of 130% compared with the wild type plant was able to extract 104% more lead from soil, whereas increased resistance of plants toward pollutants also can improve their action during phytoremediation (Gleba *et al*., 1999).



The introduction and expression of bacterial genes in plants resulting in enzymes involved in the conversion of xenobiotics is, when the legislative problems for using such plants are disregarded, also a promising tool. Bizily and associates (2000) described the use of *Arabidopsisthaliana* plants transformed with bacterial genes in order to transform and detoxify organic mercury, and Iimura and associates (2002) described the expression of a manganese-peroxidase gene in transgenic tobacco.

### 2.6.2 **Organic pollutants**

The second group of pollutants which can be targeted for phytoremediation is that of the organic pollutants, such as polychlorinated biphenyls, polycyclic aromatic compounds, nitro aromatics, or linear halogenated hydrocarbons. This group of pollutants can be mineralized completely using poplar trees, willow, alfalfa, and different grass varieties. Although the knowledge of the degradation of pollutants by plant metabolic systems is still limited compared with what is known for bacteria, various reports confirm the importance of phytoremediation as a newly emerging tool.

### 2.6.3 **Rhizoremediation**

Rhizoremediation involves the breakdown of contaminants in soil resulting from microbial activity that is enhanced in the plant root zone (rhizosphere) (Kuiper *et al.,* 2004).

The term rhizosphere refers to the environment influenced by plant roots in which elevated bacterial activity is observed. Rhizoremediation (the degradation of recalcitrant pollutants by bacteria in the rhizosphere) is an attractive process since plant roots provide a large surface area for a significant population of bacteria and transport the root-colonizing, remediating microorganism to pollutants 10 m to 15 m deep in the soil.

The roots supply nutrients (amino acids, carbohydrates, and organic acids) so no exogenous carbon source must be added, and they may also supply bacteria with cofactors required for the activation of bacterial enzymes involved in the pollutant degradation pathway.

The objective of rhizoremediation is increased microbial numbers and activity, and the exploitation of that increased microbial activity to enhance biotreatment.

Rhizoremediation is suggested to be the primary mechanism responsible for hydrocarbon degradation in phytoremediation efforts (Frick *et al.*, 1999; Hutchinson *et al.*, 2003).

Microbial densities in the rhizosphere are suggested to be 1 to 4 orders of magnitude higher than in bulk soil. Consequently, rhizoremediation appears to be an aesthetically pleasing, low-cost, minimal-maintenance, in situ treatment for pollutants in surface soils.

Rhizoremediation has shown promise based on the use of wild-type bacteria in their native environments to degrade a variety of pollutants (Pilon-Smits, 2005).

Plants have shown the capacity to withstand relatively high concentrations of organic chemicals without toxic effects, and they can uptake and convert chemicals quickly to less toxic metabolites in some cases. In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes, and the build-up of organic carbon in the soil.

The products of rhizoremediation (e.g. alcohols, acids, carbon dioxide and water) are generally less toxic and less persistent in the environment than the parent compounds (Kuiper *et al.*, 2004).

Many bacteria in the rhizosphere are stimulated by the presence of some plants. The main source of this stimulation is the availability of complex carbohydrates on plants for accumulation and growth. Plants on the other hand benefit from nitrogen fixing compounds either fixed or released from organic material by bacterial metabolism.

For instance, *Azatobacter vinelandii,* a free – living nitrogen fixing bacterium ultimately depends on carbon fixed by plants. While plants growing in low nitrogen soil colonize by *A. vineelandii* benefit from fixed nitrogen (Yevdokimov *et al.*, 2005).

The search for effective methods to restore polluted sites in less expensive, less labor intensive, safe and environmentally friendly way is required. Such an alternative method is rhizoremediation.

Plant enzymes establish the degradation of pollutants during phytoremediation; whereas, during natural attenuation or bioremediation, the (indigenous) microbial population performs the degradation. In many of these studies, an important contribution to degradation of pollutants is ascribed to microbes present in the rhizosphere of plants used during phytoremediation.

This contribution of the rhizomicrobial population is referred to as rhizoremediation making this alternative the best among other applications and technologies.

By the synergistic reaction of the plant and microorganisms, rhizoremediation shows a higher degradation rate of petroleum pollutants as compared to microbial remediation and phytoremediation (Escalante – Espinosa *et al.*, 2005. Gurska *et al.*, 2009; Xin *et al.*,2008)

Several plant species have been proven to be more effective in degrading TPH including sorghum, maize, Bermuda grass, rice, legumes and sorghum (Merkl *et al.*, 2005)

The addition of crude oil results in an immediate change in bacterial community structure, increasing abundance of hydrocarbon – degrading microorganisms and a rapid rate of oil degradation, which suggests

the presence of a pre-adapted oil-degrading microbial community and sufficient supply of nutrients ( Coulon *et al.,* 2006; Hamamura *et al.,* 2008).

The degradation rate of microbial remediation and phytoremediation differ greatly depending on different conditions. Microbial degradation can be accomplished by different species of microorganisms both native to the soil and added as effective degrading strains.

The microbial degradation is generally higher than 40% within a one year of disposal and may be as high as over 70% in some cases (Sathishkumar *et al.,* 2008).

Influencing factors for microbial remediation include soil moisture content, temperature, soil pH, oxygen supply, nutrient, oxidation-reduction potential, soil texture and structure (Riser-Roberts, 1998).

In most situations, plants that are native to the region of contamination have been shown to be most appropriate for rhizoremediation (Merkl *et al.,* 2005). Differences in environmental conditions and restrictions on species importation mean that each country may need to identify indigenous plants to use for rhizoremediation (Robson *et al.,* 2003). The introduction of non-native plants into any agricultural ecosystem should not be taken lightly. Species chosen for rhizoremediation must be well adapted to the soil and climatic conditions of the region.

This means that average temperature, annual rainfall, and length of growing season are important considerations in rhizoremediation planning (Frick *et al.,* 1999).

Practical considerations such as cost and availability of seed are also important.

In this study, vegetative parts of *Paspalum* spp. were used to ascertain the effectiveness of vegetative parts in this application. The use of vegetative part comes with its own advantages and disadvantages.

## 2.7 Advantages of Vegetative Propagation

The offspring's are genetically identical and therefore advantageous traits can be preserved.

Only one parent is required which eliminates the need for special mechanisms such as pollination, etc.

It is faster. For example, bacteria can multiply every 20 minutes. This helps the organisms to increase in number at a rapid rate that balances the loss in number due to various causes.

Many plants are able to tide over unfavourable conditions. This is because of the presence of organs of asexual reproduction like the tubers, corm, bulbs, etc.

Vegetative propagation is especially beneficial to the agriculturists and horticulturists. They can raise crops like bananas, sugarcane, potato, etc. that do not produce viable seeds. The seedless varieties of fruits are also a result of vegetative propagation.

The modern technique of tissue culture can be used to grow virus-free plants.



## 2. 8 Disadvantages of Vegetative Propagation

The plants gradually lose their vigour as there is no genetic variation. They are more prone to diseases that are specific to the species. This can result in the destruction of an entire crop.

Since many plants are produced, it results in overcrowding and lack of nutrients (http://www.tutorvista.com, 2010).

Plant tolerance to high contaminant concentrations is also a very important factor to keep in mind. The phytotoxicity of petroleum hydrocarbons is a function of the specific contaminant composition, its concentration, and the plant species used. Major adverse effects typically include reduced germination and growth if contaminant concentrations are sufficiently high. In general, TPH values of 15 percent or greater can result in significant reductions in plant growth and in some cases mortality. Compared with uncontaminated soil, soils with 2% TPH reduced alfalfa yields by 32 percent (Wiltse *et al*., 1998).

Production of biomass by ryegrass was reduced 46 percent at a soil concentration of 0.5 percent (5000 mg/kg) hydrocarbons (Gunther et al, 2003).

It was found that plants pre-grown in clean soil and subsequently transplanted to the contaminated soil grew nearly as well as the control, showing that toxicity was associated with germination and/or early

plant growth. Similarly, poor rooting of ryegrass compared to legumes appeared to adversely affect the removal of TPH from Gulf War-contaminated soils (Yateem et al., 1998).

Although the germination of sunflower seeds and beans was greater than that of maize, vegetative growth was greater for maize than beans, demonstrating that germination and later plant growth may be affected differently (Chaineau et al., 1997).

Aged spills tend to be much less phototoxic than fresh ones, possibly because of the lower bioavailability of toxic compounds in the aged spills. However, the speciation of petroleum hydrocarbons is also very important in determining phytotoxicity.

## 2.9 **Interaction between plants and microorganisms**

The interaction between plants and microbial communities in the rhizosphere are complex and has evolved to the mutual benefit of both organisms. Plants sustain large microbial populations in the rhizosphere by secreting substances such as carbohydrates and amino acids through root cells and by sloughing root epidermal cells.

Also, root cells secret mucigel, a gelatinous substance that is a lubricant for root penetration through the soil during growth. Using this supply of nutrients, soil microorganisms proliferate to form the plant rhizosphere (Anderson *and Coats*, 1993).

The type of root exudates can influence the type of interaction between plants and soil microorganisms (Westover *et al*., 2002). Specific interaction occurs when the plant exudes a specific compound in response to the presence of the contaminant.

Non-specific interactions occur when typical plant exudates are chemically similar to the organic contaminant, resulting in an increased microbial activity and an increased degradation of contaminants (Donnelly *et al*., 1994).

