SUBSTRATE ULTILIZATION FOR BIOREMEDIATION OF HYDROCARBON COMPOUNDS

By

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(BSc. Mathematics)

A Thesis submitted to the Department of Mathematics

Kwame Nkrumah University of Science and Technology

in partial fulfillment of the requirement for the degree

of

MASTER OF PHILOSOPHY

College of Science

May 2012

DECLARATION

I hereby declare that this submission is my own work towards the M.Phil., and that, to the best of my knowledge, it contains no material previously published or presented by another person or material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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ACKNOWLEDGEMENT

I express my profound gratitude to the Almighty God for His guidance and grace for seeing me through this course.

I am also grateful to my supervisor Dr. S. K. Amponsah for his guidance, support and his contribution towards my work.

Besides, I am thankful to all demonstrators in the Mathematics, Statistics and Actuarial Science Department, Kwame Nkrumah University of Science and Technology for their support and encouragement.

I will also wish to express my sincere thanks to my dearest friend Ama Kwansima Nyarku a student in the Mathematics department for her time she devout to the editing and typing as well as her suggestions towards the writing of this script and to my colleague Philemon Baah, a demonstrator in the Mathematics department for his support

Finally, I also wish to express my kind appreciation to my family, especially my mother Grace Abena Korkor and my father Prince Patrice Afrifah Yamoah-Ponkoh and to all the Afrifah and de-Graft Johnson's families.

DEDICATION

This project is dedicated to my siblings, Jeffrey; de-Graft Johnson Junior, Esther, Rose and my niece Henrietta Serwaah Princess

ABSTRACT

We design, evaluate and compare the biodegradation performance of the application of compost (made up of some dead plants), Poultry manure (Organic) and inorganic chemical fertilizer (Urea) in the bioremediation of soil contaminated with petroleum hydrocarbon. Four different levels of nitrogen application were augmented for each of the substrates. A combination of treatments consisting of the application of poultry manure, Chemical fertilizer, and Compost was evaluated ex situ during a period of 8 weeks of remediation. The aim was to (i) find the best performance of substrate for the process, (ii) the level of nitrogen which stimulate performance (ii) find if there exists a significant difference in both the substrate and the level of nitrogen (iv) the best combination of substrate and level of nitrogen good for bioremediation process using ANOVA and Tukey's test of difference. Contaminated soil containing oil and total petroleum hydrocarbon with different levels was bioremediated by blending of the hydrocarbon contaminated soil with portions of compost, poultry manure and fertilizer. After eight weeks of remediation, the most efficient contributor to hydrocarbon decomposition was poultry manure, followed by compost and fertilizer respectively. Moreover, in all the four (4) fixed nitrogen levels, it was found that, the higher the level of nitrogen the better the rate of degradation. For the substrate used, poultry manure-hydrocarbon blend recorded the least residual of Oil/ grease and TPH values followed by the compost substrate and with fertilizer blend recording the highest.

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CHAPTER 1

INTRODUCTION

1.0 Introduction

This chapter represents the background of the study of bioremediation, the problem statement, and the objectives of the study, the research questions associated with the objectives, the justification and the organization of the study.

This study involves the use of an environmentally procedure to help decontaminate the environment engulf by hydrocarbon compounds. The study involves the use of different substrate to serves an alternative procedure for the expensively procedure of volatilization current used by mining industries in Ghana to decontaminate the soil off hydrocarbon.

1.1 Background of the Study

Millions of barrels of oil spillage over the last century have call for an increase in environmental law to governing methods of drilling across the globe. Scientists have also continuously looking for a more environmental friendly approach in dealing with the oil spillage characterizing the business activities of the multi-billion dollar oil industry. One of most recently use methods is facilitation of the natural process of decomposition of organic and semi-organic substances is a process known as bioremediation.

Coastal environments are threatened by petroleum spills ranging from low-level discharges to catastrophic accidents. Large spills commonly are followed by clean-up efforts, but complete containment is rare. In all cases, remediation ultimately depends on microbial degradation. The rate of this natural bioremediation varies with physical and biological factors (temperature, wind and wave action, macro-ecology, and microbial community diversity), all of which have been

extensively studied and reviewed Atlas and Bartha. (1973), Atlas (1981), Harayama, Kasai, and Hara. (2004). Head and Swannell. (1999),

The involvement of microorganisms in the degradation of petroleum hydrocarbons in the environment has been established as an economic, efficient, versatile, and environmentally friendly treatment method (Margesin and Schinner, 2001; Yakubu, 2007).One promising method that has been researched into is the application of chemical fertilizers to augment for the mineral element, particularly nitrogen and phosphorus, limitations in the soil during biodegradation (Margesin and Schinner, 1999; Ayotamuno et al., 2006). The effectiveness of this treatment method has, however, been conflicting (Cunningham and Philip, 2000; Lindstrom and Braddock, 2002; Okolo et al., 2005). This might be due to the heterogeneity of soils and crude oil as well as possible interactions between the soil amendments and the natural soil constituents (Knaebel et al., 1994). Nonetheless, in developing countries, fertilizers are not sufficient for agriculture, let alone for cleaning oil spills in most mining and petrochemical sites. It therefore necessitates the search for the most utilized substrate and the optimum nitrogen level that are environmentally friendly options of enhancing petroleum hydrocarbon degradation.

1.1.1 Bioremediation

Bioremediation is the use of microorganism metabolism to remove pollutants. Bioremediation allows natural processes to clean up harmful chemicals in the environment.

Microscopic “bugs” or *microbes* that live in soil and groundwater like to eat certain harmful chemicals, such as those found in gasoline and oil spills. When microbes completely digest these chemicals, they change them into water and harmless gases such as carbon dioxide.

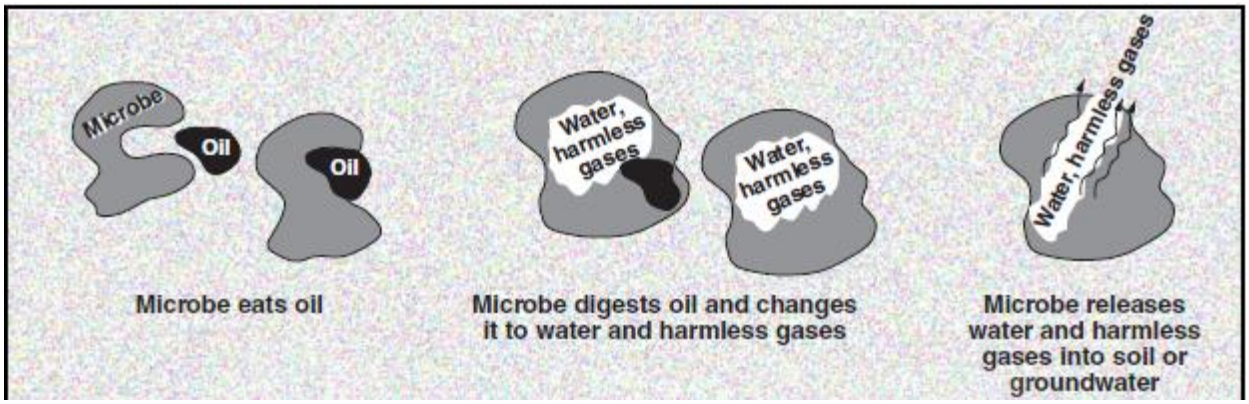


Figure 1.1: Microbe reactions on hydrocarbon. Sources: EPA, USA

Technologies for bioremediation can be generally classified as 'in situ' or 'ex situ'. In situ bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some of these bioremediation technologies are phytoremediation, bioventing, bioleaching, land farming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators, Tera Nova Environmental Resources (2009).

Meagher, (2000), reveals that not all contaminants, however, are easily treated by bioremediation using microorganisms, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. The assimilation of metals such as mercury into the food chain may worsen matters. Phytoremediation is useful in these circumstances because natural plants or

transgenic plants are able to bioaccumulation these toxins in their above-ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use.

The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes, Diaz (2008). This has call for the use of genetic engineering to create organisms specifically designed for bioremediation. Lovley, (2003) finds that, the bacterium *Deinococcus radiodurans* (the most radioresistant organism known) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste Brim H, McFarlan et al, (2000). Most commonly, the process is misunderstood. The microbes are ever-present in any given context and are generally referred to as "normal microbial flora". During bioremediation (biodegradation) processes, fertilizer/nutrient supplementation is introduced to the environments in efforts to maximize growth and production potential. Common misbelief is that microbes are transported and dispersed into an unadulterated environment.

1.1.2 Mycoremediation

Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area. The term *mycoremediation* refers specifically to the use of fungal mycelia in bioremediation. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed

of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain strains have been reported to successfully degrade the nerve gases VX and sarin.

1.1.3 Mycofiltration

Mycofiltration is a similar process, using fungal mycelia to filter toxic waste and microorganisms from water in soil.

1.1.4 Bioaugmentation

Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water. Usually the steps involve studying the indigenous varieties present in the location to determine if biostimulation is possible. If the indigenous variety do not have the metabolic capability to perform the remediation process, exogenous varieties with such sophisticated pathways are introduced, Andrea Leeson, Bruce Alleman, Pedro, Alvarez, Victor Magar (2001). Bioaugmentation is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of Microbial cultures, containing all necessary microorganisms (*B. licheniformis*, *B. thurengensis*, *P. polymyxa*, *B. sterothemophilus*, Penicillium sp., Aspergillus sp., Flavobacterium, Arthrobacter, Pseudomonas, Streptomyces, Saccaromyces, Triphoderma, etc.). Whereas activated sludge systems are generally based on microorganisms like bacteria, protozoa, nematodes, rotifers and fungi capable to degrade bio degradable organic matter.

Bioaugmentation of chlorinated solvents

At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, bioaugmentation is used to ensure that the *in situ* microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic. This is typically only applicable to bioremediation of chlorinated ethenes, although there are emerging cultures with the potential to biodegrade other compounds including chloroethanes, chloromethanes, and MTBE, hence it is typically performed in conjunction with the addition of electron donor (biostimulation) to achieve geochemical conditions in groundwater that favor the growth of the dechlorinating microorganisms in the bioaugmentation culture (Donald 2000).

1.1.5 Biostimulation

Biostimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation. It is done by addition of various forms of rate limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (e.g. in the form of molasses). Additives are usually added to the subsurface through injection wells, although injection well technology for biostimulation purposes is still emerging. Removal of the contaminated material is also an option, albeit an expensive one. Biostimulation is greatly enhanced by bioaugmentation. This process, overall, is referred to as bioremediation and is an EPA-approved method for reversing the presence of oil or gas spills mainly across the globe. Tera Nova Environmental Resources (2009).

Recently a number of products have been introduced which allow popular use of bioremediation using biostimulative methods. They may harness local bacteria using biostimulation by creating a hospitable environment for hydrocarbon-devouring microorganisms, or they may introduce

foreign bacteria into the environment as a direct application to the hydrocarbon. While the jury is out as to whether either is particularly more effective than the other, prima fascie consideration suggests the introduction of foreign bacteria to any environment stands a chance of mutating organisms already present and affecting the biome.

Investigations to determine subsurface characteristics (such as natural groundwater velocity during ambient conditions, hydraulic conductivity of the subsurface, and lithology of the subsurface) are important in developing a successful biostimulation system. In addition, a pilot-scale study of the potential biostimulation system should be undertaken prior to full-scale design and implementation.

However, some biostimulative agents may be used in chaotic surfaces such as open water and sand so long as they are (oleophilic), meaning that they bond exclusively to hydrocarbons, and basically sink in the water column, bonding to oil, where they then float to the water's surface, exposing the hydrocarbon to more abundant sunlight and oxygen where greater micro-organic aerobic activity can be encouraged. Some consumer-targeted biostimulants bond possess this quality, others do not. With the introduction of therapeutic lasers, biostimulation also refers to the application of photon energy to injured tissue, in order to achieve a stimulatory and regenerative effect at the molecular level.

1.1.6 Phytoremediation

Phytoremediation it describes the treatment of environmental problems (bioremediation) through the use of plants that mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere. It consists of mitigating pollutant

concentrations in contaminated soils, water, or air, with plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them. Hence, it refers to the natural ability of certain plants called hyperaccumulators to bioaccumulate, degrade,or render harmless contaminants in soils, water, or air. Contaminants such as metals, pesticides, solvents, explosives Mendez, Maier (2008), and crude oil and its derivatives, have been mitigated in phytoremediation projects worldwide. Many plants such as mustard plants, alpine pennycress and pigweed have proven to be successful at hyperaccumulating contaminants at toxic waste sites.

Phytoremediation is considered a clean, cost-effective and non-environmentally disruptive technology, as opposed to mechanical cleanup methods such as soil excavation or pumping polluted groundwater. Over the past 20 years, this technology has become increasingly popular and has been employed at sites with soils contaminated with lead, uranium, and arsenic Burken, (2004).

1.1.8 Rhizofiltration

Rhizofiltration is a form of bioremediation that involves filtering water through a mass of roots to remove toxic substances or excess nutrients.

Rhizofiltration is a type of phytoremediation, which refers to the approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants. It also filters through water and dirt.

The 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. Many plant species naturally uptake heavy metals and excess nutrients for a variety of reasons: sequestration, drought resistance, disposal by leaf abscission, interference with other plants, and defense against pathogens and herbivores, (Boyd 1998) Some of these species are better than others and can accumulate extraordinary amounts of these contaminants. Identification of such plant species has led environmental researchers to realize the potential for using these plants for remediation of contaminated soil and wastewater.

1.2 Problem Statement

The manufacture, transportation, and distribution of petroleum and chemical products during the last century have resulted in hydrocarbon-contamination becoming a major environmental problem. Petroleum has contaminated waters, threatening human health and damaging the environment. Hydrocarbons have leaked from tankers into the oceans and from underground storage tanks into soils and ground waters. Although it is the large marine oil spills with the pictures of dead seabirds that attract public attention, most environmental hydrocarbon contaminants originate from much smaller leakages, such as improper disposal of waste motor oils and leaking underground storage. Most of the environmental inputs of petroleum are accommodated largely due to the capacities of microorganisms to biodegrade hydro-carbons. The environmental applications of hydrocarbon biodegradation have become a focus of study through the late 1960s and early 1970s, largely through projects supported by the US Office of Naval Research. Before the establishment of the US Environmental Protection Agency (EPA), the US Navy had been in charge of cleaning up the seas. Ronald, Atlas and Carl (1995). A new treatment technology grew out of these fundamental studies on hydrocarbon biodegradation. Called bioremediation, which is the use of living organisms, especially microorganisms, to degrade pollutants and restore environmental quality. The original studies on the bio-chemistry

and diversity of hydro-carbon biodegradation pathways were part of basic research on the physiology of microorganisms, which includes work elucidating the chemical pathways of metabolism of a variety of compounds. At the time of those studies, petroleum was inexpensive and viewed as a candidate substrate for microorganisms to be used to produce single-cell protein as a food for the burgeoning world population. Bioremediation extends the natural processes by which microorganisms consume organic molecules, including hydrocarbons. The micro-organisms convert organic molecules to cell biomass and products such as carbon dioxide and water that can be readily accommodated in the environment (Atlas and Pramer 1990). Bioremediation of petroleum pollutants aims to increase the natural rates of hydrocarbon biodegradation that produces nontoxic end products.

Many of the current problems of contamination and pollution (presence of chemicals from human activities that exert untoward effects on organisms and their ecology) with petroleum hydrocarbons at specific sites, such as those sites contaminated by underground leaking storage tanks, can be treated by microbiological processes based on native organisms. Many petroleum hydrocarbons, including aromatic and aliphatic hydrocarbons, are readily degraded by native organisms when oxygen is available. Some hydrocarbons are easily degraded; others are more slowly and/or less completely degraded; still other compounds found in petroleum are totally non-biodegradable. The method of bioremediation has constantly be experiments with the introduction of statistical approach to obtain the optimum ingredients needed to speed up the rate of metabolism of micro-organism in the soil to help increase the rate of breakdowns of hydrocarbons. Hence as more and more spills over the gulf and the sites of petrochemical industries emerges, different methodologies are still under constant scientific research to help

come out with a well defined environmental friendly process for the bioremediation process across the globe.

1.3 Objectives of the Study

This study intends to find the strategies in utilizing the best substrate and the needed level of nitrogen to speed up the metabolism of the ex situ process of the bioremediation. Among other things, the study seeks to establish the following objectives:

(i) To established, if there is significant differences among the treatments (weeks) for the three substrates (compost, poultry manure and fertilizer).

(ii) To established, if there is significant differences among the four nitrogen levels (0.4, 1.0, 1.6, 2.2) used for the three substrates (compost, poultry manure and fertilizer).

(iii) To established, if the interaction between the two factors have effect on the degradation of hydrocarbon compounds.

(iv) To find the best nitrogen level that optimizes the bioremediation for each substrate by using the Tukey's multiple comparison procedure.

(v) To find the best substrate that is suitable and influences rapidly the bioremediation process by using the Tukey's multiple comparison procedure.

1.4 Research Questions

In order to arrive at the best solution for the experimental processes, the following questions were asked to serve as a guide.

(i) There exist evidence of significant differences among the treatment means (weeks) for the three substrates?

(ii) There exists differences among the various levels of nitrogen concentration used for the three substrates?

(iii) What is the best nitrogen level that facilitates the bioremediation process?

(iv) What is the best substrate that influences rapid degradation for the bioremediation processes?

1.5 Methodology

As new technology give rise to solving complex problems, this study also sort to bring to bear other strategies of dealing with the well established procedures of the bioremediation, where by newly ways of considering different levels of Nitrogen content, applying to different substrates. The data from this experimental project gathered was analyzed using the Generalized Linear Models (GLM), a statistical tool to analyze the difference in means of the different treatments at the different levels of nitrogen.

The experiment was conducted using a design approach, of which Randomized Block Design was utilized for three different substrates (Fertilizer, Poultry Manure and Compost) at four different Nitrogen addition levels (0.4, 1.0, 1.6 and 2.2). Each experiment involves three replicates with one control. Data was analyzed using the statistical software "Minitab Version 16" to find the optimum level of nitrogen and best substrate needed to increase the rate of hydro-carbon degradation in the a contaminated soil.

1.6 Justification of the Study

In Ghana, the activities of the Tema Oil Refinery (TOR), the various mining industries across the regions and the emerging oil and petro chemical industries recently has given rise to a major concern to the treatments of oil spillage on sites of these heavy industries. Mainly, most mining

industries in Ghana are mainly concern with the removal of toxic metals found in the debris of their activities and constantly developing and applying new technologies to avert the situation and to control their pollution to the environment. Moreover, recent reports over the past decade from the mining sector shows a drastically increase in pollution of the environment caused by the spillage of oil and other hydrocarbon chemicals on site of operations, this has cause for incorporating strategies of environmental bioremediation techniques available to curb and remediate the pollution cause to the environment. This study intends to add up to literature, to produce a cost effective, easy managed and environmentally friendly techniques of the bioremediation process for use in the bioremediation processes.

1.7 Organization of the Study

This study has been organized into five chapters; with each chapter dedicated to a different line of procedure apply in the entire study.

Chapter 1 presents the background of the study, the problem statement, objectives of the study, brief methodology, justification and the thesis organization. Chapter 2 deals with the existing literature on the subject, from the view of the different areas combined in this study such as biochemistry, environmental engineering and statistics.

Chapter 3 deals with the methodology adopted for the study, it comprises the experimental procedure and the theoretical backbone of the GLM. Chapter 4 presents data analysis and discussions and chapter 5 which is the last chapter is devoted for conclusion and recommendations of the study.

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

This chapter deals with the various bioremediation process projects undertaken by various stakeholders in the environmental engineering process such as the US Marine and host of others, other works which make use of randomization in clinical trials as the well as the work of Fisher which gave birth to the analysis of variance are discussed.

2.1 Bioremediation of Soil

The use of micro-organism for remediation has been exhaustibly researched. However, application of different levels of stimulants for different substrates which comes as a new technique in approaching the bioremediation process has literally not been discussed much literature,. According to Margesin and Schinner (2001); Yakubu (2007), microorganisms' involvement in the degradation of petroleum hydrocarbons in the environment has been found to be economic, efficient, versatile, as well as environmentally friendly. One promising method that has been researched into is the application of chemical fertilizers to augment for the mineral element, particularly nitrogen and phosphorus, Margesin and Schinner (1999); Ayotamuno et al. (2006). The effectiveness of this treatment method has, however, been conflicting with the work of Cunningham and Philip (2000); Lindstrom and Braddock (2002) and Okolo et al. (2005). The latter works attribute the differences to the heterogeneity of soils and crude oil as well as possible interactions between the soil amendments and the natural soil constituents as has been explained by Knaebel et al. (1994).

On the other hand, in developing and most emerging economic countries, fertilizers are not sufficient for agriculture, let alone for cleaning oil spills. It therefore necessitates the search for

cheaper and environmentally friendly options of enhancing petroleum hydrocarbon degradation. One such option is the use of poultry and piggery manure as biostimulation agents. Even though, these techniques do not have adequate literatures on the potential use of animal manures as biostimulating agents, few works such as Ijah and Antai (2003); Okolo et al. (2005) and Yakubu (2007) have investigated the potential of these two different manures in the cleanup of soil contaminated with petroleum hydrocarbons and were found to enhance petroleum hydrocarbon biodegradation in a polluted environment. Nevertheless, their rate of biodegradation performance has not been evaluated and compared with inorganic chemical fertilizer. Furthermore, there is no literature or data on the use of goat manure in the biodegradation of petroleum hydrocarbon in a contaminated environment. Therefore, the purpose of the present study was to evaluate and compare the biodegradation performance of the application of compost inorganic chemical fertilizer (Urea) and Poultry Manure, in the bioremediation of soil contaminated with petroleum hydrocarbon.

According to Agarry et al. (2010), a combination of treatments consisting of the application of poultry manure, piggery manure, goat manure, and chemical fertilizer was evaluated in situ during a period of 4 weeks of remediation. Each treatment contained petroleum hydrocarbon mixture (kerosene, diesel oil, and gasoline mixtures) (10% *w/w*) in soil as a sole source of carbon and energy. After 4 weeks of remediation, the results showed that poultry manure, piggery manure, goat manure, and NPK (nitrogen, phosphorous, and potash [potassium]) fertilizer exhibited 73%, 63%, 50%, and 39% total petroleum hydrocarbon degradation, respectively. Thus, all the biostimulation treatment strategies showed the ability to enhance petroleum hydrocarbon microbial degradation. However, poultry manure, piggery manure, and goat manure

treatments showed greater petroleum hydrocarbon reductions than NPK fertilizer treatment. A first-order kinetic equation was fitted to the biodegradation data and the specific degradation rate constant (*k*) values obtained showed that the order of effectiveness of these biostimulating strategies in the cleanup of soil contaminated with petroleum hydrocarbon mixtures (mixture of kerosene, diesel oil, and gasoline) is NPK fertilizer < goat manure < piggery manure < poultry manure. Their work has indicated that the application of poultry manure, piggery manure, goat manure, and chemical fertilizer could enhance petroleum hydrocarbon degradation with poultry manure, showing a greater effectiveness and thus could be one of the severally sought environmentally friendly ways of remediating natural ecosystem contaminated with crude oil. This result was also confirmed by Daniel (2011) in his work of bioremediation process.

Moore, Cooper and Kröger (2007) in their search on practices aimed at decreasing nutrient contributions to receiving aquatic ecosystems as a form of remediating the aqua ecosystem. They examined the use of rice (*Oryza sativa*) for luxury uptake of nitrogen and phosphorus components associated with agricultural storm runoff. Mesocosms (379 L) planted with rice were exposed to two concentrations (5 and 10 mg/L) of nitrate, ammonia, and ortho-phosphorus. Results from these mesocosms were compared to un-vegetated controls (also amended with 5 or 10 mg/L nitrate, ammonia, and orthophosphorus) to determine efficiency of rice in remediating nutrient runoff. Statistically significant differences in ammonia and nitrate retention of vegetated mesocosms amended with 5 mg/L versus vegetated mesocosms amended with 10 mg/L were noted after the first exposure. Although rice is a nutrient-dependent aquatic plant, this study suggests that more efficient mitigation is possible at lower inflow concentrations as opposed to higher inflow concentrations.

2.2 Biodegradation of Naphthalene in Marine Environment

Soniassy et al. (1994) in their work found that, polycyclic aromatic hydrocarbons (PAHs) are considered as important environmental pollutants, of which latter Garcıa et al. (1998), finds that, the marine environment is subjected to contamination by PAHs from a variety of sources, mainly of anthropogenic nature as well as by large-scale oil spills. In quantitative terms, crude oil is one of the most important sources of contamination and it has been estimated that such pollution represents between 1.7 and 8.8 × 106 tons of petroleum hydrocarbons that impact marine waters and estuaries annually, Head and Swannell (1999). Naphthalene is a PAH that has been classified as a potential human carcinogen by international agencies (the International Agency for Research on Cancer [IARC], the US Environmental Protection Agency [US EPA], and the Deutsche Forschungs Gemeinschaft [DFG]) , Preuss et al. (2003). Naphthalene is among the most toxic components in the water-soluble fraction of crude oils and has been shown to be concentrated in vertebrate and invertebrate marine organisms, Sharanagouda and Karegoudar (2001). Its toxicity, together with its chemical persistence, means that this compound is extremely dangerous as an environmental contaminant, Bamforth and Singleton (2005). Biological methods of treatment have proved to offer good alternatives for the degradation of naphthalene. Several reports on the naphthalene degradation by different microorganisms have appeared, Manohar and Karegoudar (1998), Abou and Maachi (2003),.

Feijoo-Siota et al. (2008), in their work to find the biodegradation of naphthalene in sea water finds that, immobilized cells can be stored at 4^{o}c for 1 month without loss of viability. The biodegradation was highly affected by the availability of nitrogen and phosphorous, so at 30^{0}c a

naphthalene concentration of 25mM was almost completely degraded (93%) by free cells in 6 days in samples supplemented with these nutrients, whereas only 42% naphthalene was consumed in the non supplemented samples. Biodegradation was much slower at 16°C than at 30°c; after 6 days of culture at 30°c, almost all naphthalene was depredated by free and immobilized cells, whereas only 22% and 34% at 16°c, respectively. The degradation rate remained unaffected when the naphthalene concentration was reduced from 25 to 10 mM. Alginate of three different viscosities was used for immobilization of cells. After 7 days of culture, beads formed with 31.4 cP alginate were fragmented, whereas beads formed with 240 and 3600 cP did not display structural changes and afforded the same degradation rate. Beads formed with high-viscosity alginate retained cells more efficiently.

2.3 Options for In situ remediation of soil contamination

However, situations where contamination is closed to residential, then an *ex situ* remediation method is mostly appropriate. A combination of features of the sites makes the study of bioremediation an interesting case for the exploration of remediation options. Lee et al. (1998) in their study, finds that, their study area was firstly, a 45,000 m^3 site being situated close to residential areas, hence an in situ remediation solution was desired by the community, the government regulator, and the industry. Secondly, the contaminants were at high concentrations, suggesting that the contaminant matrix was highly toxic. Thirdly, and most importantly, the contaminant profile was mixed. Perchloroethene (PCE) has been the subject of countless abiotic and biological investigations into its in situ degradation, thereby becoming a mainstay of the bioremediation industry, Lee et al. (1998), Smidt and de Vos, (2004). Similarly, the degradation of hexachlorobenzene (HCB) has received extensive attention and is listed in the Stockholm

Convention. In contrast hexachloro-1,3-butadiene (HCBD), although widely distributed globally, appears rarely in the abiotic or biological degradation literature (Booker and Pavlostathis 2000; Bosma et al. 1994).

Environments contaminated with mixtures of chlorinated hydrocarbons represent a formidable challenge for bioremediation because biodegradation of all components of the mixture must be demonstrated. In the study of Adrian et al. (2007), a soil site contaminated with hexachloro-1,3-butadiene (HCBD), hexachlorobenzene (HCB), and perchloroethene (PCE) was investigated. Environmental parameters (including toxicity) and microbial community composition were characterized. The lack of scientific literature on HCBD biodegradation led to attempts to develop HCBD-respiring enrichment cultures and to test the hypothesis that known PCE-degrading cultures could dechlorinate HCBD. No HCBD dechlorination was observed. An alternative approach, using electron shuttles to degrade the mixture of chlorinated hydrocarbons, was compared with the activity of zero-valent iron. The authors conclude that electron shuttles offer promise for the in situ treatment of mixtures of chlorinated hydrocarbons which was purely a bioremediation method.

2.4 Removal of Heavy metals by bioremediation influenced by fertilizer application

According to Lombi et al. (2001), a large number of sites worldwide are contaminated by heavy metals as a result of human activities. The industrial revolution in the developed countries of Europe and America after the Second World War and the ever increasing mining activities in Asia and Africa led to heavy metal pollution of soils Cordon and Jules (1976). Despite increase in the public awareness and attention on environmental pollution in recent times, very scanty information is available on heavy metal pollution of the tropical ecosystem and its effects.

Apart from the in situ values of these heavy metals in the soil as a result of the underlying parent material and the pedogenic processes in soil formation, these chemicals may rise to toxicity levels through continuous human activities and indiscriminate use of pesticides and other agrochemicals, Zhu and Alva (1993), dumping of industrial and municipal wastes or sludge, Kuzel et al. (1994), Preer et al. (1995). The menace of soil pollution is a global problem, which causes serious concern from major stakeholders in environmental, agricultural, and health sectors.

Unraveled the contamination of soils by effluents from industries is on the increase. There is the possibility of remediating these contaminated soils through the use of certain plants. Adewole, et al. (2010), investigated the remediating ability of *Helianthus annuus* and *Tithonia diversifolia* on the soil polluted with effluents from a paint industry in Ibadan, Nigeria. The experiment consisted of three treatments (*H. annuus, T. diversifolia*, and control) each replicated three times in a factorial combination of four different fertility managements, viz mineral fertilizer (MF); Grade A organomineral fertilizer (OMF); control plants without fertilizer application; and contro, where no fertilizer and no crop was planted using randomized complete block design. A total of 12 plots of 2×4 m^2 each per phytoplant were obtained. Each plot was planted with the viable seeds of the phytoplant at a spacing of 60×30 cm^2 and at the seed rate of four seeds per hole. The seedlings were thinned to two stands per hole two weeks after planting (WAP) and also weeded two times (2 and 5 WAP). They found that, after in situ second successive cultivation, percentage removal of heavy metals by *Helianthus annuus* with MF and OMF, respectively, were Cu 32.5 and 41.6; Pb 30.3 and 42.8; and Cd 44.5 and 56.7. *Tithonia diversifolia*, similarly, removed, respectively, Cu 16.9 and 23.4; Pb 36.9 and 43.7; and Cd 20.1 and 35.1. Lower

percentages were removed in the controls where no fertilizer was applied. In the shoot of *H. annuus* with OMF, significantly ($p < .05$) higher values of 0.27, 1.72, and 0.11 mg kg^{-1} of Cu, Pb, and Cd, respectively, were removed and stored at second cultivation as against 0.21, 3.39 and 0.08mg kg−1 in the shoot of *T diversifolia*. Lower values of Cu, Pb, and Cd were removed with MF, and also at first cultivation with OMF and MF. This study therefore recommends the use of sunflower plants, whether hybrids or wild-types along with the application of OMF for the effective remediation of soils contaminated with heavy metals, particularly in tropical climate.

2.5 Comparison of substrates for removal of heavy metals

Phytoremediation, an emerging technology that uses plants to remove heavy metals from the environment, finds to be cost-effective and environmentally friendly technique and appears to be a promising alternative, Lopez et al. (2009). Phytoextraction relies on the ability of plants to translocate contaminants from their roots to the above-ground biomass for storage, Morikawa and Erkin (2003) and involves the fundamental processes of mobilization, sorption, uptake, translocation, and sequestration, Johnson et al. (2009). Environmental impacts associated with the exploration and exploitation of crude oil, Nwaichi et al. (2010) has been of utmost concern in Nigeria in the recent time. Agricultural application of amendments restores degraded or marginal soils, offers the possibility of recycling nitrogen (N), phosphosrus (P), and other nutrients. Elemental analysis has revealed that spectra due to metal elements such as Ca Fe, Mg, Cu, Cd, Zn, Na, Ni, K, and Mo were recorded using laser-induced breakdown spectroscopy (LIBS) technique, Gondal et al. (2006) in crude oil. The presence or absence of mineral elements influences the physical characteristics and agricultural quality of a soil. However, the concentration and availability of potentially toxic elements in amendments represent a risk of

soil contamination. Another problem arising from the land application of urea is the potentially phytotoxic nature of the compounds generated as a result of the intense organic matter mineralization, such as ammonia, ethylene oxide, low-molecular-weight organic acids, or organic pollutants such as phenolic compounds. In the soil, it hydrolizes to ammonia and carbon dioxide. The ammonia is oxidized by bacteria in the soil to nitrate, which can be absorbed by the plants. Urea is also used in many multi component solid fertilizer formulations. Urea is highly soluble in water and is, therefore, also very suitable for use in fertilizer solutions (in combination with ammonium nitrate, e.g., in "foliar feed" fertilizers). For fertilizer use, granules are preferred over prills because of their narrower particle size distribution, which is an advantage for mechanical application. The most common impurity of synthetic urea is biuret, which impairs plant growth. Another thing to be aware of with chemical fertilizers is the kind of nutrients they contain and the way these nutrients are extracted. For example, the kind of nitrogen typically found in chemical fertilizers dissolves very quickly in water, unlike their organic counterpart. This means that excess nitrogen may find its way into groundwater and freshwater sources and contaminate the water. *Mucuna pruriens* has been used in urea-amended soils, Nwaichi et al. (2009) and as cover crops, Eteka et al. (1998) in stressed environments. There are no such reports with *Sphenostylis sternocarpa* hence the benchmark evaluation. Clear comparison, however, need to be drawn among selected amendments for more effective phytoextraction designs using these species.

