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DEPARTMENT OF ENVIRONMENTAL SCIENCE

COLLEGE OF SCIENCE

BIOREMEDIATION OF HYDROCARBON CONTAMINATED SOIL - A CASE STUDY AT NEWMONT GHANA GOLD LIMITED – AHAFO KENYASI

BY

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A Thesis Submitted to the Department of Theoretical and Applied Biology in partial fulfillment of the requirement for the award of the Master of Science Degree in Environment Science

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DECLARATION

I, **Daniel Osei-Twumasi**, hereby certify that this report is a true outcome of the research carried out at Newmont Ghana Gold Limited (NGGL) Ahafo Kenyasi on Bioremediation of hydrocarbon contaminated soil at the vol.pad facility of the company to accelerate the rate of degradation of hydrocarbons. I hereby declare that, except for reference to other people's work which has been duly acknowledged, this research work consists of my own work produced from research undertaken under the supervision of Dr. Bernard Fei–Baffoe (Department of Theoretical and Applied Biology –K.N.U.S.T.) and that no part has been presented for any degree elsewhere. This report is submitted in partial fulfillment for the award of MSc. (Hons.) Environmental Science.

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ABSTRACT

The study investigated the degradation rate of hydrocarbon contaminated soil using indigenous micro-organism. Contaminated soil containing oil and grease level and total petroleum hydrocarbon levels (mg/kg) of 3.43×10^4 and 2.15×10^4 respectively was bioremediated by blending 2Kg of the hydrocarbon contaminated soil with portions of compost, topsoil and fertilizer (fertilizer). The soil was inoculated with the above mentioned nitrogen sources and monitored for a period of seven(7) weeks until significant degradation occurred for the compost and topsoil hydrocarbon contaminated soil blend and eight(8) weeks for the fertilizer-hydrocarbon contaminated soil blend by which time there had been significant degradation. Specific portions of compost, topsoil and fertilizer were mixed with the hydrocarbon contaminated soil to achieve 0.2%, 0.8%1.4%, and 2.0% nitrogen levels within the various blends.

The samples were placed under wooden structure covered with polythene sheets. The experiment was replicated three times in randomized complete block design. Each block contained 13 different treatments with one Control sample.

At the end of the experiment, the concentrations of most of the selected treatments were reduced by up to 95% degradation. Oil and grease and Total petroleum hydrocarbons (TPH) recorded 8.09 and 8.27 % degradation respectively in the control experiments. Microbial activities were shown to correlate with the reduction in hydrocarbon contents of the soil. Statistically ($p<0.05$), there were significant differences in all the treatments within the compost, topsoil and fertilizer hydrocarbon contaminated soil blends. Soil analyses shows that augmenting the nitrogen levels in the various treatments have potentials to ameliorate hydrocarbon levels with increases in microbial numbers. Among all the blends, the nitrogen levels 0.2% , 0.8% , 1.4% and 2.0% of the compost gave the best performance on the hydrocarbon degradation.

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ACRONYMS

Vol. Pad	Volatilization Pad
NGGL	Newmont Ghana Gold Limited
TPH	Total Petroleum Hydrocarbon
HPC	Heterotrophic plate count
HC	Hydrocarbon
PCB	PolyChlorinated Biphenyls
PCE	Polychloroethlyene
TCE	Trichloroethlyene
DDT	Dichlorodiphenyltrichloroethane
BTEX	Benzene, Toluene, Ethylbenzene, and Xylenes
GEMs	Genetically engineered microbes
PHC	Petroleum hydrocarbons
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
PAH	Polycyclic Aromatic Hydrocarbon
TPH	Total Petroleum Hydrocarbons
DNA	Deoxyribonucleic acid
NAH	Naphthalene oxidation
CFU	Colony forming units
PLFA	Phospholipids fatty acids

rDNA	ribosomal Deoxyribonucleic acid
PCR	Polymerase chain reaction
TOL	Toluate oxidation
CIL	Carbon In Leach
Mtpa	Mega tonnes per annum
Kg	Kilogram
N- level	Nitrogen level
Wks	weeks
STDev	Standard deviation

CHAPTER ONE

1.0 INTRODUCTION

Large quantities of organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Mueller *et al*., 1996).

Micro-organisms degrade or transform contaminants by a variety of mechanisms. Petroleum hydrocarbons for example are converted to carbon dioxide and water or are used as a primary food source by bacteria, which use the energy to generate new cells. The impressive capabilities of micro-organisms and plants to degrade and transform contaminants should provide tremendous benefits in the clean-up of pollutants from spills and storage sites. These remediation ideas have provided the foundation for many ex situ waste treatment processes (including sewage treatment) and a host of in situ bioremediation methods that are in practice today (Hinchee *et al*., 1994). Bioremediation of HC-contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contamination, has been established as an efficient, economic, versatile, and environmentally sound treatment (Norris, 1994). On-site–off-site and in situ systems may be used. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation (Vidali, 2001).