## 2.10 The Rhizoshere: Exudates, Nutrients and Root Colonization

The exudation of nutrients by plant roots creates a nutrient – rich environment in which microbial activity is stimulated. Plant root exudates contain sugars, organic acids and amino acids as main components (Vancura and Hovadik, 1965). A list of compounds found in these exudates is shown in (table 1.0) over leaf. In addition, the mucigel secreted by root cells, lost root cap cells, the starvation of root cells or decay of complete roots provides nutrients (Lynch , 1990). It is known that the rhizosphere is dominated by gram- negative rods such as *Pseudomonas* species (Kuiper *et al*., 2004).

The ability to efficiently colonize plant roots depends on different strains, indicating rhizosphere competence abilities.

**Table1.0 Compounds found in Root Exudates (Schnoor, 2002)**

| **Compounds** | **Example of Compounds** |
|---|---|
| Carbohydrates | Glucose, fructose, sucrose, maltose, galactose, xylos, oligosaccharides |
| Amino Acids | PAH's dissipation during phytoremediation, glucose, fructose, sucrose, maltose, galactose |
| Organic Compounds | Acetic acid, propionic acid, citric acid, butyric acid |

| | |
|---|---|
| Volatile Compounds | Ethanol,methanol,formaldehyde,acetone,acetaldehyde,propionaldehyde |
| Vitamins | Thiamine, biotin, niacin, riboflavin, pyridoxine |
| Aromatics | Phenols,1-carvone,p-cymene,limonene |
| Enzymes | Phosphatase, dehydrogenase, peoxidase, dehalogenase |



### 2.11 Effects of the Rhizosphere Microbial Population on Plants

Just as plant roots have a direct effect on the surrounding microbial populations; microorganisms in the rhizosphere have a marked influence on the growth of plants.

In the absence of appropriate microbial populations in the rhizosphere, plant growth may be impaired (Atlas and Bartha, 1998).

Microbial populations in the rhizosphere may benefit the plant by increasing recycling and dissolving of mineral nutrients, synthesis of vitamins, amino acids, auxins and gibberellins that stimulate plant growth (Atlas and Bartha, 1998). Microorganisms release antagonistic substances in the rhizosphere that allows the plant to enter an amensal relationship with other plants.

Another role played by microorganisms involves their ability to reduce the phytotoxicity of contaminants to the point where plants can grow in adverse soil conditions, thereby stimulating the degradation of the pollutants (Walton *et al*., 1994).

Microorganisms in the rhizosphere influence the availability of nutrients to the plants by solubilizing materials that are unavailable to plants like phosphorous.

Some microorganisms produce organic chelating agents that increase the solubility of manganese and iron compounds making them more available to plants.

Microorganisms in the rhizosphere produce high concentrations of carbon dioxide that increase the solubility of calcium, which significantly increase the uptake of calcium by roots (Atlas and Bartha, 1998).

## 2.12 Rhizoremediation and Depth of Contamination

Rhizoremediation is most effective at sites with shallow (i.e., root accessible) contaminated soils where contaminants can be treated in the rhizosphere.

Roots of some trees can be expected to grow at least 3meters into a soil profile, and it is possible to encourage rooting to a depth of 5 meters or more using the tree-in-a-well concept . On the other hand, roots of some grasses (alfalfa, switch grass, tall fescue) can reach soil depths of only 0.25-0.4 m.

Buffel grass roots to a depth of 0.75 m but has been observed to have dense rooting pattern within 0.3 m from the topsoil layer.

Hawaiian plants, Milo and Kou which were used to remediate saline soils contaminated with TPHs, rooted to a depth of more than 1.5 m by growing through the brackish water table into a zone of concentrated contaminants (US Army Corps, 2003).

Optimizing irrigation patterns can also facilitate biodegradation of contaminants by creating an "expanded rhizosphere" due to translocation of organic root exudates and inorganic nutrients to relatively deep soil layers.

Rhizoremediation can therefore influence soils to the depth where irrigation water reaches, even if the roots are sparse in the contamination zone.

Soil composition and 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.

It is therefore critical to use amendments to improve the quality of soil before planting. Common limitations are poor moisture-holding capacity, insufficient aeration, low permeability and nutrient deficiencies. Agronomic soil analysis and preliminary greenhouse or pilot scale experiments can help identify these constraints.

For example, nutrient analysis of contaminated soils from a site at the Unocal Bulk Storage Terminal at Superior, Wisconsin (Rentz *et al*., 2003) indicated general deficiencies in nitrogen, phosphorus, potassium, and zinc.

To decrease the soil pH, an addition of sulfur was also recommended. This information was subsequently used in greenhouse treatability studies, from which a formula of 50 lb/ac phosphorus, 225 lb/ac zinc, and 50 lb/ac potassium was identified as optimum for growth of native grasses.

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 (Rentz *et al.*, 2003).

## 2.13 Effects of Weather conditions

Rhizoremediation might be best suited for tropical countries where plant growth occurs all year round. In temperate climates; the active contribution of rhizoremediation is restricted to the growing period only. Winter operations may pose problems for rhizoremediation when deciduous vegetation loses its leaves, transformation and uptake cease, and soil water is no longer transpired. ( Huxtable , 1997).

## 2.14 Time scale of clean-up

Degradation of organics may be limited by mass transfer, i.e., desorption and mass transport of chemicals from soil particles to the aqueous phase may become the rate determining step.

Therefore, rhizoremediation may require more time to achieve clean-up standards than other more costly alternatives such as excavation or ex-situ treatment, especially for hydrophobic pollutants that are tightly bound to soil particles.

## 2.15 **Effects of Irrigation**

Results suggest that irrigation can enhance bioremediation of certain diesel components. For terrestrial rhizoremediation applications, it is often desirable to include irrigation costs on the order of 10-20 inches of water per year, in the design.

Spray irrigation is less efficient than drip irrigation as it encourages the growth of weeds that compete for nutrients with plants and hinder their delivery to the contaminated zone. Irrigation of the plants is especially important during the start of the project.



## 2.16 **Effect of chemical fertilizer addition on the remediation process**

As both microbial activity and plant growth can be affected by addition of fertilizer, fertilizer addition is an important factor that may affect the efficiency of bioremediation process.

Contaminated soils are usually deficient in macro- and micro-nutrients necessary for establishing healthy vigorously growing plants and stimulating microbial contaminant degradation. (Tang *et al.,* 2010). Organic sources of nitrogen are better than inorganic sources.

This is probably because organic nitrogen sources provides a low release source of nitrogen, and also help to improve soil structure and soil water relationships for plant growth. For example it's been observed that poultry manure increased the growth of corn in a soil containing 3 percent weight per volume crude oil more than an inorganic fertilizer containing nitrogen, phosphorus, and potassium (Amadi et al., 1993).

The addition of sawdust alone improved germination by decreasing oil contact with seeds, but accentuated the adverse effect of the oil on later growth, apparently by further widening the carbon-to-nitrogen ratio (Amadi *et al.,* 1993) with respect to TPH degradation, nutrient addition during rhizoremediation was

observed to have yielded mixed results. Hutchinson *et al*. (2001) observed better degradation of TPH using grasses with N/P amendments than without inorganic amendments (Hutchinson et al., 2001).

Joner *et al*. (2001) reported improved degradation of 3 and 4 ringed PAHs with the addition of N/P, but diminished degradation of 5 and 6 ringed PAHs.

Finally, Palmroth *et al*. (2002) observed no improved degradation of diesel fuel with nutrient amendments during phytoremediation with pine, poplar, or grasses. Microbial bioremediation of TPH contaminants with nutrient addition also produced widely varying results.



Diesel fuel degradation was stimulated with the addition of N/P using cold region soils (Walworth and Ferguson, 2002) and P amendments stimulated creosote degradation.

However, Graham and Curtis (2003) 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 additions results in an increase in soil salinity and this increases the osmotic stress and suppresses the activity of hydrocarbon-degrading organisms (Walworth and Ferguson, 2002). Carmichael and Pfander (1997) observed slower degradation of 3 and 4 ringed PAHs with N addition and no effects for P addition (Carmichael and Pfander 1997).

Johnson and Scow (1999) reported similar results indicating N/P addition inhibited or did not change phenanthrene degradation (3ringed PAH) (Johnson and Scow1999). Their results 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.

Thus, addition of nutrients should be considered on a site-by-site basis and a balance should be considered between biodegradation and plant growth. Application of amendments exclusively for plant growth may result in diminished contaminant degradation, the ultimate goal of rhizoremediation.

## 2.17 **Oxygen requirements**

Soil oxygen is required for optimal aerobic microbial degradation of petroleum hydrocarbon contaminants. Similar to nutrient deficiencies, oxygen depletion is caused by natural microbial respiration of contaminants.

Within phytoremediation, plants may be a net positive or negative oxygen source (Lee *et al*., 2000). Plants may improve soil oxygen through two mechanisms.

First, specially adapted plants use parenchyma, channels of reduced air resistance, to transport oxygen to the root zone, enhancing aerobic biological degradation (Shimp, 1993; Erickson, 1993). Secondly, soil dewatering and fracturing increases soil porosity, allowing increased diffusion of atmospheric oxygen (US EPA, 2001).

Plant roots can also be a net oxygen sink within petroleum-contaminated soils.Rentz *et al.* (2003) observed stimulation of hybrid poplar growth and increased poplar root density with the addition of Oxygen Release Compound (ORC) when plants were grown in petroleum smear zone soils there was high biochemical oxygen demand.