In the study of Nwaichi et al. (2010), Cadmium (Cd) solubilization in soil and uptake by *Mucuna pruriens* var. pruriens and *Sphenostylis stenocarpa* was used in response to the chicken manure and urea fertilizers application types. In thier study, 0.8 g each of the amendments was applied to

petroleum-contaminated soil in a pot experiment. Results indicate that the chicken manure application at fourteen (14) days before planting gave significantly higher shoot dry matter than its urea counterpart under conditions of Cd stress. Chicken manure application resulted in less Cd solubilization as compared with urea fertilizer dosing. The chicken manure application also significantly increased the shoot Cadmium accumulation despite its lesser effect on Cadmium solubilization; thus, it is expected to minimize the risk of groundwater contamination. Chicken manure amended treatment showed greater Cadmium tolerance for the two species investigated and *S. stenocarpa* did not support Cadmium phytoextraction. Although the amendments gave marked reduction in Cadmium phtotoxicity, those of the urea fertilizer gave only rapid, but short, growth support.

2.6 Bacterial Community Dynamics and Hydrocarbon Degradation during a Field-Scale Evaluation of Bioremediation

From the work of Ian et al. (2004). A field-scale experiment with a complete randomized block design was performed to study the degradation of buried oil on a shoreline over a period of almost one year. Four treatments were examined in three replicate blocks: two levels of fertilizer treatment of oil-treated plots, one receiving a weekly application of liquid fertilizer and the other treated with a slow-release fertilizer; and two controls, one not treated with oil and the other treated with oil but not with fertilizer. Oil degradation was monitored by measuring carbon dioxide evolution and by chemical analysis of the oil. Buried oil was degraded to a significantly greater extent in fertilized plots, but no differences in oil chemistry were observed between the two different fertilizer treatments, although carbon dioxide production was significantly higher in the oil-treated plots that were treated with slow-release fertilizer during the first fourteen (14)

days of the experiment. Bacterial communities present in the beach sediments were profiled by denaturing gradient gel electrophoresis (DGGE) analysis of PCR amplified 16S rRNA gene fragments and 16S rRNA amplified by reverse transcriptase PCR. Similarities between the DGGE profiles were calculated, and similarity matrices were subjected to statistical analysis. It was discovered that although significant hydrocarbon degradation occurred both in plots treated with oil alone and in the plots treated with oil and liquid fertilizer, the bacterial community structure in these plots was, in general, not significantly different from that in the control plots that were not treated with oil and did not change over time. In contrast, the bacterial community structure in the plots treated with oil and slow-release fertilizer changed rapidly, and there were significant differences over time, as well as between blocks and even within plots. The differences were probably related to the higher concentrations of nutrients measured in interstitial water from the plots treated with slow-release fertilizer. Bacteria with 16S rRNA sequences closely related (>99.7% identity) to *Alcanivorax borkumensis* and *Pseudomonas stutzeri* sequences dominated during the initial phase of oil degradation in the plots treated with slow-release fertilizer. Field data were compared to the results of previous laboratory microcosm experiments, which revealed significant differences.

2.7 Quantifying Microbial Utilization of Petroleum Hydrocarbons in Salt Marsh Sediments by Using the ^{13}C Content of Bacterial rRNA_

Natural remediation of oil spills is catalyzed by complex microbial consortia. Dekas et al. (2008) took a whole community approach to investigate bacterial incorporation of petroleum hydrocarbons from a simulated oil spill. They utilized the natural difference in carbon isotopic abundance between a salt marsh ecosystem supported by the ^{13}C-enriched C_4 grass *Spartina*

alterniflora and ^{13}C depleted petroleum to monitor changes in the ^{13}C content of biomass. Magnetic bead capture methods for selective recovery of bacterial RNA were used to monitor the ^{13}C content of bacterial biomass during a two-week experiment. The data show that by the end of the experiment, up to 26% of bacterial biomass was derived from consumption of the freshly spilled oil. The results contrast with the inertness of a nearby relict spill, which occurred in 1969 in West Falmouth, MA. Sequences of 16S rRNA genes from thier experimental samples also were consistent with previous reports suggesting the importance of *Gamma-* and *Deltaproteobacteria* and *Firmicutes* in the re-mineralization of hydrocarbons. The magnetic bead capture approach makes it possible to quantify uptake of petroleum hydrocarbons by microbes in situ. Although employed here at the domain level, RNA capture procedures can be highly specific. The same strategy could be used with genus-level specificity, something which is not currently possible using the ^{13}C content of biomarker lipids.

Recently Nikita et al. (2011), sample a significant portion of oil from the recent Deepwater Horizon (DH) oil spill in the Gulf of Mexico and transported to the shoreline, where it may have severe ecological and economic consequences. Thier objectives were (i) to identify and characterize predominant oil-degrading taxa that may be used as model hydrocarbon degraders or as microbial indicators of contamination and (ii) to characterize the *in situ* response of indigenous bacterial communities to oil contamination in beach ecosystems. This study was conducted at municipal Pensacola Beach, FL, where chemical analysis revealed weathered oil petroleum hydrocarbon (C_8 to C_{40}) concentrations ranging from 3.1 to 4,500 mg kg^{-1} in beach sands. A total of twenty four (24) bacterial strains from fourteen (14) genera were isolated from oiled beach sands and confirmed as oil-degrading microorganisms. Isolated bacterial strains were

primarily *Gammaproteobacteria*, including representatives of genera with known oil degraders (*Alcanivorax*, *Marinobacter*, *Pseudomonas*, and *Acinetobacter*). Sequence libraries generated from oiled sands revealed phylotypes that showed high sequence identity (up to 99%) to rRNA gene sequences from the oil-degrading bacterial isolates. The abundance of bacterial SSU rRNA gene sequences was 10-fold higher in oiled (0.44-107 to 10.2-107 copies g) versus clean (0.024-107 to 1.4-107 copies g) sand. Community analysis revealed a distinct response to oil contamination, and SSU rRNA gene abundance derived from the genus *Alcanivorax* showed the largest increase in relative abundance in contaminated samples. Hence, they conclude that oil contamination from the DH spill had a profound impact on the abundance and community composition of indigenous bacteria in Gulf beach sands, and our evidence points to members of the *Gammaproteobacteria* (*Alcanivorax*, *Marinobacter*) and *Alphaproteobacteria* (*Rhodobacteraceae*) as key players in oil degradation there.

2.8 Microbial Population Dynamics Associated with Crude-Oil Biodegradation in Diverse Soils

In the work of William et al. (2006), Soil bacterial population dynamics were examined in several crude-oil-contaminated soils to identify those organisms associated with alkane degradation and to assess patterns in microbial response across disparate soils. Seven soil types obtained from six geographically distinct areas of the United States (Arizona, Oregon, Indiana, Virginia, Oklahoma, and Montana) were used in controlled contamination experiments containing 2% (wt/wt) crude oil spiked with ($^{1-}$ ^{14}C) hexadecane. Microbial populations present during hydrocarbon degradation were analyzed using both 16S rRNA gene sequence analysis and by traditional methods for cultivating hydrocarbon-oxidizing bacteria. After a 50-day incubation, all seven soils showed comparable hydrocarbon depletion, where >80% of added

crude oil was depleted and approximately 40 to 70% of added (^{14}C) hexadecane was converted to $^{14}CO2$. However, the initial rates of hydrocarbon depletion differed up to 10-fold, and preferential utilization of shorter-chain-length *n*-alkanes relative to longer-chain-length *n*-alkanes was observed in some soils. Distinct microbial populations developed, concomitant with crude-oil depletion. Phylogenetically diverse bacterial populations were selected across different soils, many of which were identical to hydrocarbon-degrading isolates obtained from the same systems (e.g., *Nocardioides albus*, *Collimonas* sp., and *Rhodococcus coprophilus*). In several cases, soil type was shown to be an important determinant, defining specific microorganisms responding to hydrocarbon contamination. However, similar *Rhodococcus erythropolis*like populations were observed in four of the seven soils and were the most common hydrocarbon-degrading organisms identified via cultivation.

2.9 Statistical Application of Randomized Block Design and the use of ANOVA

Lan et al. (2011), in their work "Comparison of dynamic block randomization and minimization in randomized trials", they agreed that, minimizing the imbalance of key baseline covariates between treatments is known to be very important to the precision of the estimate of treatment effect in clinical research. Dynamic randomization allocation techniques have been used to achieve balance across multiple baseline characteristics. However, empirical data are limited on how these techniques compare in terms of balance and efficiency. Hence they were motivated by a newly funded randomized controlled trial, in which they have the option of choosing between two methods of randomization at the subject level: (1) randomizing individual subjects consecutively as they are enrolled, using Pocock and Simon's minimization method, and (2) simultaneously randomizing blocks of subjects once all subjects in a block have been enrolled,

using a balance algorithm originally developed for cluster randomized trials. The main purpose of their work was to compare dynamic block randomization and minimization in terms of balance on baseline covariates and statistical efficiency. Simple randomization was included as a reference. Their result demonstrates that dynamic block randomization outperforms minimization with regard to achieving balance and maximizing efficiency. Nevertheless, the differences across the three randomization strategies were modest. They also found that, statistical advantages associated with dynamic block randomization need to be considered in relation to the planned sample size and the practical issues for its implementation in deciding the preferred method of randomization for a given trial (e.g., the time required to accrue blocks of subjects of adequate size as balanced against the need to commence intervention/treatment immediately in those randomized to that experimental condition).

Moreover, Christof et al. (2001), also study the Relationship of ANOVA models with random effects. Their article shows how different experimental designs arise out of the variation of three basic distinctions: block versus treatment factors, fixed versus random factors, and crossed versus nested factors. Once it is understood how each distinction influences the statistical analysis, the amount of experimental designs can be considerably reduced, because sometimes seemingly different experimental designs are essentially equivalent. This was shown by an example comparing a two-way analysis of variance model to a three-factor partially nested design. Furthermore, the way each distinction influences the statistical analysis of an experimental design can simplify the computational effort of the analysis because virtually every basic ANOVA procedure implemented in common statistical software packages can be used to fit more complex ANOVA models that are usually analyzed using special computer modules. They conclude that, when claiming that the distinctions between block and treatment factors as

well as between crossed and nested factors determine to a large extent the model formula, they referred to the way in which ANOVA models are usually presented and explained in textbooks. For instance, if Factor B is nested within Factor A, the interaction between these two factors is not included in the model formula. Likewise, the model formula for a randomized complete block design does not contain effects for the interaction between blocks and treatments if there are no replications for each block-treatment combination, as is usually the case. Therefore, the treatment structure together with the block structure of an experimental design readily leads to a standard model formula, which is used to partition the total sums of squares. For this reason, the presentation of model formulas in experimental designs is sometimes completely omitted in textbooks and the discussion focuses only on building F ratios for testing standard hypothesis. This practice has two serious disadvantages. First, seeing ANOVA merely from a hypothesis-testing perspective can lead to a superficial understanding of the statistical technique. It becomes difficult to see how designs with different labels are sometimes related to each other. Second, when dealing with more complex situations for instance, designs having many factors and/or unbalanced designs the modeling perspective on ANOVA is, in our opinion, more sensible because it encourages formulating a model for the data. Substantive considerations can be taken into account. For instance, if factors do not have equal status in an experiment or an observational study, it might be worthwhile fitting a hierarchical analysis of variance for unbalanced data. If many factors are involved, it might be known, perhaps from earlier research or pilot studies, that certain interactions between factors are negligible. In these cases, it would be unreasonable to rely on a standard model including all interactions.

Reed (2003), also in his study of ANOVA, considers a study in which 2 new treatments are being compared with a control group. One way to compare outcomes would simply be to compare the 2 treatments with the control and the 2 treatments against each using 3 student *t* tests (*t test*). If we were to compare 4 treatment groups, then we would need to use 6 *t* tests. He finds that, the difficulty with using multiple *t* tests is that as the number of groups increases, so will the likelihood of finding a difference between any pair of groups simply by change when no real difference exists by definition a Type I error. If we were to perform 3 separate *t* tests each at $\alpha = .05$, the experimental error rate increases to .14. As the number of multiple *t* tests increases, the experiment-wise error rate increases rather rapidly. The solution to the experimental error rate problem is to use analysis of variance (ANOVA) methods. Three basic ANOVA designs are reviewed that give hypothetical examples drawn from the literature to illustrate single-factor ANOVA, repeated measures ANOVA, and randomized block ANOVA.

2.10 The Birth of Randomization from Fisher

The importance of the analysis of variance procedure in the development of Fisher's ideas of experimental design becomes apparent from "Studies in Crop Variation. Fisher and Mackenzie (1923), in which the analysis of variance was made explicit, and the analysis of variance table appeared for the first time. In introducing this method of analysis, Fisher made it conditional on randomization. The experiment was essentially run in triplicate. Having divided the sums of squares of all the deviations from the general mean into two parts, one measuring the variation between parallel plots within triplicates similarly treated and the other the variation between means of the triplicates differently treated, he wrote, "*If all the plots are un- differentiated, as if the numbers had been mixed up and written down in random* order", then the average value of

each of the two sums of squares would be proportional to the number of their respective degrees of freedom. The statement rested on Fisher's understanding of the underlying distribution theory. His justification could have relied directly on normal theory assumptions if he had been prepared to assume that the observations had been drawn independently from a normally distributed population. But the assumption of independence was obviously not justified in any ordinary field experiment; observations of the fertility of adjacent plots were known to be not independent but highly correlated. Fisher perceived, however, that the random allocation of plot treatments would simulate the effect of independence in the distribution of the variance ratio, so that the analysis of variance and test of significance appropriate under normal theory assumptions would be approximately valid, provided the allocation of treatments to plots had been made deliberately at random. Fisher tested this result informally, using data from uniformity trials. His confidence in the result, however, depended on the geometric representation that was by then second nature to him. The author could picture the distribution of *n* results as a pattern in *n*-dimensional space, and he could see that randomization would produce symmetry in that pattern rather like that produced by a kaleidoscope, and which approximated the required spherical symmetry available, in particular, from standard normal theory assumptions. Thus, Fisher's first principle of experimental design arose at least partly from considerations not readily accessible to experimental scientists. The principle tied together what was done in the field and what could be learned from analysis of the results in a single logical package. What had been an empirical art of the experimenter was thus brought into the domain of the statistician; the role of the statistician was necessarily extended from the analysis of data to embrace the whole conduct of experimental inquiry. Randomization was not readily accepted either by the mathematicians or by the experimenters, Box (1978).

Russell (1926) referred to it as "a further refinement now being introduced at Rothamsted"; he then gave a correct argument for randomization as the guarantee of the validity of the estimate of error. But he continued: "In practice this (randomization) is impossible. A compromise has to be made between what is desirable and what is practicable. The best practicable arrangement is to have as many repetitions as there are treatments, to set the plots out in chessboard fashion in a 'Latin Square'." From Russell's earlier discussion and examples, it is clear that the Latin square was acceptable to him because, randomization or no, it retained the principle of balance in the design. His aim was to make the experiment as precise as possible. Therefore he advocated systematically balanced designs that could counteract fertility trends of the field. His simplest example was a row crop with two treatments applied to strips of the field in a sandwich arrangement ABBAABBA. With more numerous treatments of a row crop, each replication was differently ordered, but in such a way as to keep the average distance between strips that had been similarly treated the same for all treatments. The same principle was extended to a two-way arrangement of plots in a chess- board design, so that similar treatments should never be put closer to each other than necessary. With such designs the real error was usually reduced. Arguing for randomization, Fisher (1926) pointed out that if the systematic arrangement resulted in smaller real errors, it must also result in an inflated estimate of error; if it resulted in larger real errors, the estimate of error would be correspondingly diminished. In either case the false estimate of error would be liable to vitiate the conclusions drawn from the experiment. As Fisher put it in correspondence, the experimenter games with the devil; he must be prepared by his layout to accommodate whatever pattern of soil fertilities the devil may have chosen in advance. A systematic arrangement is prepared to deal only with a certain sort of devilish plan. But the

devil may have chosen any plan, even the one for which the systematic arrangement is least appropriate. To play this game with the greatest chance of success, the experimenter cannot afford to exclude the possibility of any possible arrangement of soil fertilities, and his best strategy is to equalize the chance that any treatment shall fall on any plot by determining it by chance himself. Then if all the plots with a particular treatment have higher yields, it may still be due to the devil's arrangement, but then and only then will the experimenter know how often his chance arrangement will coincide with the Design of Experiments (1935) opens with an example: A lady declares that by tasting a cup of tea made

with milk she can discriminate whether the milk or the tea infusion was first added to the cup. We will consider the problem of designing an experiment by means of which this assertion can be tested. The example was actually taken from Fisher's experience at tea time one day at Rothamsted in 1921 or 1922, when he drew a cup of tea from the urn, then added the milk and offered the cup to Bristol. The author refused it, maintaining that it made a difference if the milk was added first. At William Roach's suggestion, they proceeded at once to test her assertion. Roach prepared the tea cups, and recalls with pride the overwhelming success of the lady (who became his wife soon after), but he does not mention randomization. In contrast, Fisher's (1935) discussion emphasized, among the first considerations in this first example of design, that randomization, "'the physical basis of the validity of the test," was "'the essential safeguard" contained in the experimental procedure for the validity of the estimate of error, and thus for the test of significance by which the result of the experiment was to be judged. Moreover, he included similar discussions of randomization in relation to each class of design introduced later in the book. In 1926 it remained to be seen whether a randomized design could be as precise as a systematic one. That year Eden was running the first randomized block design; Fisher used it as

an example even before the results were known. On analysis (Eden and Fisher 1927), this experiment proved admirably precise. Despite this success, many of the older experimenters continued to doubt the accuracy of randomized de- signs. As late as 1936, W.S. Gosset ("Student" 1936) expressed the view that "Since the tendency of deliberate randomization is to increase the error, a balanced arrangement like the half-drill (a systematic sandwich design) is best."

2.11 Replication and Blocking

The need for replication was widely acknowledged before Fisher clarified its essential role. As Russell (1926) put it, "Variations in soil can be overcome only by repeating the experiment on the same field at the same time. This is now well recognized, and duplicate experiments have long been the rule." Russell's examples of strip and chessboard designs actually contain triplicates of each treatment. Three or four replications were usual in contemporary designs. Eden's design (Fisher and Mackenzie 1923) was conceived as triple replication, but was not wholly so. The aim was to inquire into the response of 12 different potato varieties to either of two potash fertilizers, with or without farmyard manure. The field was first divided into two equal areas, one of which received farmyard manure. Each half was then divided into 36 plots on which the 12 varieties were planted in triplicate in a chessboard arrangement. Finally, the plots were subdivided into three patches, which received either the basal dressing only or the basal dressing with either sulfate or chloride of potash. Some of the disadvantages of the design were obvious. When Gosset saw the analysis, he wrote to Fisher, "The experiment seems to me to be quite badly planned; you should give them a hand in that." In taking Gosset's advice, Fisher showed how many lessons in design such an experiment had to teach. In his initial analysis of variance, Fisher made the mistake of using a single estimate of error for all the comparisons. He

quickly discovered where he had gone wrong and published the correct analysis in Statistical Methods for Research Workers (1925). Carefully explaining his reasoning, he derived the separate estimates of error and made the separate analyses required for the plots and for the patches. He considered data from one half of the field only, because without replication of the treatment on half- fields, no applicable estimate of error could be de- rived by which to assess the effect of farmyard manure.

Thereafter (1935), the author said of replication that "its main purpose, which there is no alternative method of achieving, is to supply an estimate of error." Eden's experiment was not run as a randomized block design. None of Russell's (1926) examples was of this kind. Russell valued designs like the Knut-Vik systematic square and Fisher's randomized Latin squares because of the supposed increased precision obtained by balance. The advantages of block designs were not apparent until, with the coming of the analysis of variance, it became possible to isolate the variance to be ascribed to block differences and see how it could be eliminated in the analysis (Fisher 1935). With Fisher's randomized Latin squares the variance could be eliminated in two directions at once, but these designs were restricted by the requirement that the number of replications had to equal the number of treatments compared. Randomized block designs, however, were not similarly restricted. Each block could be a small and compact arrangement on relatively homogeneous land, and the number of replications on different blocks could be increased to any desired amount without adversely affecting the precision of the experimental results. Fisher's (1926) example used eightfold replication in blocks of 12 treatments each. In Design of Experiments (1935) Fisher explained the ways in which a larger number of replications in blocks served to diminish the error.

2.12 Collaboration in the Field

A report (Eden and Fisher, 1929) on a series of manurial trials on potatoes tells its own story of the interplay between practical needs and theoretical solutions in the rapid development of ideas on the design of agricultural experiments. In 1925 two experiments were run, a systematic 4 x 4 square to test qualitative differences, and a systematic arrangement of an incomplete factorial, in four blocks or strips running the length of the field, to test quantitative differences. In 1926 no change was made in the square design. In the block design, although there were still only four blocks, they were arranged to quarter the field; they contained a complete factorial of 16 treatments, and the treatments were randomized in each block. The results of this experiment, though improved, were rather inaccurate. More replications and smaller blocks were needed, but practical considerations forbade the use of a larger number of plots. The problem was overcome by amalgamating the two experiments. In 1926 there had been 80 plots in all, 16 in the square and 64 in the four blocks of 16. In 1927 there were 81 plots, with nine blocks of 9 plots each. For this nine fold replication, the highest of the four levels of dressing both with nitrogen and with potash was dropped; there were now three levels of ammonium sulfate dressing to be tested at three levels of potash of three kinds: sulfate, muriate, and low-grade salt. Fisher treated the three levels of nitrogen and potash (the quantitative factors) as the basic block of nine treatments. He explained: The actual position of a plot considered only as representing potash and nitrogen interactions was determined entirely by chance. The element of chance also operated largely in the disposition of the qualitative factor, but there was one restriction. The restriction provided that any particular variety of potash manure should occur in the total of the nine blocks in conjunction with every amount of nitrogen three times. In every other way the distribution was at random. In 1929, he pointed out that the amount of replication varied with each factor or

interaction of factors concerned and listed the number of replications for each of the eight classes of comparison. He did not explain how he had arrived at this layout. The actual scheme of randomization was not important to his readers. It might merely have bewildered them to be told that it was in fact a 9 x 9 Latin square whose rows appear as the nine experimental blocks (treatment combinations at three levels of phosphate and three levels of nitrogen) and whose columns appear as the nine other factorial arrangements (of the three sorts of phosphate at three levels of nitrogen), which are necessarily orthogonal not only to each other but also to the blocks. Even today it comes as a surprise that so complex a design was planned the same year that Fisher's Latin square and factorial designs first saw print. What was important to his readers was to show how "The large and complex type of experiment finally adopted thus supplied more precise information on both heads (qualitative and quantitative) than could previously be obtained, and in addition to a more thorough exploration of the different combinations possible". The size and complexity of the new designs was, at first, their most embarrassing feature. Russell (1926) declared "no experiment should involve more than four or five" treatments. Of the 22 factorial in duplicate, he wrote: "The set involves 16 plots, but the agricultural operations can be managed without much difficulty"; and of the Latin square: "Obviously the method requires a considerable number of plots. Its use at Rothamsted necessitates special arrangements for harvesting, thrashing, weighing and recording, which, however, are too intricate to be dealt with here". Nevertheless, Russell gave Eden, and Fisher, a free hand, and allowed their complex designs to be ran on Rothamsted Farm. Although doing so entailed many adjustments in field operations, the practical running of the experiments proved quite manageable, and in time the extra trouble came to seem a small price to pay for the highly satisfactory results.

CHAPTER 3

METHODOLOGY

3.0 Introduction

This chapter presents the mathematical methodology used for the analysis of the data gathered from the field of experiment, Moreover, the methodology also deals with the procedural measures and routine the experiment followed in the laboratory and on the field of experiment.

3.1 Factorial Design of Experiment

An experiment is just a test or series of tests. Experiments are performed in all engineering and scientific disciplines and are an important part of the way we learn about how systems and processes work. The validity of the conclusions that are drawn from an experiment depends to a large extent on how the experiment was conducted. Therefore, the design of the experiment plays a major role in the eventual solution of the problem that initially motivated the experiment.

Experiments may include two or more factors that the experimenter thinks may be important. The factorial experimental design is as a powerful technique for this type of problem. Generally, in a factorial experimental design, experimental trials (or runs) are performed at all combinations of factor levels.

Most of the statistical concepts used for single-factor experiments can be extended to the factorial experiments. The analysis of variance (ANOVA), in particular, is continued to be used as one of the primary tools for statistical data analysis.

3.2 Analysis Of Data

The type of statistical analysis employed in factorial design is analysis of variance (ANOVA). Computationally, statistical software packages such as GENSTAT; SAS; PROC.ANOVA; MINITAB; EXCELL; R-Gui are usually employed for the analysis of variance. However, for conceptual and theoretical knowledge and understanding of the method of analysis of two-factor factorial design being used, manual process is required.

Table 3.1: A generalized arrangement of a two factor factorial design

Factor A	Factor B: 1	2	...	b
1	$y_{111}, y_{112}, \ldots, y_{11n}$	$y_{121}, y_{122}, \ldots, y_{12n}$		$y_{1b1}, y_{1b2}, \ldots, y_{1bn}$
2	$y_{211}, y_{212}, \ldots, y_{21n}$	$y_{221}, y_{222}, \ldots, y_{22n}$		$y_{2b1}, y_{2b2}, \ldots, y_{2bn}$
⋮				
a	$y_{a11}, y_{a12}, \ldots, y_{a1n}$	$y_{a21}, y_{a22}, \ldots, y_{a2n}$		$y_{ab1}, y_{ab2}, \ldots, y_{abn}$

Furthermore, the observations in factorial experiment can be described by a model. Dealing with two factors design, illustrated in Table 3.1. The equation for each observation can be written as the means model:

$$\boldsymbol{y_{ijk}} = \mu_{ij.} + \boldsymbol{E_{ijk}}, \quad \text{----------} \quad 3.1$$

where $\boldsymbol{i = 1, 2, \ldots a}$, $\boldsymbol{j = 1, 2, \ldots b}$ and $\boldsymbol{k = 1, 2, \ldots n}$.

Also, $\boldsymbol{E_{ijk}}$= the residual or random error (that is, measures of deviations of the observed values ($\boldsymbol{y_{ijk}}$) in the (ij)th cell from the population mean effect for the (ij)th cell, $\boldsymbol{\mu_{ij.}}$ **.** The population mean effect for the (ij)th cell, $\boldsymbol{\mu_{ij.}}$, can also be expressed as:

$$\mu_{ij.} = \mu + \boldsymbol{\alpha_i} + \boldsymbol{\beta_j} + (\propto \boldsymbol{\beta})_{ij}, \text{----------3.2}$$

where $\boldsymbol{i = 1, 2, \ldots a}$ and $\boldsymbol{j = 1, 2, \ldots b}$.

Substituting this into the means model of equation3.1,we have the effect model:

$$\boldsymbol{y_{ijk}} = \mu + \boldsymbol{\alpha_i} + \boldsymbol{\beta_j} + (\propto \boldsymbol{\beta})_{ij} + \boldsymbol{E_{ijk}}. \text{ ----------3.3}$$

Where:

$$\boldsymbol{\mu} = \frac{\boldsymbol{1}}{\boldsymbol{ab}} \sum_{\boldsymbol{i=1}}^{\boldsymbol{a}} \sum_{\boldsymbol{j=1}}^{\boldsymbol{b}} \mu_{\boldsymbol{ij.}}, \text{ .....3.4}$$

which is the overall population mean effect;

$$\boldsymbol{\alpha_i} = \mu_{\boldsymbol{i..}} - \mu, \text{ .....3.5}$$

which is the effect of the ith level of the row factor **A**;

$$\boldsymbol{\beta_j} = \mu_{\boldsymbol{.j.}} - \mu, \text{ ..... 3.6}$$

which is the effect of the jth level of the column factor **B**;

$$(\boldsymbol{\alpha\beta})_{\boldsymbol{ij}} = \mu_{\boldsymbol{ij.}} - \mu_{\boldsymbol{i..}} - \mu_{\boldsymbol{.j.}} + \mu, \text{ .....3.7}$$

which is the interaction effect between the ith level of factor **A** and the jth level of factor **B**;

and

$$\boldsymbol{E_{ijk}} = \boldsymbol{y_{ijk}} - \mu_{\boldsymbol{ij}\cdot}, \ldots\ldots\ldots 3.8$$

which is the residual or random error.

$\mu_{\boldsymbol{i}\cdot\cdot}$ = mean effect for the ith level of factor A

$$\mu_{\boldsymbol{i}\cdot\cdot} = \frac{\boldsymbol{1}}{\boldsymbol{b}}\sum_{\boldsymbol{j=1}}^{\boldsymbol{b}} \mu_{\boldsymbol{ij}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.9$$

$\mu_{\cdot\boldsymbol{j}\cdot}$ = mean effect for the ith level of factor B

$$\mu_{\cdot\boldsymbol{j}\cdot} = \frac{\boldsymbol{1}}{\boldsymbol{a}}\sum_{\boldsymbol{i=1}}^{\boldsymbol{a}} \mu_{\boldsymbol{ij}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.10$$

This shows that the effects model of equation 3.3 is partitioned into five under the consideration of the assumptions made about the population under the discussion of two-factor factorial design in section 3.1.1 above.