1.1 JUSTIFICATION

Over two billion tons of petroleum is produced annually worldwide (Gogoi *et al*., 2003). Oil pollution of the oceans and coastal environment has been a problem ever since man began to transport and use fossil fuel (Minas *et al.,* 1995). Environmental contamination with petroleum introduces a myriad of hydrocarbons, causing a variety of problems (Atlas and Philp, 2005). Activities such as periodic engine oil changes for light& heavy vehicles, mobile equipment, oil leakages from poorly maintained vehicles and mobile equipment occasionally spill oil into soil especially at mining sites.

It is therefore incumbent upon mining companies to have a very comprehensive hydrocarbon management practice.

Hydrocarbon management as a best practice effort is a fundamental indicator in environmental management, in gold Mining Company that deals with large volumes of hydrocarbons and its hydrocarbon related waste (Newmont Waste management plan, 2009).

In view of this and for best practice, Newmont Ghana Gold Limited (NGGL) Ahafo mine has embarked on Volatilization of hydrocarbon contaminated waste which commenced full operation in March 2009 (Newmont waste management plan, 2009).

Available data indicates that the rate of breakdown is not as fast as expected.

Therefore, this project work seeks to assess the efficiency of bioremediation technique of the hydrocarbon contaminated soil as an alternative to the volatilization process.

1.2 OBJECTIVES

The overall objective is to investigate the extent of degradation of hydrocarbon contaminated soil using autochonous (indigenous) microorganisms in amended soil.

Specific objectives

- To design a bioremediation set up of fertilizer/compost/topsoil blend.
- To determine the levels of TPH and oil/grease of sample taken from site.
- To determine appropriate monitoring parameters/regimes for the degradation process.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HYDROCARBON CONTAMINATION AND OTHER REMEDIATION TECHNOLOGIES

Contamination of the environment by petroleum hydrocarbons is potentially widespread because modern society uses so many petroleum-based products (for example, gasoline, kerosene, fuel oil, mineral oil and asphalt). Hydrocarbons are quantitatively the most important constituents of petroleum, and arise from natural as well as anthropogenic sources (Law *et al.,* 1994 and Medeiros *et al.,* 2005). Human-mediated sources of petroleum hydrocarbons include offshore oil production, mining activities, marine transportation, atmospheric or aerial depositions from combustion of coal and gas flaring, direct ocean dumping, coastal, municipal and industrial wastes, and runoff (NRC Oil in the Sea, 2000).

However, among the anthropogenic sources, point discharges, contamination by urban run-offs, refineries and other coastal effluents are in aggregate substantial and are important in causing local, chronic pollution in the vicinity of estuaries, land, creeks, harbours and coastal settlements (Abu-Hilal *et al.,* 1994).

Many factors affect the selection of potential remediation technologies. These include:

contaminant type and characteristics, (properties, volume, location, exposure risk), site characteristics (soil types, permeability, surface and ground water properties, climate, site infrastructures, topography, location), costs (capital, operating, maintenance),regulatory and public acceptance and remediation schedule (Malroz Engineering Inc, 1994).

Some of the other known remediation technologies are: Physical, Chemical Treatment Technologies, and Thermal Treatment Technologies. Physical/chemical treatment includes soil vapour extraction, solidification/stabilization, soil flushing, excavation and landfilling. Chemical oxidation, and electrokinetic separation are examples of chemical treatment. Thermal methods include, incineration and thermal desorption (Malroz Engineering Inc, 1994).

2.1.1 SOIL WASHING

Soil washing involves an on-site set-up to scrub soil and remove Hydrocarbons which are then treated separately. Soil washing can be carried out with the aid of surfactants. Emulsifiers and other additives to increase hydrocarbon solubility (Kosaic, 1993). The major drawback with this technology is that, abrasive additives can harm the natural microbial flora and damage the soil environment (Loss of minera1 cycling capacities) (Atlas and Bartha, 1993). Additional steps to remove soi1 additives after clean-ups, non-specificity of cleaning agents. High labour requirements and low treatrnent volumes may also serve to reduce efficiency and increase costs of soil washing (Malroz Engineering Inc, 1994).

2.1.2 EXCAVATION AND LAND FILLING

This option involves excavating hydrocarbon contaminated soil with heavy equipment and placing it in a regulated landfill. When on-site land filling is not feasible, soil must be containerized and shipped to a licensed institution. These factors plus the need for ongoing monitoring to control fugitive leachate emissions make excavating and landfilling costly and logistically difficult to implement. ((Malroz Engineering Inc, 1994).