Flux of oxygen into soil by plants could be offset by root turnover and root exudation that provides microbial populations with simple carbon sources that could deplete soil oxygen when metabolized (Lynch, 1990). Furthermore, plant roots are known to require oxygen (Neuman et al., 1996). For soils with a high biochemical oxygen demand, oxygen addition may be required to promote plant growth and stimulate microbial degradation.

Passive methods of oxygen delivery are suggested to keep costs of rhizooremediation low and include the following. Perforated aeration tubes, placed next to cuttings, can supply oxygen to roots along a vertical axis Perforated ADS tubing, placed at depth prior backfilling the planting trench provides oxygen on a

horizontal plane. Gravel used to backfill planting trenches allows permeation of oxygen on vertical and horizontal axis.

Finally, the use of solid peroxides (e.g. Oxygen Release Compound®) can provide oxygen to soils when in contact with water (Koenigsberg and Noms, 2003).

## 2.18 **Importance of Oxygen**

The importance of oxygen in the biological remediation of petroleum contaminants, especially saturated aliphatics (i.e. diesel), is well documented (Frick *et al.,* 1999; Olson *et al.,* 2003).

Plants may enhance the oxygenation of contaminated soils improving remediation potential.

Roots can act as physical channels which transport oxygen to the root zone, enhancing aerobic conditions for biological degradation.

Roots also increase the soil porosity allowing increased diffusion of atmospheric oxygen (Rentz *et al.,* 2003). Vegetation cover can also moderate temperature and moisture conditions, which influences availability of oxygen (Gunther *et al.,* 1996).

Plant root systems may increase the moisture content of soil by promoting an effective circuit for water movement (Jing *et al.,* 2008). Jing *et al.* (2008) showed that soil moisture content increased by 5% in petroleum polluted soil planted with grasses.

## 2.19 **Cost**

Rhizoremediation is usually less costly than competing alternatives such as soil excavation, pump-and-treat, soil washing, or enhanced extraction.

Apart from costs incurred during installation of vegetation at the site, a field-scale rhizoremediation project involves expenditure on design, site preparation, reporting, monitoring, and operation and maintenance. It would be prudent to include preliminary greenhouse experiments along with agronomic soil testing during the design phase to ensure vigorous plant growth at the field-site.

Mathematical modeling may be necessary to demonstrate the effectiveness of the technology to regulatory agencies.

### 2.20 Factors Influencing Petroleum Hydrocarbon Degradation

A number of limiting factors have been recognized to affect the biodegradation of petroleum hydrocarbons. The composition and inherent biodegradability of the petroleum hydrocarbon pollutant is the first and foremost important consideration when the suitability of a remediation approach is to be assessed. (Brusseau, 1998). Among physical factors, temperature plays an important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Temperature also affects the solubility of hydrocarbons (Foght *et al*., 1996). Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. The highest rate of degradation rate generally occur in the range 30–40°C in soil environments, 20–30 °C in some freshwater environments and 15–20 °C in marine environments (Bartha and Bossert,1984). Venosa and Zhu. (2003) reported that ambient temperature of the environment affected both the properties of spilled oil and the activity of the microorganisms.

Significant biodegradation of hydrocarbons have been reported in psychrophilic environments in temperate regions of oil pollutant (Pelletier *et al*., 2004). On the other hand, excessive nutrient concentrations can also inhibit the biodegradation activity (Chaillan *et al*., 2006). Several authors have reported the negative effects of high NPK levels on the biodegradation of hydrocarbons especially on aromatics (Carmichael *et al.,* 1997). The effectiveness of fertilizers for the crude oil bioremediation in subarctic intertidal sediments was studied by (Pelletier *et al*., 2004). The use of poultry manure as organic fertilizer in contaminated soil was also reported (Okolo *et al*., 2005) and biodegradation was found to be enhanced in the presence of poultry manure alone.

Maki *et al.* (2005) reported that photo-oxidation increased the biodegradability of petroleum hydrocarbon by increasing its bioavailability and thus enhancing microbial activities.

## 2.21 Mechanism of Petroleum Hydrocarbon Degradation

The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. The initial intracellular attack of organic pollutants is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases. Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various biosynthesis and growth are synthesized by gluconeogenesis (Fritsche and Hofricher , 2000).

The degradation of petroleum hydrocarbons can be mediated by specific enzyme system. Other mechanisms involved are (1) attachment of microbial cells to the substrates and (2) production of biosurfactants (Hommel, 1996). The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but production of biosurfactants has been well studied.

## 2.22 Enzymes Participating in Degradation of Hydrocarbons

Cytochrome P450 alkane hydroxylases constitute a super family of ubiquitous Heme-thiolate Monooxygenases which play an important role in the microbial degradation of oil, chlorinated hydrocarbons, fuel additives, and many other compounds (Van Beilen and Funhoff, 2005). Depending on the chain length, enzyme systems are required to introduce oxygen in the substrate to initiate biodegradation. Higher eukaryotes generally contain several different P450 families that consist of large number of individual P450 forms that may contribute as an ensemble of isoforms to the metabolic conversion of given substrate. In microorganisms such P450 multiplicity can only be found in few species

(Zimmer *et al*., 1996). Cytochrome P450 enzyme systems has been found to be involved in biodegradation of petroleum hydrocarbons.

The capability of several yeast species to use n-alkanes and other aliphatic hydrocarbons as a sole source of carbon and energy is mediated by the existence of multiple microsomal Cytochrome P450 forms.

These cytochrome P450 enzymes had been isolated from yeast species such as Candida maltosa, Candida tropicalis, and Candida apicola (Miya and Firestone, 2000).

The diversity of alkaneoxygenase systems in prokaryotes and eukaryotes that are actively participating in the degradation of alkanes under aerobic conditions like Cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g., alkB), soluble di-iron methane monooxygenases, and membrane-bound copper containing methane monooxygenases have been discussed by Van Beilen and Funhoff.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 STUDY AREA

Newmont Ghana Gold Limited operates a surface mine at Kenyase- Ahafo in the Brong - Ahafo Region of Ghana.

Some soils at the site have been impacted over the years with hydrocarbons, particularly aliphatics such as diesel and lube oils, from machinery used in the mining process. An area noted for its semi deciduous vegetation with an average annual rainfall of 23000mm. Figure1.0 shows the location of the study area.


Figure 1.0 Location Map showing Ahafo Mine Site

### 3.2 Sample preparation and Set up

Three different treatments of Nitrogen were used in this study namely; topsoil, compost and fertilizer (urea).

Topsoil (0-15 cm) with no previous history of diesel and hydraulic lubricant contamination was collected from Newmont Ghana Gold Ltd.'s mine site with paper bags. Compost was taken from the Newmont Ghana Gold limited compost plant. Fertilizer of strength 46% (urea) was used for this study. The fertilizer was bought from the open market.

Three set ups involving mixings with 3kg hydrocarbon contaminated soil with portions of fertilizer, compost and topsoil was prepared. The samples were placed under an erected structure roofed with plastic sheets as shown in Plate 1.

The fertilizer/compost/topsoil was used to adjust the Nitrogen (N) - level to the optimum soil condition suitable for plant growth. This amendment was done based on the Nitrogen (N) - levels of the hydrocarbon contaminated soil. Nitrogen (N) levels in the hydrocarbon contaminated soil was as low as 0.01% according to baseline analysis that was carried out.

Nitrogen (N) levels were amended to 0.2%, 0.5% and 0.8% based on calculations considering levels of N in compost, topsoil and urea as well as the depleted N level in each 3 kg hydrocarbon contaminated soil in each pot.

Laboratory assay of the N- level was carried out to verify whether the levels were consistent with the calculated values. The experiment was replicated three times in Randomized Complete Block Design (RCBD). Each block contained ten (10) different treatments (Table 2.0). Eight (8) vegetative parts of *Paspalum* spp. was planted in each bowl.

### 3.3 **Plant Selection Criteria**

Newmont Ghana Gold Ltd.'s mine site has an established seed bank consisting of plant species native to the area. The seed bank list of plant species was considered first to establish if any would be suitable for the rhizoremediation application. Plant species available in seed banks were dominated by large tree species such as *Triplochiton scleroxylon,* and other small to medium plants such as *Centrosema* Spp. and *Paspalum* Spp.

The requirement for dense plant coverage coupled with rapid growth rate in successful rhizoremediation disqualified the use of higher plants. The grass species available in the seed bank was selected for inclusion in the study. The next step in plant selection involved a desk-top survey using evidence in the literature combined with geographical and botanical information. This enabled the development of selection criteria for 'ideal candidates' for the rhizoremediation of hydrocarbon contaminated soil. Adopting this approach, Ghanaian native grass species belonging to the (*Poaceae*) family was selected for the current study based on the following desirable criteria:



• Dense and fibrous root system for maximum surface area and enhanced microbial activity,

• Native,

• Rapid growth,

• Dense coverage to provide good soil cover and prevent soil erosion,

• Easy to establish and maintain,

• Hardy and drought tolerant,

• Suitable as site restoration species (long term stability),

• Tolerance of low nitrogen and phosphorus availability, as is commonly found with hydrocarbon contaminated soil,

• Capacity to excrete organic and/or nitrogenous compounds into the rhizosphere,

• Suitable for livestock feeding,

• Wide growth distribution across the continent,

• High hydrocarbon tolerance, as determined by screening procedures in contaminated soil (Merkl *et al.*, 2005).