Sending μ in equation 3.3 to the left side of the equal sign (=), we have:

$$\boldsymbol{y_{ijk}} - \mu = \boldsymbol{\alpha_i} + \boldsymbol{\beta_j} + (\propto \boldsymbol{\beta})_{\boldsymbol{ij}} + \boldsymbol{E_{ijk}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.11$$

Substituting equations 3.5, 3.6, 3.7 and 3.8 into equation 3.11, we have:

$$\boldsymbol{y_{ijk}} - \mu = (\mu_{\boldsymbol{i}\cdot\cdot} - \mu) + \left(\mu_{\cdot\boldsymbol{j}\cdot} - \mu\right) + \left(\mu_{\boldsymbol{ij}\cdot} - \mu_{\boldsymbol{i}\cdot\cdot} - \mu_{\cdot\boldsymbol{j}\cdot} + \mu\right) + (\boldsymbol{y_{ijk}} - \mu_{\boldsymbol{ij}\cdot})$$

Replacing each of the theoretical means, $\mu, \mu_{\boldsymbol{i}\cdot\cdot}, \mu_{\cdot\boldsymbol{j}\cdot}$ and $\mu_{\boldsymbol{ij}\cdot}$ by their unbiased estimators,

$\bar{\boldsymbol{y}}_{\ldots}, \bar{\boldsymbol{y}}_{\boldsymbol{i}\cdot\cdot}, \bar{\boldsymbol{y}}_{\cdot\boldsymbol{j}\cdot}$ and $\bar{\boldsymbol{y}}_{\boldsymbol{ij}\cdot}$ thus mathematically, the various sum of observations are expressed as follows:

$y_{ij\cdot} = \sum_{k=1}^{n} y_{ijk}$	$\bar{y}_{ij\cdot} = \frac{y_{ij\cdot}}{n}$
$y_{i\cdot\cdot} = \sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}$	$\bar{y}_{i\cdot\cdot} = \frac{y_{i\cdot\cdot}}{bn}$
$y_{\cdot j\cdot} = \sum_{i=1}^{a}\sum_{k=1}^{n} y_{ijk}$	$\bar{y}_{\cdot j\cdot} = \frac{y_{\cdot j\cdot}}{an}$
$y_{\cdots} = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}$	$\bar{y}_{\cdots} = \frac{y_{\cdots}}{abn}$

respectively, we have:

$$(y_{ijk} - \bar{y}_{\cdots}) = (\bar{y}_{i\cdot\cdot} - \bar{y}_{\cdots}) + (\bar{y}_{\cdot j\cdot} - \bar{y}_{\cdots}) + (\bar{y}_{ij\cdot} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} + \bar{y}_{\cdots}) + (y_{ijk} - \bar{y}_{ij\cdot})$$

Squaring and summing over i, j and k, we have obtain the corrected sum of squares identity:

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{\cdots})^2$$

$$= \sum_{i=1}^{a}[(\bar{y}_{i\cdot\cdot} - \bar{y}_{\cdots}) + (\bar{y}_{\cdot j\cdot} - \bar{y}_{\cdots}) + (\bar{y}_{ij\cdot} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} + \bar{y}_{\cdots}) + (y_{ijk} - \bar{y}_{ij\cdot})]^2$$

$$= bn\sum_{i=1}^{a}(\bar{y}_{i\cdot\cdot} - \bar{y}_{\cdots})^2 + an\sum_{j=1}^{b}(\bar{y}_{\cdot j\cdot} - \bar{y}_{\cdots})^2 +$$

$$n\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{y}_{ij\cdot} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} + \bar{y}_{\cdots})^2 + \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij\cdot})^2 \quad \ldots\ldots\ldots 3.12$$

Let SS_T = total sum of squares in the data (measures of total variability in the data),

SS_A = sum of squares due to rows or factor **A** (measure of variability in data attributable to the use of different levels of factor **A**),

SS_B = sum of squares due to columns of factor **B** (measure of variability in data attributable the use of different levels of factor **B**),

SS_{AB}= sum of squares due the interaction between factors **A** and **B** (measure of variability in data due to interaction between the levels of factors **A** and **B**), and

SS_E = sum of squares due to residual or random error (measure of variability in data due to random or unexplained sources)

The sum of squares identity is written in a corresponding order in equation 3.12 as:

$$SS_T = SS_A + SS_B + SS_{AB} + SS_E, \quad \text{----------} \quad 3.13$$

Considering a generalized arrangement for a two factor factorial design table

for easy manual computation directly from the general data layout for two-factor factorial design as presented in Table 3.1, the following formulas are used:

$$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn} \quad \ldots\ldots\ldots\ldots\ldots\ldots 3.14$$

$$SS_A = \frac{1}{bn}\sum_{i=1}^{a} y_{i..}^2 - \frac{y_{...}^2}{abn} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.15$$

$$SS_B = \frac{1}{an}\sum_{j=1}^{b} y_{.j.}^2 - \frac{y_{...}^2}{abn} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.16$$

For $\boldsymbol{SS_{AB}}$, the sum of squares between the **ab** cells totals, that is, the treatment sum of squares ($\boldsymbol{SS_{Tr}}$) which contains $\boldsymbol{SS_A}$, $\boldsymbol{SS_B}$ and $\boldsymbol{SS_{AB}}$, must be computed first and this is given by:

$$\boldsymbol{SS_{Tr}} = \frac{\mathbf{1}}{\boldsymbol{n}}\sum_{\boldsymbol{i=1}}^{\boldsymbol{a}}\sum_{\boldsymbol{j=1}}^{\boldsymbol{b}} \boldsymbol{y_{ij.}^2} - \frac{\boldsymbol{y_{...}^2}}{\boldsymbol{abn}} \text{ ... ... 3.17}$$

And since $\boldsymbol{SS_{Tr}}$ contains$\boldsymbol{SS_A}$, $\boldsymbol{SS_B}$ and$\boldsymbol{SS_{AB}}$, it is mathematically expressed as:

$$\boldsymbol{SS_{Tr}} = \boldsymbol{SS_A} + \boldsymbol{SS_B} + \boldsymbol{SS_{AB}} \text{ .....3.18}$$

This implies:

$$\boldsymbol{SS_{AB}} = \boldsymbol{SS_{Tr}} - \boldsymbol{SS_A} - \boldsymbol{SS_B} \quad \text{---------- 3.19}$$

Hence making $\boldsymbol{SS_E}$ the subject in equation 3.13, we have:

$$\left.\begin{aligned} \boldsymbol{SS_E} &= \boldsymbol{SS_T} - \boldsymbol{SS_A} - \boldsymbol{SS_B} - \boldsymbol{SS_{AB}} \\ &= \boldsymbol{SS_T} - (\boldsymbol{SS_A} + \boldsymbol{SS_B} + \boldsymbol{SS_{AB}}) \\ &= \boldsymbol{SS_T} - \boldsymbol{SS_{Tr}} \end{aligned}\right\} \text{..... 3.20}$$

3.2.1 Degrees of Freedom and Mean Squares

Degrees of freedom simply depict the number of independent pieces of information available for computing variability. Generally, it is the sample size (**n**) minus one, that is ***n–1***. According to Gordor and Howard (2000), "for sum of squares, degrees of freedom are the number of independent elements in the sum of squares concerned. Also, the use of degrees of freedom is to make the sum of squares being calculated an unbiased estimator of its population value". For example, assuming $\boldsymbol{SS} = \sum_{\boldsymbol{i=1}}^{\boldsymbol{n}}(\boldsymbol{y_i} - \boldsymbol{\bar{y}})$ has **n** elements of $(\boldsymbol{y_1} - \boldsymbol{\bar{y}})$, $(\boldsymbol{y_2} - \boldsymbol{\bar{y}})$, $(\boldsymbol{y_3} - \boldsymbol{\bar{y}})$,- - - $(\boldsymbol{y_n} - \boldsymbol{\bar{y}})$, these elements are not independent because they sum up to zero, that is$\sum_{\boldsymbol{i=1}}^{\boldsymbol{n}}(\boldsymbol{y_1} -$

$\bar{y}) = \mathbf{0}$. Hence, only **n**-1 of them is independent, meaning sum of squares has **n**-1 degrees of freedom.

The degrees of freedom associated with the sum of squares in equation 3.13 in a corresponding order are:

$$(\boldsymbol{abn} - \mathbf{1}) = (\boldsymbol{a} - \mathbf{1}) + (\boldsymbol{b} - \mathbf{1}) + (\boldsymbol{a} - \mathbf{1})(\boldsymbol{b} - \mathbf{1}) + \boldsymbol{ab}(\boldsymbol{n} - \mathbf{1})$$

Conceptually, since the sample size for the data is **abn**, the degree of freedom for $\mathbf{SS_T}$ is **abn**-1. Also, the main effects, factors A and B, have **a** and **b** levels respectively, implying that $\mathbf{SS_A}$ and $\mathbf{SS_B}$ have $(\boldsymbol{a} - 1)$ and $(\boldsymbol{b} - 1)$ degrees of freedom respectively. The interaction's degrees of freedom are simply the product of the degrees of freedom for the two main effects, factors **A** and **B**, that is $(\boldsymbol{a} - 1)(\boldsymbol{b} - 1)$. Finally, each of the **ab** cells has **n**-1 degrees of freedom between the **n** observations (replicates), hence the degrees of freedom for $\mathbf{SS_E}$ is $\boldsymbol{ab}(\boldsymbol{n} - 1)$.

Dividing each of the sum of squares on the right side of the sum of squares identity, that is equation 3.13, by their corresponding number of degrees of freedom, we have:

$$\boldsymbol{MS_A} = \frac{\boldsymbol{SS_A}}{\boldsymbol{a} - 1}, \qquad \boldsymbol{MS_B} = \frac{\boldsymbol{SS_B}}{\boldsymbol{b} - \mathbf{1}}, \quad \boldsymbol{MS_{AB}} = \frac{\boldsymbol{SS_{AB}}}{(\boldsymbol{a} - \mathbf{1})(\boldsymbol{b} - \mathbf{1})}, \qquad \boldsymbol{MS_E} = \frac{\boldsymbol{SS_E}}{\boldsymbol{ab}(\boldsymbol{n} - \mathbf{1})}$$

where $\boldsymbol{MS_A}$= sample variances for factor A effects (mean square for factor A effects)

$\boldsymbol{MS_B}$ = sample variance for factor B effects (mean square for factor B effects)

$\boldsymbol{MS_{AB}}$ = mean square for interaction effects between factors A and B

$\boldsymbol{MS_E}$ = sample variance for the data (mean square for random error effects)

All these are variance estimates and are independent estimates of $\boldsymbol{\sigma^2}$ under the condition that there are no effects $\boldsymbol{\alpha_i}$, $\boldsymbol{\beta_j}$, and $(\boldsymbol{\alpha\beta})_{\boldsymbol{ij}}$.

Also,

$$\boldsymbol{MS_{Tr} = \frac{SS_{Tr}}{ab - 1}}$$

where $\boldsymbol{MS_{Tr}}$ is the mean square for treatment effects.

3.2.2 Null Hypothesis Testing

In hypothesis or significance testing, a specific idea concerning a parameter, $\boldsymbol{\emptyset}$, is available before a study, and the purpose of the study is to collect a data sample to confirm, or dispute, this idea. Consequently, there are two hypothesis of interest: the hypothesis being proposed by the experimenter and the negation of this hypothesis. The former, denoted by $\boldsymbol{H_1}$, is called the alternative hypothesis or research hypothesis; the latter is called the null hypothesis and it is denoted by $\boldsymbol{H_o}$. Therefore, the purpose of the experiment is to decide whether the evidence based on the available data tends to refute the null hypothesis ($\boldsymbol{H_o}$).

Since the decision at the end of the study will be either to reject the H_o or to fail to do so, this decision is made by observing the value of some statistic whose probability distribution is known under the assumption that the null or estimated value ($\boldsymbol{\emptyset_o}$) is the true value of the parameter ($\boldsymbol{\emptyset}$). Such a statistic is called the test statistic. If the test statistic assumes a value that is rarely seen when $\boldsymbol{\emptyset = \emptyset_o}$ and tends to lend credence to H_1, then H_o is rejected in favour of $\boldsymbol{H_1}$, otherwise, $\boldsymbol{H_o}$ is not rejected. This means that at the end of a study, the experimenter is likely to make exactly one of the following decisions:

i. Rejecting $\boldsymbol{H_o}$ when it is true, hence committing a ***Type I error***

ii. Rejecting $\boldsymbol{H_o}$ when it is false, hence making the right decisions

iii. Not rejecting $\boldsymbol{H_o}$ when it is false, hence committing a ***Type II error***

iv. Not rejecting $\boldsymbol{H_o}$ when it is true, hence making the right decision.

In stating the null hypothesis ($\boldsymbol{H_o}$) and the alternate hypothesis ($\boldsymbol{H_1}$), the following guidelines must be followed.

i. Equality must be included in the statement of the H_o about the value of the parameter ($\boldsymbol{\theta}$) in relation with the null value ($\boldsymbol{\theta_o}$). For example;

   i. $\boldsymbol{H_o : \theta = \theta_o}$,

   ii. $\boldsymbol{H_o : \theta \leq \theta_o}$,

   or *iii.* $\boldsymbol{H_o : \theta \geq \theta_o}$

ii. Whatever is to be supported or detected by the experimenter is the alternate hypothesis ($\boldsymbol{H_1}$)

iii. Since the research hypothesis is the alternate hypothesis ($\boldsymbol{H_1}$), it is hoped that the evidence leads to the rejection of the null hypothesis ($\boldsymbol{H_o}$) and thereby accepting the alternate hypothesis ($\boldsymbol{H_1}$).

In two-factor factorial design involving fixed factors, random factors or mixed factors, the null hypothesis to be tested first is the null hypothesis of "no interaction between the factors **A** and **B**". If this hypothesis is not rejected, then the test for main effects follows, that is testing the null hypothesis of no difference among the levels of factors **A** and **B**. If the null hypothesis of no interaction is rejected, then the levels of factor **A** do not behave consistently across the levels of factor **B**. Thus, it is difficult to make generalizations about the behaviour of factors **A** and **B**.

Thus:

Test for Interaction effect: Factor A vs. Factor B

H_0: All interaction means are zero (no interaction effect)

H_1: Not all interaction means are zero (interaction effect)

Construct the F statistic as follows:

$$F = \frac{MS_{AB}}{MS_E}$$

Reject H_0 if $F > F_{\propto,(a-1)(b-1),ab\,(n-1)}$.

Test for the main effects of factor A

Construct the F statistic as follows:

$$F = \frac{MS_A}{MS_E}$$

Reject H_0 if $F > F_{\propto,a-1,ab\,(m-1)}$

Test for the main effects of factor B

Construct the F statistic as follows:

$$F = \frac{MS_B}{MS_E}$$

Reject H_0 if $F > F_{\propto,b-1,ab\,(n-1)}$

To understand exactly what is going on, there is the need to compare levels of factor **A** to each level of factor **B** and vice versa. This approach is called multiple comparison methods. Examples of multiple comparison method are graphical method, contrasts method, orthogonal contrasts, Scheffe's method, paired comparisons (by Turkey's test, Duncan's test or Newman Keul's test), control method and others.

For simplicity and convenience sake, the differences among treatment combinations are checked when the null hypothesis ($\boldsymbol{H_o}$) of no interaction is rejected.

The test statistic for two-factor factorial design with fixed factors, random factors or mixed factors is the ***F***-ratio. The ***F***-ratio, simply termed "calculated ***F***", is normally compared with the value of ***F*** from the statistical table for ***F*** distribution (F_α), also simply termed "table ***F***" and if the calculated ***F***" is greater than the "table ***F***" (F_α), the null hypothesis (H_o) is rejected, otherwise H_o is not rejected. α is the level of significance or probability of rejecting H_o.

3.2.3 ANOVA for Fixed Factors

The expected mean squares for two-factor factorial design involving fixed factors are:

$$E(MS_A) = E\left(\frac{SS_A}{a-1}\right) = \sigma^2 + \frac{bn\sum_{i=1}^{a} \propto_i^2}{a-1}$$

$$E(MS_B) = E\left(\frac{SS_B}{b-1}\right) = \sigma^2 + \frac{an\sum_{j=1}^{b} \beta_j^2}{b-1}$$

$$E(MS_{AB}) = E\left(\frac{SS_{AB}}{(a-1)(b-1)}\right) = \sigma^2 + \frac{n\sum_{i=1}^{a}\sum_{j=1}^{b}(\alpha\beta)_{ij}^2}{(a-1)(b-1)}$$

$$E(MS_E) = E\left(\frac{SS_E}{ab(n-1)}\right) = \sigma^2$$

As already noted, the first null hypothesis to be tested should be the null hypothesis (H_o) of no interaction, that is:

$$H_o: (\propto\beta)_{ij} = 0 \text{ , i = 1, 2,..., a ; j = 1, 2, ...,b}$$

$$H_1: \text{at least one } (\alpha\beta)_{ij} \neq 0$$

If this hypothesis is not rejected then the analysis is continued by testing for main effects

For factor A:

$$H_o: \propto_1 = \propto_2 = \cdots \propto_a = 0$$

$$H_1 : \text{at least one } \propto_i \neq 0$$

And for factor B:

$$H_o \beta_1 = \beta_2 = \cdots \beta_b = 0$$

$$H_1 : \text{at least one } \beta_j \neq 0$$

:

If $H_o : (\propto \beta)_{ij} = 0$ is rejected, then the null hypothesis for equal treatment combination means is tested:

$$H_o : \mu_{11.} = \mu_{12.} = \cdots = \mu_{2b.}$$

$$H_1: \text{at least one } \mu_{ij.} \neq 0 \; i = 1, 2, \ldots, a; \; j = 1, 2, \ldots, b$$

(i) To test $H_o : (\propto \beta)_{ij} = 0$, that is the interaction effects are all equal to zero, the ***F-ratio***:

$$F_{AB} = \frac{MS_{AB}}{MS_E}$$

the value of a random variable having the ***F***–distribution with $(a-1)(b-1)$ and $ab(n-1)$degrees of freedom when $H_o : (\propto \beta)_{ij} = 0$ is true calculated. $H_o : (\propto \beta)_{ij} = 0$ is rejected (when $F_{AB} > F_{\propto[(a-1)(b-1),\ ab(n-1)]}$) and it is concluded that interaction is present.

(ii) To test $H_o : \propto_1 = \propto_2 = \cdots \propto_a = 0$, that the effects of factor **A** are all equal, we calculate:

$$F_A = \frac{MS_A}{MS_E}$$

the value of a random variable having the ***F***–distribution with $(a-1)$ and $ab(n-1)$ degrees of freedom when$H_o : \propto_1 = \propto_2 = \cdots \propto_a = 0$is true. However, $H_o := \propto_1 = \propto_2 = \cdots \propto_a = 0$ is rejected at α – level of significance when $F_A > F_{\propto[(a-1),\ ab(n-1)]}$ and it is concluded that some differences exist between the effects of factor **A**.

(iii) Similarly, to test $\boldsymbol{H_o: \beta_1 = \beta_2 = \cdots \beta_b = 0}$, that the effects of factor **B** are all equal to zero, we compute:

$$\boldsymbol{F_B = \frac{MS_B}{MS_E}}$$

the value of a random variable having the ***F***–distribution with $\boldsymbol{(b-1)}$ and $\boldsymbol{ab\,(n-1)}$ degrees of freedom when $\boldsymbol{H_o: \beta_1 = \beta_2 = \cdots \beta_b = 0}$ is true. $\boldsymbol{H_o: \beta_1 = \beta_2 = \cdots \beta_b = 0}$ is rejected at $\boldsymbol{\alpha}$–level of significance when $\boldsymbol{F_B > F_{\propto[(a-1)(b-1),\ ab(n-1)]}}$ and we conclude that some differences between the effects of factor **B** exist.

(iv) If the null hypothesis of no interaction ($\boldsymbol{H_o: (\propto \beta)_{ij} = 0}$) is rejected, then the ***F*** statistic:

$$\boldsymbol{F_{Tr[(ab-1),\ ab(n-1)]} = \frac{MS_{Tr}}{MS_E}}$$

is used to test null hypothesis of no differences among treatment combination. Or any of the methods of multiple comparisons could be employed.

The initial region for ***F***-ratio will be the upper tail of the ***F***-distribution.

The analysis of variance (ANOVA) table for two-factor factorial design with fixed factors is displayed in table 3.2 below. The table is summarized with the columns containing the sources of variation, sum of squares, degrees of freedom, mean squares, expected mean squares and calculated ***F*** (the test statistic); and the rows containing the sources of variation due to treatment effect, factor **A** effect (**A**), factor **B** effect (**B**), interaction effect (**AB**), error effect and the total effect.

Table 3.2: Analysis of variance (ANOVA) table for two-factor factorial design with fixed factors

Source of variation	*Sum of squares*	*Degrees of freedom (DF)*	*Mean squares (MS)*	*Expected mean squares [E(MS)]*	*Calculated F*
Treatment	$SS_{Tr} = \frac{1}{n}\sum_{i=1}^{a}\sum_{j=1}^{b} y_{ij.}^2 - \frac{y_{...}^2}{abn}$	$ab-1$	$MS_{Tr} = \frac{SS_{Tr}}{ab-1}$	$E(MS_{Tr}) = \sigma^2 + \frac{n}{ab-1}\sum_{i=1}^{a}\sum_{j=1}^{b}(\mu_{ij.} - \mu)^2$	$F_{Tr} = \frac{MS_{Tr}}{MS_E}$
A	$SS_A = \frac{1}{bn}\sum_{i=1}^{a} y_{i..}^2 - \frac{y_{...}^2}{abn}$	$a-1$	$MS_A = \frac{SS_A}{a-1}$	$E(MS_A) = \sigma^2 + \frac{bn\sum_{i=1}^{a}\propto_i^2}{a-1}$	$F_A = \frac{MS_A}{MS_E}$
B	$SS_B = \frac{1}{an}\sum_{j=1}^{b} y_{.j.}^2 - \frac{y_{...}^2}{abn}$	$b-1$	$MS_B = \frac{SS_B}{b-1}$	$E(MS_B) = \sigma^2 + \frac{an\sum_{j=1}^{b}\beta_j^2}{b-1}$	$F_B = \frac{MS_B}{MS_E}$
AB	$SS_{AB} = SS_{Tr} - SS_A - SS_B$	$(a-1)(b-1)$	$MS_{AB} = \frac{SS_{AB}}{(a-1)(b-1)}$	$E(MS_B) = \sigma^2 + \frac{n}{(a-1)(b-1)}\sum_{i=1}^{a}\sum_{j=1}^{b}(\propto\beta)_{ij}^2$	$F_{AB} = \frac{MS_{AB}}{MS_E}$
Error	$SS_E = SS_T - SS_{Tr}$	$ab(n-1)$	$MS_E = \frac{SS_E}{ab(n-1)}$	$E(MS_E) = \sigma^2$	
Total	$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn}$	$abn-1$			

3.3 Generalized Linear Model

Model building entails the development of prediction equations (statistical models) by statistical or mathematical method from experimental data.

3.3.1 Regression Models

The regression model representation of the two-factor factoriall experiment could be written as:

$$\boldsymbol{y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2 + E} \quad \text{.....21}$$

where $\boldsymbol{y}$ is the response, the $\boldsymbol{\beta}'s$ are the parameters whose values are to be determined, $\boldsymbol{x_1}$ is a variable that represents factor **A**, $\boldsymbol{x_2}$ is a variable that represent factor **B** and $\boldsymbol{E}$ is a random error term. The variables x_1 and x_2 are defined on a coded scale from -1 to+1 (the low and high) levels of factors **A** and **B**, and $\boldsymbol{x_1 x_2}$ represents the interaction between $\boldsymbol{x_1}$ and $\boldsymbol{x_2}$.

Let the smallest and biggest values among the values under the first level of factor **A** be 110 and 250 respectively; let the smallest and biggest values among the values under the last level of factor A be 150 and 300 respectively; let the smallest and biggest values in the first

level of **B** be 110 and 150 effect of **A** is:

$$\mathbf{A} = \frac{150 + 300}{2} - \frac{110 + 250}{2}$$

$$= 225 - 180$$

$$= 45$$

That is, increasing factor **A** from the first level to the last level causes an average response increase of 45 units. Similarly, the effect of **B** is:

$$\mathbf{B} = \frac{250 + 300}{2} - \frac{110 + 250}{2}$$

$$= 275 - 130$$

$$= 145$$

To estimate the parameters (which are related to the effect estimates) in the regression model, that is the estimates of $\boldsymbol{\beta_1}$ and $\boldsymbol{\beta_2}$, we have:

$$\widehat{\boldsymbol{\beta}}_1 = \frac{45}{2} = 22.5$$

$$\widehat{\boldsymbol{\beta}}_2 = \frac{145}{2} = 72.5$$

The effect of factor **A** at the first level of factor **B** is

$$\mathbf{A} = 150 - 110 = 40$$

The effect of factor **A** at the last level of factor **B** is:

$$\mathbf{A} = 300 - 250 = 50$$

Hence the interaction effect is given by:

$$\mathbf{AB} = \frac{50 - 40}{2} = 5$$

Therefore, the estimate of $\boldsymbol{\beta_3}$ is given by:

$$\widehat{\boldsymbol{\beta}}_3 = \frac{5}{2} = 2.5$$

The estimate of $\boldsymbol{\beta_o}$ is given by:

$$\widehat{\boldsymbol{\beta}}_{\boldsymbol{o}} = \frac{110+250+150+300}{4} = 202.5$$

Therefore the fitted regression model is:

$$\boldsymbol{y = 202.5 + 22.5x_1 + 72.5x_2 + 2.5x_1x_2}$$

However, since $\widehat{\boldsymbol{\beta}}_{\boldsymbol{3}} = 2.5$ is very small compared with the other estimates, interaction is negligible. Hence dropping the term $\boldsymbol{2.5x_1x_2}$, we have:

$$\boldsymbol{y = 202.5 + 22.5x_1 + 72.5x_2}$$

3.3.2 Effect Models

As captured above under reaction of this chapter, effect models could be fixed-effect models, random-effect models or mixed-effect models. The formulas for all these three models are identical as:

$$\boldsymbol{y_{ijk} = \mu + \alpha_i + \beta_j + (\propto \beta)_{ij} + E_{ijk}}\ ,$$

where $\boldsymbol{i = 1, 2, \ldots a; j = 1, 2, \ldots b}$; and $\boldsymbol{k = 1, 2, \ldots n}$. Their differences lie in the definition of the effect terms.

(i)For fixed-effect models, $\boldsymbol{\mu}$ is the overall mean effect, $\boldsymbol{\alpha_i}$ and $\boldsymbol{\beta_j}$ are the fixed treatment effects of factors A and B respectively, and defined as the deviations from the overall mean effect $\boldsymbol{\mu}$. Hence $\sum_{i=1}^{a} \boldsymbol{\alpha_i = 0}$ and $\sum_{j=1}^{b} \boldsymbol{\beta_j = 0}$. Also, $(\propto \boldsymbol{\beta})_{ij}$ is the fixed interaction effects of factors **A** and **B** in the ith and jth cell, defined as the deviation from $\boldsymbol{\mu}$such that $\sum_{i=1}^{a} \boldsymbol{\alpha_i = 0}$ and $\sum_{j=1}^{b} \boldsymbol{\beta_j = 0}$.

$\boldsymbol{E_{ijk}}$ is the measure of the deviations of the observed value, $\boldsymbol{y_{ijk}}$, in the (ij)th cell from $\boldsymbol{\mu_{ij}}$.

However, the sum of squares corresponding to the model, symbolically given by:

$$SS_{m-odel}=SS_A+SS_B+SS_{AB}\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.22$$

could be used. This is verified

by the value of co − efficient of determination $(\boldsymbol{R^2})$, also given by:

$$\boldsymbol{R^2} = \frac{\boldsymbol{SS_{model}}}{\boldsymbol{SS_T}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.23$$

That is if $\boldsymbol{R^2 = r}$ %, then $\boldsymbol{r}$ % of the variability of the response in equation 3.3 is explained by factor **A,** factor **B** and the interaction between factors **A** and **B.**

3.3.3 The Estimation of Model Parameters

The estimation of the parameters of the effect model (equation 3.3):

$$\boldsymbol{y_{ijk} = \mu + \alpha_i + \beta_j + (\propto \beta)_{ij} + E_{ijk}}$$

is done by using the least squares method. In summary, if there are **a** levels of factor **A** and **b** levels of factor **B**, then the model has **1+a+ab** parameters to be estimated, and **1+a+b+ab** normal equations which are given by:

$$\boldsymbol{\mu}: \boldsymbol{abn\hat{\mu} + bn\sum_{i=1}^{a}\hat{\alpha}_i + an\sum_{j=1}^{b}\hat{\beta}_j + n\sum_{i=1}^{a}\sum_{j=1}^{b}(\widehat{\propto \beta})_{ij} = y}\ldots\ldots\ldots\ldots\ldots 3.24$$

$$\propto_i : \boldsymbol{bn\hat{\mu} + bn\hat{\alpha}_i + n\sum_{j=1}^{b}\hat{\beta}_j + n\sum_{j=1}^{b}(\widehat{\propto \beta})_{ij} = y_{i..}}\ , \ldots\ldots\ldots\ldots\ldots 3.25$$

where $\boldsymbol{i = 1,2,\ldots a}$

$$\boldsymbol{\beta_j}: \boldsymbol{an\hat{\mu} + n\sum_{i=1}^{a}\hat{\alpha}_j + an\hat{\beta}_j + n\sum_{j=1}^{a}(\widehat{\propto \beta})_{ij} = y_{.j.}}\ , \ldots\ldots\ldots\ldots\ldots 3.26$$

where $j = 1,2,\ldots b$

$$(\propto \boldsymbol{\beta})_{ij} : \; n\hat{\mu} + n\hat{\alpha}_i + n\hat{\beta}_j + n(\widehat{\propto \beta})_{ij} = y_{ij.},\ldots\ldots\ldots\ldots 3.27$$

where $i=1,2,\ldots a$ and $j=1,2,\ldots b$

Applying the assumptions:

$$\sum_{i=1}^{a} \hat{\alpha}_i = 0 \ldots\ldots\ldots\ldots\ldots 3.28$$

$$\sum_{j=1}^{b} \hat{\beta}_i = 0 \ldots\ldots\ldots\ldots\ldots 3.29$$

$$\sum_{i=1}^{a} (\widehat{\propto \beta})_{ij} = 0 \ldots\ldots\ldots\ldots 3.30$$

and

$$\sum_{j=1}^{b} (\widehat{\propto \beta})_{ij} = 0, \ldots\ldots\ldots\ldots 3.31$$

we obtain:

$$\hat{\mu} = \bar{y} \ldots\ldots\ldots\ldots\ldots 3.32$$

$$\hat{\alpha}_i = \bar{y}_{i..} - \bar{y} \ldots\ldots\ldots\ldots\ldots 3.33$$

$$\hat{\beta}_j = \bar{y}_{.j.} - \bar{y} \ldots\ldots\ldots\ldots\ldots 3.34$$

$$(\widehat{\propto \beta})_{ij} = \bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y} \ldots\ldots\ldots\ldots\ldots 3.35$$

Hence substituting equations (i), (ii), (iii) and (iv) into

$$\hat{y}_{ijk} = \hat{\mu} + \hat{\alpha}_i + \hat{\beta}_j + (\widehat{\propto \beta})_{ij} \ldots\ldots\ldots\ldots 3.36$$

we obtain:

$$\hat{y}_{ijk} = \bar{y}_{...} + (\bar{y}_{i..} - \bar{y}_{...}) + (\bar{y}_{.j.} - \bar{y}_{...}) + (\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...}) = \bar{y}_{ij}. \quad \ldots\ldots\ldots\ldots 3.37$$

This means, the *kth* observation in the *(ij)th* cell is estimated by the average of the **n** observations (replicates) in that cell.

3.3.4 Model Adequacy Checking

Before the conclusions from the analysis of variance are adopted, the adequacy of the model should be checked. The primary diagnostic tool for model adequacy checking is the residual analysis which is mostly done by graphically analysis in different forms and simply called residual plots. In Walpole et al., (2007), a residual is essentially an error in the fit of a model.

The residuals for two-factor factorial model are given by:

$$E_{ijk} = y_{ijk} - \hat{y}_{ijk}, \quad \ldots\ldots\ldots\ldots 3.38$$

where $\hat{y}_{ijk}$ is the estimator of y_{ijk} ,

$$\hat{y}_{ijk} = y_{ij\cdot} \quad \ldots\ldots\ldots\ldots 3.39$$

This implies that:

$$E_{ijk} = y_{ijk} - \bar{y}_{ij} \quad \ldots\ldots\ldots\ldots 3.40$$

The residual plots are: the normal probability plot of the model; residuals plot in time sequence, used to check independence assumption on the errors; and the plot of residuals versus fitted values ($\hat{y}_{ij.}$), used to check consistency of the variance.

For the normal probability plot of residuals (shown in figure3.1), if the underlying error distribution is normal, then the plot exhibit some kind of linearity, hence the adequacy of the model. In the case of residual plots in time sequence (shown in figure 3.2), when the points in the graph are uniformly spread out about the mean of the residuals, zero, then there is no reason to suspect any violation of the independence assumption, hence the adequacy of the model. With the plot of residuals versus fitted values ($\hat{y}_{ij.}$) -figure 3.3- when the points are uniformly scattered about the mean, zero, and do not portray any obvious pattern, then the variance is constant and the model is adequate.

Montgomery (2001) determined that, if the model is adequate, the residuals should be structureless; that is, they should contain no obvious patterns. However, a very common defect that often shows up on the normal probability plots is one residual being much larger than the others, and this can seriously distort the analysis of variance. This residual is called an outlier. Mostly, the cause of the outlier is such human error as calculation error, date coding error or copying error. However, a suspected outlier could be checked by examining the standardized residuals value ($\boldsymbol{d_{ijk}}$) given by:

$$\boldsymbol{d_{ijk}} = \frac{\boldsymbol{E_{ijk}}}{\sqrt{\boldsymbol{MS_E}}}. \quad \text{.....3.41}$$

A residual value ($\boldsymbol{d_{ijk}}$) bigger than 3 in absolute is a potential outlier which can cause a serious distortion to the conclusion drawn from the ANOVA.

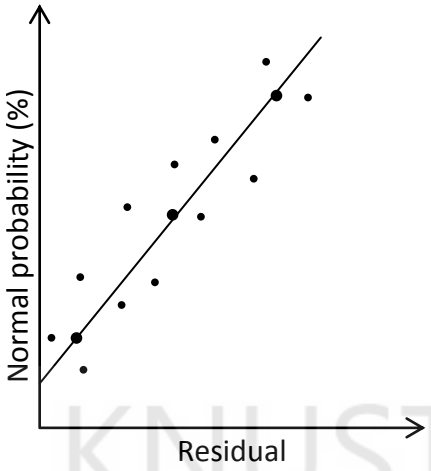


Figure 3.1: Normal probability plot of residuals

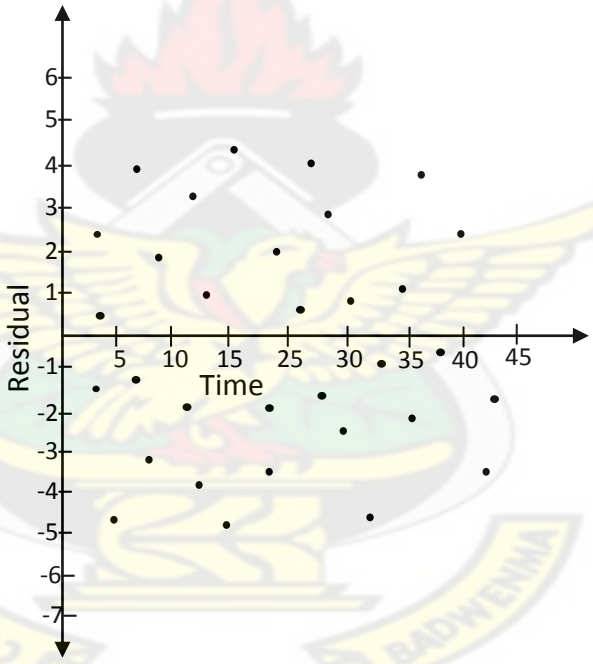


Figure 3.2: Plot of residuals versus time.

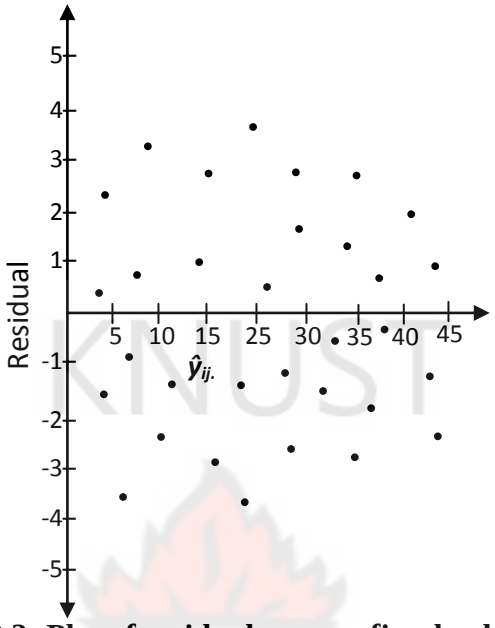


Figure 3.3: Plot of residuals versus fitted values ($\hat{y}_{ij.}$)

3.4 Multiple Comparison Test

When analysis of variance indicates that row or column means differ, it is usually of interest to make comparison between the individual rows or column means to discover the specific differences. The multiple comparison methods are useful in this regard to establish the difference that lead to the rejection of the null hypothesis.