2.1.3 INCINERATION AND THERMAL DESORPTION

Thermal desorption and incineration use heat to volatilize and destroy hydrocarbon contaminants. Incineration uses a closed-vessel combustion unit to completely destroy hydrocarbon components at high temperature, whereas thermal desorption can be carried out in or ex situ and uses lower temperature ranges to volatilize hydrocarbon components from the soil. Volatilized components are then captured and or treated. Influent/effluent streams for both processes face varying regulatory restrictions and monitoring requirements (Kostecki and Calabrese, 1990). These factors combined with low treatment volumes reduce efficiency and increase costs for large-scale treatment, making incineration and/or thermal desorption inappropriate.

2.1.4 VACUUM EXTRACTION

In vacuum extraction, a pump draws air through wells constructed above the water table within the contaminated soil. Contaminants volatilize into the vapour phase where they are then

captured, treated or exhausted. This in situ treatment method removes the need for excavation and ex situ remediation. It is not possible, however for treatrnent of soils with tight formations (clay) thin unsaturated zones Permafrost or the presence of oils and non-volatile components (Kostecki and Calabrese, 1990).

2.1.5 CHEMICAL REMEDIATION

Chemical oxidation is a promising innovative process for degrading an extensive variety of hazardous compounds in remediation of soil at waste disposal and spill sites. Chemical oxidation can be applied both in situ (to soil in place) and ex situ (after soil excavation). Hydrogen peroxide is one of the most successfully used remedial chemical for contaminated soil remediation. Oxidation with hydrogen peroxide can be direct and/or through the generation of free radicals (hydroxyl radicals OH*). The latter relies on the decomposition of hydrogen peroxide catalysed by most ions of transition metals (Fe, Cu, Zn, etc.) and by natural minerals of those metals (hematite, goethite, etc.) present in soil.

Treatment contaminants are treated in situ, converted to innocuous and/or natural occurring compounds (e.g. H_2O, CO_2, O_2, halide ions). By acting/reacting up on the contaminant in place, the reagent serves to eliminate the possibility of contaminant vertical movement other than resulting from the act of vertical injection itself, which is often a concern in other remediation technologies (Technology and Regulatory Cooperation Work Group, 2001). As a side advantage, aerobic biodegradation of contaminants can benefit from the presence of oxygen released during H_2O_2 decomposition, if large quantities of chemical needed to be applied. Hydrogen peroxide can be electrochemically generated on site, which may further increase the economic feasibility and effectiveness of this process for treated contaminated sites. (Technology and Regulatory Cooperation Work Group, 2001). Natural iron oxide minerals (hematite -Fe_2O_3, goethite FeOOH, magnetite Fe_3O_4 and ferrihydrite) present in soil can catalyse hydrogen peroxide oxidation of organic compounds (Watts *et al.,* 1992 and Kong *et al.,* 1998). Thus, the treatment of contaminated soil would require no addition of soluble iron catalyst. Hydrogen peroxide has several advantages over other soil remediation methods. Hydrogen peroxide oxidation is relatively fast, taking only days or weeks. The disadvantages include the need for pH control in some cases and difficulties controlling in situ heat and gas production (ThermoRetec Consulting Corporation *et al.,* 1999). The efficacy of hydrogen peroxide oxidation may be limited by low

soil permeability, incomplete site delineation, subsurface heterogeneities, and highly alkaline soil where carbonate ions are free radical scavengers (Technology and Regulatory Cooperation Work Group, 2001)

In the present study hydrogen peroxide treatment was applied for remediation of soil contaminated with chlorophenols, PAH, diesel and transformer oil. The influence of hydrogen peroxide dosage, ferrous iron catalyst addition and a manner of hydrogen peroxide application on the removal of the contaminants in soil has been investigated.

2.2 WHAT IS BIOREMDIATION?

Bioremediation which occurs without human intervention other than monitoring is often called natural attenuation. This natural attenuation relies on natural conditions and behaviour of soil microorganisms that are indigenous to soil (Biobasic, 2006). Biological treatment techniques fall into two categories, biostimulation and bioaugmentation. (Rahman *et al.*, 2003). Biostimulation uses indigenous microbial populations to remediate contaminated soils. It consists of adding nutrients and other substances to soil to catalyze natural attenuation processes. Several studies of the effects of biostimulation with mainly N-P-K or oleophilic fertilizers have reported positive effects on oil decontamination (Morgan *et al*., 1989).

Bioaugmentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms (Biobasics, 2006). Bioaugmentation is a method to improve degradation and enhance the transformation rate of hydrocarbons by the injection (seeding) of specific microbes, able to degrade the hydrocarbon of interest. Many microbes are described to have the genetic tools to mineralize recalcitrant pollutants such as PAHs, chlorinated aliphatics and aromatics, nitroaromatics, and long-chain alkanes (Cerniglia, 1993 and Grosser *et al*., 1991). These microbes can be wild-type isolates, but also can be genetically modified strains equipped with catabolic plasmids, containing the relevant degradation genes (Dixon, 1996, King *et al.*, 1990 and Yee *et al.*, 1998). Genetically engineered microbes (GEMs) have been constructed in order to degrade those pollutants. These GEMs can be equipped with new metabolic routes which, for example, are constructed by combining parts of known pathways or by optimizing the known pathways; for example, by overexpression of certain genes or operons (Chakrabarty,

1996, Timmis and Pieper, 1999). An example of a field release is the genetically engineered Pseudomonas fluorescences HK44 (King *et al*., 1990). However, both the inability to improve the action of microbial consortia and the restriction of degrading only a few pollutants limit the use of GEMs. In addition, legislative problems arise when GEMs are introduced in the environment (Hamer, 1993, Sayler and Ripp, 2000).

2.2.1 PHYTOREMEDIATION

It is the green technology that uses plants to remediate contaminated soil, sediment and surface water. (Pradhan *et al.,* 1998) It is a cost-effective, ecologically compatible tool for the environmental clean-up of a wide array of contaminants such as petroleum hydrocarbons, chlorinated solvents, pesticides and metals (Anderson *et al.,* 1993 and Schwab *et al.,* 1995). Plants preserve the natural structure and texture of soil using solar energy and hence this technology is suitable to diverse regions and climates. Phytoremediation process (a) Uptake of organic compounds from soil and water; (b) Accumulation or processing of these chemicals via lignifications, volatilization, metabolization, mineralization; (c) Use of enzymes to break down complex organic molecules into simpler molecules (ultimately carbon dioxide & water) and (d) Increase the carbon and oxygen content of soil around roots (and so promote microbial/fungal activity) and decay of root tissues.

2.2.2 RHIZOMEDIATION

Plant enzymes establish the degradation of pollutants during phytoremediation; whereas, during natural attenuation or bioaugmentation, the (indigenous) microbial population performs the degradation. In many of these studies, an important contribution to the degradation of pollutants is ascribed to microbes present in the rhizosphere of plants used during phytoremediation or of plants which are emerging as natural vegetation on a contaminated site. This contribution of the rhizomicrobial population is referred to as rhizoremediation (Anderson *et al.,* 1993, Schwab and Banks, 1994). In some cases, rhizosphere microbes are even the main contributors to the degradation process. A plant can be considered to be a solar-driven biological pump and treatment system, attracting water with its root system, accumulating water-soluble pollutants in the rhizosphere, and concluding with the degradation or translocation of the pollutant (Erickson,

1997). Although the importance of the rhizosphere community for degradation of pollutants has been recognized, very little is known about the exact composition of the degrading population.

The first studies toward degradation of compounds in the rhizosphere mainly focused on the degradation of herbicides and pesticides (Hoagland *et al.*, 1994, Jacobsen 1997, Zablotowicz *et al.*, 1994). These studies suggested that plants are protected against these compounds by the degrading bacteria.

2.3 LIMITING FACTORS OF HYDROCARBON BIODEGRADATION

Hydrocarbon biodegradation in soil can be limited by many factors, for example: microorganism type, nutrients, pH, temperature, moisture, soil properties and contaminant presence/concentration, pressure (Leahy and Colwell, 1990)

2.3.1 NUTRIENTS

Carbon, nitrogen and phosphorus (C, N, and P) are the primary nutrients required for biosynthesis and cell growth of microbes involved in bioremediation processes. Studies of contaminant degradation have shown that normal indigenous have the capability to degrade HC contaminants more rapidly when supplemented with nutrients in the form of fertilizers (Hutchinson *et al.*, 1994 and Mohn, 1998). Research into crude oil degradation supports the hypothesis that nutrient addition is the most effective way to stimulate HC breakdown (Hutchinson *et al.*, 1994). There has been a demonstration that nitrogen and phosphorus contents greatly affect the microbial degradation of hydrocarbons.(van Hamme *et al.*, 2003). It is further suggested that adjustment of the ratios of these two elements ratios by the addition of nitrogen and phosphorus in the form of slow releasing fertilizers stimulated the biodegradation of crude.oil. Studies done elsewhere also supported the stimulated degradation of hydrocarbons in the top soil and the aquifer sand following the addition of inorganic nitrogen and phosphorous (Breedveld and Sparrevik, 2000).

2.3.2 pH

Soil pH can be highly variable, ranging from 2.5 in mine spoils to 11.0 in alkaline deserts (Bossert *et al.*, 1994). Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients.

Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the acceptable range is pH 6 – 8 (US EPA, 2006).

2.3.3 OXYGEN

The initial steps in the catabolism of aliphatic (Singer *et al.,* 1984), cyclic (Perry *et al.,* 1984), and aromatic (Cerniglia *et al.,* 1984) hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases, for which molecular oxygen is required. Aerobic conditions are therefore necessary for this route of microbial oxidation of hydrocarbons in the environment. Conditions of oxygen limitation normally do not exist in the upper levels of the water column in marine (Floodgate, 1984) and freshwater (Cooney *et al.,* 1985) environments. Aquatic sediments, however, are generally anoxic except for a thin layer at the surface of the sediment (Cooney *et al.,* 1984 and Hambrick *et al.*, 1980). The availability of oxygen in soils is dependent on rates of microbial oxygen consumption, the type of soil, whether the soil is waterlogged, and the presence of utilizable substrates which can lead to oxygen depletion (Bossert *et al.,* 1984). The concentration of oxygen has been identified as the rate-limiting variable in the biodegradation of petroleum in soil (von Wedel, 1988) and of gasoline. However, the microbial degradation of halogenated aromatic compounds such as the halobenzoates (Suflita *et al.,* 1982), chlorophenols (Boyd *et al.,* 1984), and polychlorinated biphenyls (Chen *et al.,* 1988) has been shown to occur under anaerobic conditions

2.3.4 TEMPERATURE

Temperature plays very important roles in biodegradation of petroleum hydrocarbons, firstly by its direct effect on the chemistry of the pollutants, and secondly on its effect on the physiology and diversity of the microbial milieu. Ambient temperature of an environment affects both the properties of spilled oil and the enzymatic activity or population of microorganisms (Venosa and Zhu, 2003). For example, short chain alkanes will be more readily volatilized at higher temperatures (van Deuren *et al.,* 1997) and within the range of 10 ^{O}C to 45 ^{O}C, the rate of microbial activity typically doubles for every 10 ^{O}C increase in temperature (Atlas and Bartha, 1998). Temperature also variously affects the solubility of hydrocarbons (Foght *et al.,* 1996)

2.3.5 MOISTURE CONTENT

Water availability in contaminated soils enhances microbial activity and growth. However, excessive water may result in blockage of soil pores and therefore limit oxygen transfer. During treatment, water content is typically retained at 50-80% of soil water holding capacity (Cookson, 1995). The failure to observe inhibition of degradation at the lower values was ascribed to a hydrocarbon-mediated reduction in the water holding capacity of the soil.

2.3.6 SURFACTANTS

The addition of surfactant soil amendments plays a key role in the bioavailability of substrate and nutrient components and optimizes aqueous phase interactions (Finnerty, 1994). Surfactants are any usable and isolatable compound that has some influence on interfaces (Kosaic, 1993) and include compounds that act as emulsifiers and dispersing agents. Surfactants aid to overcome physical forces present at air-water oil water, and solid-liquid interfaces are the primary qualities of surfactants (Kosaic, 1993).

Many organic compounds are highly insoluble and hydrocarbon degrading microorganisms are forced to develop cellular mechanisms to increase their solubility and promote their bioavailability and uptake. As a result, surfactants are commonly found in biological systems where microorganisms are grown on insoluble substrates (Thangamani and Shreve, 1994). Naturally occurring agents (biosurfactants) and their synthetic counterparts (synthetic surfactants) can be isolated and applied to bioremediation processes. In soil remediation applications, synthetic and natural surfactants are both commonly used for the cleanup of oil spills, soil contamination and for in situ "pump and treat" Processes (Kosaric, 1993). Surfactants help to displace pollutants which are adsorbed to the soil (or aquifer) matrix or formed into discrete organic phase mixtures (Non Aqueous Phase Liquids) (Thangamani and Shreve, 1994).

2.3.7 PRESSURE

Leahy and Colwell. (1990) reported that pressure may have positive impacts on the breakdown of certain hydrocarbons. For instance, they reported that “at 4 °C, 94% of the hexadecane was utilized only after a 40-week incubation under conditions of high pressure, as compared to 8 weeks at 1 atm” (Leahy and Colwell, 1990).

2.3.8 ECOLOGICAL CONSIDERATION OF BIOREMEDIATION

To achieve effective bioremediation of crude oil polluted environment a consortium of microbial communities is required. An ecological balance of the key microbes required in all aspects of bioremediation of crude oil polluted ecosystem, including cometabolising bacteria, is very important. Some workers have co-optimized biological nitrogen fixation (Paerl *et al.,* 1996) or microbial nitrogen fixation (Onwurah, 1999b) with biodegradation of petroleum hydrocarbons in the coastal environment and soil systems respectively. The capability of simultaneous existence of heterotrophic and adapted autotrophic bacteria, (*Pseudomonas sp* and *A. vinelandii*) within oil polluted environment has been demonstrated (Onwurah, 1999b). Also very important is the use of high inoculum of adapted microbial population in bioremediation of oil-polluted environment.