**Table 2.0: Amendment Levels and Contaminated soil mixings displayed as Treatments**

| |
|---|
| 0.2% urea + 3 kg HCS |
| 0.5% urea + 3 Kg HCS |
| 0.8% urea + 3 kg HCS |
| 0.2% topsoil + 3 kg HCS |
| 0.5% topsoil + 3 kg HCS |
| 0.8% topsoil + 3 kg HCS |
| 0.2% compost + 3 kg HCS |
| 0.5% compost + 3 kg HCS |
| 0.8% compost+ 3 kg HCS |
| 3 Kg HCS(Control) |

HCS- hydrocarbon contaminated soil

**Plate 1.0: Rhizoremediation Set up**

**Plate 2.0: Sign of Growth After three (3) Weeks**

### 3.4 Determination of moisture content

The moisture content of the topsoil/compost/fertilizer blend was measured weekly by using the gravimetric method as described by the Standard Book for water and waste water treatment (20th edition). Water was added as required to achieve the acceptable 40%-60% level range.

Procedure

A container was cleaned, dried and weighed (W1).

100 g of the soil sample was taken and weighed (W2).

The sample was dried to constant temperature at 105 $^{o}$C for a period of 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 % moisture content in the soil is given by

$$W\ (\%) = [(W_2-W_3)/ (W_3-W_1)]\acute{}100$$

### 3.5 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

### 3.6 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. $H_2SO_4$ was added.

It was digested until clear and was further allowed to cool. The fluid was decanted into a 100 ml volumetric flask and made up to the mark with distilled water. This was followed by the distillation and titration process.

**DISTILLATION**

An aliquot of 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 10 ml of 4% Boric acid and three (3) drops of mixed indicator in a 500 ml conical flask for 4 minutes. The presence of Nitrogen gave a light blue colour. This was followed by titration and preparation of soil extract.

**TITRATION**

Collected distillate (about 100 ml) was titrated with 0.l N HCl till blue colour changed to grey and then suddenly flashed to pink.

A blank determination was carried out without the soil sample.

## 3.7 PREPARATION OF SOIL EXTRACT

Ten grams of soil was weighed into an extraction bottle. Hundred (100) ml of 1.0 N $NH_4OAc$ solution was added. Bottle with contents was placed in a mechanical shaker for 2 hours. The supernatant solution was filtered through No. 42 whatman filter paper. 10 ml aliquot of it was taken and read for K or Na on a Flame Photometer after calibration of Photometer with prepared standards. The flame photometer reading for soil was determined. Using the meter reading standard curve, the concentration of K in the soil extract was determined. (FAO, 2008)

### 3.8 Rhizosphere sample collection

After eight (8) weeks of planting, tap water was used to wet the soil in all thirty (30) bowls before samples were taken.

Some of the grass growing in the bowls were selected randomly and slowly pulled out of the various substrates making sure that the roots of the grasses were not broken and then shaken to remove the soil attached to the roots.



The rhizosphere soil samples from the various levels of compost + hydrocarbon contaminated soil, fertilizer + hydrocarbon contaminated soil, and topsoil + hydrocarbon contaminated and hydrocarbon contaminated soils were collected in separate sample bags.

### 3.9 Oil and Grease

Thirty grams of hydrocarbon contaminated soil (rhizosphere) was weighed into a 250 mL Schott bottle. Two or three teaspoons of anhydrous $Na_2S0_4$ was added.

Thirty (30) ml of solvent (n-hexane) and 2 ml concentrated HCl was added to the sample in Schott bottle. The lid was replaced and shaken vigorously to break up any aggregates. The sample was sonicated for 2 hours.

The supernatant liquid was poured off into a phase separator filter set in a glass funnel with approximately 10 g sodium sulphate and run into a pre-weighed beaker with glass boiling chips added.

Thirty (30) ml of solvent was further added to the Schott bottle and sonicated one more time and filtering process repeated to ensure total extraction of all hydrocarbons present.

The beaker containing the solvent extract was evaporated to dryness on a hotplate at 70 °C.

The desiccated beaker and its residue were cooled to a constant weight. And the weight recorded. Duplicate samples, solvent and sand blank were run for every sample. This was done to ensure that extraction was done with precision

**CALCULATION**

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

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



### 3.10 Total petroleum hydrocarbon analysis

**Diesel oil extraction**

Approximately 20 g of soil was weighed into a 16 oz. French square bottle with minimum exposure, along with 50 ml of distilled water and was adjusted to a pH of 3 with HCL. The bottle was capped tightly using a Teflon line cap and shaken mildly to disperse the soil for 1 to 2 minutes.

**Readings from Infra-Red Spectrometer**

After shaking, 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.

10 ml of Freon was pipetted from the upper layer and filtered through 5 grams of activated silica gel and 1 g of sodium sulphate into a reference cell.

Instrument was calibrated with working standards prepared from reference oil. The analyzer was blanked and cells with samples were inserted into the calibrated analyzer and read. (Gunther *et al*., 2003).

### 3.11 Procedure for quantifying heterotrophic plate counts

About 16 sterile test tubes were arranged in a test tube rack. One gram of rhizosphere soil was weighed on a calibrated Mettler Toledo balance into the first sterile test tube. The test tube was filled with 10 mls of sterile distilled water and capped. It was then mixed thoroughly to ensure a homogeneous mixture.

One (1) ml of the sample in the first test tube was pipetted into the second test tube and topped up with 9 ml of sterile distilled water. This became the dilution factor of 10. The procedure was replicated for the rest of the test tubes.

The media was hydrated by filling media vessel to the 100 ml mark with sterile diluent (deionized water). It was re-capped and shaken to dissolve. Using a sterile tip, 1ml of sample was pipetted to the center of a simplate.

Nine (9) ml of rehydrated media was directly pipetted onto the sample in the center of the plate. Plate was covered with lid and swirled gently to distribute sample into each well. Plate was tilted 90 $^{o}$ – 120 $^{o}$ to drain excess liquid into absorbent pad.

Plate was inverted and incubated for 48 hours at 35 $\pm$ 0.5 $^{o}$C.

The step was repeated using 10 ml of rehydrated media.

After incubation time, the number of wells showing fluorescence was counted by putting the sample under 6 watts, 365 nm UV light. Special UV goggles were worn.

The Most Probable Number (MPN) table was used to determine the MPN of heterotrophic plate count bacteria in the original sample (Gunther *et al*., 2003).

### 3.12 Data Analysis

Data was analysed using the SPSS software and 5% confidence interval was taken as significant. Treatment mean values which showed significance using two way ANOVA were separated using Tukey's method of Multiple Comparisons test which was run at 0.05 level of significance.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 **Levels of TPH, Oil and Grease in media**

Contaminated soil, compost and topsoil collected were analysed for TPH and oil and grease to establish the levels that already existed in these media. Results are displayed in table 3.0.



**Table 3.0: Initial Mean TPH, Oil and Grease levels in Media**

| Sample ID | Oil and Grease(mg/kg) | TPH(mg/kg) |
|---|---|---|
| HCS | 35,652 ± 9335 | 19576.58 ± 525. 19 |
| Topsoil | <10 | <10 |
| Compost | <10 | <10 |

### 4.2 **Hydrocarbon Degrader community changes- Urea + Hydrocarbon Contaminated Soil**

Results of MPN analyses of hydrocarbon degrading microbial populations over time in rhizosphere were obtained for all treatments. The microbial population trends in the rhizoshere over the period are below;

With an initial microbial population of $30 \times 10^2$ in the hydrocarbon contaminated soil, amendment with fertilizer (urea) saw microbial population reducing appreciably.

By the third week of sampling microbial populations in the rhizosphere had increased steadily for all levels of treatments (0.2%, 0.5% and 0.8% N in Urea + 3kg HCS) as shown in table 4.0.

Microbial populations by the fourth and fifth weeks of sampling were stable showing clearly that the populations had peaked.

Comparatively the control experiment saw a steady and gradual rise in microbial populations in the rhizosphere up to the second week of sampling.

Populations became stable up to about the third week of sampling and declined by the fourth to the fifth week of sampling.

**Table 4.0: Mean Microbial population build up trend in Rhizosphere of urea + 3kg HCS**

| Period / Treatments | Wk1 | Wk2 | Wk3 | Wk4 | Wk5 |
|---|---|---|---|---|---|
| | Concentration (Log MPN) | | | | |
| Urea 0.2 % + 3kg HCS | 5.71 | 5.85 | 5.95 | 7.02 | 7.00 |
| Urea 0.5% + 3kg HCS | 5.74 | 5.89 | 7.06 | 8.12 | 8.08 |
| Urea 0.8% + 3Kg HCS | 5.71 | 6.02 | 6.08 | 7.31 | 6.67 |
| Control ( 3kg HCS) | 5.60 | 7.75 | 7.79 | 7.00 | 6.90 |

Wk- Week

### 4.2.1 **Microbial Populations in Topsoil + Hydrocarbon Contaminated Soil**

Topsoil with an initial microbial population of 70 X $10^{15}$ upon blending with 3 kg contaminated soil at different N percentage levels of (0.2, 0.5 and 0.8) showed an initial dip in microbial populations.

The first to the third week of sampling saw an increase in microbial populations with the 0.8% blend level showing the highest microbial population.

Populations remained steady up to the fifth week of sampling as shown in table 5.0.