3.4.1 Tukey's - Kramer Multiple Comparisons Method

Tukey's multiple comparison method is one efficient procedure designed to identify the specific differences that exist among mean responses to several treatments, after the ANOVA has concluded such differences do exist. This result might be useful in supporting decision making. The procedure is based on simultaneous comparisons of all the pairs of the sample means. We set up the hypotheses

H_0: $\mu_j = \mu_k$

H_1: $\mu_j \neq \mu_k$

for all the pairs (j, k).

3.4.1.1 Multiple comparisons for the single factor ANOVA

Run the one-way ANOVA, and test whether there is sufficient evidence at least one of the mean values is different. If so, we calculate a critical value for the difference between every two means as follows:

$$Critical\ difference(i,j) = q_{\propto}(b, N-b)\sqrt{\frac{MSE}{2}\left(\frac{1}{n_j}+\frac{1}{n_k}\right)}$$

'q' is taken from the "Studentized Range" table and is determined by alpha, c (the number of treatments), and N (the total number of observations); n_j and n_k are the sample sizes of the treatments compared; and MSE is taken from the ANOVA output. If the desired degrees of freedom cannot be found, we could interpolate; alternatively we could use any other source such as a computer package or an online source.

The decision rule:

1. If $|\bar{X}_j - \bar{X}_k| > Critical\ difference$ then μ_j and μ_k are different at $\alpha\%$ significant level. Furthermore, the population mean whose sample mean is larger is greater than the population mean whose sample mean is smaller.

2. Repeat this procedure for all the possible pairs.

Nevertheless, If the two samples sizes are the same, $n_j = n_k = n$, the equation becomes

$$Critical\ difference(i,j) = q_{\propto}(b, N-b)\sqrt{\frac{MSE}{n}}$$

As a result if all the samples have the same size the critical difference is calculated once because it is common to all the pairs.

3.4.1.2 Tukey's Procedure for a Two-Way ANOVA

In case there is no significant interaction effect identified, but differences in the main factor(s) are statistically significant we can use Tukey's method adjusted to this experimental design.

Here are the particulars of this procedure:

$$Critical\ Range\ (A) = q_{\propto}(b, ab(n-1))\sqrt{\frac{MSE}{an}}$$

$$Critical\ Range(B) = q_{\propto}(a, ab(m-1))\sqrt{\frac{MSE}{bn}}$$

Tukey's Procedure for a Two-Way ANOVA

In case there is no significant interaction effect identified, but differences in the main factor(s) are statistically significant we can use Tukey's method adjusted to this experimental design.

Here are the particulars of this procedure:

$$Critical\ Range\ (A) = q_{\propto}(b, ab(n-1))\sqrt{\frac{MSE}{an}}$$

$$Critical\ Range(B) = q_{\propto}(a, ab(n-1))\sqrt{\frac{MSE}{bn}}$$

CHAPTER 4

DATA COLLECTION, ANALYSIS AND DISCUSSION OF RESULTS

4.0 Introduction

This chapter presents the data collection for the analysis of the study. The data is mainly the week to week data extraction from on site of the ex situ site for the experiment. Statistical Package "Minitab" version 16.0 was used to perform the analysis and Microsoft Excel 2007 was used to generate the tables to explain the results.

4.1 Analysis of Substrates for the Bioremediation Processes

Three substrates were used, of which each substrate was involved in a different experiment of three replicates in a randomized block design with a control, the nitrogen levels and the weeks serves as factors for each substrate, which render the analysis to be a factorial design. All the three replicates were involved in analysis for each level of nitrogen.

4.1.1 Fertilizer and Hydrocarbon Contaminated Soil Blend

Fertilizer, which is an inorganic material, was used as a substrate for the different levels of nitrogen. Preliminary categorization of the site discovered Oil and Grease (mg/kg) and Total Petroleum Hydrocarbon (TPH mg/kg) levels of 34278.00 and 21514.85respectively.

The average degradation of oil/grease (mg/kg) and TPH (mg/kg) soil samples taken from the sampling site reveals that the 0.4% nitrogen level recorded the highest value of 100.00 and 390.00 for oil/grease and TPH respectively by the end of the experiment as shown in Table 4.1.

Table 4.1 Mean results of Oil and grease and TPH for 0.4% Nitrogen level in Hydro-Carbon/Fertilizer blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	23006.67	20931.67	32.79	2.710617453
Week 2	21750.00	19545.67	36.44	9.152678965
Week 3	18743.00	12558.00	45.19	41.63101842
Week 4	17676.67	12013.33	48.29	44.16260296
Week 5	11130.33	7688.00	67.33	64.26654480
Week 6	8238.23	4849.00	75.75	77.46208061
Week 7	5486.89	2740.50	83.75	87.26228747

Table 4.1, shows that, there was a sharp degradation of Oil and Grease in the first three weeks as compared to the Total Petroleum Hydrocarbon (TPH). Within the first week, 32.79% degradation has occurred in Oil and Grease whereas only 2.71% occurred in TPH, in spite of the early stages of sharp degradation in the 0.4 level of nitrogen, TPH recorded highest of 87.462% as compared to 83.75% for Oil and Grease occurred at the end Week 7 thus the end of the experimental period.

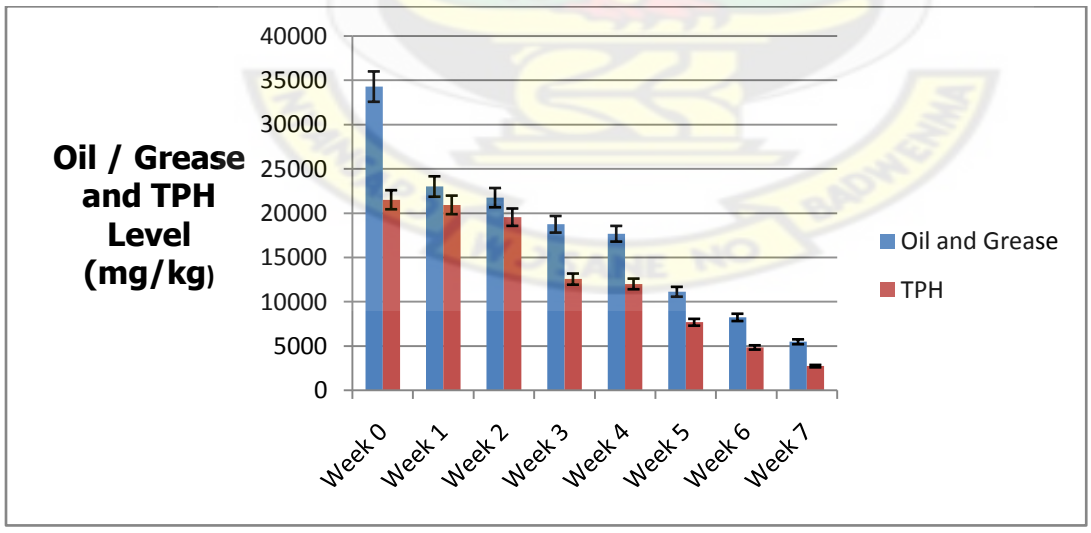


Figure 4.1: Levels of Oil/Grease and TPH degradation in Weeks of 0.4 level of Nitrogen in HC/Fertilizer blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 0.4% nitrogen level within the Hydrocarbon/Fertilizer blend is shown in Figure 4.1, by week 6, TPH was just around the 5000 mg/kg bar line on the graph, whiles Oil/Grease did not obtain this level throughout the experiment, this shows that, TPH latter degrade faster in the 0.4% of nitrogen level in the Hydrocarbon/Fertilizer blend than the Oil/Grease.

Table 4.2 Mean results of Oil and grease and TPH for 1.0 % Nitrogen level in Hydro-Carbon/Fertilizer blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	22174.13	19814.67	35.21	7.902379869
Week 2	20338.67	19544.33	40.55	9.158876234
Week 3	14661.33	11760.00	57.06	45.34008414
Week 4	11426.67	7176.00	66.47	66.64629624
Week 5	9819.33	6662.67	71.15	69.03224495
Week 6	6304.00	3965.00	81.37	81.57087021
Week 7	3454.67	2033.00	89.66	90.55071353

The degradation rate of Oil/Grease and TPH soil when the nitrogen level of Hydrocarbon/Fertilizer was augment to 1.0 is as shown in Table 4.2. Evidently, the degradation of Oil/Grease was much faster in the early stages than that of TPH. By week 2, Oil/Grease had recorded 40.55% whiles TPH had recorded only 9.16% of degradation. Nevertheless, the end of week 7, TPH had recorded a total degradation of 90.55% and Oil/Grease had achieved 89.66% in the 1.0 nitrogen augment level for hydrocarbon/fertilizer blend.

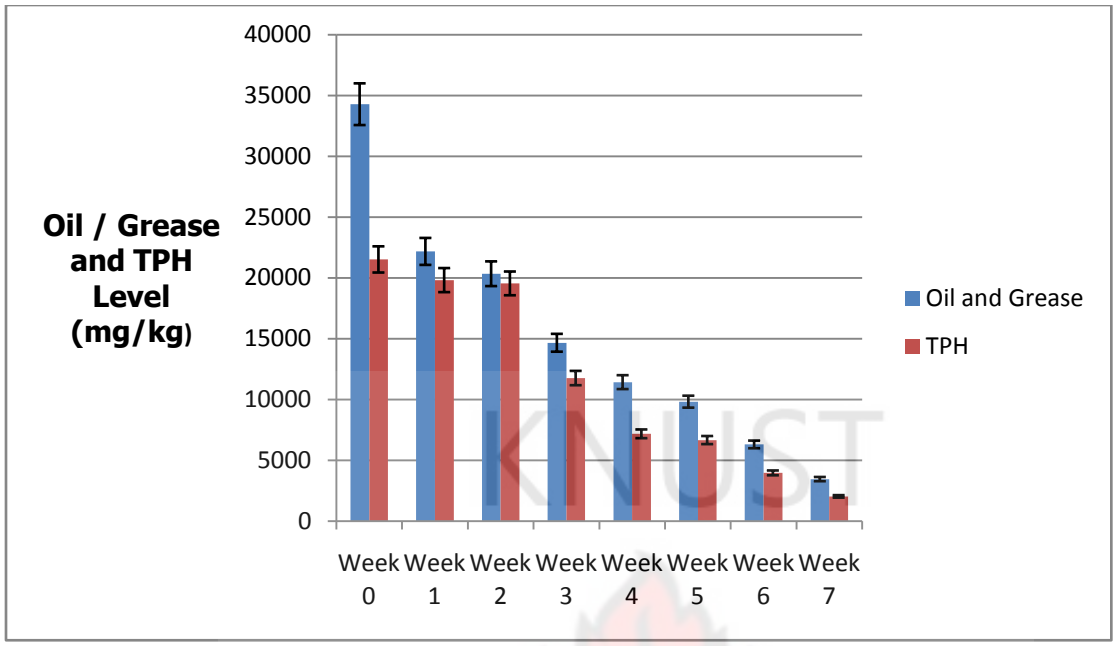


Figure 4.2: Levels of Oil/Grease and TPH degradation in Weeks of 1.0 level of Nitrogen in HC/Fertilizer blend

The graphical view of level of degradation in the augment 1.0 level of nitrogen to stimulate the Hydrocarbon/Fertilizer blend shows that, by week 6, TPH recorded less than 5000 mg/kg of which Oil/Grease was able to achieve this in week 7, which shows a much improvement upon the 0.4 level of nitrogen augment in Figure 4.1.

Table 4.3 Mean results of Oil and grease and TPH for 1.6 % Nitrogen level in Hydro-Carbon/Fertilizer blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	21080.67	19676.00	38.39	8.546895884
Week 2	19572.00	15991.33	42.78	25.67304979
Week 3	13829.33	9843.00	59.48	54.25020818
Week 4	9720.00	5331.67	71.44	75.21866910
Week 5	8865.33	4500.67	73.92	79.081117240
Week 6	6228.33	3971.00	81.59	81.54298249
Week 7	4031.00	2356.67	87.98	89.04632639

The rate of degradation for Hydrocarbon contaminated soil for an augment of 1.6 level of nitrogen for stimulation in the Hydrocarbon/Fertilizer blend shows a much more improvement in both Oil/Grease and TPH. Even though early stages of degradation show a sharp degradation for Oil/Grease, by week 6 both Oil/Grease and TPH have achieved a degradation of more than 81%, which shows a much more degradation than the early two augment level of nitrogen of 0.4 and 1.0. However, by week 7, TPH has recorded a little over 89% whiles Oil/Grease recorded 87.98% of total degradation in the Hydrocarbon/Fertilizer blend.

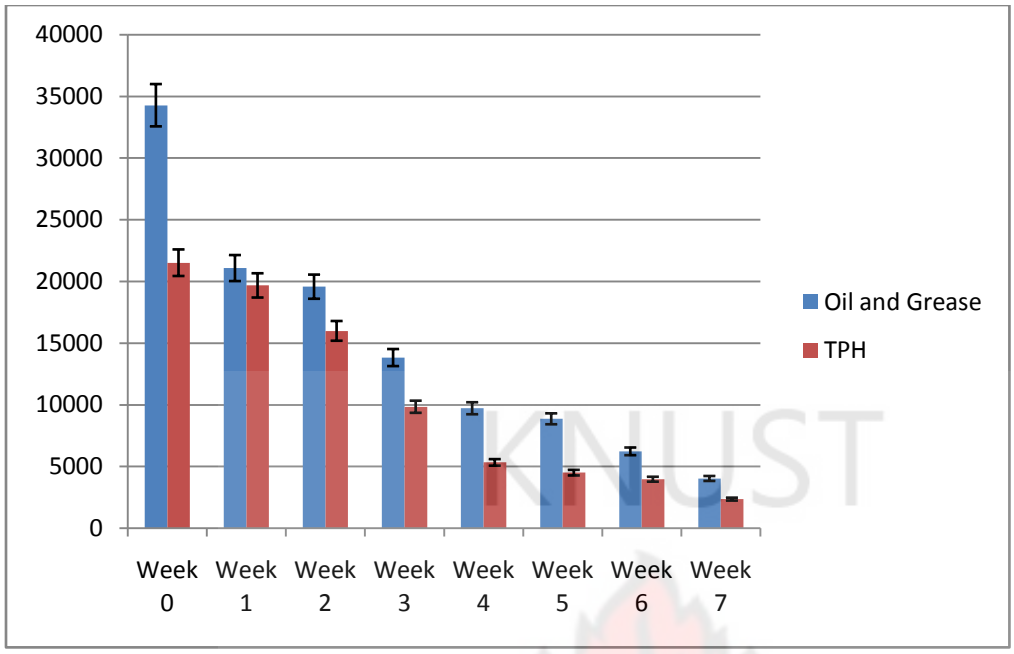


Figure 4.3: Levels of Oil/Grease and TPH degradation in Weeks of 1.6 level of Nitrogen in HC/Fertilizer blend

The graphical presentation of level of degradation in the augment 1.6 level of nitrogen to stimulate the Hydrocarbon/Fertilizer blend shows that, by week 4, TPH recorded less than 5000 mg/kg of which Oil/Grease was able to achieve this in week 7, which shows a much improvement for a better degradation.

Table 4.4 Mean results of Oil and grease and TPH for 2.2 % Nitrogen level in Hydro-Carbon/Fertilizer blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	19774.00	16574.67	42.19	22.96174444
Week 2	14569.33	12598.33	57.33	41.44355103
Week 3	9844.67	5902.67	71.07	72.56468849
Week 4	7504.00	4871.00	77.88	77.35982567
Week 5	4105.33	2515.33	87.77	88.30885133
Week 6	1984.00	997.00	93.94	95.36599183
Week 7	804.00	128.67	97.37	99.40196351

Degradation rate for a level of 2.2 augment of nitrogen for the bioremediation process saw a magnificent improvement in the biostimulation process for the bioremediation in the Hydrocarbon/fertilizer blend. From Table 4.4, by week week5 both the Oil/Grease and TPH have achieved more than 87% of hydrocarbon degradation. By week 7, there were a dramatic degradation of 97.37% for Oil/Grease and 99.40% for TPH.

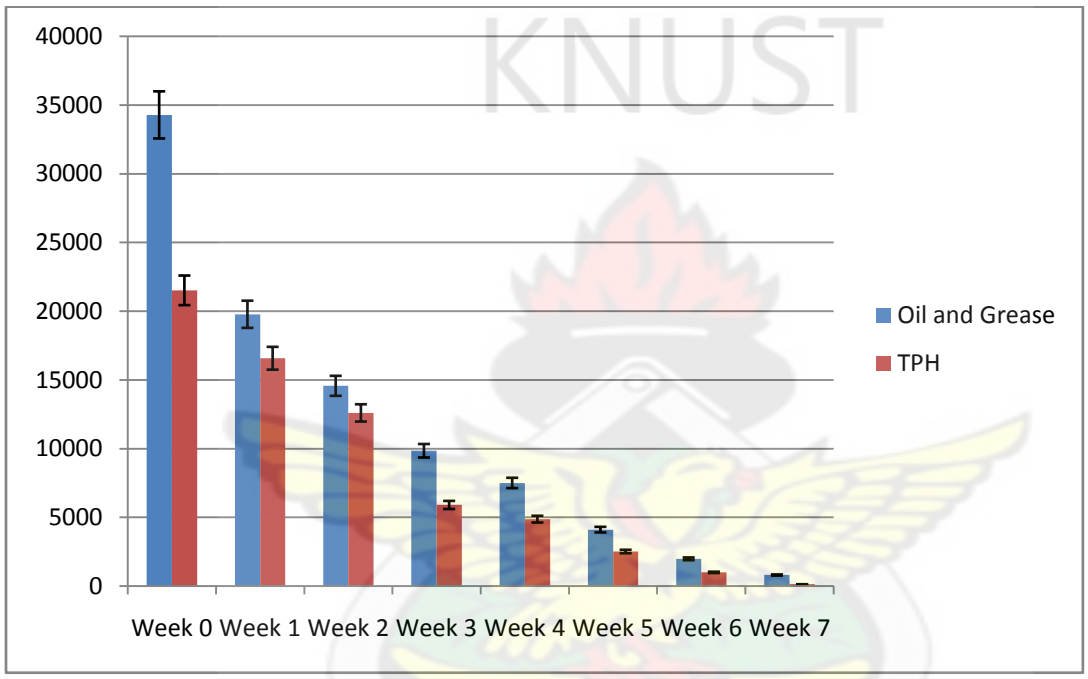


Figure 4.4: Levels of Oil/Grease and TPH degradation in Weeks of 2.2 level of Nitrogen in HC/Fertilizer blend

The graphical representation for the 2.2 level of nitrogen augment for stimulation shows that, by week 5, both Oil/Grease and TPH have left with it less than 5000mg/kg level of hydrocarbon contamination in the soil. This graph indicates that, a higher level of nitrogen augmentation increases tremendously the rate of bioremediation by providing a good condition for the microbes in soil for the bioremediation.

4.1.1.1 Test of Hypothesis for Hydrocarbon/Fertilizer Blend

Test of differences among the means of the weeks for degradation and that of the different levels of augmentation of nitrogen for biostimulation was done to establish if there exist differences among them.

H_{10}: There is no significant difference between the average degradation of hydrocarbon among the different levels of the weeks

H_{11}: There is a significant difference between the average degradation of hydrocarbon among different levels of the weeks

H_{20}: There is no significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{21}: There is a significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{30}: There is no significant difference for the interactions of weeks and the nitrogen level

H_{31}: There is a significant difference between for the interaction of weeks and the nitrogen levels.

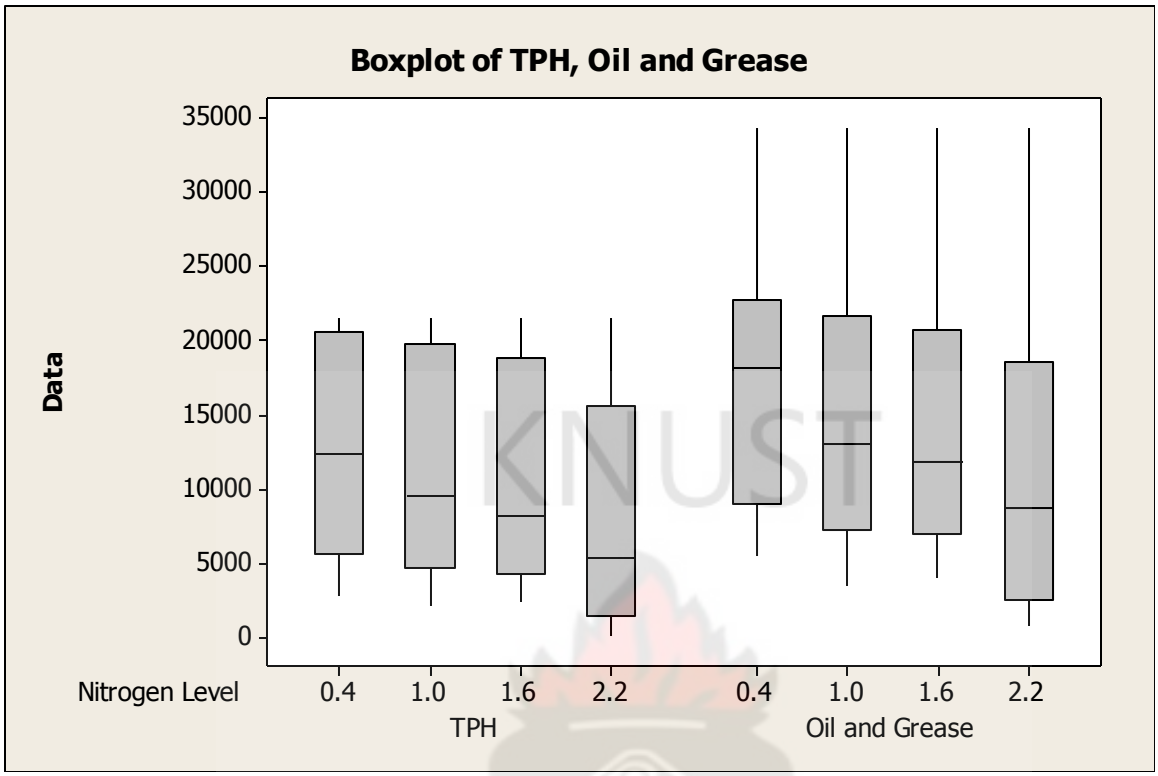


Figure 4.5: Box plot for Different levels of Nitrogen for Oil/Grease and TPH in HC/Fertilizer blend

The box plot for the hydrocarbon soil contamination and the fertilizer blend shows that, different levels of nitrogen augmentation have different levels of the rate of degradation. As evident in Figure 4.5, as the level of nitrogen for augmentation increases, lead to a higher rate of degradation of hydrocarbon in both the Oil/Grease and the TPH, hence lower level of hydrocarbon residue. Nevertheless, the rate of degradation in the TPH was much higher than that of Oil/Grease; this must be attributed to the different elemental composition of hydrocarbon in the TPH and the Oil/Grease.

Table 4.5: General Linear Model: Oil and Grease, TPH versus Weeks, Nitrogen Level

Factor	Type	Levels	Values
Weeks	**fixed**	**8**	**Week 0, Week 1, Week 2, Week 3, Week 4, Week 5,Week 6, Week 7**
Nitrogen Level	**fixed**	**4**	**0.4, 1.0, 1.6, 2.2**

The experimental data consist of two fixed factors (Weeks for degradation and the nitrogen levels), of which the weeks consist of 8 levels (Week 0 – Week 7) and that of the nitrogen consists of 4 levels (0.4, 1.0, 1.6 and 2.2).

Table 4.6: Analysis of Variance for Oil and Grease, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	8458814684	8458814684	1208402098	840478.71	0.000
Nitrogen Level	3	431124487	431124487	143708162	99953.20	0.000
Weeks*Nitrogen	21	146570334	146570334	6979540	4854.47	0.000
Error	64	92016	92016	1438		
Total	95	9036601521				

S = 37.9177 R-Sq = 100.00% R-Sq(adj) = 100.00%

The generalized linear model and the analysis of variance for Oil/Grease show that, the mean square of the weeks (1,208,402,098) and that of Nitrogen levels (143,708,162) as well as for the interaction (6,979,540) are many times larger than the within treatment or error mean square (1,438). These indicate that, it is unlikely that, the treatment means for the weeks and the nitrogen levels are equal, whereas the interaction also shows a significant contribution to the total breakdown of the hydrocarbons. Moreover, with a p-value for both the weeks and the nitrogen level less than 0.05. Its conclude that, the mean treatments of both the weeks and the nitrogen levels differ, that is weeks affects the rate of Oil/Grease degradation as well as the level of nitrogen for a biostimulation. Moreover, it is clearly evident that, the effect of the breakdown cannot be attributing to the two factors alone; however, the interaction between the weeks and the nitrogen level also contributes to the total breakdown of the compound.

Table 4.7: Analysis of Variance for TPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	4748032027	4748032027	678290290	3319697.57	0.000
Nitrogen Level	3	273386548	273386548	91128849	446004.06	0.000
Weeks*Nitrogen	21	116982789	116982789	5570609	27263.75	0.000
Error	64	13077	13077	204		
Total	95	5138414441				

S = 14.2942 R-Sq = 100.00% R-Sq(adj) = 100.00%

The generalized linear model and the analysis of variance for TPH did not show difference as compared to the Oil/Grease contaminate soil, the mean square of the weeks (678,290,290) as well as Nitrogen levels (91,128,849) accounts for larger variations than the within treatment or error mean square (204). It was also realized that, the interactions also had a mean square being far larger than the error mean sum of squares. It shows an unlikely that, the treatment means for the weeks and the nitrogen levels are equal. P-values for both the weeks and the nitrogen level less than 0.05. Hence the conclusion, mean treatments of both the weeks and the nitrogen levels differ, that is weeks and nitrogen levels affect the rate of TPH contaminated soil degradation. However, these differences that lead to the major breakdown cannot be attribute to the two factors alone, but its interaction also has a great contribution towards the breakdown.

Table 4.8: Grouping Information Using Tukey's Method and 95.0% Confidence for Oil and Grease

Weeks	N	Mean	Grouping
Week 0	12	34276.3	A
Week 1	12	21507.6	B
Week 2	12	19051.7	C
Week 3	12	14240.5	D
Week 4	12	11577.8	E
Week 5	12	8493.3	F
Week 6	12	5689.2	G
Week 7	12	3440.9	H

Means that do not share a letter are significantly different.

Table 4.9: Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Nitrogen			
Level	**N**	**Mean**	**Grouping**
0.4	24	17541.6	A
1.0	24	15293.6	B
1.6	24	14696.2	C
2.2	24	11607.3	D

Means that do not share a letter are significantly different.

Grouping of information using the Tukey's test for the biodegradation of Oil/Grease indicate a significantly different degradation for all the weeks, the mean values shows a higher rate of decomposition of hydrocarbon as the weeks increase.

Moreover, for Oil/Grease contaminated soil decomposition, there exist much significant differences among all the nitrogen levels. Preferably, 2.2 level of nitrogen shows a higher rate of decomposition among all the other levels since it gives the lowest mean.

Table 4.10: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Weeks	N	Mean	Grouping
Week 0	12	21514.9	A
Week 1	12	19248.3	B
Week 2	12	16926.6	C
Week 3	12	10013.5	D
Week 4	12	7350.6	E
Week 5	12	5840.0	F
Week 6	12	3445.6	G
Week 7	12	1813.7	H

Means that do not share a letter are significantly different.

Table 4.11: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Nitrogen			
Level	**N**	**Mean**	**Grouping**
0.4	24	12728.5	A
1.0	24	11557.4	B
1.6	24	10651.4	C
2.2	24	8139.2	D

Means that do not share a letter are significantly different.

On the part of TPH, grouping of information using the Tukey's test for the biodegradation of shows a significantly different degradation for all the weeks, this might be due to the fact that, levels of hydrocarbon in the contaminated soil decreases and the rate of chemical excretion and respiration of the living microbes increases with better environmental stimulus, hence higher rate of decomposition of the hydrocarbons. Furthermore, different levels of nitrogen show a much significant differences for the bioremediation processes. All the levels show a significantly different decomposition rate among them.

4.1.1.2 Main Effect Plots

Plotting of the main effects is very essential for the entire analysis of the study; this is done in order to come out with the preferred level of nitrogen that will be needed to stimulate the

condition in the contaminated soil needed for higher microbial activities for decomposition of hydrocarbons in the soil.

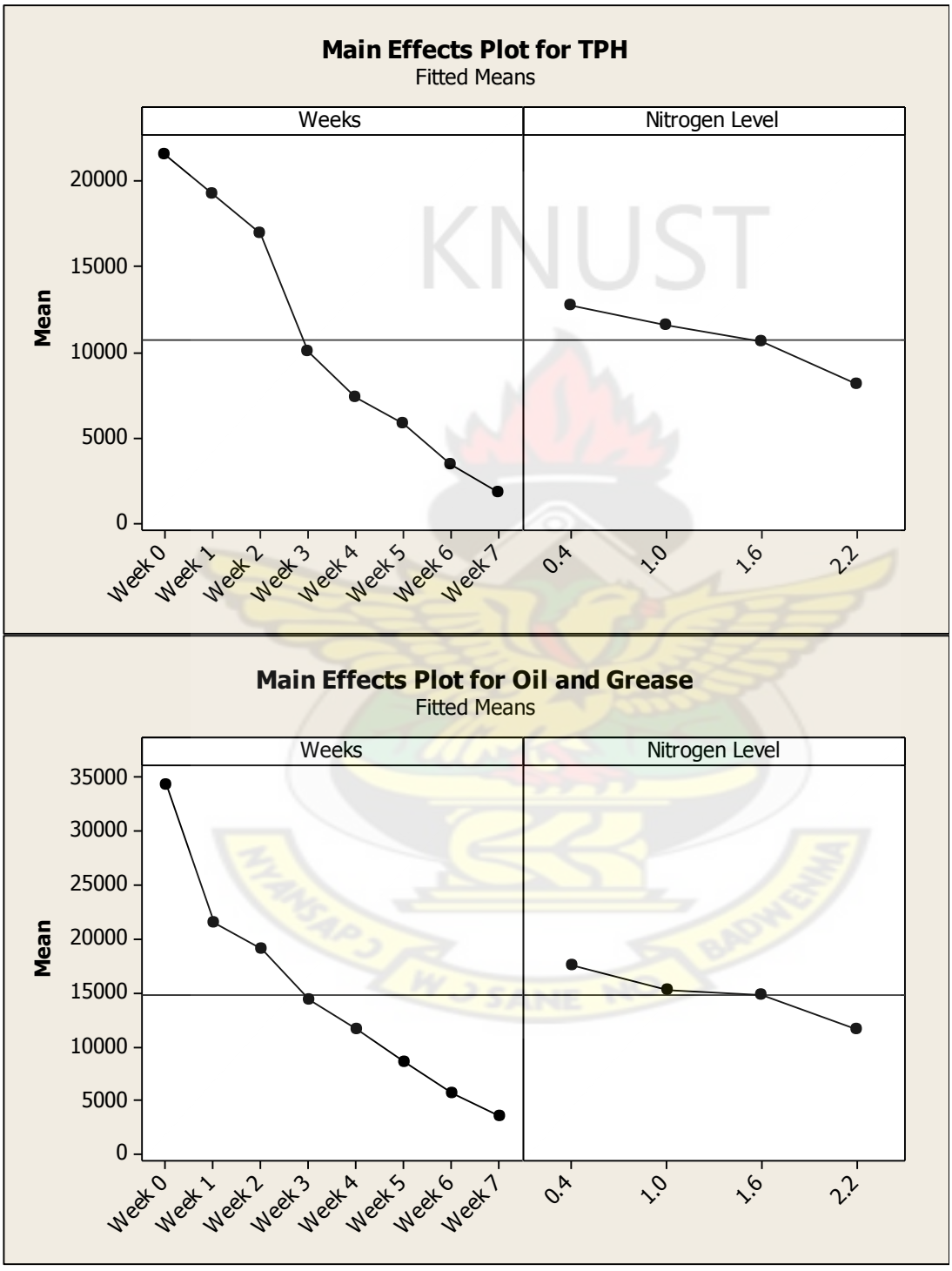


Figure 4.6: Residual Plot of Nitrogen and Weeks for Oil/Grease and TPH in HC/Fertilizer blend

The main effect plots of the TPH and the Oil/Grease gives evidence to believe that, in both cases, weeks contributes significantly to the total degradation and decomposition of the hydrocarbon. Additionally, nitrogen levels contribute immensely to total degradations. Figure 4.6 shows that, the higher the level of nitrogen the better the rate of degradation. Plots in both treatments shows that for the Hydrocarbon/Fertilizer blend a level of performance from the highest level are 2.2, 1.6, 1.0, and 0.4 respectively. This confirms the earlier findings that, the higher the level of nitrogen in the blend the better the degradation process and the higher the rate of decomposition, whereas the longer the degradation process, the better it leads to a total degradation of the hydrocarbon.