When adapted microbial strains taken from contaminated soil are introduced into a new oil spill location at high cell density, they can alter the genetic capabilities of the different bacteria in this new environment (Smets *et al.,* 1990). This was demonstrated when *A. vinelandii* was isolated from a previously oil contaminated site and introduced into a newly oil polluted site, whereby nitrogen fixation and co-metabolism contributed in enhanced bioremediation (Onwurah, 1999b). Gene distribution within strains could provide a level of community structure that can superimpose on the natural ecological structure from the mixed adapted inoculate populations.

2.3.9 PHYSICAL STATE OF THE OIL OR HYDROCARBONS

As a result of wind and wave action, oil-in-water or water in-oil ("mousse") emulsions may form (Cooney *et al.,* 1984). Some components in crude oils spilled are easily degraded; others are more slowly and/or less completely degraded; and some compounds are totally non-biodegradable (recalcitrant).The greater the complexity of the hydrocarbon structure (i.e. the higher number of alkyl-branched substituents or condensed aromatic rings), the slower the rates of degradation and the greater the likelihood of accumulating partially oxidized intermediary metabolites.

These factors, as well as others such as volatility, set the practical operational limits for the application of bioremediation strategies. There is advantage to bioremediate a spill of light hydrocarbons such as gasoline, since it would evaporate rapidly (Lee and Merlin, 1999).

2.3.10 CONCENTRATION OF OIL OR HYDROCARBON

The rates of uptake and mineralization of many organic compounds by microbial populations in the aquatic environment are proportional to the concentration of the compound, generally conforming to Michaelis-Menten kinetics (Pfaender *et al.,* 1982). Michaelian kinetics has been demonstrated for the microbial uptake and oxidation of toluene (Button *et al*., 1986 and Robertson *et al.,* 1987), a low molecular- weight aromatic hydrocarbon of relatively high water solubility, but may not apply to the more insoluble hydrocarbons. The rates of mineralization of the higher molecular-weight aromatic hydrocarbons, such as naphthalene and phenanthrene are related to aqueous solubilities rather than total substrate concentrations (Thomas *et al.,* 1986) High concentrations of hydrocarbons can be associated with heavy, undispersed oil slicks in water, causing inhibition of biodegradation by nutrient or oxygen limitation or through toxic effects exerted by volatile hydrocarbons. Fusey *et al.* (1984) reported that contamination of seashore sediments with crude oil above a threshold concentration prevented biodegradation of the oil because of oxygen and/or nutrient limitation.

2.4 ADAPTION-EFFECT OF PRIOR EXPOSURE

Prior exposure of a microbial community to hydrocarbons, either from anthropogenic sources such as accidental oil spills, petroleum exploration and transportation activities, and waste oil disposal, or from natural sources such as seeps and plant-derived hydrocarbons (Bartha *et al.*, 1984), is important in determining how rapidly subsequent hydrocarbon inputs can be biodegraded. This phenomenon, which results from increases in the hydrocarbon-oxidizing potential of the community, is known as adaptation (Spain *et al*., 1980). The three interrelated mechanisms by which adaptation can occur are (i) induction and/or depression of specific enzymes, (ii) genetic changes which result in new metabolic capabilities, and (iii) selective enrichment of organisms able to transform the compound or compounds of interest (Spain *et al.*, 1980 and Spain *et al.,* 1983). Selective enrichment has been widely observed in studies of hydrocarbon and petroleum degradation in the environment. Floodgate *et al*. (1984), Cooney *et al.* (1984), and Bossert *et al*. (1984.), have shown that the numbers of hydrocarbon-utilizing microorganisms and their proportion in the heterotrophic community increase upon exposure to petroleum or other hydrocarbon pollutants and that the levels of hydrocarbon utilizing microorganisms generally reflect the degree of contamination of the ecosystem. Whereas in other