**Table 5.0: Mean Microbial population build up trend in Rhizosphere of Topsoil+ 3kg HCS**

| Period / Treatments | Wk1 | Wk2 | Wk3 | Wk4 | Wk5 |
|---|---|---|---|---|---|
| | Concentration (Log MPN) | | | | |
| Topsoil 0.2 % + 3kg HCS | 6.12 | 7.23 | 7.33 | 8.48 | 8.42 |
| Topsoil 0.5% + 3kg HCS | 6.22 | 6.42 | 6.55 | 8.64 | 8.59 |
| Topsoil 0.8% + 3Kg HCS | 6.48 | 7.59 | 8.67 | 8.74 | 8.71 |



### 4.2.2 **Microbial Populations in Composts + Hydrocarbon Contaminated Soil**

Microbial populations in the compost hydrocarbon contaminated soil blend at (0.2, 0.5 and 0.8 N) blend levels showed rapid increase in microbial populations in the rhizosphere from the first week of sampling to the third week of sampling as shown in table 6.0 below.

The fourth to the fifth week of sampling saw a steady and sustained increase in microbial populations in the rhizosphere. The blends that showed the highest populations were (0.5% N level in Compost + 3 kg HCS and 0.8% N level in compost + 3 kg HCS).

In using the analysis of variance we require that the response variables be normally distributed. When this failed, a nonparametric test for two factors (treatments and blocks) was applied. This is the Friedman Test which is sensitive to analysis of variance. The hypotheses being that:

$H_0$: The treatment effects are all equal.

$H_1$: The treatment effects are all not equal, at 5% level of significance.

After which a multiple comparison was carried out to establish which of the treatments performed significantly the same as shown in table 7.0 below.

**Table 6.0: Mean Microbial population build up trend for Compost amended Contaminated Soil**

| Period / Treatments | Wk1 | Wk2 | Wk3 | Wk4 | Wk5 |
|---|---|---|---|---|---|
| | Concentration (Log MPN) | | | | |
| Compost 0.2 % + 3kg HCS | 6.53 | 7.35 | 8.55 | 8.62 | 8.55 |
| Compost 0.5% + 3kg HCS | 7.32 | 7.42 | 9.59 | 9.67 | 9.62 |
| Compost 0.8% + 3Kg HCS | 7.36 | 7.57 | 9.74 | 9.87 | 9.79 |



**Table 7.0: Multiple comparisons to detect which MPN pairs are significantly different**

| Treatments | Mean rank | Different (P<0.05) from variable nr |
|---|---|---|
| A) Urea 0.2 % + 3kg HCS | 2.2000 | (4) (6) (7) (8) (9) |
| B) Urea 0.5% + 3kg HCS | 4.0000 | (6) (7) (8) (9) |
| C) Urea 0.8% + 3Kg HCS | 2.8000 | (4) (6) (7) (8) (9) |
| D) Topsoil 0.2 % + 3kg HCS | 5.8000 | (1) (3) (7) (8) (9) (10) |
| E) Topsoil 0.5% + 3kg HCS | 4.0000 | (6) (7) (8) (9) |
| F) Topsoil 0.8% + 3Kg HCS | 6.4000 | (1) (2) (3) (5) (7) (8) (9) (10) |
| G) Compost 0.2 % + 3kg HCS | 8.6000 | (1) (2) (3) (4) (5) (6) (10) |
| H) Compost 0.5% + 3kg HCS | 8.6000 | (1) (2) (3) (4) (5) (6) (10) |
| I) Compost 0.8% + 3Kg HCS | 9.4000 | (1) (2) (3) (4) (5) (6) (10) |
| J) Control ( 3kg HCS) | 3.2000 | (4) (6) (7) (8) (9) |

If a treatment is significantly different from other variables, those variables are indicated against it in the last column. Rhizospheres of treatments (Compost 0.2 % + 3kg HCS), (Compost 0.5% + 3kg HCS) and

(Compost 0.8% + 3Kg HCS) had the highest microbial populations over time and hence had the highest degradation rates.

### 4.3 **Mean Residual TPH % in Rhizosphere (Urea + HCS)**

Tukey's multiple comparison showed that treatment (0.2% urea + 3kg HCS) is significantly different in performance compared with treatment (0.5% urea + 3Kg HCS) and (0.8% urea +3kg HCS) which performed significantly the same. Table 8.0 shows the percentage TPH concentration remaining in the rhizosphere for the three levels of Urea and hydrocarbon contaminated soil (HCS) blend as at the fifth week of sampling.



**Table 8.0: Mean Residual TPH % in Rhizosphere over sampling period (Urea + HCS)**

| Treatments / Sampling period | 0.2% urea + 3kg HCS | 0.5% urea + 3kg HCS | 0.8% urea + 3kg HCS |
|---|---|---|---|
| **WKI** | 82.32 ±5.39% | 77.59 ±2.93% | 71.27 ±1.55% |
| **WK2** | 69.64 ±4.39% | 72.52 ±1.47% | 62.11 ±7.36% |
| **WK3** | 54.46 ±21.81% | 61.47 ±1.17% | 52.27 ±1.17% |
| **WK 4** | 51.89 ±9.07% | 56.1 ±7.30% | 51.82 ±1.47% |
| **WK 5** | 48.03 ±3.07% | 50.56 ±4.59% | 45.26 ±9.29% |

Wk- Week

Fig. 2.0 below shows the levels of TPH concentration in the rhizosphere of *Paspalum spp.* that grew in media with different urea concentrations in combination with 3kg (HCS) mixings after the fifth week of sampling.

With an initial TPH concentration of 19576.58 mg/kg before mixing, the first week of sampling gave concentrations in the regions of about 16000 (mg/kg) a whiles final TPH concentration readings for the fifth week of sampling ranged between 10655 (mg/kg) and 8818 (mg/kg) as shown in fig.4.0.

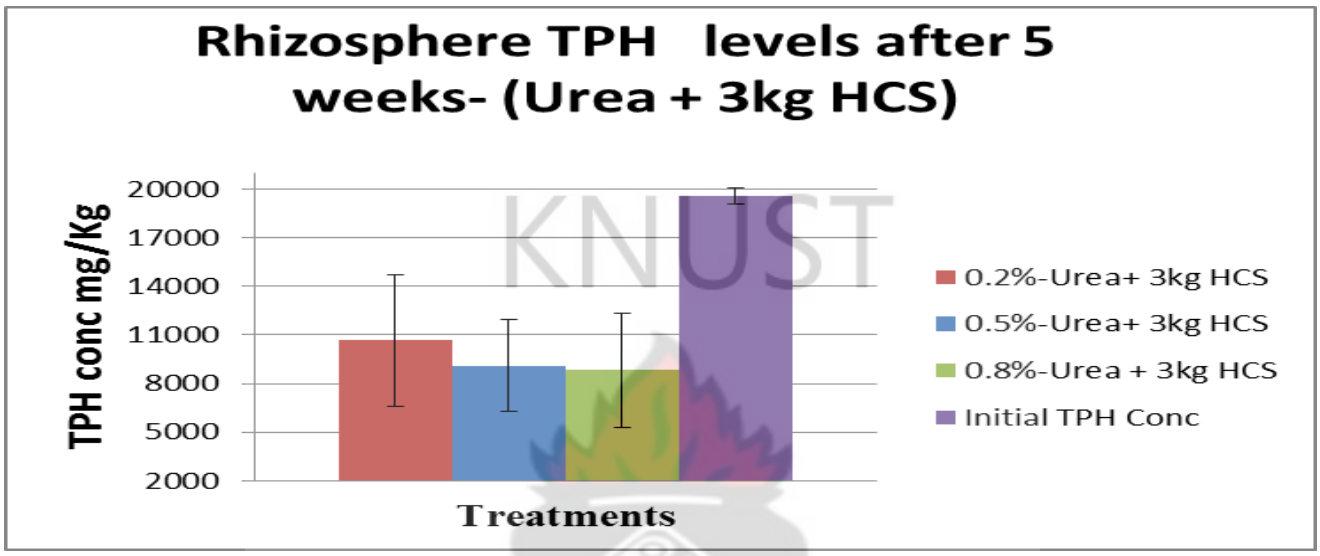


**Figure 2.0: Rhizoshere TPH degradation levels after 5weeks –Urea + 3kg HCS**

### 4.4 Mean Residual TPH % in Rhizosphere (Topsoil + HCS)

Tukey's multiple comparison showed that treatment D (0.2% N (level) in Topsoil + 3kg HCS)) is significantly different in performance compared with treatment E (0.5% N (level) in Topsoil + 3Kg HCS)) and F (0.8% N (level) in Topsoil +3kg HCS) which also performed significantly the same. Table 9.0 shows the percentage TPH concentration remaining in the rhizosphere for the three levels of topsoil and hydrocarbon contaminated soil (HCS) blend as at the fifth week of sampling.

**Table 9.0: Mean Residual TPH % in Rhizosphere over sampling period** -Topsoil+HCS

| Treatments / Sampling Period | 0.2% topsoil + 3kg HCS | 0.5% topsoil + 3kg HCS | 0.8% topsoil + 3kg HCS |
|---|---|---|---|
| WKI | 77.45 ±0.51% | 48.00 ±7.70 % | 47.99 ± 17.84 % |
| WK2 | 61.26 ±0.58 % | 40.60 ±5.88 % | 33.03 ± 6.47 % |
| WK3 | 50.93 ±8.83 % | 36.12 ±4.41 % | 27.12 ± 1.76 % |
| WK 4 | 40.59 ±5.88 % | 19.41 ±1.47 % | 17.55 ± 1.47 % |
| WK 5 | 32.59 ±4.12 % | 12.40 ±1.53 % | 11.45 ± 1.53 % |
| WK 5 | 77.45 ±0.51% | 48.00 ±7.70 % | 47.99 ± 17.84 % |

Wk-Week

Fig. 3.0 shows the levels of TPH concentration in the rhizosphere of *Paspalum spp.* that grew in media with topsoil + 3kg (HCS) mixings after the fifth week of sampling.