4.1.1.3 Residual Plots for Hydrocarbon/Fertilizer Blend

Residual plots of the associated model was plotted to ascertain if it fulfills the assumptions of the model.

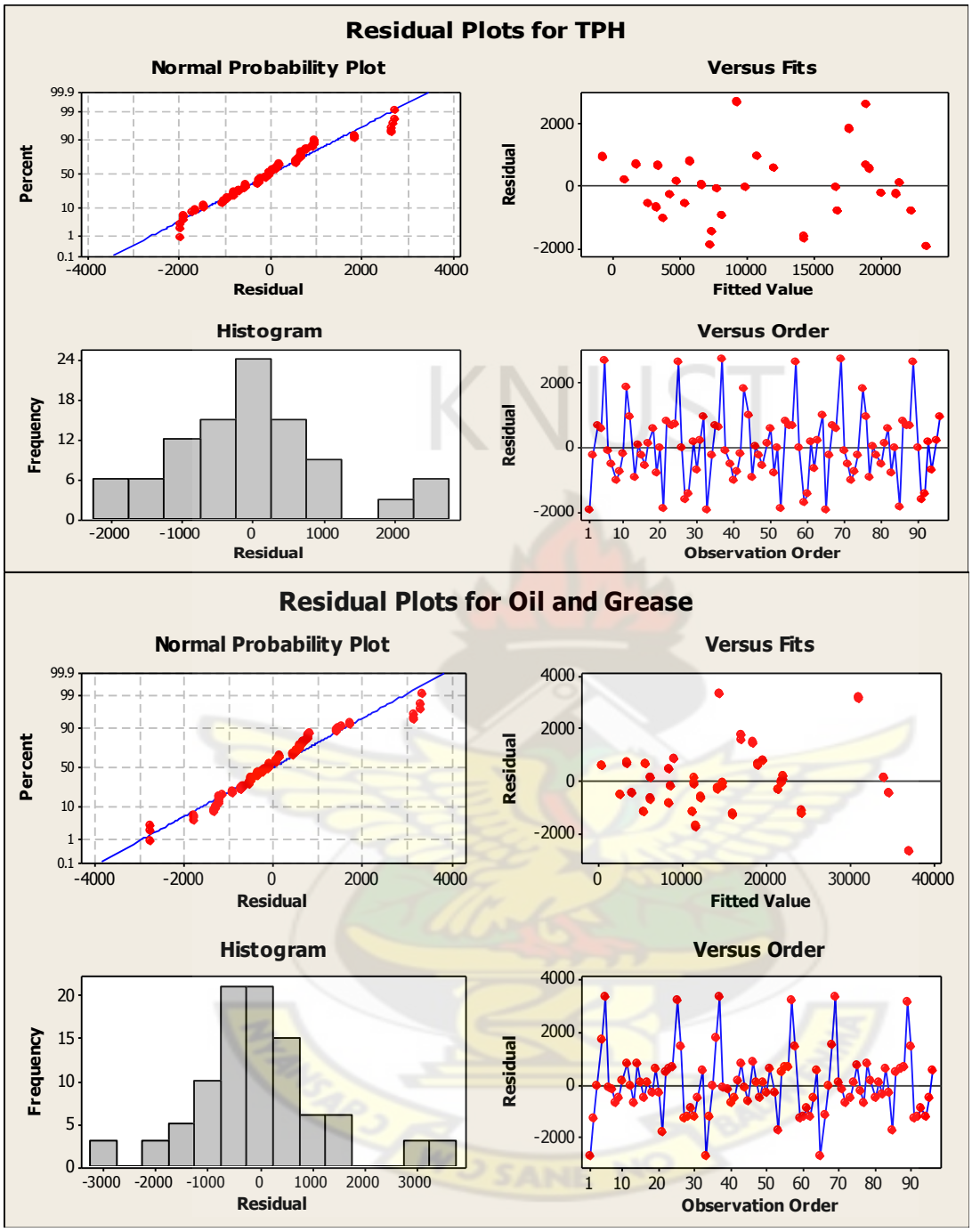


Figure 4.7: Residual Plot for TPH and Oil/Grease in Hydrocarbon/Fertilizer blend

1. The Diagnostic of the residual of hydrocarbon/fertilizer blend is shown above. The first upper left plot is the normal probability plot of the standardized residuals. It's been noticed that most of

the residuals falls on the straight line with the exception of a few residual deviating from normality. This is due to a sharp degradation between some weeks. Therefore the normality assumption is satisfied and so the residuals appear to be normally distributed

2. The upper right plot shows the plot of residuals in time sequence, which is helpful in detecting correlation between the residuals and to check if the independence assumption on the errors has been violated. These plots of residuals for both the TPH and Oil/Grease, there is no reason to suspect any violation of the independence or constant variance assumption

3. At the left side of the bottom plots shows confirms the normality assumption with normal residual histogram plot, which does not, shows any skew of the residuals.

4. The bottom part of the diagnostic is the residuals versus observation plot of observation order. It is clearly seen that it is not significant deviation in the order of plot.

4.1.2 Poultry Manure and Hydrocarbon Contaminated Soil Blends

Poultry manure was also incorporate as an alternative substrate for the bioremediation process, in the process of finding the best substrate that provides a suitable environment for effective microbial activities.

Table 4.12 Mean results of Oil/Grease, TPH for 0.4% Nitrogen level in Hydro-Carbon/Poultry Manure blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	17685.33	13144.23	48.41	38.90626
Week 2	13711.33	8911.00	60.00	58.58210
Week 3	7860.77	5084.33	77.07	76.36826
Week 4	6212.33	3583.39	81.88	83.34459
Week 5	5104.67	2804.33	85.11	86.96559
Week 6	3407.33	1950.00	90.06	90.93649
Week 7	1010.00	393.33	97.05	98.17181

Table 4.12 shows the degradation rate of hydrocarbon soil when the nitrogen level in the HC/ poultry manure blend was augmented to 0.4%. By week five 5, 0.4% had recorded more than 85% percent degradation for both oil/grease and TPH and achieved more than 97% of total degradation by week 7 as shown in Table 4.12.

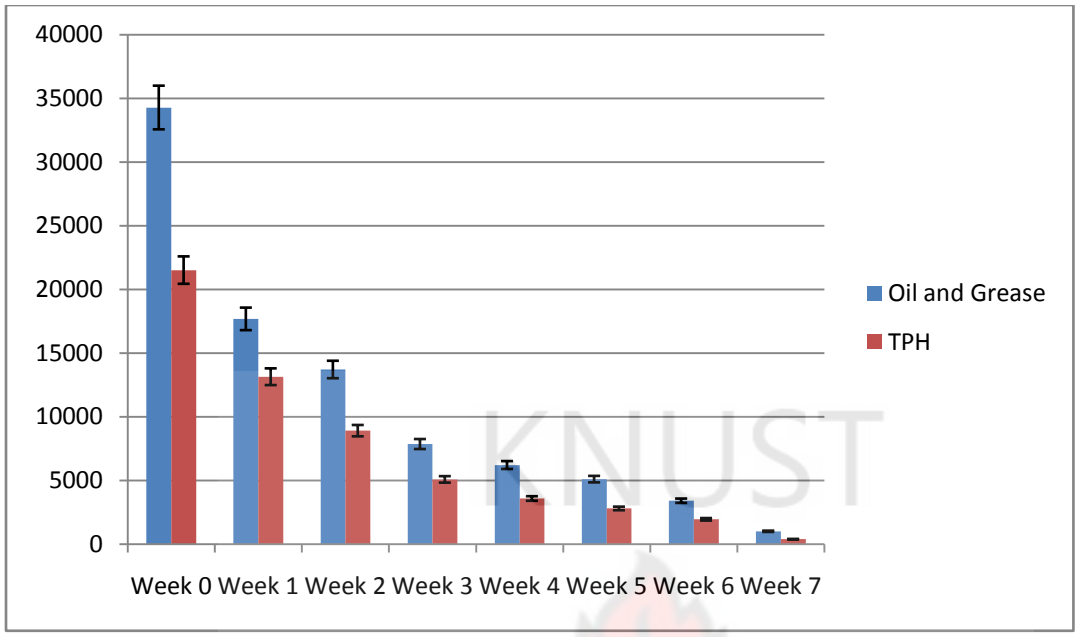


Figure 4.8: Levels of Oil/Grease and TPH degradation in Weeks of 0.4 level of Nitrogen in HC/Poultry manure blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 0.4 % nitrogen level within the poultry manure/HC blend. From figure 4.8, by week five 3, TPH had dropped significantly below 5000 mg/kg bar line, whiles Oil/Grease dropped to the sample level in week 5.

Table 4.13 Mean results of Oil/Grease, TPH for 1.0% Nitrogen level in Hydro-Carbon/Poultry Manure blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	12016.56	7446.67	64.94	65.38824
Week 2	10746.00	7316.33	68.65	65.99403
Week 3	6506.00	4482.33	81.02	79.16633
Week 4	4948.67	3464.33	85.56	83.89794
Week 5	2469.67	1256.00	92.80	94.16217
Week 6	982.33	258.00	97.13	98.80083
Week 7	235.00	47.67	99.31	99.77845

Table 4.13 shows the degradation rate of HC soil when the nitrogen level in the HC poultry manure blend was augmented to 1.0%.By week five 5 there had been a percent degradation of more than 92% for both oil/grease and TPH, which shows a much more faster rate of degradation and achieved a little more than 99% for both oil/grease and TPH.

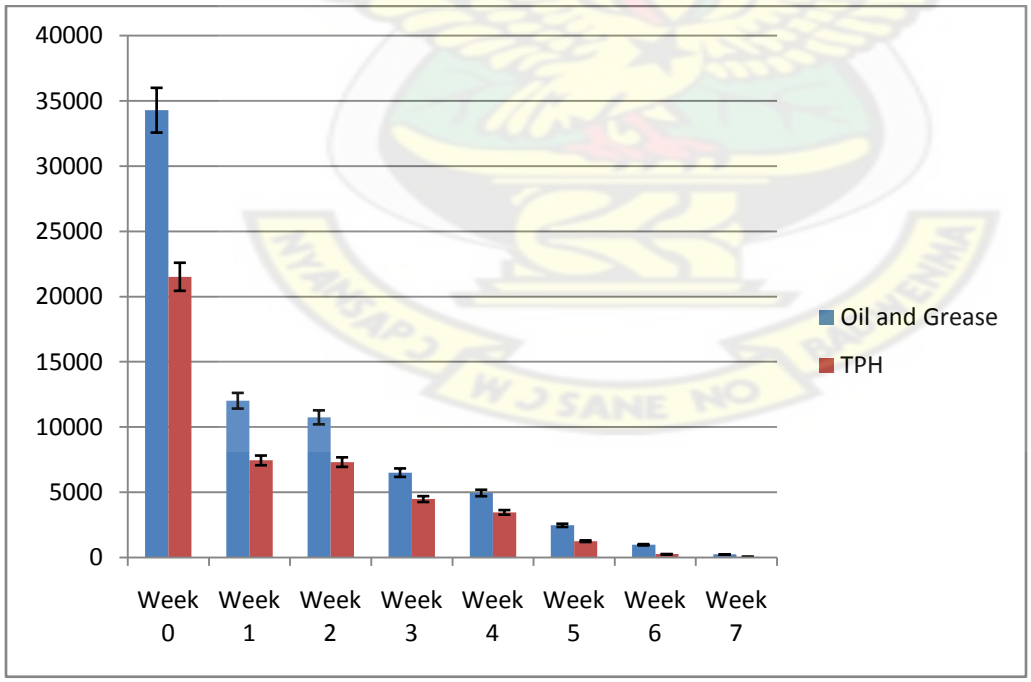


Figure 4.9: Levels of Oil/Grease and TPH degradation in Weeks of 1.0 level of Nitrogen in HC/Poultry manure blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 1.0% nitrogen level within the poultry manure HC blend is shown in figure 4.9, by week five 4, oil/grease and TPH had dropped below 5000 mg/kg bar line.

Table 4.14 Mean results of Oil and grease, TPH and HPC for 1.6% Nitrogen level in Hydro-Carbon/Poultry Manure blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	11457.67	7366.00	66.57	65.76319
Week 2	8750.00	5563.33	74.47	74.14189
Week 3	4797.00	3510.33	86.01	83.68414
Week 4	3502.33	2310.67	89.78	89.26013
Week 5	780.53	443.33	97.72	97.93941
Week 6	118.67	46.33	99.65	99.78464
Week 7	95.67	40.67	99.72	99.81098

The degradation rate of HC soil when the nitrogen level in the HC/topsoil blend was augmented to 1.6% is as shown in Table 4.14.By week five 5 there had been a percent degradation of more than 97% for both oil/grease and TPH.

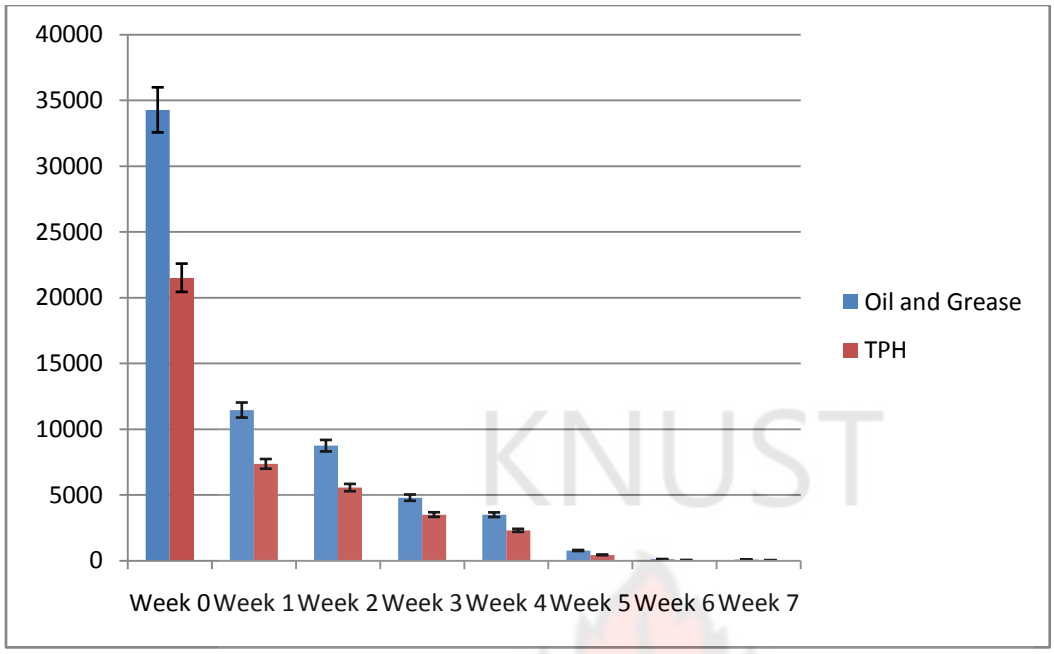


Figure 4.10: Levels of Oil/Grease and TPH degradation in Weeks of 1.6 level of Nitrogen in HC/Poultry manure blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 1.6% nitrogen level within the poultry manure/HC blend is shown in figure 4.10, by week five 3, oil/grease and TPH had dropped below 5000 mg/kg bar line.

Table 4.15 Mean results of Oil and grease, TPH and HPC for 2.2% Nitrogen level in Hydro-Carbon/Poultry Manure blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	10609.67	6348.33	69.05	70.49325
Week 2	5706.33	3763.00	83.35	82.50976
Week 3	3053.00	2249.00	91.09	89.54676
Week 4	1549.33	875.33	95.48	95.93149
Week 5	111.00	99.33	99.68	99.53830
Week 6	96.00	46.33	99.72	99.78464
Week 7	81.67	40	99.76	99.81408

From Table 4.15, when the nitrogen level was augment to 2.2%, by week 5, both the Oil/grease and the TPH had achieved a degradation of more than 99.5% which shows a me improving and a better rate of degradation for the hydrocarbons, it should be acknowledge that, not all the elements found in the hydrocarbon can be degrade due to its chemical compositions and the bondage or bonds between their compounds.

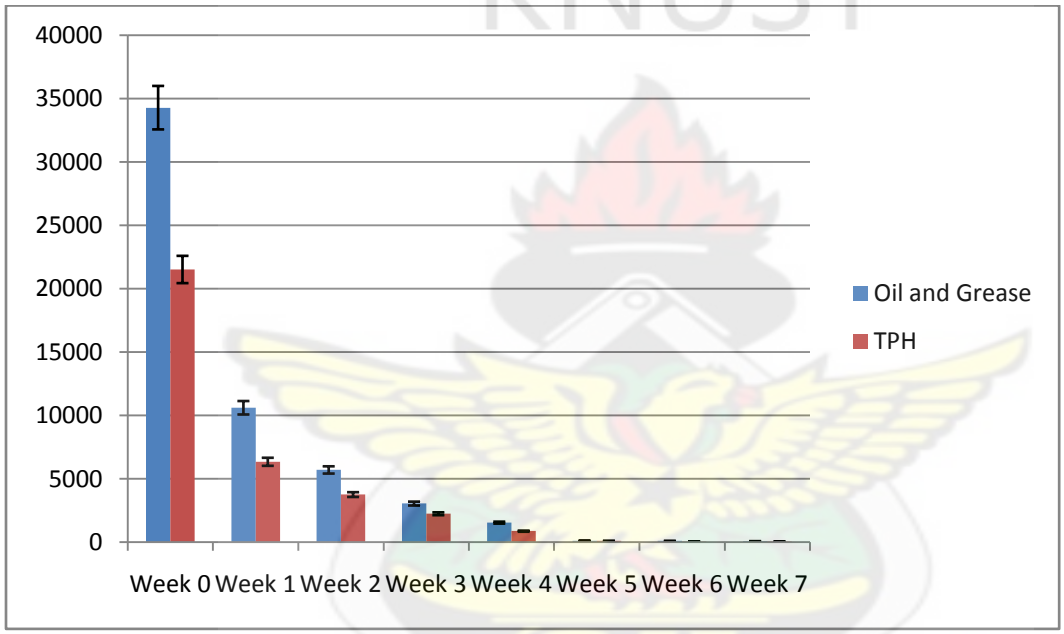


Figure 4.11: Levels of Oil/Grease and TPH degradation in Weeks of 2.2 level of Nitrogen in HC/Poultry manure blend

The graphical presentation with the error bars shows a much more improving situation for the HC/Poultry manure blend for the biodegradation processes. Clearly, by week 2, TPH has achieved a tolerable level of being less than 5000mg/kg of the remains of hydrocarbon in the contaminated soil; whiles by week 3 oil/grease had also achieved the same level of tolerable of less than 5000 mg/kg of hydrocarbon left in the contaminated soil. This shows a mass improvement of a better rate of degradation for the bioremediation process.

4.1.2.1 Test of Hypothesis for Hydrocarbon/Poultry Manure Blend

Again for the bioremediation processes, test of differences among the means of the weeks for degradation and that of the different levels of augmentation of nitrogen for biostimulation was done to establish if there exist differences among them for augment with poultry manure.

H_{10}: There is no significant difference between the average degradation of hydrocarbon among the different levels of the weeks

H_{11}: There is a significant difference between the average degradation of hydrocarbon among different levels of the weeks

H_{20}: There is no significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{21}: There is a significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{30}: There is no significant difference for the interactions of weeks and the nitrogen level

H_{31}: There is a significant difference between for the interaction of weeks and the nitrogen levels.

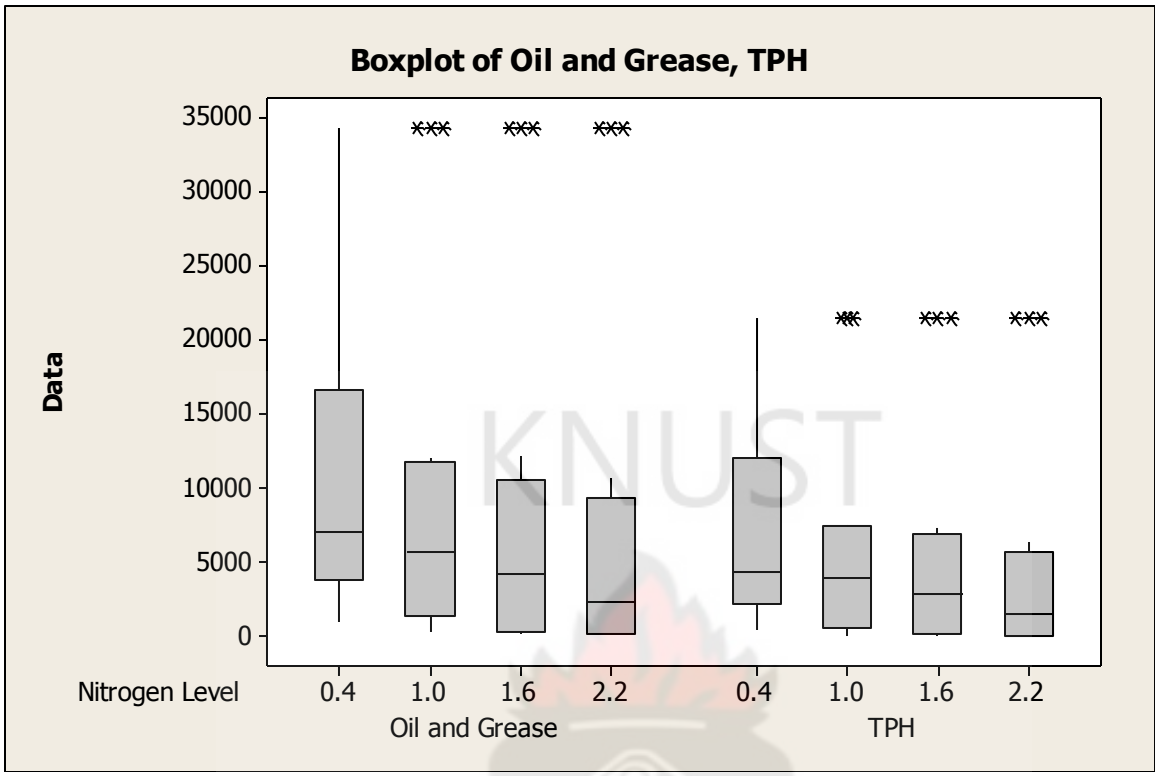


Figure 4.12: Box plot for Different levels of Nitrogen for Oil/Grease and TPH in HC/Poultry manure blend

The box plot for contaminated hydrocarbon soil and the poultry manure blend shows lower means for both oil/grease and the TPH. As an unmistakable in Figure 4.12, as the level of nitrogen for augmentation increases, lead to a higher rate of degradation of hydrocarbon in both the Oil/Grease and the TPH hence a recorded lower mean associated with it. Obviously, the rate of degradation in the TPH was much higher than that of Oil/Grease; and might be attributed to the different elemental composition of hydrocarbon in the TPH and the Oil/Grease.

Table 4.16: General Linear Model: Oil and Grease, TPH versus Weeks, Nitrogen Level

Factor	Type	Levels	Values
Weeks	fixed	8	Week 0, Week 1, Week 2, Week 3, Week 4, Week 5, Week 6, Week 7
Nitrogen Level	fixed	4	0.4, 1.0, 1.6, 2.2

The experimental data consist of two fixed factors (Weeks for degradation and the nitrogen levels), of which the weeks consist of 8 levels (Week 0 – Week 7) and that of the nitrogen consists of 4 levels (0.4, 1.0, 1.6 and 2.2).

Table 4.17: Analysis of Variance for Oil and Grease, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	10496333421	10496333421	1499476203	141474.00	0.00
Nitrogen Level	3	234499904	234499904	78166635	7374.94	0.00
Weeks*Nitrogen	21	104105982	104105982	4957428	467.73	0.00
Error	64	678333	678333	10599		
Total	95	10835617639				

S = 102.951 R-Sq = 99.99% R-Sq(adj) = 99.99%

The generalized linear model and the analysis of variance for Oil/Grease show that, the mean square of the weeks (1,499,476,203) and that of Nitrogen levels (78,166,635)as well as the interaction between the weeks and the nitrogen level (4,957,428) is many times larger than the within treatment or error mean square (10599). It is unlikely for, the treatment means for the weeks and the nitrogen levels to be equal. However, it is evident from the interactions that, the weeks and nitrogen level are not the only factors that contributes to the degradation but the interaction of these two factors also contributes immensely to the total degradation process. Furthermore, with p-values for the weeks, nitrogen level and the interaction being less than 0.05. Hence, the mean treatments of both weeks and the nitrogen levels differ, as well as its interaction, that is weeks and nitrogen levels affects the rate of Oil/Grease degradation as well as the interaction between these two factors.

Table 4.18: Analysis of Variance for TPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	4201443241	4201443241	600206177	5371199.81	0.000
Nitrogen Level	3	102255064	102255064	34085021	305024.28	0.000
Weeks*Nitrogen	21	76930038	76930038	3663335	32782.91	0.000
Error	64	7152	7152	112		
Total	95	4380635494				

S = 10.5710 R-Sq = 100.00% R-Sq(adj) = 100.00%

The generalized linear model and the analysis of variance for TPH did not show difference as compared to the Oil/Grease contaminate soil in terms of degradation, the mean square of the weeks (600,206,177), Nitrogen levels (34,085,021) and the interaction of weeks and nitrogen levels (3,663,335) accounts for larger variations than the within treatment or error mean square (112). It shows an unlikely for the treatment means of weeks and nitrogen levels to be equal. P-values for both the weeks and the nitrogen levels are less than 0.05. Hence the conclusion, mean treatments of both the weeks and the nitrogen levels differ, that is weeks and nitrogen levels affect the rate of TPH contaminated soil degradation. Moreover, these factors interaction also contributes to the difference in the mean values of the factors and also accounts for some of the degradation processes.

Table 4.19: Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Weeks	N	Mean	Grouping
Week 0	12	34278.0	A
Week 1	12	12942.3	B
Week 2	12	9728.4	C
Week 3	12	5554.2	D
Week 4	12	4053.2	E
Week 5	12	2116.5	F
Week 6	12	1151.1	G
Week 7	12	355.6	H

Means that do not share a letter are significantly different.

Table 4.20: Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Nitrogen			
Level	**N**	**Mean**	**Grouping**
0.4	24	11158.7	A
1.0	24	9022.8	B
1.6	24	7972.5	C
2.2	24	6935.6	D

Means that do not share a letter are significantly different.

Using the Tukey's test for the biodegradation of Oil/Grease indicate a significantly different degradation for all the weeks, the mean values shows a higher rate of decomposition of hydrocarbon as the weeks increase.

Similarly, for Oil/Grease contaminated soil decomposition, there exist much significant differences among all the levels (0.4, 1.0, 1.6, 2.2). Preferably, 2.2 level of nitrogen shows a higher rate of decomposition among all the other levels since it gives the lowest mean.

Table 4.21: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Weeks	**N**	**Mean**	**Grouping**
Week 0	12	21515.4	A
Week 1	12	8576.3	B
Week 2	12	6388.4	C
Week 3	12	3831.5	D
Week 4	12	2558.4	E
Week 5	12	1150.8	F
Week 6	12	575.2	G
Week 7	12	130.4	H

Means that do not share a letter are significantly different.

Table 4.22: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Nitrogen			
Level	**N**	**Mean**	**Grouping**
0.4	24	7173.2	A
1.0	24	5723.6	B
1.6	24	5099.4	C
2.2	24	4367.0	D

Means that do not share a letter are significantly different.

On the part of TPH, Tukey's test for the biodegradation shows a significantly different degradation for all the weeks. Additionally, different levels of nitrogen show a much significant differences for the bioremediation processes. All the levels show a significantly different decomposition rate among them.

4.1.2.2 Main Effect Plots

Plotting of the main effects is very crucial for the analysis; it is done in order to come out with the preferred level of nitrogen that will be needed to stimulate the condition in the contaminated soil needed for higher microbial activities for decomposition of hydrocarbons in the soil.

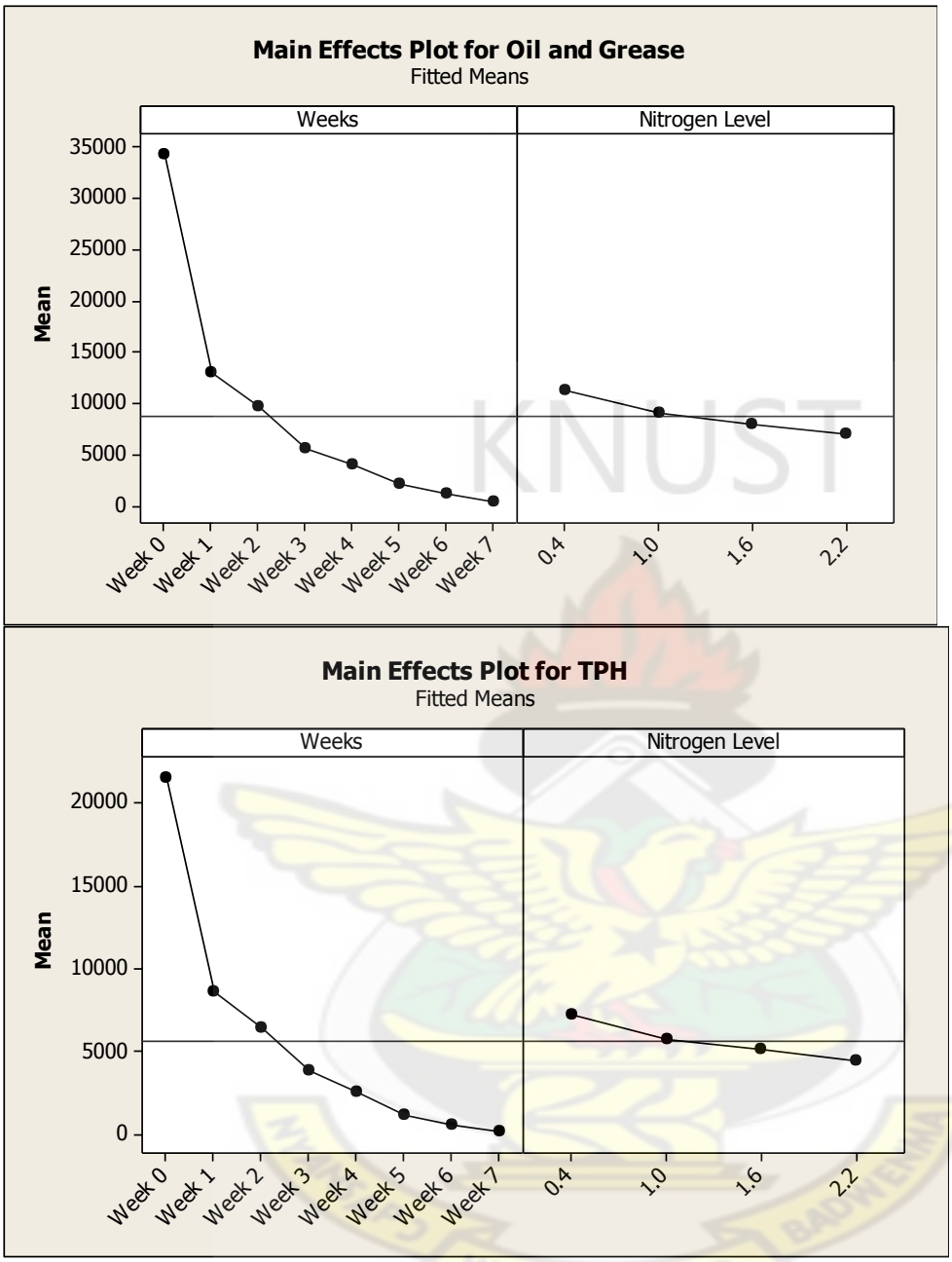


Figure 4.13: Residual Plot of Nitrogen and Weeks for Oil/Grease and TPH in HC/Fertilizer blend

The main effect plots of the TPH and the Oil/Grease gives confirmation to believe that, in both cases, weeks contributes significantly to the total degradation and decomposition of the hydrocarbon. Additionally, nitrogen levels contribute immensely to total degradations. Figure 4.13 shows that, the higher the level of nitrogen the better the rate of degradation. Plots in both

treatments show that, for Hydrocarbon/Poultry manure blend, the levels of performance from the highest level are 2.2, 1.6, 1.0, and 0.4 respectively. This confirms the earlier findings that, the higher the level of nitrogen in the blend the better the degradation process and the higher the rate of decomposition, whereas the longer the degradation process, the better it leads to a total degradation of the hydrocarbon.