studies the diversity of heterotrophic populations was shown to be unchanged (Olsen *et al.*, 1982). Sherrill *et al*. (1980) observed increased phenanthrene-biodegradative capacity in water samples from two reservoirs receiving industrial and domestic wastes compared with a reservoir not receiving wastes. Wong *et al.* (1980) concluded that sediment microbial populations within the Athabasca oil sands were more capable of oxidizing hydrocarbons than were populations from control sites, based on respiration rates of radiolabeled hexadecane and naphthalene. Sayler*et al.* (1983) showed that exposure of freshwater sediments to a synthetic oil accelerated the rate of polyaromatic hydrocarbon (PAH) mineralization. Cooney *et al.* (1985) found that water-sediment mixtures from an oil-contaminated area of a freshwater lake exhibited higher rates of degradation of marker hydrocarbons in kerosene than did samples from the non-oiled area of the lake. Bauer *et al.* (1985) and Kerr *et al.* (1988) provided evidence for "cross-acclimation" of sediment microbial communities to PAHs, in which exposure to one compound, such as phenanthrene, effects an increase in metabolism rates of a compound of similar structure, such as naphthalene. The occurrence of this phenomenon was attributed to the broad specificity of selected microbial populations for PAHs and/or the existence of common pathways for PAH catabolism (Bauer *et al*., 1988).

2.5 ADAPTATION OF THE GENETIC COMPOSITION OF THE MICROBIAL COMMUNITY

Of the three mechanisms for adaptation of microbial communities to chemical contaminants, induction and depression of enzymes, genetic changes, and selective enrichment, it is only selective enrichment that has been examined in detail. This has been primarily a result of limitations imposed by available methods, which have, until recently, restricted the study of adaptation of microbial communities to the phenomenon of selective enrichment, in which the numbers or proportion of microorganisms that can utilize the compound of interest increase within the community and can be enumerated by their ability to grow on a medium containing the compound as the sole carbon source. The primary genetic mechanism for the adaptation of the microbial community is the amplification, by means of selective enrichment and gene transfer and mutation, of genes which are involved in the metabolism of the chemical contaminant (Barkay *et al.*, 1988 and Spain *et al*., 1983). Direct monitoring of this process with respect to adaptation to hydrocarbons has recently been made possible by the development of DNA probes specific for the genes encoding hydrocarbon-catabolic pathways (Trevors *et al.*,

1985). Sayler *et al.* (1985), using the colony hybridization technique, showed a correlation between the enhanced rates of PAH mineralization in oil-contaminated sediments and an increase in the number of colonies containing DNA sequences which hybridized to TOL (toluate oxidation) and NAH (naphthalene oxidation) plasmid probes. The colony hybridization procedure, however, has the disadvantage of requiring the growth of organisms on laboratory media, which limits sensitivity and does not allow detection of DNA sequences in viable but nonculturable microorganisms (Roszak *et al.,* 1987). Dot blot hybridization, in which DNA is extracted from environmental samples and then probed (Holben *et al.,* 1988), can be used to detect specific DNA sequences in the environment without the need for isolation and culture of microorganisms. The newly described polymerase chain reaction technique can improve the sensitivity of the dot blot method by 3 orders of magnitude, permitting the detection of one cell per g of sediment sample (Steffan *et al*., 1988). The use of these methods in conjunction with nucleic acid probes for genes involved in hydrocarbon metabolism will allow measurement of the frequency of those genes within the microbial community (Trevors *et al.,* 1985). This will permit assessment of the relative degree of adaptation of the community as well as a more detailed analysis of the dynamics of gene amplification associated with adaptation.

2.6 SEEDING

Seeding had variable success in stimulating the breakdown of organic contaminants in nature. This was attributed to the following: (i) the concentration of the contaminant may be too low to support growth of the inoculants, (ii) the concentration of the contaminant may be toxic to the inoculant, (iii) the added microorganisms may be susceptible to naturally occurring toxins/ predators in the environment, and/or (iv) the inoculant may be unable to move through the environment to the contaminant. These are difficulties that could be encountered when seeding is used to treat oil spills, except that sufficient oil is normally present to support the added microorganism (Goldstein *et al*., 1985).

Mixed cultures has been most commonly used as inocula for seeding because of the relative ease with which microorganisms with different and complementary hydrocarbon degrading capabilities can be isolated. The potential for creating, through genetic manipulation, microbial strains able to degrade a variety of different types of hydrocarbons has been demonstrated by Floodgate *et al.* (1984). They successfully produced a multiplasmid-containing Pseudomonas