With an initial TPH concentration of 19576.58 mg/kg before mixing the first week of sampling gave concentrations in the regions of about 14909 (mg/kg) to 15000 (mg/kg) whiles final TPH concentration readings for the fifth week of sampling ranged between 8671 (mg/kg) and 5202 (mg/kg) as shown in fig.5.0.

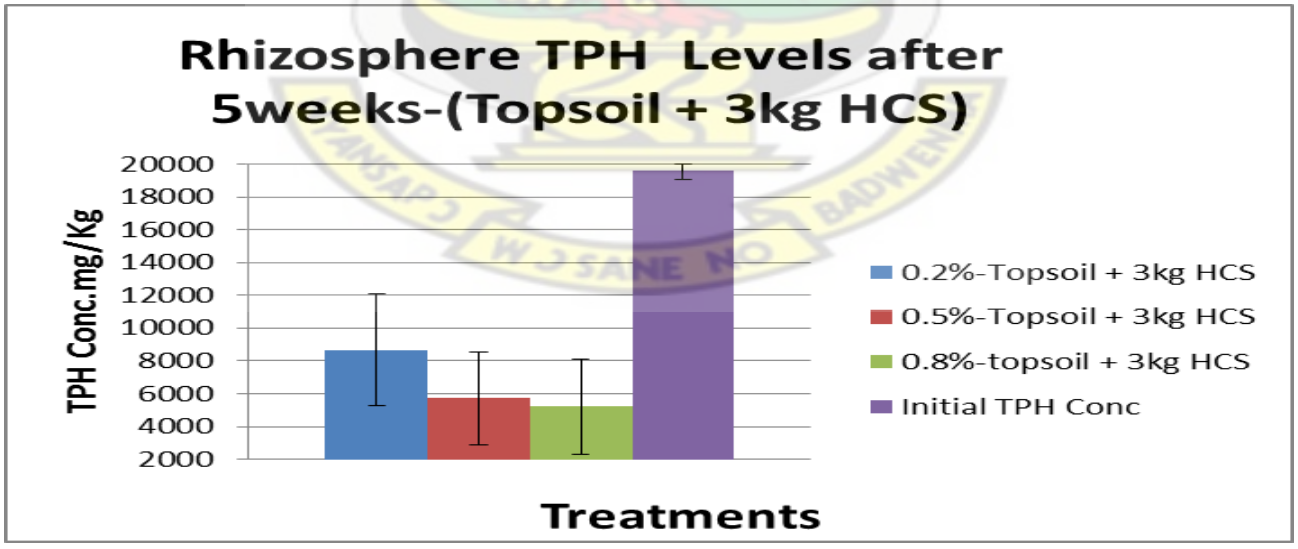


Figure 3.0**: Rhizosphere TPH degradation levels after 5 weeks- Topsoil + 3kg HCS**

## 4.5 **Mean Residual TPH % in Rhizosphere (Compost + HCS)**

Tukey's multiple comparison showed that treatment G (0.2% N (level) in Compost + 3kg HCS)) is significantly different in performance compared with treatment H (0.5% N level in Compost + HCS) and C (0.8%N level in Compost + 3kg HCS) which performed significantly the same. Table 10.0 shows the percentage TPH concentration remaining in the rhizosphere for the three levels of compost and hydrocarbon contaminated soil (HCS) blend throughout the sampling period.



**Table 10.0: Mean Residual TPH % in Rhizosphere over sampling period**
(Compost+ HCS)

| Treatments / Sampling Period | 0.2% Compost +3kg HCS | 0.5% Compost +3kg HCS) | 0.8% Compost +3kg HCS)N | 3kg HCS |
|---|---|---|---|---|
| **WK1** | 53.36 ±6.10% | 67.07 ±13.39% | 42.50±6.80% | 77.75 ±3.34% |
| **Wk2** | 47.71 ±7.30% | 42.36 ±13.54% | 35.91 ±16.48% | 75.10 ±12.66% |
| **WK3** | 27.39 ±3.82% | 32.70 ±0.27% | 25.88 ±1.47% | 69.14 ±6.47% |
| **WK4** | 22.70 ±0.29% | 21.81 ±2.65% | 16.38 ±1.03% | 65.72 ±1.17% |
| **WK5** | 12.78 ±1.28% | 11.99 ±2.06% | 9.15 ±3.07% | 48.07 ±5.61% |

Wk - Week

Fig. 4.0 shows the levels of TPH concentration in the rhizosphere of *Paspalum spp.* that grew in media with compost + 3kg (HCS) mixings after the fifth week of sampling.

With an initial TPH concentration of 19576.58mg/kg the first week of sampling gave concentrations in the regions of about 13000 (mg/kg) to 8000 (mg/kg) whiles final TPH concentration readings for the fifth week of sampling ranged between 6753 (mg/kg) and 4975 (mg/kg) as shown in fig.6.0.

**Table 11.0: Multiple comparisons to detect which pair of treatments is significantly different-(TPH)**

| Treatment | N | Mean | Grouping |
|---|---|---|---|
| Urea 0.2 % + 3kg HCS | 15 | 10654.0 | B |
| Topsoil 0.2 % + 3kg HCS | 15 | 10119.0 | B |
| Urea 0.5% + 3kg HCS | 15 | 9112 | C |
| Urea 0.8% + 3Kg HCS | 15 | 8817.3 | C |
| Compost 0.5% + 3kg HCS | 15 | 6773.3 | D |
| Compost 0.2 % + 3kg HCS | 15 | 6311.7 | DE |
| Topsoil 0.5% + 3kg HCS | 15 | 5787.9 | EF |
| Topsoil 0.8% + 3Kg HCS | 15 | 5202.1 | F |
| Compost 0.8% + 3Kg HCS | 15 | 4972.8 | F |
| Control ( 3kg HCS) | 15 | 12497.6 | A |

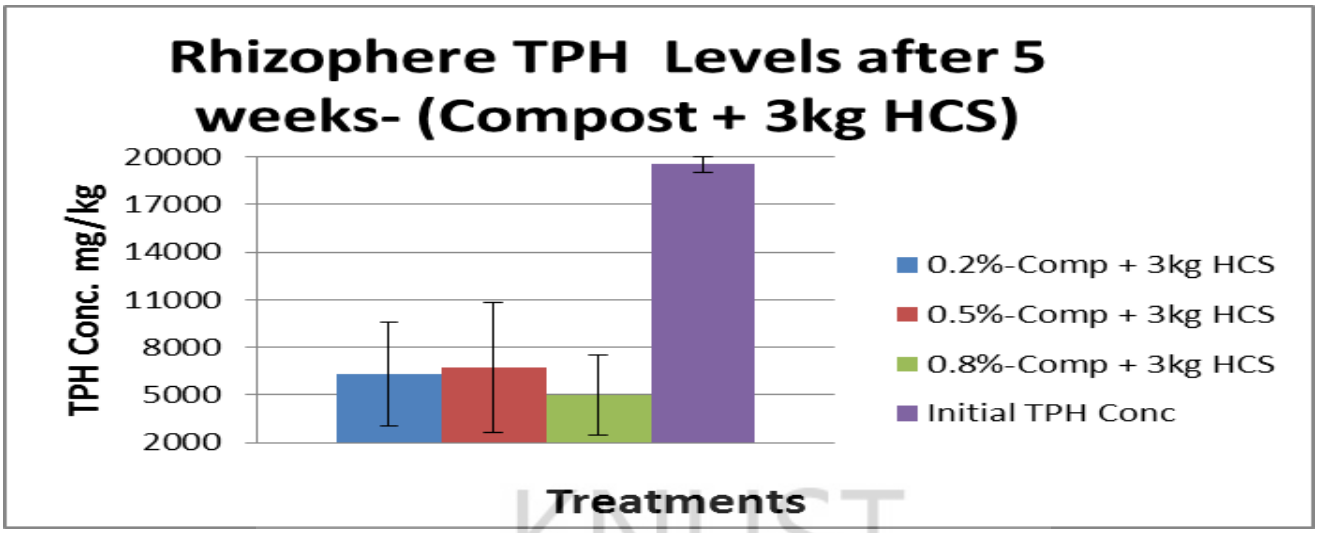


Figure 4.0**: Rhizosphere TPH degradation levels after 5 weeks – (Compost + HCS)**

Results of TPH analyses of hydrocarbon degradation over time in rhizosphere soil were obtained for each treatment.

Table 11.0 shows grouping of treatments using Tukey's method at 95.0% confidence for TPH degradation levels. Means that do not share same letter are significantly different.

## 4.4 Oil and Grease Level degradation

**Table 12.0: Multiple comparisons to detect which pair of treatments is significantly different- (Oil and Grease)**

| Treatment | N | Mean | Grouping |
|---|---|---|---|
| Control ( 3kg HCS) | 15 | 23968.4 | A |
| Urea 0.2 % + 3kg HCS | 15 | 21143.8 | B |
| Compost 0.2 % + 3kg HCS | 15 | 19841.2 | B |
| Urea 0.5% + 3kg HCS | 15 | 17867.1 | C |
| Urea 0.8% + 3Kg HCS | 15 | 17288.7 | C |
| Compost 0.5% + 3kg HCS | 15 | 13553.1 | D |
| Compost 0.2 % + 3kg HCS | 15 | 12912.9 | DE |
| Compost 0.5% + 3kg HCS | 15 | 11629.0 | EF |
| Compost 0.8% + 3Kg HCS | 15 | 10632.7 | FG |
| Topsoil 0.8% + 3Kg HCS | 15 | 10200.3 | G |

Table 12.0 shows treatment mean separation using Tukey's method at 95.0% Confidence for Oil and Grease degradation levels. Means that do not share a letter are significantly different.