4.1.2.3 Residual Plots for Hydrocarbon/Poultry Manure Blend

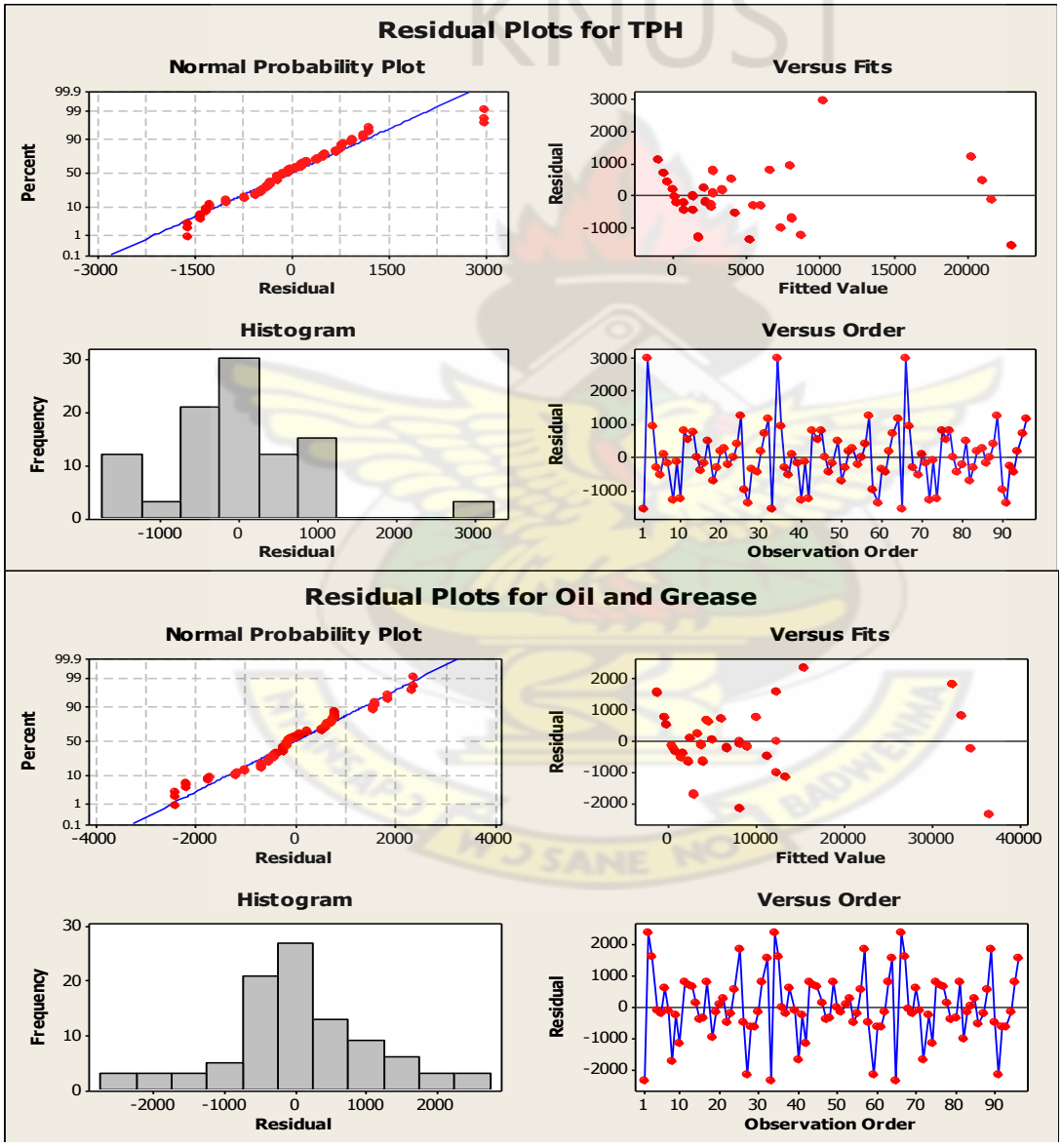


Figure 4.14: Residual Plot for TPH and Oil/Grease in Hydrocarbon/Poultry Manure Blend

1. The Diagnostic of the residual of hydrocarbon/poultry manure blend is shown above. The first upper left plot is the normal probability plot of the standardized residuals. It's been noticed that most of the residuals falls on the straight line with the exception of a few residual deviating from normality. This is due to a sharp degradation between some weeks. Therefore the normality assumption is satisfied and hence the residuals appear to be normally distributed

2. The upper right plot shows the plot of residuals in time sequence, which is helpful in detecting correlation between the residuals and to check if the independence assumption on the errors has been violated. These plots of residuals for both the TPH and Oil/Grease, there is no reason to suspect any violation of the independence or constant variance assumption

3. At the left side of the bottom plots shows confirms the normality assumption with normal residual histogram plot, which does not, shows any skew of the residuals.

4. The bottom part of the diagnostic is the residuals versus observation plot of observation order. It is clearly seen that it is not significant deviation in the order of plot.

4.1.3 Compost and Hydrocarbon Contaminated Soil Blend

In order to have a thorough understanding of the substrate utilization, a compost blend was also used as a substrate to find out the effects of different substrates with the fixed factors on degradation of hydrocarbon compounds.

Table 4.23 Mean results of Oil and grease, TPH and HPC for 0.4% Nitrogen level in Hydro-Carbon/Compost blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	21251.67	17254.33	38.00	19.80269
Week 2	18149.64	12847.33	47.05	40.28621
Week 3	12840.00	8851.67	62.54	58.85788
Week 4	9357.67	5988.67	72.70	72.16496
Week 5	7810.67	5307.33	77.21	75.33177
Week 6	4906.67	2717.00	85.69	87.37151
Week 7	2353.33	1219.67	93.13	94.33105

Table 4.23 shows the rate of degradation level in a carbon contaminated soil with compost blend, when it is augmented with a 0.4 level of nitrogen, by week 5, the rate of degradation has reached a minimum of 77% and proof to be consistently decreasing for the weeks ahead, by week 7 both the oil/grease and TPH have achieved a degradation of more than 90% indicating a success of using compost in the bioremediation process of hydrocarbon.

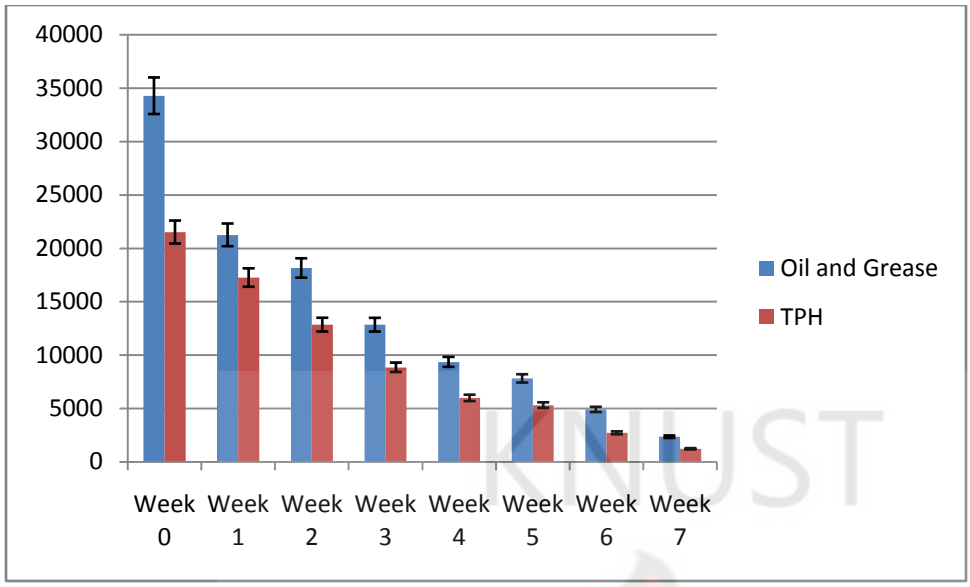


Figure 4.15: Levels of Oil/Grease and TPH degradation in Weeks of 0.4 level of Nitrogen in HC/Compost blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with 0.4% nitrogen level within the compost HC blend is shown in figure 4.15, by week 5 TPH had recorded a less than 5000mg/kg whiles Oil/grease achieved such a level in week 6.

Table 4.24 Mean results of Oil and grease, TPH and HPC for 1.0% Nitrogen level in Hydro-Carbon/Compost blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	21253.00	17251.67	38.00	19.81508
Week 2	18155.31	12825.67	47.04	40.38692
Week 3	12837.67	8853.00	62.55	58.85168
Week 4	9361.00	5985.33	72.69	72.18046
Week 5	7812.67	5314.67	77.21	75.29768
Week 6	3915.33	2018.67	88.58	90.61733
Week 7	920.33	387.33	97.32	98.19969

Degradation of hydrocarbon with an augmentation of 1.0 level of nitrogen, both TPH and oil/grease achieved a more than 75% of degradation by week 5. Moreover, a degradation rate of more than 97% was recorded by both different hydrocarbon compounds at the week 7. This

shows the high rate of facilitation compost exert on the decomposition of hydrocarbons in the contaminated soil.

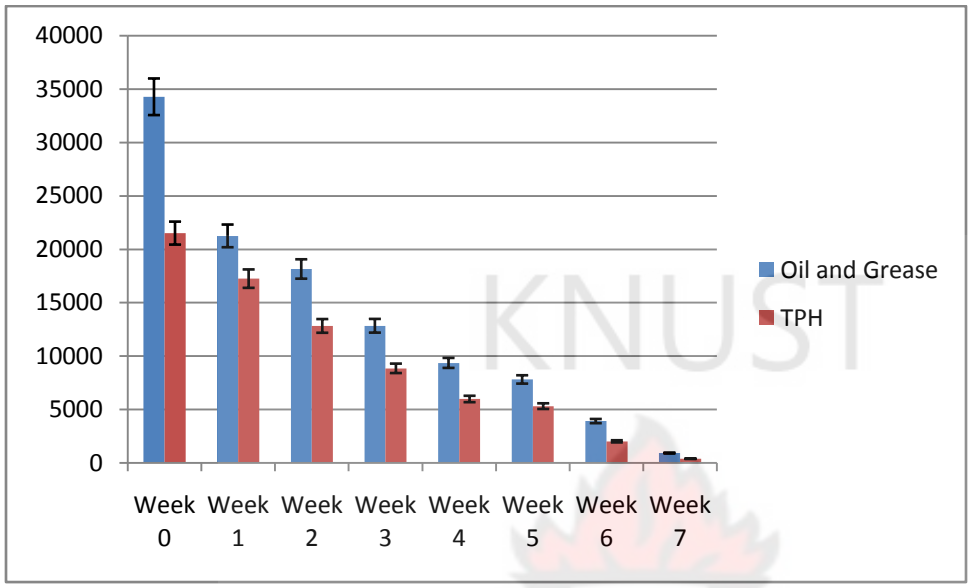


Figure 4.16: Levels of Oil/Grease and TPH degradation in Weeks of 1.0 level of Nitrogen in HC/Compost blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) shows a higher degradation rate for TPH than its counterpart Oil/grease, by week 5 the TPH has achieved a higher degradation with only a less than 5000mg/kg of TPH left, on the other hand, Oil/grease achieved this level of less than 500mg/kg at week 6.

Table 4.25 Mean results of Oil and grease, TPH and HPC for 1.6% Nitrogen level in Hydro-Carbon/Compost blend.

Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	20842.33	15843.00	39.20	26.3625
Week 2	10386.64	6848.33	69.70	68.16928
Week 3	5434.00	3750.39	84.15	82.56837
Week 4	3226.99	1834.33	90.59	91.47411
Week 5	2544.33	1282.17	92.58	94.04052
Week 6	1217.67	448.33	96.45	97.91617
Week 7	318.17	38.33	99.07	99.82183

An augment level of 1.6% nitrogen shows a much faster rate of degradation than its previous levels of compost blend. By week 5, TPH and Oil/grease have achieved a degradation rate of more than 92%. Moreover, nearly a total degradation for hydrocarbon was obtained in week 7 of which the degradation have achieved more than 99% for TPH and Oil/grease.

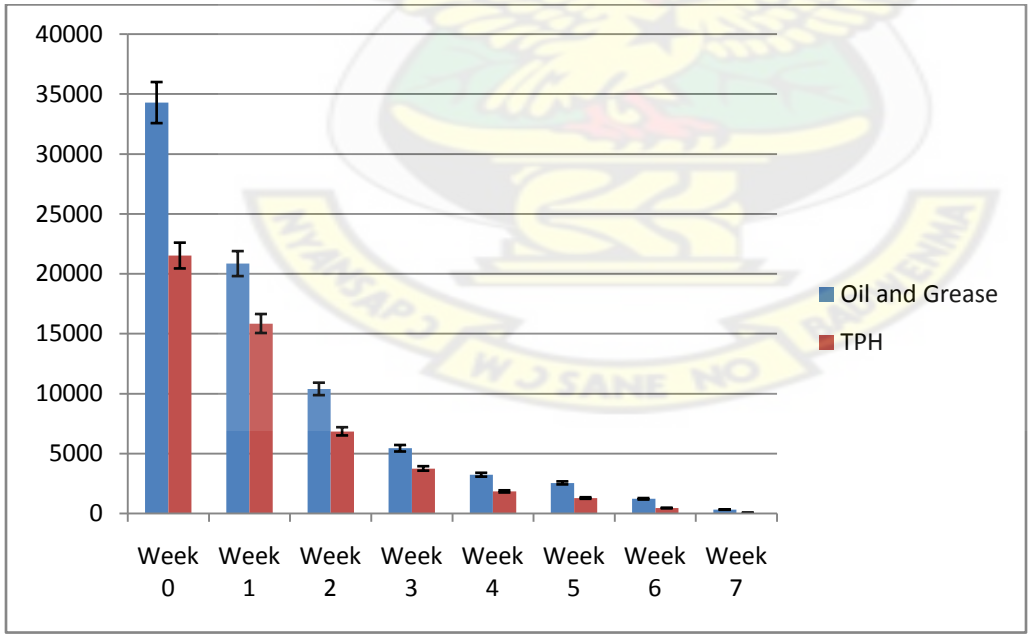


Figure 4.17: Levels of Oil/Grease and TPH degradation in Weeks of 1.0 level of Nitrogen in HC/Compost blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 1.6% nitrogen level within the Hydrocarbon/compost blend is shown in Figure 4.17, by week 3, TPH was just less around the 5000 mg/kg bar line on the graph, whiles Oil/Grease did not obtain this level for that particular week, however it was achieved in week 4, this shows that, TPH degrade faster in the 1.6% of nitrogen level tha in the Hydrocarbon/compost blend than the Oil/Grease.

Table 4.26 Mean results of Oil and grease, TPH and HPC for 2.2% Nitrogen level in Hydro-Carbon/Compost blend.

. Parameters Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	% degradation of oil/grease	% degradation TPH
Week 0	34278.00	21514.85	0.00	0.00
Week 1	13810.33	9585.27	59.71	55.44814
Week 2	7565.00	5979.33	77.93	72.20835
Week 3	3779.67	2443.13	88.97	88.64443
Week 4	3480.67	2081.73	89.85	90.32420
Week 5	2128.67	1433.62	93.79	93.33661
Week 6	1014.67	111.00	97.04	99.48408
Week 7	194.00	37.33	99.43	99.82648

Degradation rate for a level of 2.2 augment of nitrogen for the bioremediation process saw a magnificent improvement in the biostimulation process for the bioremediation in the Hydrocarbon/compost blend. From Table 4.26, by week week 5 both the Oil/Grease and TPH have achieved more than 93% of hydrocarbon degradation. By week 7, there were a dramatic degradation of 99.43% for Oil/Grease and 99.82% for TPH.

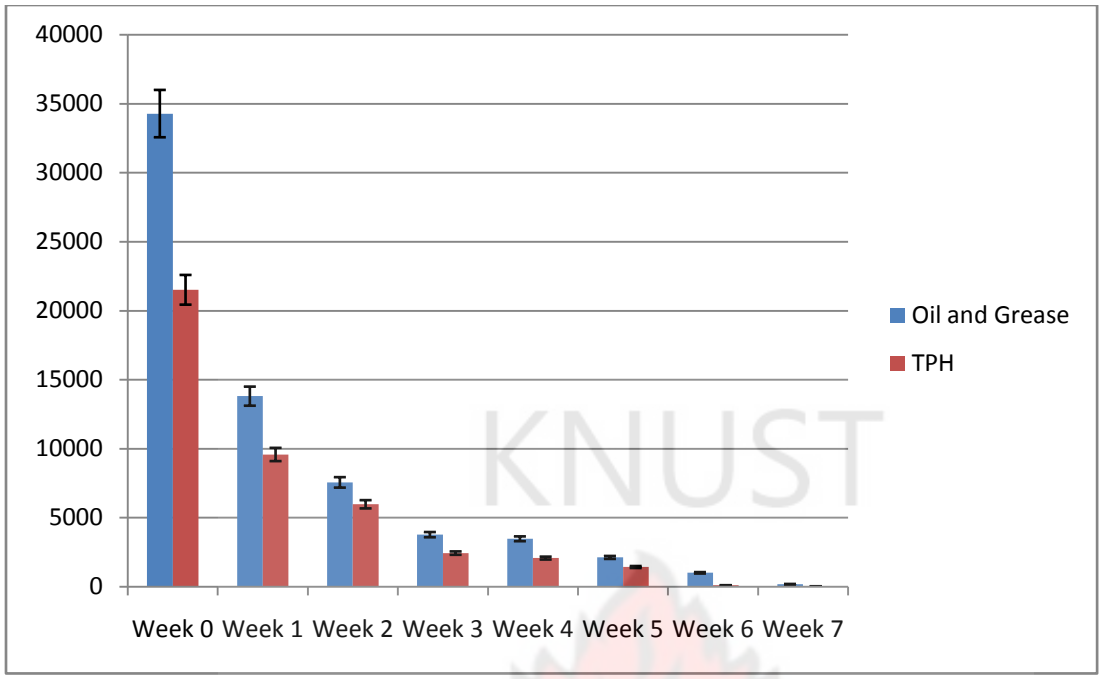


Figure 4.18: Levels of Oil/Grease and TPH degradation in Weeks of 1.0 level of Nitrogen in HC/Compost blend

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 2.2% nitrogen level within the Hydrocarbon/compost blend is shown in Figure 4.18, by week 2, TPH was just around the 5000 mg/kg bar line on the graph, whiles Oil/Grease did not obtain this level for that particular week, however it was achieved in week 3, and slowly dies off as the week pass by to a nearly total degradation for the hydrocarbon contaminated soil.

4.1.3.1 Test of Hypothesis for Hydrocarbon/Compost Blend

Test of differences among the means of the weeks for degradation and that of the different levels of augmentation of nitrogen for biostimulation was done to establish if there exist differences among them. By and large the test of hypothesis is to test the following:

H_{10}: There is no significant difference between the average degradation of hydrocarbon among the different levels of the weeks

H_{11}: There is a significant difference between the average degradation of hydrocarbon among different levels of the weeks

H_{20}: There is no significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{21}: There is a significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{30}: There is no significant difference for the interactions of weeks and the nitrogen level

H_{31}: There is a significant difference between for the interaction of weeks and the nitrogen levels.

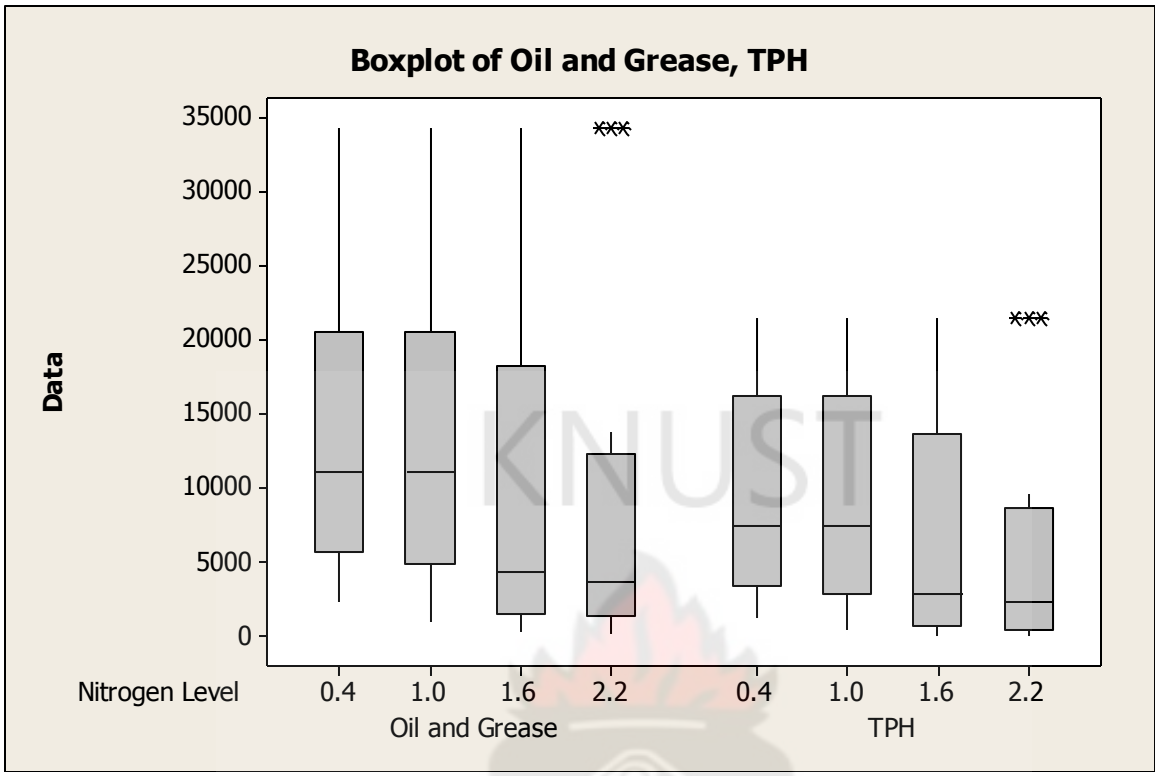


Figure 4.19: Box plot for Different levels of Nitrogen for Oil/Grease and TPH in HC/Compost blend

Clearly, the box plot for the hydrocarbon soil contamination and the compost blend indicates that, different levels of nitrogen augmentation have different levels of the rate of degradation as was realized in the previous two substrate situations. As the level of nitrogen for augmentation increases, lead to a higher rate of degradation of hydrocarbon in both the Oil/Grease and the TPH. Nevertheless, the box plot above shows no sign of differentiations among the 0.4 and 1.0 for both oil/grease and TPH as well as for 1.6 and 2.2 for both cases in the compost blend substrate.

Table 4.27: General Linear Model: Oil and Grease, TPH versus Weeks, Nitrogen Level

Factor	Type	Levels	Values
Weeks	**fixed**	8	Week 0, Week 1, Week 2, Week 3, Week 4, Week 5, Week 6, Week 7
Nitrogen Level	**fixed**	4	0.4, 1.0, 1.6, 2.2

In the compost blend substrate experimental data, its also consist of two fixed factors (Weeks for degradation and the nitrogen levels), of which the weeks consist of 8 levels (Week 0 – Week 7) and that of the nitrogen consists of 4 levels (0.4, 1.0, 1.6 and 2.2).

Table 4.28: Analysis of Variance for Oil and Grease, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	10161689727	10161689727	1451669961	10493209.57	0.00
Nitrogen Level	3	555168103	555168103	185056034	1337653.74	0.00
Weeks*Nitrogen	21	278636816	278636816	13268420	95909.07	0.00
Error	64	8854	8854	138		
Total	95	10995503500				

S = 11.7620 R-Sq = 100.00% R-Sq(adj) = 100.00%

In the compost blend substrate for biostimulation, the generalized linear model and the analysis of variance for Oil/Grease demonstrate that, the mean square of the weeks (1,451,669,961) , Nitrogen levels (185,056,034) and the interaction of weeks and the nitrogen levels (13,268,420) are far larger than the within treatment or error mean square (138). Which indicate a more explanatory of the degradation being accounted for by the nitrogen levels, weeks and the interaction. Therefore, it is unlikely for, the treatment means for the weeks and the nitrogen levels to be equal in terms of average decomposition. Moreover, these changes might also be caused by the interactions of the factors. Besides, with p-values for both the weeks and the nitrogen level less than 0.05. Hence, the mean treatments of both the weeks and the nitrogen levels differ, that is weeks affects the rate of Oil/Grease degradation as well as the level of nitrogen as a biostimulation method.

Table 4.29: Analysis of Variance for TPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	4525871989	4525871989	646553141	8460015.77	0.000
Nitrogen Level	3	298284977	298284977	99428326	1300999.33	0.000
Weeks*Nitrogen	21	161572933	161572933	7693949	100673.75	0.000
Error	64	4891	4891	76		
Total	95	4985734791				

S = 8.74212 R-Sq = 100.00% R-Sq(adj) = 100.00%

additionally, the analysis of variance for TPH hydrocarbon demonstrate that, the mean square of the weeks (646,553,141) and that of Nitrogen levels (99,428,326) and the interaction (7693949) are far larger than the within treatment or error mean square (4891). Therefore, it is unlikely for, the treatment means for the weeks and the nitrogen levels to be equal in terms of average decomposition. Whereas it also found that, the interaction is somehow also responsible for the differences. Besides, with p-values for both the weeks and the nitrogen level less than 0.05. Hence, the mean treatments of both the weeks and the nitrogen levels differ, that is weeks affects the rate of TPH degradation as well as the level of nitrogen as a biostimulation method, which leads to the rejection of the hypothesis H_{10} and H_{20}.

Table 4.30: Grouping Information Using Tukey's Method and 95.0% Confidence for Oil and Grease

Weeks	N	Mean	Grouping
Week 0	12	34278.0	A
Week 1	12	19289.3	B
Week 2	12	13564.1	C
Week 3	12	8722.8	D
Week 4	12	6356.6	E
Week 5	12	5074.1	F
Week 6	12	2763.6	G
Week 7	12	946.5	H

Means that do not share a letter are significantly different.

Table 4.31: Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Nitrogen Level	N	Mean	Grouping
0.4	24	13868.5	A
1.0	24	13566.7	A
1.6	24	9781.0	B
2.2	24	8281.4	C

Means that do not share a letter are significantly different.

Using Tukey's test for the biodegradation, it shows a significantly different degradation for all the weeks. Additionally, for the different levels of nitrogen, the levels 0.4 and 1.0 do not show any significant difference, this confirms the outcome of the box plot for the oil/grease, however, levels 1.6 and 2.2 shows a significant difference among its rates of biodegradation.

Table 4.32: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Weeks	N	Mean	Grouping
Week 0	12	21514.9	A
Week 1	12	14983.6	B
Week 2	12	9625.2	C
Week 3	12	5974.5	D
Week 4	12	3972.5	E
Week 5	12	3334.4	F
Week 6	12	1323.7	G
Week 7	12	420.7	H

Means that do not share a letter are significantly different.

Table 4.33: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Nitrogen			
Level	**N**	**Mean**	**Grouping**
0.4	24	9462.6	A
1.0	24	9268.9	B
1.6	24	6445.0	C
2.2	24	5398.3	D

Means that do not share a letter are significantly different.

Again using Tukey's test for the biodegradation shows a significantly different degradation for all the weeks. Furthermore, for the different levels of nitrogen, all levels show a significant difference among its rates of biodegradation.

4.1.3.2 Main Effect Plots

The plotting of the main effects is done in order to come out with the preferred level of nitrogen that will be needed to stimulate the condition in the contaminated soil needed for higher microbial activities for decomposition of hydrocarbons in the soil.

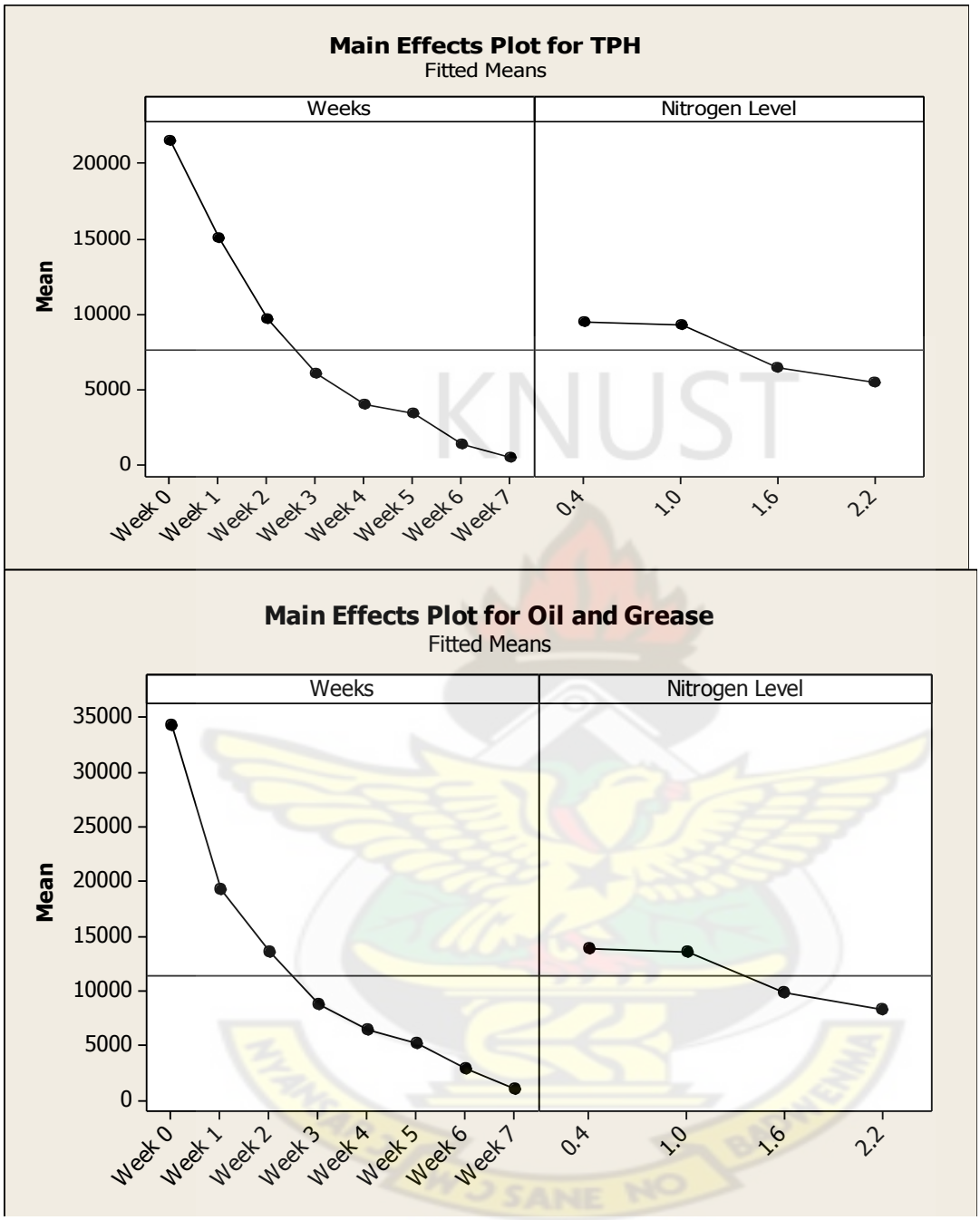
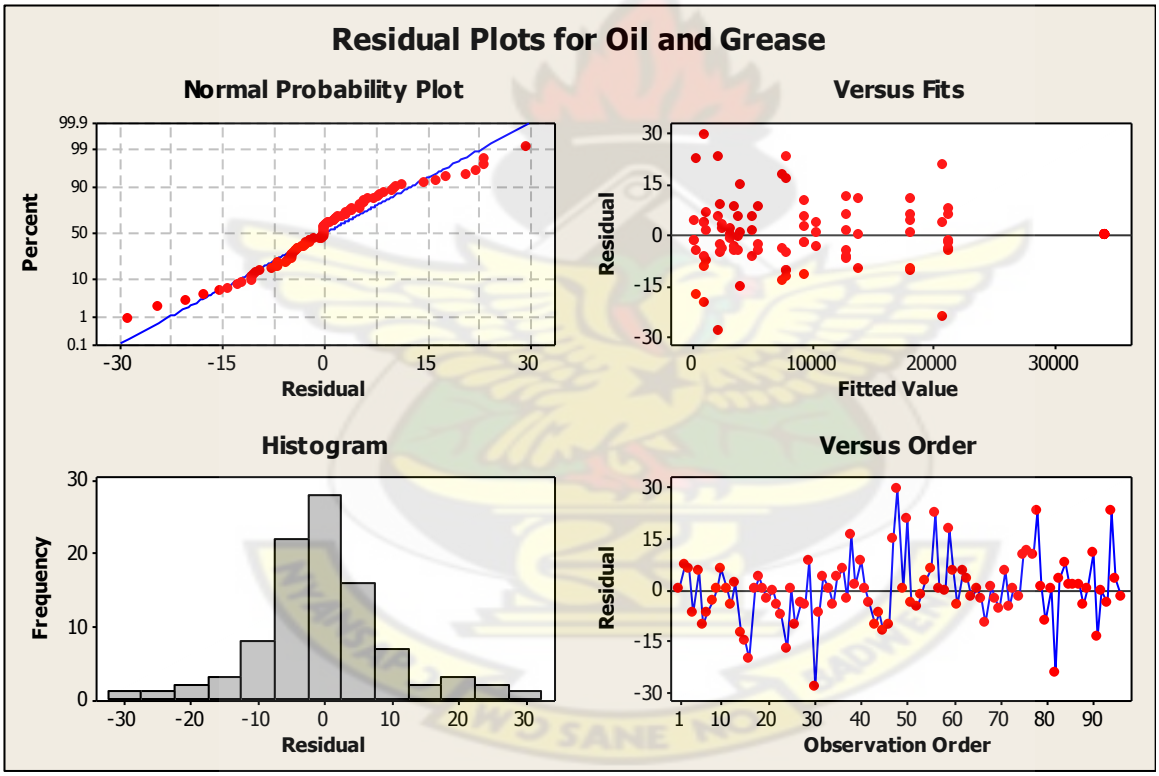


Figure 4.20: Residual Plot of Nitrogen and Weeks for Oil/Grease and TPH in HC/Compost blend

The main effect models for both TPH and oil/grease indicate weeks contributes significantly to the total degradation and decomposition of the hydrocarbon. Additionally, nitrogen levels

contribute immensely to total degradations. Figure 4.20 point out, the higher the level of nitrogen the better the rate of degradation. Plots in both treatments illustrate that, for Hydrocarbon/compost blend as a substrate, the levels of performance from the highest level are 2.2, 1.6, 1.0, and 0.4 respectively. This confirms the earlier findings that, the higher the level of nitrogen in the blend the better the degradation process and the higher the rate of decomposition, whereas the longer the degradation process, the better it leads to a total degradation of the hydrocarbon.