# CHAPTER FIVE

## 5.0 DISCUSSION

### 5.1 Levels of TPH and Oil and grease in Compost, HCS and Topsoil

Topsoil and compost samples did not show any concentrations of TPH and oil and grease contamination apparently because there was no past history of hydrocarbon contamination of the sources of these samples.



### 5.2 Hydrocarbon Degrader community changes

In the current study, compost and topsoil blend rhizosphere samples displayed a similar trend, after an initial increase in the number of hydrocarbon degrading microorganisms equilibrium in microbial abundance was observed for all treatments as shown in table. 5.0 and 6.0. However it should be noted that metabolic activities continued throughout the experimental period. There are three possible explanations for this observation. First, soil conditions (nutrient) no longer permitted the proliferation of the hydrocarbon degrading microorganisms, but supported the metabolic activity of the existing population. Secondly, changes in plant activity with shifting growth phase (such as exudation patterns) may have influenced the metabolic activity of the rhizosphere microbial population (Maila and Randime, 2005).

Finally, changes in enzyme activity may be a response to the depletion of short-chain aliphatics and then degradation of long-chain more hydrophobic compounds (Riffaldi *et al.*, 2006). These three observations might have contributed immensely to rhizospheric microbial influence on TPH/ Oil and grease degradation in compost and topsoil blends in this current study.

### 5.2.1. Urea+ Hydrocarbon Contaminated Soil Rhizosphere

Microbial population build up trends observed for Urea (inorganic fertilizer) and 3kg hydrocarbon contaminated soil (HCS) was quite different.

Merkl *et al*. (2005) observed that the addition of nitrogen fertilizer (urea) enhances the remediation of hydrocarbons in soil but not all rhizoremediation systems respond to the addition of inorganic nutrients as asserted by Kirkpatrick *et al.* (2006). Some researchers observed diminished rates of microbial degradation after the addition of inorganic nitrogen and phosphorus amendments (Rentz *et al.*, 2003); this is attributed to inhibition of oligotrophic degraders or the stimulation of non-competent bacteria (Olson *et al.*, 2003).

In addition, there is an adaptive trait found in many plants (grasses) where low soil fertility and low nutrient conditions are optimal for growth (Huxtable, 1997). This suggests that the addition of inorganic fertilizer commonly practiced in phytoremediation may not be suitable for rhizoremediation application in some instances. In this current study, table 4.0 shows the effect of the addition of inorganic fertilizer and the impact it had on rhizospheric microbial population build up.

Graham and Curtis (1999) also 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 can result in an increase in soil salinity and this increases osmotic stress and suppresses the activity hydrocarbon-degrading organisms (Walworth and Ferguson, 2002). This phenomenon might have affected the growth rate of *Paspalum* Spp. that grew in (urea+ 3kg HCS) as demonstrated in the percentage degradation of TPH levels in (table 8.0).

## 5.3 TPH/ Oil and Grease Degradation in Rhizosphere

Compost and topsoil blends in the presence of *Paspalum Spp*. had greater reduction in TPH and oil and grease concentration.

The enrichment of hydrocarbon degrading microorganisms in the rhizosphere was greater in compost and topsoil amended contaminated soils.

The presence of hydrocarbons in soil has been shown to stimulate some microbial populations (Gaskin and Bentham, 2005), but plants appear to add additional influence. Plants have been shown to increase the microbial numbers in the rhizosphere (Kirk *et al.*, 2004).

In this current study *Paspalum spp*. rhizosphere growing in hydrocarbon contaminated soil amended with 0.2%, 0.5% and 0.8% N per volume of compost demonstrated the greatest extent of TPH /Oil and grease concentration reduction of 87%, 88% and 91% respectively.



The explanation being that organic amendments such as compost can be used to increase the water-holding capacity and also improve soil structure of a contaminated soil as was also asserted by (Rentz *et al*., 2004).

This resulted in a boost in the growth of plants species that grew in this medium which contributed to the higher levels of degradation recorded for contaminated soils amended with compost.

Hydrocarbon contaminated soils amended with 0.2%, 0.5% and 0.8% N per volume of topsoil also demonstrated reduction in TPH at 68%, 87% and 89% respectively.

Plants have been shown to encourage hydrocarbon contaminant reduction principally by providing an optimum environment for microbial proliferation in the rhizosphere (Adam and Duncan, 1999).

The control in this experiment reduced TPH concentration by about 50%. In a study by Gunther *et al.* (2003) hydrocarbon contaminated soil amended with nutrients but unplanted resulted in about 50% hydrocarbon removal.

This indicates nutrient limitation to TPH biodegradation in the absence of plants. This confirms the fact that amendments alone may not be able to break down TPH levels at a fast rate.

The current study confirms the assertion by Gerhardta *et al*. (2009) that the combined effect of microbes and selected plant species is the best application for the breakdown of hydrocarbons in contaminated soils. There are also several literatures that support the fact that plants can contribute to the removal of pollutants from soil. Firstly, the direct interaction of plant roots with hydrocarbons in the soil by sorption, uptake and transport (Gunther *et al.*, 2003).

Secondly, microbial populations aide in degradation of pollutant due to rhizosphere interactions (Gatliff, 1994). This is likely to be the primary role influencing the fate of diesel/oil in soil in this current study.

Thirdly, a more general effect of vegetation cover on environmental conditions and soil properties (Germida *et al.*, 2002). Any of these factors could have contributed to the outcomes in this current study

# CHAPTER SIX

## 6.0 Conclusion and Recommendations

### 6.1 Conclusion

The success of rhizoremediation depends on establishing plant cover at a site that has sufficient root-soil contact to produce the desired contaminant degradation.

The outcome of this experiment clearly shows that the rate of hydrocarbon degradation in rhizoremediation application presents a better alternative for dealing with hydrocarbon contaminated soils compared with the rates of degradation in the Vol. Pad facility.

In the current study, *Paspalum* Spp. enhanced TPH and Oil and Grease removal relative to planted controls without the need for nutrient addition. Addition of nitrogen (N- 0.8% Compost) to the system improved the removal efficiencies further. Alternatively, nutrient addition to a rhizoremediation system involving native species which prefer low soil nutrients for growth may inhibit biodegradation efficiencies.

### 6.2 Recommendations

Other grass species in Ghana belonging to the Poaceae family should be tested for their rhizoremediation capabilities. These grasses should be used in this application over a longer period of time without the use of any amendments. This is because literature has it that grasses belonging to this family are the best candidates for this application. Newmont Ghana Gold should concentrate on using plant species which can found in its locality in order to avoid invasion of plants that might be introduced from elsewhere for this application.

Legumes which are also possible candidates for this application should also be assessed. Further studies to ascertain the actual composition of microbial communities associated with Hydrocarbon degradation will be beneficial.

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# APPENDICES

## APPENDIX I: Baseline Conditions

**(i) .** Baseline conditions before fertilizer/compost/topsoil blend

| **Sample date** | **Moisture content (%)** | **Temp$^0$ /C** | **Total Nitrogen (%)** | **Available Phosphorus(mg/kg)** | **Potassium (mol/Kg)** |
|---|---|---|---|---|---|
| 17-10-10 | 19.00 | 24.80 | 0.1 | 5.02 | 0.20 |
| 17-10-10 | 30.00 | 26.00 | 0.4 | 64.2 | 0.25 |
| 17-10-10 | 25.20 | 25.20 | 0.7 | 1.05% | 0.45 |

| **Sample date** | **Sample ID** | **Oil n Grease(mg/kg)** | **TPH(mg/kg)** | **plate count/(mpn)** |
|---|---|---|---|---|
| 17-10-10 | HC | 35,652 | 19576.58 | $30*10^2$ |
| 17-10-10 | Topsoil | <10 | <10 | $70*10^{15}$ |
| 17-10-10 | Compost | <10 | <10 | Nil |

**(ii). Table Showing the Level of Hydrocarbon Contamination after one week of Amendment**

| SAMPLE | NITROGEN (%) | TPH(mg/kg) | Oil and Grease(mg/kg) | MPN |
|---|---|---|---|---|
| COMPOST + CONT.SOIL (0.2%) | 0.07 | 11200kg/mg | 24500kg/mg | $17 * 10^4$ |
| COMPOST+CONT. SOIL (0.5%) | 0.19 | 12320kg/mg | 23500kg/mg | $21 * 10^6$ |
| COMPOST+CONT.SOIL (0.8%) | 0.22 | 11250kg/mg | 22000kg/mg | $30 * 10^{10}$ |
| CONT. +TOP SOIL (0.2%) | 0.11 | 16340kg/mg | 30126kg/mg | $26 * 10^6$ |
| TOPSOIL + CONT.SOIL (0.5%) | 0.15 | 15481kg/mg | 25342 kg/mg | $35 * 10^6$ |
| TOPSOIL + CONT.SOIL (0.8%) | 0.21 | 13240kg/mg | 26246 kg/mg | $30 * 10^8$ |
| FERT+CONT. SOIL (0.2%) | 0.11 | 18342kg/mg | 31234 kg/mg | $23 * 10^{10}$ |

| | | | | |
|---|---|---|---|---|
| **FERT+CONT.SOIL (0.5%)** | **0.13** | **14320kg/mg** | **32342 kg/mg** | **$8 * 10^{6}$** |
| **FERT+CONT.SOIL (0.8%)** | **0.14** | **13371kg/mg** | **29452 kg/mg** | **$21 * 10^{10}$** |

## APPENDIX II: MEDIA MIXINGS

**(i).Plate showing measurement and mixing of media at the commencement of Experiment**


**(ii) Plate showing set up showing randomized complete block design**

**APPEN DIX III: Plates Showing Vegetative Parts, sample collection and Sample reading**

**(i) Plate showing *Paspalum spp.***

**(ii) Plate showing Collection of Rhizosphere samples for HPC**

**(iii) Plate showing TPH samples prepared for reading in the TPH meter.**


**(vi). Plate showing TPH readings being done by Infra-red Spectroscopy**

**APPENDIX V: STATISTICAL ANALYSIS**

**(iii). Table Showing Correlation Analysis showing strength of relationship exiting among oil and grease, TPH and MPN**

| PARAMETER | TPH | OIL AND GREASE |
|---|---|---|
| OIL AND GREASE | 0.994 | - |
| MPN | -0.343 | -0.350 |



**(iv) .Table showing Descriptive Statistics**

| Variable | Treatment | Mean | StDev |
|---|---|---|---|
| **TPH** | A | 10655 | 4089 |
| | B | 9118 | 2856 |
| | C | 8818 | 3532 |
| | D | 10119 | 3389 |
| | E | 5720 | 2824 |
| | F | 5202 | 2884 |
| | G | 6308 | 3298 |
| | H | 6753 | 4111 |
| | I | 4975 | 2518 |
| | J | 12499 | 4098 |

**OIL AND GREASE**

| | | |
|---|---|---|
| A | 21145 | 8429 |
| B | 17878 | 5600 |
| C | 17290 | 6926 |
| D | 19841 | 6645 |
| E | 11495 | 5819 |
| F | 10201 | 5656 |
| G | 12906 | 7325 |
| H | 13513 | 8198 |
| I | 10637 | 6492 |
| J | 23972 | 7026 |

| | | | |
|---|---|---|---|
| **MPN** | A | 4422000 | 5284735 |
| | B | 52916000 | 67005003 |
| | C | 5268000 | 8551978 |
| | D | 120796000 | 148274192 |
| | E | 167645320 | 227353751 |
| | F | 314245980 | 270997215 |
| | G | 16094742000 | 20564589706 |
| | H | 2603520000 | 2278074110 |
| | I | 3907078000 | 3460198802 |
| | J | 5658000 | 3265780 |

**(v). Table Showing Analysis of variance for TPH**

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 9 | 898529775 | 99836642 | 201.78 | 0.000 |
| Week | 5 | 1325399876 | 331349969 | 669.68 | 0.000 |
| Error | 136 | 67291466 | 494790 | | |



**(vi). Table Showing Analysis of variance for OIL AND GREASE**

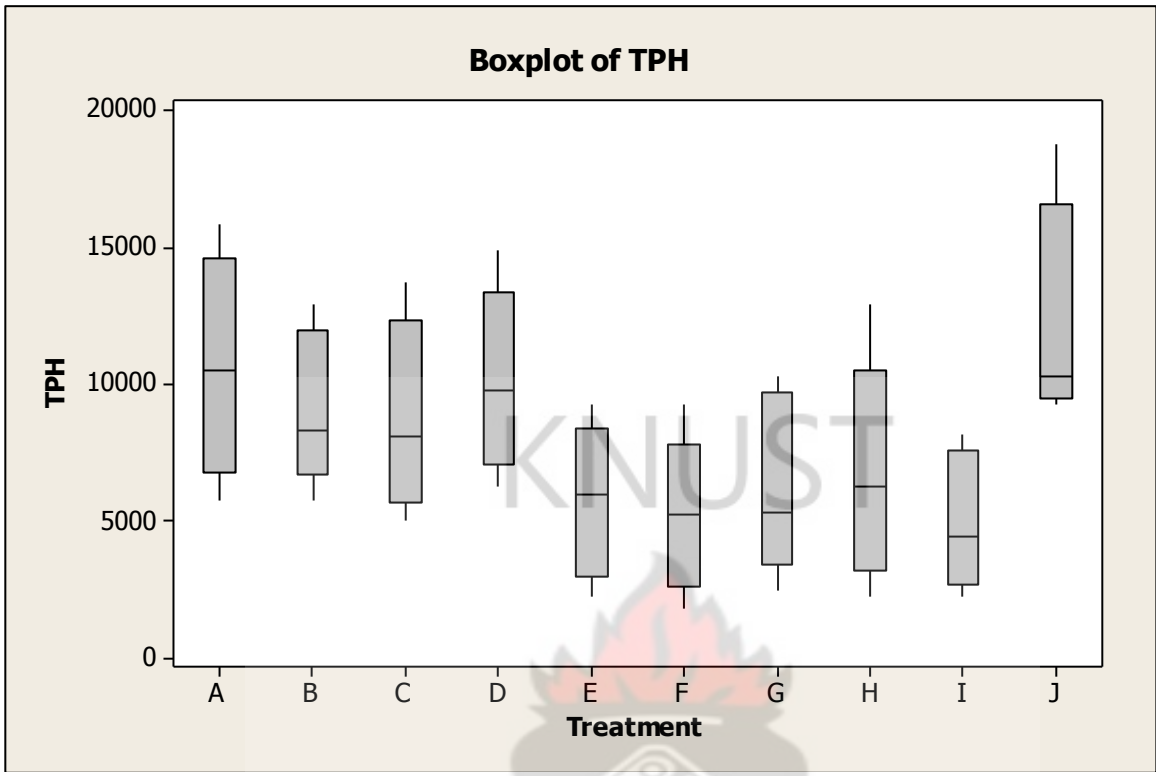
| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 9 | 3102462449 | 344718050 | 260.88 | 0.000 |
| Week | 5 | 5493512356 | 1373378089 | 1039.38 | 0.000 |
| Error | 136 | 179702992 | 321346 | | |

**(vii). Table Showing Friedman Test for MPN**

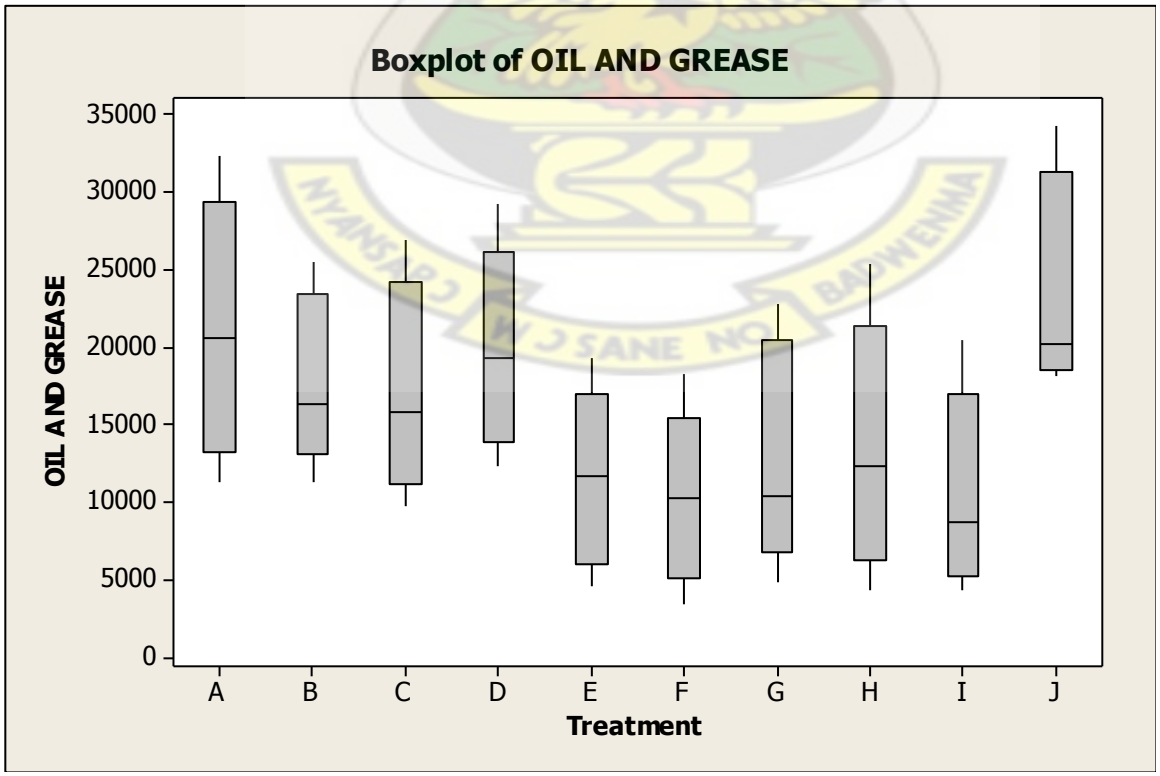
| | |
|---|---|
| F | 13.1875 |
| DF 1 | 9 |
| DF 2 | 36 |
| P | <0.001 |

## APPENDIX VI: COMPARISON OF TREATMENTS

### (i) Figure Showing Box plots of TPH




### (ii) Figure Showing Boxplot of Oil and Grease



**(ii) Figure Showing Boxplot of MPN**