4.1.3.3 Residual Plots for Hydrocarbon/Compost Blend



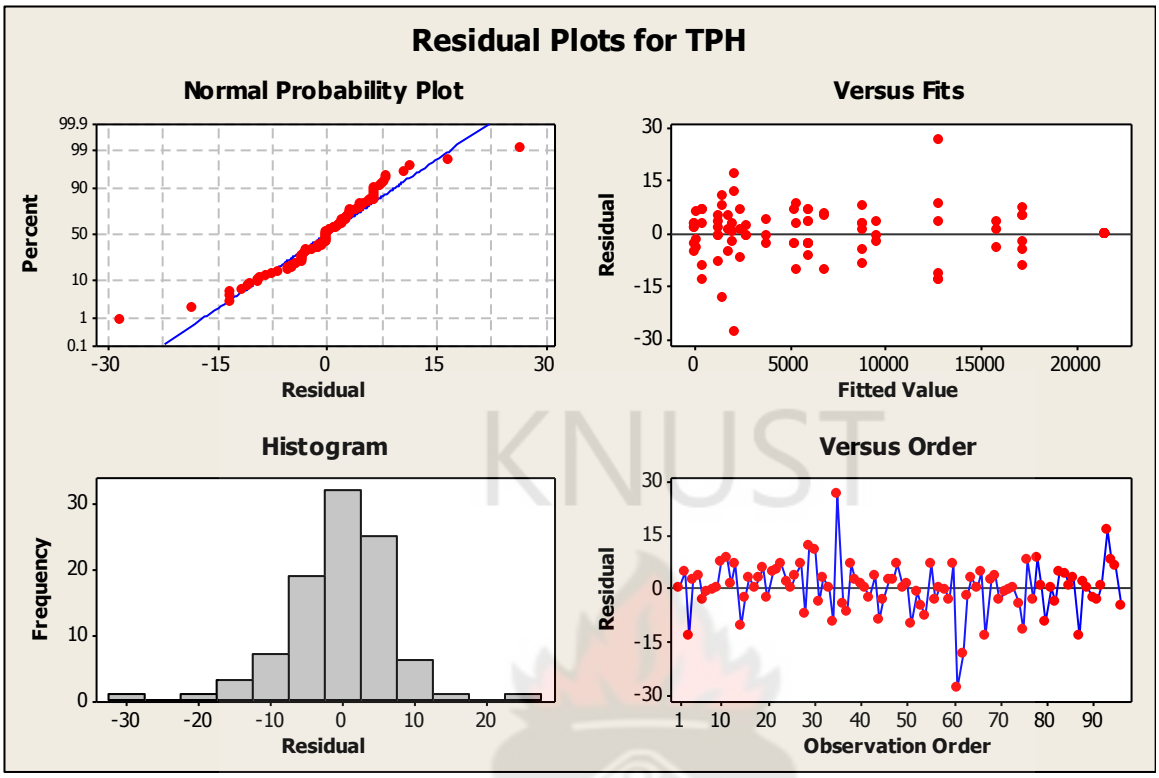


Figure 4.21: Residual Plot for TPH and Oil/Grease in Hydrocarbon/compost blend

1. The Diagnostic of the residual of hydrocarbon/poultry manure blend is shown above. The first upper left plot is the normal probability plot of the standardized residuals. It's been noticed that most of the residuals falls on the straight line with the exception of a few residual deviating from normality. This is due to a sharp degradation between some weeks. Therefore the normality assumption is satisfied and hence the residuals appear to be normally distributed

2. The upper right plot shows the plot of residuals in time sequence, which is helpful in detecting correlation between the residuals and to check if the independence assumption on the errors has been violated. These plots of residuals for both the TPH and Oil/Grease, there is no reason to suspect any violation of the independence or constant variance assumption

3. At the left side of the bottom plots shows confirms the normality assumption with normal residual histogram plot, which does not, shows any skew of the residuals.

4. The bottom part of the diagnostic is the residuals versus observation plot of observation order. It is clearly seen that it is not significant deviation in the order of plot.

4.2 Blend of Substrates

Different blend analysis was carried out to find and to compare the most efficient substrate that facilitates and help bioremediation process, additionally, all the fixed factor levels of different treatment were also considered to factor in the various levels of nitrogen in order to come out with the best substrate as well as the best level of nitrogen as a combine substrate to help in the bioremediation at a lower cost.

4.2.1 Test of Hypothesis for Hydrocarbon and Blend Of Substrates

Test of differences among the means of the weeks, nitrogen levels and for different substrates for biostimulation was done to establish if there exist differences among them. With the following hypothesis to be tested.

H_{10}: There is no significant difference between the average degradation of hydrocarbon among the different levels of the weeks

H_{11}: There is a significant difference between the average degradation of hydrocarbon among different levels of the weeks

H_{20}: There is no significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{21}: There is a significant difference between the average degradation of hydrocarbon among the different levels of nitrogen

H_{30}: There is no significant difference between the average degradation of hydrocarbon among the different substrates

H_{31}: There is a significant difference between the average degradation of hydrocarbon among the different substrates

H_{40}: There is no significant difference for the interactions of weeks and the nitrogen level

H_{41}: There is a significant difference between for the interaction of weeks and the nitrogen levels.

H_{50}: There is no significant difference for the interactions of weeks and the different blend

H_{51}: There is a significant difference between for the interaction of weeks and the different blends.

H_{60}: There is no significant difference for the interactions of different blends and the nitrogen level

H_{61}: There is a significant difference between for the interaction of different blends and the nitrogen levels.

H_{70}: There is no significant difference for the interactions of weeks, blend and the nitrogen level

H_{71}: There is a significant difference between for the interaction of weeks, blend and the nitrogen levels.

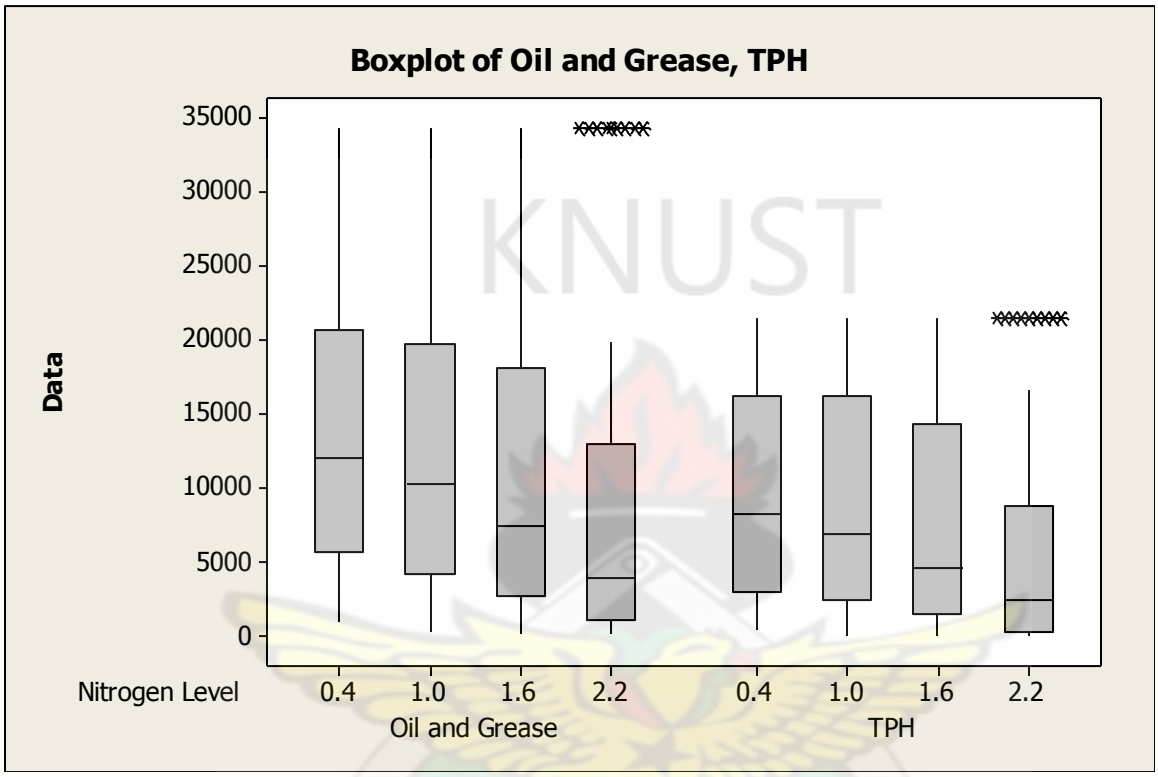


Figure 4.22: Box plot for Different levels of Nitrogen for Oil/Grease and TPH in HC/Blend of substrate

For the blend of different substrates, it was realized from Figure 4.22 that, different levels of nitrogen still contributes significantly to the bioremediation process, clearly, it has been shown that, the higher the level of nitrogen for augmentation in the biostimulation process, the higher the rate of biodegradation of the hydrocarbon compounds. As shown above, the box plots depicts different rate and significantly different from each of the levels of nitrogen considered in the process. This difference were the same for both TPH and Oil/grease hydrocarbon compounds, this it gives a fair view of failing to accept the hypothesis that, there is no significant different of bioremediation process of the different levels of hydrocarbon.

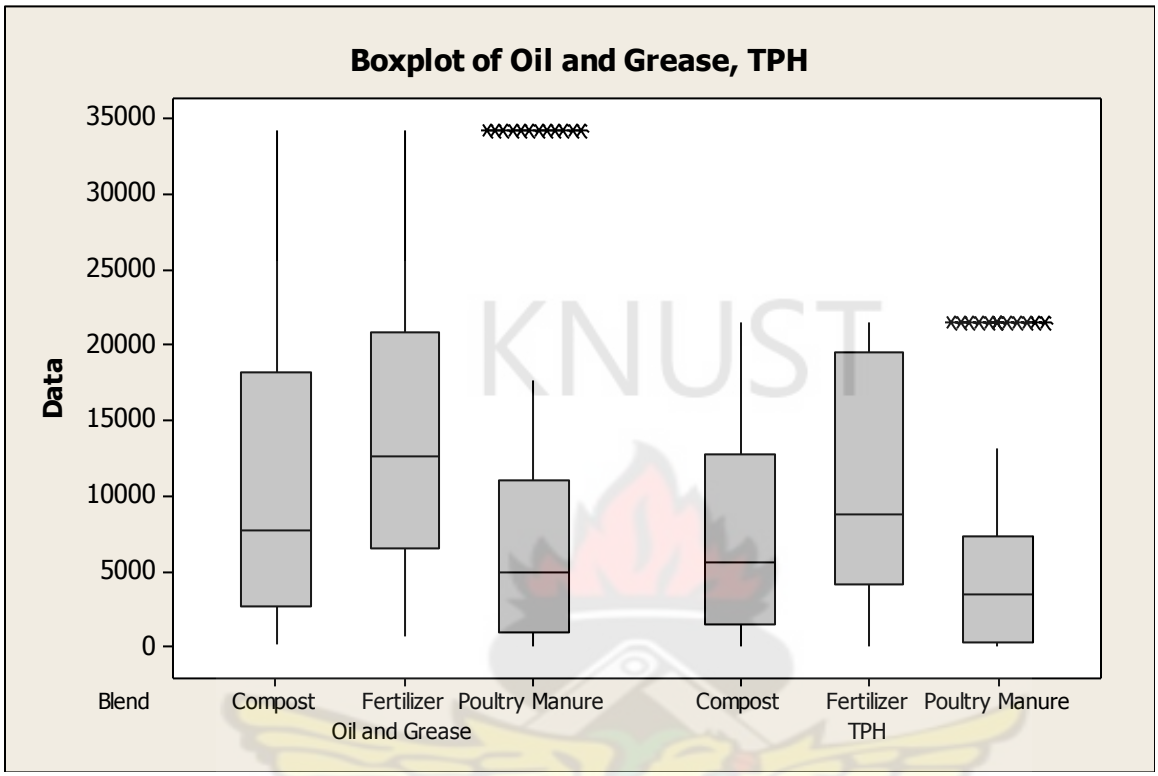


Figure 4.23: Box plot for Different levels of Nitrogen for Oil/Grease and TPH in HC/Blend of substrate

in as much as the consideration of the different levels of nitrogen was concern, it was also part of the main objectives to ascertain the best substrate necessary for the entire decomposition of hydrocarbon at a lowest cost. As shown in Figure 4.23 in both TPH and Oil/grease, fertilizer blend shows the least degradation rate with a higher mean, followed by compost and then poultry manure blend. Hence the box plot gives as some sort of evidence to come out with the best substrate suitable for a higher bioremediation process in the ex situ experiment conducted to ascertain the rate of bioremediation process of hydrocarbon compounds.

Table 4.34: General Linear Model: Oil and Grease, TPH versus Weeks, Nitrogen Level and Blend

Factor	Type	Levels	Values
Weeks	fixed	8	Week 0, Week 1, Week 2, Week 3, Week 4, Week 5, Week 6, Week 7
Nitrogen Level	fixed	4	0.4, 1.0, 1.6, 2.2
Blend	fixed	3	Compost, Fertilizer, Poultry Manure

The analysis of the blend of substrates consists of three fixed factors (Weeks for degradation, nitrogen levels and blend of substrates), of which the weeks consist of 8 levels (Week 0 – Week 7) nitrogen consists of 4 levels (0.4, 1.0, 1.6 and 2.2) and blend of substrates consists of 3 levels (compost, fertilizer and poultry manure).

Table 3.35: Analysis of Variance for Oil and Grease, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	28604803909	28604803909	4086400558	1006911.76	0.00
Blend	2	1745519893	1745519893	872759947	215052.89	0.00
Nitrogen Level	3	1111410594	1111410594	370470198	91285.91	0.00
Weeks*Blend	14	512033923	512033923	36573852	9012.00	0.00
Weeks*Nitrogen Level	21	334285718	334285718	15918368	3922.37	0.00
Blend*Nitrogen Level	6	109381900	109381900	18230317	4492.05	0.00
Weeks*Blend*Nitrogen Lev	42	195027413	195027413	4643510	1144.19	0.00
Error	192	779203	779203	4058		
Total	287	32613242553				

S = 63.7052 R-Sq = 100.00% R-Sq(adj) = 100.00%

Blend of substrate shows some observations as portray by the earlier results. All the fixed factors indicate a strong to the total degradation of hydrocarbon compounds. As indicate in Table 3.35, the adjusted mean squares values for the fixed factors (weeks, nitrogen and blend) have a higher values as compare with the error mean sum of squares, the adjusted mean sum of squares also shows a higher values for the various interactions of the fixed factors, which can be explain that, in terms of bioremediation process, the substrates used contributes greatly to the total breakdown process for the decomposition as well as the interaction of the substrates with number of weeks

used for degradation. The weeks, the substrates and the level of nitrogen as well as their interactions all contribution to total breakdown, however, all the factors contributions to the general breakdown is not as strongly as the number of weeks. It is clearly established that, interms of total breakdown, the weeks play a major role, followed by the blend (substrate), the nitrogen levels and the various interactions of the fixed factors.

Moreover, the associated p-values for the three factors lead to the conclusion that, we fail to accept the hypothesis the treatment means for the weeks, nitrogen levels and the blend as well for the interaction are equal in terms of average decomposition and hence the fixed factors affect the rate of Oil/Grease degradation as well as being affected by their interactions.

Table 4.36: Analysis of Variance for TPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Weeks	7	12802212860	12802212860	1828887551	13979015.99	0.000
Blend	2	1305536308	1305536308	652768154	4989402.69	0.000
Nitrogen Level	3	605503515	605503515	201834505	1542712.55	0.000
Weeks*Blend	14	673134397	673134397	48081028	367505.08	0.000
Weeks*Nitrogen Level	21	238896790	238896790	11376038	86952.21	0.000
Blend*Nitrogen Level	6	68423074	68423074	11403846	87164.76	0.000
Weeks*Blend*Nitrogen Lev	42	116588970	116588970	2775928	21217.67	0.000
Error	192	25120	25120	131		
Total	287	15810321034				

S = 1997.33 R-Sq = 93.06% R-Sq(adj) = 92.76%

In the analysis of variance for TPH, it does not show any difference from the previous table 4.35, clearly it was shown that, the adjusted mean sum of squares for the three fixed factors were higher than the error adjusted sum of squares. Whereas their interactions also show a higher mean sum of squares than the error mean sum of squares.

Additionally, the associated p-values for the three factors and their interactions lead to the conclusion that, we fail to accept the hypothesis the treatment means for the weeks, nitrogen levels and the blend are equal in terms of average decomposition and hence the fixed factors affect the rate of TPH degradation

Table 4.37: Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Weeks	N	Mean	Grouping
Week 0	36	34277.4	A
Week 1	36	17913.1	B
Week 2	36	14114.7	C
Week 3	36	9505.8	D
Week 4	36	7329.2	E
Week 5	36	5228.0	F
Week 6	36	3201.3	G
Week 7	36	1581.0	H

Means that do not share a letter are significantly different.

Table 4.38: Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Nitrogen			
Level	N	Mean	Grouping
0.4	72	14189.6	A
1.0	72	12627.7	B
1.6	72	10816.6	C
2.2	72	8941.4	D

Means that do not share a letter are significantly different.

The Tukey's test for the weeks shows a significantly difference for the rate of degradation for the weeks at a 95% confidence level. The longer the weeks used for degradation, the better the decomposition as shown in Table 4.37.

Again from Table 4.38, the level of nitrogen also shows a significantly different rate of decomposition for each level, clearly as shown the higher the level of nitrogen the faster the rate of decomposition.

Table 4.39: Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Blend	N	Mean	Grouping
Fertilizer	96	14784.7	A
Compost	96	11374.4	B
Poultry Manure	96	8772.4	C

Means that do not share a letter are significantly different.

Grouping information for the blend shows a significant contribution of various blends (substrates) to the bioremediation process. From table 4.39, it's been found out the poultry manure has the lowest mean value showing a better substrate for the bioremediation process, this is followed by the compost and them the fertilizer for the Oil/Grease decomposition.

Table 4.40: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Weeks	N	Mean	Grouping
Week 0	36	21515.0	A
Week 1	36	14269.4	B
Week 2	36	10980.1	C
Week 3	36	6606.5	D
Week 4	36	4627.2	E
Week 5	36	3441.7	F
Week 6	36	1781.5	G
Week 7	36	788.3	H

Means that do not share a letter are significantly different.

Table 4.41: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Nitrogen			
Level	**N**	**Mean**	**Grouping**
0.4	72	9788.1	A
1.0	72	8850.0	B
1.6	72	7398.6	C
2.2	72	5968.2	D

Means that do not share a letter are significantly different.

Similarly, the Tukey's test for the weeks shows a significantly difference for the rate of degradation for the weeks at a 95% confidence level all the weeks differ in its mean of degradation. However, the longer the weeks used for degradation, the better the decomposition.

once more from Table 4.41, the level of nitrogen also shows a significantly different rate of decomposition for each level, clearly as shown the higher the level of nitrogen the faster the rate of decomposition.

Table 4.42: Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Blend	**N**	**Mean**	**Grouping**
Fertilizer	96	10769.1	A
Compost	96	7643.7	B
Poultry Manure	96	5590.8	C

Means that do not share a letter are significantly different.

Correspondingly, grouping information for the blend shows a significant contribution of various blends (substrates) to the bioremediation process. From table 4.42, it's been found out the poultry manure has the lowest mean value showing a better substrate for the bioremediation process, this is followed by the compost and them the fertilizer for the Oil/Grease decomposition.

4.2.2 Main Effect Plots

The main effect plots show the preferred level of nitrogen and blend that will be needed to stimulate the condition in the contaminated soil needed for higher microbial activities for decomposition of hydrocarbons in the soil.

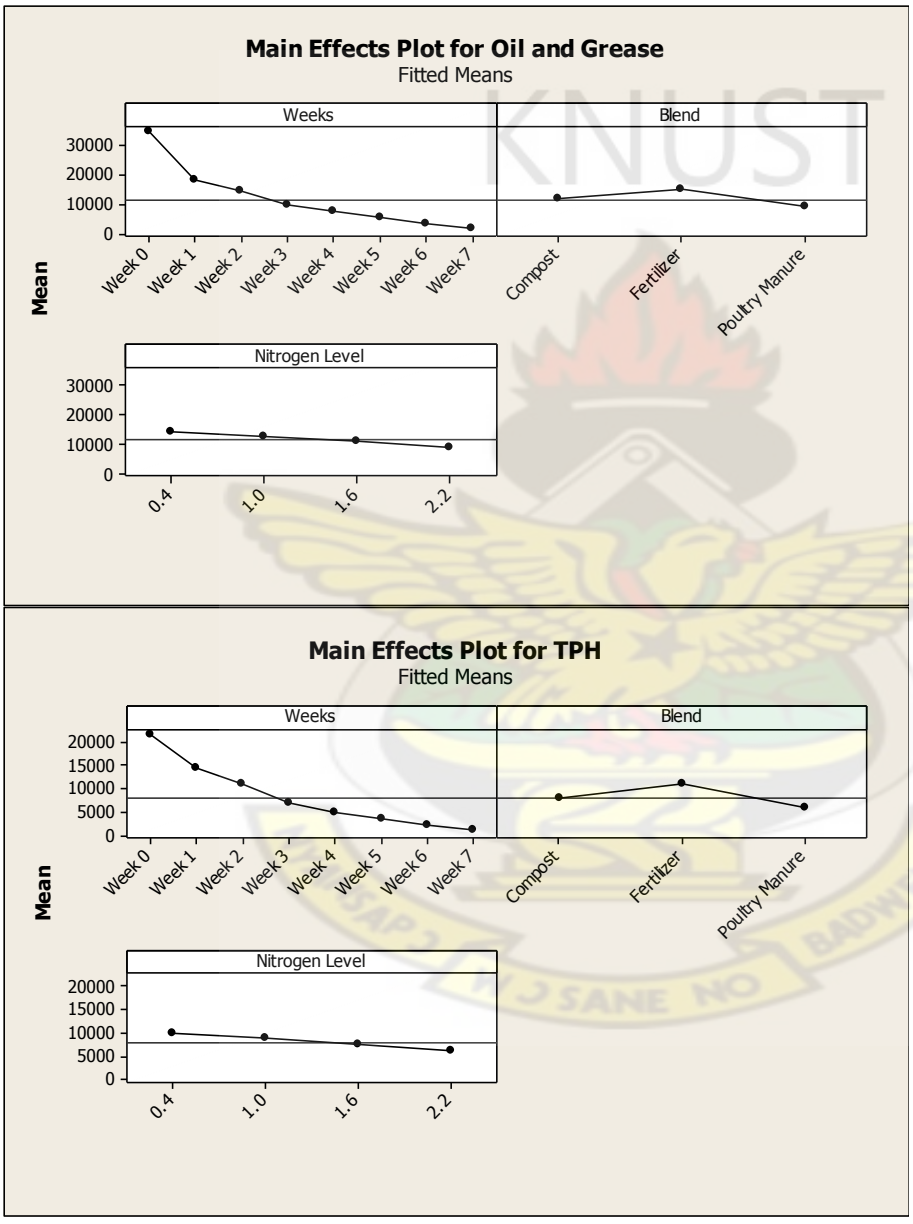


Figure 4.24: Residual Plot of Nitrogen and Weeks for Oil/Grease and TPH in HC/Blend of substrates

The main effect models for both TPH and oil/grease indicate weeks contributes significantly to the total degradation and decomposition of the hydrocarbon. Additionally, nitrogen levels contribute immensely to total degradations. Figure 4.24 identify, the higher the level of nitrogen the better the rate of degradation. Plots in both treatments illustrate that, for Hydrocarbon/compost blend as a substrate, the levels of performance from the highest level are 2.2, 1.6, 1.0, and 0.4 respectively. This confirms the earlier findings that, the higher the level of nitrogen in the blend the better the degradation process and the higher the rate of decomposition, whereas the longer the degradation process, the better it leads to a total degradation of the hydrocarbon.

Again, for the substrate, it was found that, poultry manure provides the best substrate for the decomposition of hydrocarbon, which is follow by compost and then the fertilizer. This analysis shows that, for a better bioremediation process for hydrocarbon decomposition, it is better to use a substrate of poultry manure and waste at a higher nitrogen level which is environmentally friendly and efficient for the processes.

4.2.3 Residual Plots of Hydrocarbon/Blend of Substrates

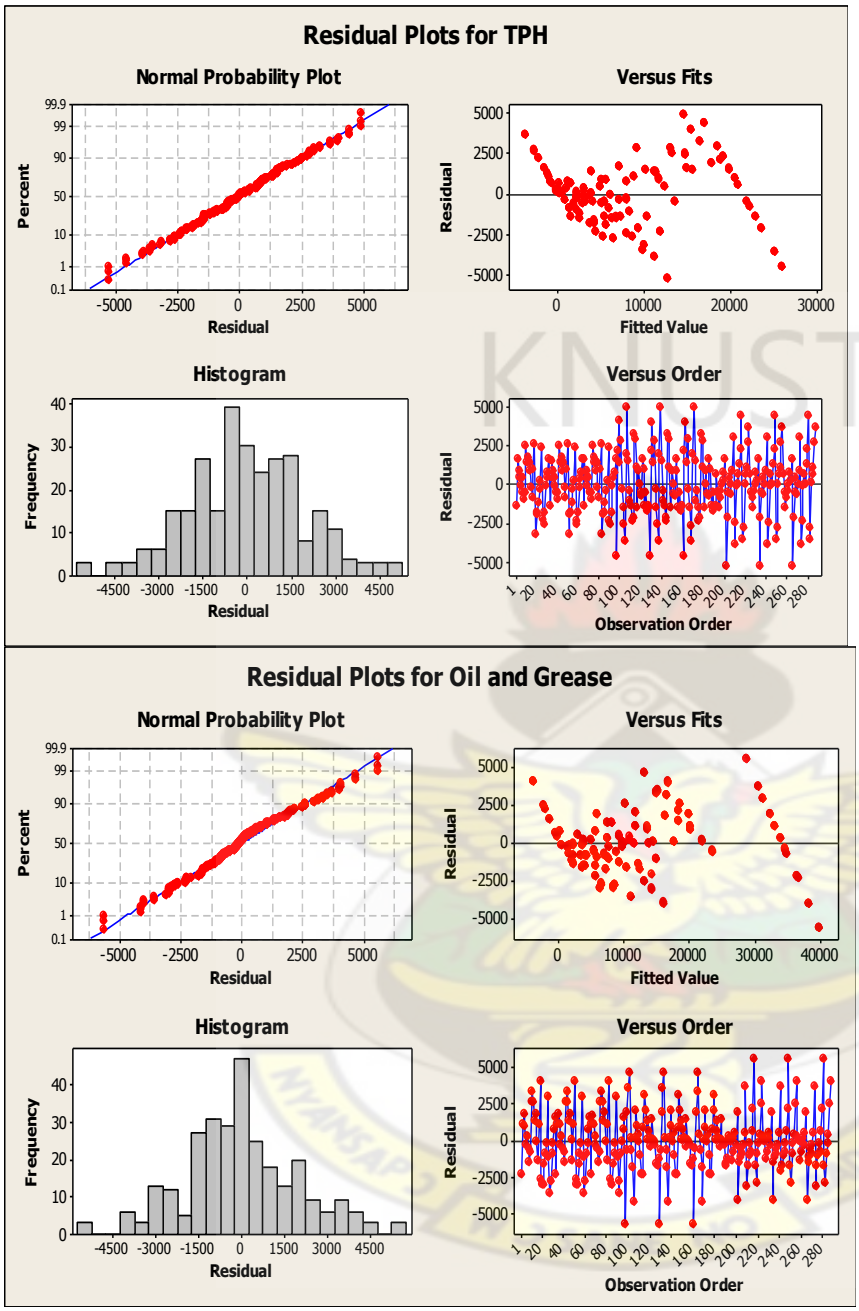


Figure 4.25: Residual Plot for TPH and Oil/Grease in Hydrocarbon/Blend of Substrates

1. The Diagnostic of the residual of hydrocarbon/poultry manure blend is shown above. The first upper left plot is the normal probability plot of the standardized residuals. It's been noticed that

most of the residuals falls on the straight line with the exception of a few residual deviating from normality. This is due to a sharp degradation between some weeks. Therefore the normality assumption is satisfied and hence the residuals appear to be normally distributed

2. The upper right plot shows the plot of residuals in time sequence, which is helpful in detecting correlation between the residuals and to check if the independence assumption on the errors has been violated. These plots of residuals for both the TPH and Oil/Grease, there is no reason to suspect any violation of the independence or constant variance assumption

3. At the left side of the bottom plots shows confirms the normality assumption with normal residual histogram plot, which does not, shows any skew of the residuals.

4. The bottom part of the diagnostic is the residuals versus observation plot of observation order. It is clearly seen that it is not significant deviation in the order of plot.

CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.0 Introduction

This chapter presents the summary of key findings of the study. Conclusions are drawn from the findings and recommendations are given to help improve the entire bioremediation process.

5.1 Summary of substrates contributions and level of nitrogen

5.1.1 Fertilizer and Hydrocarbon Contaminated Soil Blend

The residual means(mg/kg) for oil and grease after week 7 for 0.4% , 1.0% , 1.6% and 2.2% nitrogen levels were 5486.89, 3454.67 ,4031.00 , 804.00 respectively with a percentage of 83.75, 89.66, 87.98 and 97.37 respectively. Similarly, TPH also recorded 2740.50, 2033.00, 2356.67 and 128.67 for 0.4 %, 1.0%, 1.6% and 2.2% nitrogen levels respectively. Percent degradation for TPH were 87.26, 90.55, 89.05and 99.40 followed the order of 0.4 % < 1.0 %,< 1.6 %< 2.2% nitrogen level. In both oil and grease and TPH, the lowest residual mean was recorded by 0.4% nitrogen level whereas 2.2% nitrogen level recorded the highest. Statistically, using Tukey's method of significant difference with $p<0.05$, all the nitrogen levels were significantly different for the oil and grease degradation, additionally TPH nitrogen levels of 0.4%, 1.0%, 1.6% and 2.2% were also recorded to be significantly different. However, the main effect model shows that, 2.2% and 1.6% are more effective in the breakdown of oil/grease than 0.4%, 1.0% nitrogen level.

5.1.2 Poultry Manure/Hydrocarbon Contaminated Soil Blend

The method of trying of different substrates for a different level of nitrogen is to support the fact that nutrient supplementation enhances the growth of indigenous micro-organisms. The order of decreasing residual oil/grease (mg/kg) in the various levels within the blends was 0.4%, 1.0%, 1.6% and 2.2% nitrogen level respectively. This coincide with the percent degradation at the end of the entire duration of the study for the various levels of nitrogen within the poultry manure hydrocarbon contaminated soil blend representing 97.05%, 99.31%, 99.72% and 99.76% respectively for the oil/grease, whereas the TPH also recorded 98.17%, 99.78%, 99.81% and 99.82% for 0.4%, 1.0%, 1.6% and 2.2% levels of nitrogen. Statistically, with $P<0.05$, using Tukey's Methods for significant test, for oil and grease, different levels of nitrogen in the poultry performed significantly different in the breakdown of oil/grease. Whiles for the TPH. There was also a significant different for all the different levels of nitrogen. Moreover, since the mean residual TPH and Oil/Grease (mg/kg) of 2.2% nitrogen level was smaller than the others, we conclude that it performed better than the others.

5.1.3 Compost/Hydrocarbon Contaminated Soil Blend

Residual oil and grease (mg/kg) after the seven weeks period for 0.4% , 1.0% , 1.6% and 2.2% nitrogen levels were 2353.33, 920.33, 318.17and 194.00 for oil/grease and 1219.67, 387.33, 38.33and 37.33 for TPH respectively.

Within the compost/hydrocarbon blend for oil and grease level, 0.4% recorded the least percent degradation (mg/kg) of 93.13% for oil/grease and 94.33% for TPH, whiles 2.2 % recorded the highest percent degradation of 99.43% for oil/grease and 99.83% for TPH by week 7.

Statistically, using Tukey's method of significant difference with $p<0.05$, 1.6% nitrogen level and 2.2% nitrogen level were significantly different from 0.4% and 1.0%.nitrogen level for the oil and grease degradation, whiles for the TPH, 0.4%, 1.0%, 1.6% and 2.2% were recorded to be significantly different. However, the main effect model shows that, 2.2% are more effective in the breakdown of oil/grease than 0.4%, 1.0% nitrogen level.

5.1.4 Comparing Different Blends: Compost, Topsoil Fertilizer Hydrocarbon Blends Oil and Grease

In the different blends (substrates) used for the bioremediation processes, poultry manure was found to be the most efficient contributor to hydrocarbon decomposition followed by compost and fertilizer respectively, this findings was in agreement with Agarry. Owabor and Yusuf (2010), whose findings reveal that poultry manure is one of the most efficient and cheapest substrate suitable for bioremediation processes,. This higher achievement for the performance of poultry manure was done to fulfill the findings of Yakubu (2007) whose work conclude that, the rate of biodegradation performance for poultry manure has not been evaluated and compared with inorganic chemical fertilizers.

Moreover, in all the four (4) fixed nitrogen levels (0.4%, 01. %, 1.6% and 2.2%) used, poultry manure-hydrocarbon blend recorded the least residual Oil and grease and TPH values with fertilizer blend recording the highest. Nevertheless, the rapid degradation of hydrocarbons in the poultry-hydrocarbon blend was highly achieved with a 2.2% level of nitrogen and was expected since poultry manure is rich in nutrients and has additional qualities such as improving soil structure, texture, and aeration capacity than compost and fertilizer.

5.2 Conclusion

It has been found statistically, that different fixed factor of substrates contributes significantly to the degradation of hydrocarbon compounds which contaminate the soil and perturb both aqua culture environment as well as terrestrial environment. From the analysis, it was found that by increasing the level of nitrogen to any substrate, there stands a high chance of facilitating the rate of biodegradation of hydrocarbon compounds. Clearly, it was shown that, with the increasing price in fertilizer, poultry manure stands to be best substrate for bioremediation process which also comes with a cheaper cost as compare to both the compost generation and the inorganic fertilizer blend. Additionally, with the experiment, it became clearly that. The higher the nitrogen level augments in the substrate, the better the rate of biodegrading. Hence poultry manure and a higher content of nitrogen is essential for bioremediation process.

5.3 Recommendation

Based on the findings and discussions, the following recommendations are made to help in tackling the bioremediation process for hydrocarbon compounds in heavy and light industrial sites. Since poultry manure is barely free and compost is generated on site, it is recommended that these two substrates are used for the bioremediation technique to substitute for the volatilization method currently used, due to its cheaper cost and easily to come by.

It is recommended that 2.2 % level of nitrogen should be used to serve as an added up augment for the bioremediation process in order to decrease the cost of augmenting the process for a suitable environment for microbial activities to take place.

Moreover, since statistical methods proved to be more sustainable in finding significant differences among group of homogeneous factors, it is recommended, there should be a

collaboration of mathematics, statistics, environmental and biological department in pursuing a cross functional approach in dealing with such situations since heterotrophic plate count process used in biological methods is not enough to place more emphasis on significance of differences.

REFERENCES

Abou Seoud, M., and R. Maachi. 2003. Biodegradation of naphthalene by free and alginate entrapped *Pseudomonas* sp. *Z. Naturforsch. C. 58*:726–731.

Adrian Low, David Schleheck, Muoi Khou, Vibeke Aagaard, Matthew Lee & Mike Manefield (2007): Options for In Situ Remediation of Soil Contaminated with a Mixture of Perchlorinated Compounds, Bioremediation Journal, 11:3,113-124. http://dx.doi.org/10.1080/10889860701548556,accessed 15th November, 2011.

Andrea Leeson, Bruce C. Alleman, Pedro J. J. Alvarez, Victor S. Magar (Hrsg.): *Bioaugmentation, biobarriers, and biogeochemistry: the Sixth International In Situ and On-Site Bioremediation Symposium.* San Diego, California, June 4-7, Battelle Press 2001, Band 6, ISBN 1574771108.

Atlas, R. M. (1981). Microbial degradation of petroleum hydrocarbons—an environmental perspective. Microbiol. Rev. 45:180–209.

Atlas, R. M., and D. Pramer. (1990). Focus on bioremediation. ASM News 56: 7.

Atlas, R. M., and R. Bartha. 1973. Stimulated biodegradation of oil slicks using oleophilic fertilizers. Environ. Sci. Technol. 7:538–541.

Ayotamuno, M. J., R. B. Kogbara, S. O. T. Ogaji, and S. D. Probert. 2006. Bioremediation of a crude oil polluted agricultural soil at Port Harcourt, Nigeria. *Appl. Energy 83*:1249–1257.

Ayotamuno, M. J., R. B. Kogbara, S. O. T. Ogaji, and S. D. Probert. 2006. Bioremediation of a crude oil polluted agricultural soil at Port Harcourt, Nigeria. *Appl. Energy 83*:1249–1257.

Bamforth, S. M., and I. Singleton. 2005. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *J.Chem. Technol. 80*:723–736.

Booker, R. S., and S. G. Pavlostathis. 2000. Microbial reductive dechlorination of hexachloro-1,3-butadiene in a methanogenic enrichment culture. *Water Res.* 34:4437–4445.

Bosma, T. N. P., F. H. M. Cottaar, M. A. Posthumus, C. J. Teunis, A. van Veldhulzen, G. Schraa, and A. J. B. Zehnder. 1994. Comparison of reductive dechlorination of Hexachloro-1, 3-butadiene in Rhine sediment and model systems with Hydroxocobalamin. *Environ. Sci. Technol.* 28:1–1128.

Box, Joan Fisher (1978), R.A. Fisher, The Life of a Scientist, New York: John Wiley & Sons.

Boyd, R. S. Plants That Hyperaccumulate Heavy Metals. Ed. R. R. Brooks. Willingford, U.K.: CAB International, 1998. 181-201.

Brim H, McFarlan SC, Fredrickson JK, Minton KW, Zhai M, Wackett LP, Daly MJ (2000). "Engineering Deinococcus radiodurans for metal remediation in radioactive mixed waste environments". *Nature Biotechnology* **18** (1): 85–90. doi:10.1038/71986. PMID 10625398.

Burken, J.G. (2004), "2. Uptake and Metabolism of Organic Compounds: Green-Liver Model", in McCutcheon, S.C.; Schnoor, J.L., *Phytoremediation: Transformation and Control of Contaminants*, A Wiley-Interscience Series of Texts and Monographs, Hoboken, NJ: John Wiley, pp. 59, doi:10.1002/047127304X.ch2, ISBN 0471394351

Christof Schuster and Alexander Von Eye (2001): The Relationship of ANOVA Models with Random Effects and Repeated Measurement Designs, Journal of Adolescent Research 2001 16: 205: DOI: 10.1177/0743558401162006: http://jar.sagepub.com/content/16/2/205 Accessed on 14/12/2011.

Cordon, R. C., and N. P. Jules. 1976. *Unwelcome Harvest. Agriculture and Pollution*. London: Earthscan Publications.

Cunningham, C. J., and J. C. Philip. 2000. Comparison of biostimulation in ex-situ treatment of diesel contaminated soil. *Land Contam. Reclam. 8*:261–269.

Cunningham, C. J., and J. C. Philip. 2000. Comparison of biostimulation in ex-situ treatment of diesel contaminated soil. *Land Contam. Reclam. 8*:261–269.

Daniel (2011): Bioremediation process: Seminar paper, Environmental Engineering process.

Daniel and Ian M. Head Francesco Fratepietro, Richard P. J. Swannell, Fabien Wilfred F. M. Röling, Michael G. Milner, D. Martin Jones, *Appl. Environ. Microbiol.* 2004, 70(5):2603. DOI: 10.1128/AEM.70.5.2603-2613.2004.

Diaz E (editor). (2008). *Microbial Biodegradation: Genomics and Molecular Biology* (1st ed.). Caister Academic Press. ISBN 1904455174. [http://www.horizonpress.com/biod.

Donald Lee Wise (Hrsg.): *Bioremediation of contaminated soils.* CRC Press, 2000, ISBN 0824703332.

E. Dekas, William D. Leavitt and Katrina J. Edwards Ann Pearson, Kimberly S. Kraunz, Alex L. Sessions, Anne *Appl. Environ. Microbiol.* 2008, 74(4):1157. DOI: 10.1128/AEM.01014-07.

E. O. Nwaichi, E. N. Onyeike & M. O. Wegwu (2010): Comparison of Chicken Manure and Urea Fertilizers as Potential Soil Amendments for Enhanced Phytoextraction of Heavy Metals,

Bioremediation Journal, 14:4, 180-188**:** http://dx.doi.org/10.1080/10889868.2010.514873, accessed 11/11/2011.
Ete` ka, A. C., R. J. Carsky, S. A. Tarawali, and T. Owoeye. 1998. Centre d'Information et d'Echanges sur les Plantes de Couverture en Afrique CIEPCANEWSLETTER.1. http://ppathw3.cals.cornell. edu/mba project/moist/CIEPCA3.html. Accessed 4 August 2010.

Fisher, Ronald Aylmer (1926), "The Arrangement of Field Experiments," Joiurnal of the Ministry of Agriculture of Great Britain, 33, 503-513, Collected Papers, 2, no. 48, and Contributions, paper 17.

Fisher, Ronald Aylmer (1925), Statistical Methods for Research Workers, Edinburgh: Oliver and Boyd

Fisher, Ronald Aylmer (1935), Design of Experiments, Edinburgh: Oliver and Boyd.

Fisher, Ronald Aylmer, and Eden, Thomas (1927), "Studies in Crop Variation. IV. The Experimental Determination of the Value of Top Dressings With Cereals," Journal of Agricultural Science, 17, 548-562, and Collected Papers, 2, no. 57.

Fisher, Ronald Aylmer, and Mackenzie, W.A. (1923), "Studies in Crop Variation. II. The Manurial Response of Different Potato Varieties," Journal of Agricultural Science, 13, 311-320, and Collected Papers, 1, no. 32.

Garcıa, E. M., I. G. Siegert, and P. Suarez. 1998. Toxicity assays and naphthalene utilization by natural bacteria selected in marine environments. *Bull. Environ. Contam. Toxicol. 61*:370– 377.

Gondal, M. A., T. Hussain, Z. H. Yamani, and M. A. Baig. 2006. Detection of heavy metals in Arabian crude oil residue using laser induced breakdown spectroscopy. *Talanta 69*:1072–1078.

Gordor, B. K. and Howard, K. N. (2000). "Elements of Statistical Analysis". Ghana Mathematics

Group. 18-71.

Harayama, S., Y. Kasai, and A. Hara. 2004. Microbial communities in oil contaminated seawater. Curr. Opin. Biotechnol. 15:205–214

Head, I. M., and R. P. J. Swannell. 1999. Bioremediation of petroleum 1164 pearson et al. appl. environ. microbiol.

Head, I. M., and R. P. Swannell. 1999. Bioremediation of petroleum hydrocarbon contaminants in marine habitats. *Curr. Opin. Biotechnol. 10*:234–239.

Ijah, U. J. and S. P. Antai. 2003. The potential use of chicken-drop microorganisms for oil spill remediation. *Environmentalist 23*:89–95.

James F. Reed III (2003): Analysis of Variance (ANOVA) Models in Lower Extremity Wounds: International Journal of Lower Extremity Wounds 2003 2: 87: DOI: 10.1177/1534734603256075 Johnson, A., B. Gunawardana, and N. Singhal. 2009. Amendments for enhancing copper uptake by *Brassica juncea* and *Lolium perenne* from solution. *Int. J. Phytoremediat.11*:215–234.

Knaebel, D. B., T.W. Federle, D. C. McAvoy, and J. R. Vestal. 1994. Effects of mineral and organic soil constituents on microbial mineralization of organic compounds in natural soil. *Appl. Environ. Microbiol. 60*:4500–4508.

Knaebel, D. B., T.W. Federle, D. C. McAvoy, and J. R. Vestal. 1994. Effectsof mineral and organic soil constituents on microbial mineralization of organic compounds in natural soil. *Appl. Environ. Microbiol. 60*:4500–4508.

Kuzel, S., V. Nydl, L. Kolar, and R. Tichy. 1994. Spatial variability of cadmium, pH, organic matter in soil and its dependence on sampling scales. *Water Air Soil Pollut. 78*:51–59.

L. Feijoo-Siota, F. Rosa-Dos-Santos, T. de Miguel & T. G. Villa (2008): Biodegradation of Naphthalene by *Pseudomonas stutzeri* in Marine Environments: Testing Cells Entrapment in Calcium Alginate for Use in Water Detoxification, Bioremediation Journal, 12:4, 185-192http://dx.doi.org/10.1080/10889860802477168.

Lan Xiao, Phillip W Lavori, Sandra R Wilson and Jun Ma (2011): Comparison of dynamic block randomization and minimization in randomized trials: a simulation study: Clin Trials 2011 8: 59, DOI: 10.1177/1740774510391683: http://ctj.sagepub.com/content/8/1/59, access on 15/12/2011

Lee, M. D., J. M. Odom, and R. J. Buhanan Jr. 1998. New perspectives on microbial dehalogenation of chlorinated solvents: Insights from the field. *Annu. Rev. Microbiol.* 52:423–452.

Lindstrom, J. and J. Braddock. 2002. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant corexit 9500. *Mar. Pollut. Bull. 44*:739–747.

Lindstrom, J. and J. Braddock. 2002. Biodegradation of petroleum hydrocarbons at low temperature in the presence of the dispersant corexit 9500. *Mar. Pollut. Bull. 44*:739–747.

Lombi, E., F. J. Zhao, S. J. Dunhan, and S. P.McGrath. 2001. Phytoremediation of heavy metal contaminated soils. Natural hyperaccumulation versus chemically enhanced phytoextraction. *J. Environ. Qual. 30:* 1919–1926.

Lopez, M. L., J. R. Peralta-Videa, J. G. Parsons, J. L. Gardea-Torresdey, and M. Duarte-Gardea. 2009. Effect of indole-3-acetic acid, kinetin, and ethylenediaminetetraacetic acid on plant growth and uptake and translocation of lead, micronutrients, and macronutrients in alfalfa plants. *Int. J. Phytoremediat.11*:131–149.

Lovley, DR (2003). "Cleaning up with genomics: applying molecular biology to bioremediation". *Nature Reviews. Microbiology.* **1** (1): 35–44. doi:10.1038/nrmicro731. PMID 15040178.

M. B. Adewole, M. K. C. Sridhar & G. O. Adeoye (2010): Removal of Heavy Metals from Soil Polluted with Effluents from a Paint Industry Using *Helianthus annuus* L. and *Tithonia diversifolia* (Hemsl.) as Influenced by Fertilizer Applications, Bioremediation Journal, 14:4, 169-179. http://dx.doi.org/10.1080/10889868.2010.51487, access date 9/12/2011

Manohar, S., and T. B. Karegoudar. 1998. Degradation of naphthalene by a *Pseudomonas* strain NGK1.Appl. *Microbiol. Biotechno. 49*:785– 792.

Margesin, R. and F. Schinner. 1999. A feasibility study for the *in situ* remediation of a former tank farm. *World J. Microbiol. Biotechnol 15*:615–622.

Margesin, R. and F. Schinner. 1999. A feasibility study for the *in situ* remediation of a former tank farm. *World J. Microbiol. Biotechnol 15*:615–62.

Margesin, R. and F. Schinner. 2001. Bioremediation (natural attenuation and biostimulation) of diesel oil contaminated soil in an Alpine Glacier Skiing area. *Appl. Environ. Microbiol. 67*: 3127–3133.

Margesin, R. and F. Schinner. 2001. Bioremediation (natural attenuation and biostimulation) of diesel oil contaminated soil in an Alpine Glacier Skiing area. *Appl. Environ. Microbiol. 67*: 3127–3133.

Meagher, RB (2000). "Phytoremediation of toxic elemental and organic pollutants". *Current Opinion in Plant Biology* **3** (2): 153–162. doi:10.1016/S1369-5266(99)00054-0. PMID 10712958.

Mendez MO, Maier RM (2008), "Phytostabilization of Mine Tailings in Arid and Semiarid Environments—An Emerging Remediation Technology", *Environ Health Perspect* 116 (3): 278–83, doi:10.1289/ehp.10608, PMC 2265025, PMID 18335091.

Montgomery, D. C. (2001). "Design and Analysis of Experiment". John Wiley and Sons, Inc.

5:13-20.

Moore, M. T., Cooper, C. M. and Kröger, R. (2007) 'Rice (Oryza sativa) as a Remediation Tool for Nutrient Runoff', Bioremediation Journal, 11:4, 165–170.: DOI: 10.1080/10889860701710529. URL: http://dx.doi.org/10.1080/10889860701710529

Morikawa, H. and O. C. Erkin. 2003. Basic processes in phytoremediation and some applications to air pollution control. *Chemosphere 52*:1553–1558.

Nikita Norton, Terry C. Hazen and Markus Huettel Green, Gina Freyer, Andy Canion, Jonathan Delgardio, Joel E. Kostka, Om Prakash, Will A. Overholt, Stefan J. (2011) *Appl. Environ. Microbiol.* 2011, 77(22):7962. DOI: 10.1128/AEM.05402-11.

Nwaichi, E. O., E. N. Onyeike, and M. O. Wegwu. 2009. Phytoextracting cadmium and copper using *Mucuna pruriens*. *Afr. J. Plant Sci. 3*:277–282.

Nwaichi, E. O., E. N. Onyeike, and M. O.Wegwu. 2010. Safety evaluation of the impacts of metals on ecological receptors. *Ann. Biol. Res. 1*:191–198.

Okolo, J. C., E. N. Amadi, and C. T. I. Odu. 2005. Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. *Appl. Ecol. Environ. Res. 3*:47–53.

Okolo, J. C., E. N. Amadi, and C. T. I. Odu. 2005. Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. *Appl. Ecol. Environ. Res. 3*:47–53

Preer, J. R., A. N. Abdi, H. S. Sekhon, and G. B. Murchison Jr. 1995. Metal in urban gardens—Effect of lime and sludge. *J. Environ. Sci. Health 30*:2041–2056.

Preuss, R., J. Angerer, and H. Drexler. 2003. Naphthalene—an environmental and occupational toxicant. *Int. Arch. Occup. Environ. Health. 76*:556–576

Ronald M. Atlas and Carl E. CernigliaReviewed (1995), Bioremediation of Petroleum pollutants, University of California Press: BioScience, Vol. 45, No. 5 (May, 1995), pp. 332-338.

Russell, Sir E. John (1926), "Field Experiments: How They Are Made and What They Are," Journal of the Ministry of Agri- culture of Great Britain, 32, 989-1001.

S. E. Agarry, C. N. Owabor & R. O. Yusuf (2010): Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbon Oil Mixtures: Evaluation of the Use of Animal Manure and Chemical Fertilizer, Bioremediation Journal, 14:4, 189-195**:** http://dx.doi.org/10.1080/10889868.2010.514965

Sharanagouda, U., and T. B. Karegoudar. 2001. Degradation of 2- methylnaphthalene by *Pseudomonas* sp. strain NGK1. *Curr. Microbiol.43*:440–443.

Smidt, H., and W. M. de Vos. 2004. Anaerobic microbial dehalogenation. *Annu. Rev. Microbiol.* 58:43–73.

Soniassy, R., P. Sandra, and C. Schlett.1994. *Water Analysis—Organic Micropollutants.* Germany: Hewlett Packard.

Student (1936), "Cooperation in Large-Scale Experiments,' Journal of the Royal Statistical Society, Supplement, 3, 115- 122.

Terra Nova's Environmental Remediation Resuources. Terranovabiosystems.com. 2009-08-31. Retrieved 2011-03-22.

William P. Inskeep Natsuko Hamamura, Sarah H. Olson, David M. Ward (2006): *Appl. Environ. Microbiol.* 2006, 72(9):6316. DOI: 10.1128/AEM.01015-06.

Yakubu, M. B. 2007. Biodegradation of Lagoma crude oil using pig dung. *Afr. J. Biotechnol. 6*:2821–282.

Yakubu, M. B. 2007. Biodegradation of Lagoma crude oil using pig dung. *Afr. J. Biotechnol. 6*:2821–282.

Zhu B., and A. K. Alva. 1993. Differential adsorption of trace metals by soils as influenced by exchangeable cations and ionic strength. *Soil Sci. 155*:61–62.

Appendix 1 Compost

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Welcome to Minitab, press F1 for help.

General Linear Model: Oil and Grease, TPH versus Weeks, Nitrogen Level

Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Weeks	Nitrogen Level	N	Mean	Grouping
Week 0	0.4	3	34278.0	A
Week 0	1.6	3	34278.0	A
Week 0	1.0	3	34278.0	A
Week 0	2.2	3	34278.0	A
Week 1	1.0	3	21253.0	B
Week 1	0.4	3	21251.7	B
Week 1	1.6	3	20842.3	C
Week 2	1.0	3	18155.3	D
Week 2	0.4	3	18149.6	D
Week 1	2.2	3	13810.3	E
Week 3	0.4	3	12840.0	F
Week 3	1.0	3	12837.7	F
Week 2	1.6	3	10386.6	G
Week 4	1.0	3	9361.0	H
Week 4	0.4	3	9357.7	H
Week 5	1.0	3	7812.7	I
Week 5	0.4	3	7810.7	I
Week 2	2.2	3	7565.0	J
Week 3	1.6	3	5434.0	K
Week 6	0.4	3	4906.7	L
Week 6	1.0	3	3915.3	M
Week 3	2.2	3	3779.7	N
Week 4	2.2	3	3480.7	O
Week 4	1.6	3	3227.0	P
Week 5	1.6	3	2544.3	Q
Week 7	0.4	3	2353.3	R
Week 5	2.2	3	2128.7	S
Week 6	1.6	3	1217.7	T
Week 6	2.2	3	1014.7	U
Week 7	1.0	3	920.3	V
Week 7	1.6	3	318.2	W
Week 7	2.2	3	194.0	X

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Weeks	Nitrogen Level	N	Mean	Grouping
Week 0	0.4	3	21514.9	A
Week 0	2.2	3	21514.9	A
Week 0	1.6	3	21514.9	A
Week 0	1.0	3	21514.9	A
Week 1	0.4	3	17254.3	B
Week 1	1.0	3	17251.7	B
Week 1	1.6	3	15843.0	C
Week 2	0.4	3	12847.3	D
Week 2	1.0	3	12825.7	D
Week 1	2.2	3	9585.3	E
Week 3	1.0	3	8853.0	F
Week 3	0.4	3	8851.7	F
Week 2	1.6	3	6848.3	G
Week 4	0.4	3	5988.7	H
Week 4	1.0	3	5985.3	H
Week 2	2.2	3	5979.3	H
Week 5	1.0	3	5314.7	I
Week 5	0.4	3	5307.3	I
Week 3	1.6	3	3750.4	J
Week 6	0.4	3	2717.0	K
Week 3	2.2	3	2443.1	L
Week 4	2.2	3	2081.7	M
Week 6	1.0	3	2018.7	N
Week 4	1.6	3	1834.3	O
Week 5	2.2	3	1433.6	P
Week 5	1.6	3	1282.2	Q
Week 7	0.4	3	1219.7	R
Week 6	1.6	3	448.3	S
Week 7	1.0	3	387.3	T
Week 6	2.2	3	111.0	U
Week 7	1.6	3	38.3	V
Week 7	2.2	3	37.3	V

Means that do not share a letter are significantly different.

Appendix 2 FERTILIZER

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General Linear Model: Oil and Grease, TPH versus Weeks, Nitrogen Level

Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Weeks	Nitrogen Level	N	Mean	Grouping
Week 0	1.6	3	34278.0	A
Week 0	1.0	3	34278.0	A
Week 0	0.4	3	34278.0	A
Week 0	2.2	3	34271.3	A
Week 1	0.4	3	23043.3	B
Week 1	1.0	3	22140.1	C
Week 2	0.4	3	21743.3	D
Week 1	1.6	3	21066.0	E
Week 2	1.0	3	20329.7	F
Week 1	2.2	3	19781.0	G
Week 2	1.6	3	19566.7	H
Week 3	0.4	3	18672.3	I
Week 4	0.4	3	17676.3	J
Week 3	1.0	3	14612.3	K
Week 2	2.2	3	14567.0	K
Week 3	1.6	3	13830.7	L
Week 4	1.0	3	11414.3	M
Week 5	0.4	3	11193.7	N
Week 3	2.2	3	9846.7	O
Week 5	1.0	3	9810.0	O P
Week 4	1.6	3	9717.7	P
Week 5	1.6	3	8865.3	Q
Week 6	0.4	3	8241.4	R
Week 4	2.2	3	7502.7	S
Week 6	1.0	3	6314.0	T
Week 6	1.6	3	6220.7	T
Week 7	0.4	3	5484.4	U
Week 5	2.2	3	4104.3	V
Week 7	1.6	3	4024.7	V
Week 7	1.0	3	3450.0	W
Week 6	2.2	3	1980.7	X
Week 7	2.2	3	804.3	Y

Means that do not share a letter are significantly different.

Weeks	Nitrogen Level	Grouping
Week 0	0.4	A
Week 0	1.6	A
Week 0	1.0	A
Week 0	2.2	A
Week 1	0.4	B
Week 1	1.0	C
Week 1	1.6	D
Week 2	0.4	E
Week 2	1.0	E
Week 1	2.2	F
Week 2	1.6	G
Week 2	2.2	H
Week 3	0.4	I
Week 4	0.4	J
Week 3	1.0	K
Week 3	1.6	L
Week 5	0.4	M
Week 4	1.0	N
Week 5	1.0	O
Week 5	1.6	P
Week 3	2.2	Q
Week 4	1.6	R
Week 4	2.2	S
Week 6	0.4	S
Week 6	1.6	T
Week 6	1.0	T
Week 7	0.4	U
Week 5	2.2	V
Week 7	1.6	W
Week 7	1.0	X
Week 6	2.2	Y
Week 7	2.2	Z

Means that do not share a letter are significantly different.

Appendix 3 Poultry Manure

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Welcome to Minitab, press F1 for help.
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General Linear Model: Oil and Grease, TPH versus Weeks, Nitrogen Level

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Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease
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Weeks	Nitrogen Level	N	Mean	Grouping
Week 0	0.4	3	34278.0	A
Week 0	1.6	3	34278.0	A
Week 0	1.0	3	34278.0	A
Week 0	2.2	3	34278.0	A
Week 1	0.4	3	17685.3	B
Week 2	0.4	3	13711.3	C
Week 1	1.0	3	12016.6	D
Week 1	1.6	3	11457.7	E
Week 2	1.0	3	10746.0	F
Week 1	2.2	3	10609.7	F
Week 2	1.6	3	8750.0	G
Week 3	0.4	3	7860.8	H
Week 3	1.0	3	6506.0	I
Week 4	0.4	3	6212.3	I
Week 2	2.2	3	5706.3	J
Week 5	0.4	3	5104.7	K
Week 4	1.0	3	4948.7	K
Week 3	1.6	3	4797.0	K
Week 4	1.6	3	3502.3	L
Week 6	0.4	3	3407.3	L
Week 3	2.2	3	3053.0	M
Week 5	1.0	3	2469.7	N
Week 4	2.2	3	1549.3	O
Week 7	0.4	3	1010.0	P
Week 6	1.0	3	982.3	P
Week 5	1.6	3	780.5	P
Week 7	1.0	3	235.0	Q
Week 6	1.6	3	118.7	Q
Week 5	2.2	3	111.0	Q
Week 6	2.2	3	96.0	Q
Week 7	1.6	3	95.7	Q
Week 7	2.2	3	81.7	Q

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Means that do not share a letter are significantly different.
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Grouping Information Using Tukey Method and 95.0% Confidence for TPH
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Weeks	Nitrogen Level	N	Mean	Grouping
Week 0	1.0	3	21517.2	A
Week 0	1.6	3	21514.9	A
Week 0	0.4	3	21514.9	A
Week 0	2.2	3	21514.9	A
Week 1	0.4	3	13144.2	B
Week 2	0.4	3	8911.0	C
Week 1	1.0	3	7446.7	D
Week 1	1.6	3	7366.0	E
Week 2	1.0	3	7316.3	F
Week 1	2.2	3	6348.3	G
Week 2	1.6	3	5563.3	H
Week 3	0.4	3	5084.3	I
Week 3	1.0	3	4482.3	J
Week 2	2.2	3	3763.0	K
Week 4	0.4	3	3583.4	L
Week 3	1.6	3	3510.3	M
Week 4	1.0	3	3464.3	N
Week 5	0.4	3	2804.3	O
Week 4	1.6	3	2310.7	P
Week 3	2.2	3	2249.0	Q
Week 6	0.4	3	1950.0	R
Week 5	1.0	3	1256.0	S
Week 4	2.2	3	875.3	T
Week 5	1.6	3	443.3	U
Week 7	0.4	3	393.3	V
Week 6	1.0	3	258.0	W
Week 5	2.2	3	99.3	X
Week 7	1.0	3	47.7	Y
Week 6	2.2	3	46.3	Y
Week 6	1.6	3	46.3	Y
Week 7	1.6	3	40.7	Y
Week 7	2.2	3	40.0	Y

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Means that do not share a letter are significantly different.
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Appendix 4 BLEND OF SUBTRATES

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Welcome to Minitab, press F1 for help.
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General Linear Model: Oil and Grease, TPH versus Weeks, Blend, ...

Weeks	Blend	Grouping
Week 0	Compost	A
Week 0	Poultry Manure	A
Week 0	Fertilizer	A
Week 1	Fertilizer	B
Week 1	Compost	C
Week 2	Fertilizer	D
Week 3	Fertilizer	E
Week 2	Compost	F
Week 1	Poultry Manure	G
Week 4	Fertilizer	H
Week 2	Poultry Manure	I
Week 3	Compost	J
Week 5	Fertilizer	K
Week 4	Compost	L
Week 6	Fertilizer	M
Week 3	Poultry Manure	N
Week 5	Compost	O
Week 4	Poultry Manure	P
Week 7	Fertilizer	Q
Week 6	Compost	R
Week 5	Poultry Manure	S
Week 6	Poultry Manure	T
Week 7	Compost	U
Week 7	Poultry Manure	V

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Weeks	Nitrogen Level	N	Mean	Grouping
Week 0	1.6	9	34278.0	A
Week 0	0.4	9	34278.0	A
Week 0	1.0	9	34278.0	A
Week 0	2.2	9	34275.8	A
Week 1	0.4	9	20660.1	B
Week 1	1.0	9	18469.9	C
Week 2	0.4	9	17868.1	D
Week 1	1.6	9	17788.7	D
Week 2	1.0	9	16410.3	E
Week 1	2.2	9	14733.7	F
Week 3	0.4	9	13124.4	G
Week 2	1.6	9	12901.1	H
Week 3	1.0	9	11318.7	I
Week 4	0.4	9	11082.1	J
Week 2	2.2	9	9279.4	K
Week 4	1.0	9	8574.7	L
Week 5	0.4	9	8036.3	M
Week 3	1.6	9	8020.6	M
Week 5	1.0	9	6697.4	N
Week 3	2.2	9	5559.8	O
Week 6	0.4	9	5518.5	O
Week 4	1.6	9	5482.3	O
Week 4	2.2	9	4177.6	P
Week 5	1.6	9	4063.4	P
Week 6	1.0	9	3737.2	Q
Week 7	0.4	9	2949.3	R
Week 6	1.6	9	2519.0	S
Week 5	2.2	9	2114.7	T
Week 7	1.0	9	1535.1	U
Week 7	1.6	9	1479.5	U
Week 6	2.2	9	1030.4	V
Week 7	2.2	9	360.0	W

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Oil and Grease

Blend	Nitrogen Level	N	Mean	Grouping
Fertilizer	0.4	24	17541.6	A
Fertilizer	1.0	24	15293.6	B
Fertilizer	1.6	24	14696.2	C
Compost	0.4	24	13868.5	D
Compost	1.0	24	13566.7	E

Fertilizer	2.2	24	11607.3	F
Poultry Manure	0.4	24	11158.7	G
Compost	1.6	24	9781.0	H
Poultry Manure	1.0	24	9022.8	I
Compost	2.2	24	8281.4	J
Poultry Manure	1.6	24	7972.5	K
Poultry Manure	2.2	24	6935.6	L

Means that do not share a letter are significantly different.

Means that do not share a letter are significantly different.

Weeks	Nitrogen Level	Grouping
Week 0	1.0	A
Week 0	1.6	A
Week 0	0.4	A
Week 0	2.2	A
Week 1	0.4	B
Week 1	1.0	C
Week 1	1.6	D
Week 2	0.4	E
Week 2	1.0	F
Week 1	2.2	G
Week 2	1.6	H
Week 3	0.4	I
Week 3	1.0	J
Week 2	2.2	K
Week 4	0.4	L
Week 3	1.6	M
Week 4	1.0	N
Week 5	0.4	O
Week 5	1.0	P
Week 3	2.2	Q
Week 6	0.4	R
Week 4	1.6	R
Week 5	1.6	S
Week 4	2.2	T
Week 6	1.0	U
Week 6	1.6	V
Week 7	0.4	W
Week 5	2.2	X
Week 7	1.0	Y
Week 7	1.6	Y
Week 6	2.2	Z
Week 7	2.2	AA

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for TPH

Blend	Nitrogen Level	N	Mean	Grouping
Fertilizer	0.4	24	12728.5	A
Fertilizer	1.0	24	11557.4	B
Fertilizer	1.6	24	10651.4	C
Compost	0.4	24	9462.6	D
Compost	1.0	24	9268.9	E
Fertilizer	2.2	24	8139.2	F
Poultry Manure	0.4	24	7173.2	G
Compost	1.6	24	6445.0	H
Poultry Manure	1.0	24	5723.6	I
Compost	2.2	24	5398.3	J
Poultry Manure	1.6	24	5099.4	K
Poultry Manure	2.2	24	4367.0	L

Means that do not share a letter are significantly different.