strain capable of oxidizing aliphatic, aromatic, terpenic, and polyaromatic hydrocarbons. The use of such a strain as an inoculum during seeding would preclude the problems associated with competition between strains in a mixed culture. However, there is considerable controversy surrounding the release of such genetically engineered microorganisms into the environment, and field testing of these organisms must therefore be delayed until the issues of safety, containment, and the potential for ecological damage are resolved (Sussman *et al*., 1988). Horowitz *et al*. (1980) found that greater losses of oil in seawater in an open flow-through system occurred when octadecane-coated bacteria were applied 2 weeks after the addition of an oleophilic fertilizer to the system than when the fertilizer alone was added. In the same study, no significant increases in the loss of gasoline from freshwater sediment were produced by seeding. Terrestrial ecosystems differ from aquatic ecosystems in that soils contain higher concentrations of organic and inorganic matter and, generally, larger numbers of microorganisms and are more variable in terms of physical and chemical conditions (Bossert *et al.,* 1984). The microbial community of soils usually includes a significant hydrocarbon-utilizing component, which readily increases in response to hydrocarbon contamination (Atlas *et al.,* 1980). The presence of indigenous microbial populations which are highly adapted to a particular soil environment would be expected to influence negatively the ability of seed microorganisms to compete successfully and survive; for this reason, soils are sometimes not widely considered to be amenable to improvements in rates of biodegradation through seeding alone (Bossert *et al.,*1984). Other potential problems associated with the inoculation of soils, reviewed by Goldstein et al. (Goldstein *et al*.,1985), include inadequate (i.e., extremely low) concentrations of the chemical of interest, the presence of inhibitory substances, predation, preferential metabolism of competing organic substrates, and insufficient movement of the seed organisms within the soil. Addition of selected pure cultures of bacteria to soil has been found to increase the rate of degradation of pesticides such as isopropyl N-phenylcarbamate. The best results from seeding experiments have been reported in studies in which the environment is controlled to some extent, such as in fermentors and chemostats. The advantages of such arrangements are clear: competition with autochthonous microflora is reduced or nonexistent, and system parameters can be optimized to achieve the highest rates of biodegradation. The disadvantages are economic costs associated with equipment and equipment transport, energy input, and the impracticality of treating spills in some environments (e.g., uncontained oil slicks) in a closed or semicontained

system (Atlas *et al.*, 1981). This approach has been used with some success in situ bioremediation of hydrocarbon contaminated soil and groundwater (von Wedel *et al.*, 1988). Microbial seeding from a chemostat has also been used in conjunction with conventional activated-sludge treatment to improve the ability of a refinery wastewater treatment plant to absorb intermittently high loads of hydrocarbons (Wong *et al.*, 1988).

2.7 CHEMISTRY AND BIODEGRADABILITY OF PETROLEUM HYDROCARBON

Hydrocarbons differ in their susceptibility to microbial attack and, in the past, have generally been ranked in the following order of decreasing susceptibility: n-alkanes >branched alkanes > low-molecular-weight aromatics > cyclic alkanes (Perry *et al.*, 1984). Petroleum has been known for several years to occur in the surface seepage and was first obtained in pre- Christian times by the Chinese. The modern petroleum industry had its beginning in Romania and in a well-sunk in Pennsylvania by Colonel E. A. Drake in 1859 (Alloway and Ayres, 1993). The principal early use of the product of the petroleum industry was for the replacement of expensive whale oil for lighting. Today, its consumption as a fuel and its dominance in the world market as a source of chemicals has diversified tremendously. Petroleum is defined as any mixture of natural gas, condensate, and crude oil. Crude oil which is a heterogeneous liquid consisting of hydrocarbons comprised almost entirely of the elements hydrogen and carbon in the ratio of about 2 hydrogen atoms to 1 carbon atom. It also contains elements such as nitrogen; sulphur and oxygen, all of which constitute less than 3% (v/v). There are also trace constituents, comprising less than 1% (v/v), including phosphorus and heavy metals such as vanadium and nickel. Crude oils could be classified according to their respective distillation residues as paraffins, naphthenes or aromatics and based on the relative proportions of the heavy molecular weight constituents as light, medium or heavy. Also, the composition of crudes may vary with the location and age of an oil field, and may even be depth dependent within an individual well. About 85% of the components of all types of crude oil can be classified as either asphalt base, paraffin base or mixed base. Asphalt base contain little paraffin wax and an asphaltic residue (Atlas, 1981). The sulphur, oxygen and nitrogen contents are often relatively higher in comparison with paraffin base crudes, which contain little or no asphaltic materials. Mixed crude oil contains considerable amount of oxides of nitrogen and asphalt. Petroleum oil biodegradation by bacteria can occur under both oxic and anoxic conditions (Zengler *et al.*, 1999), albeit by the action of different consortia of

organisms. In the subsurface, oil biodegradation occurs primarily under anoxic conditions, mediated by sulfate reducing bacteria (Holba *et al.*, 1996) or other anaerobes using a variety of other electron acceptors as the oxidant. On a structural basis, the hydrocarbons in crude oil are classified as alkanes (normal or iso), cycloalkanes, and aromatics. Alkenes, which are the unsaturated analogs of alkanes, are rare in crude oil but occur in many refined petroleum products as a consequence of the cracking process. Increasing carbon numbers of alkanes (homology), variations in carbon chain branching (iso-alkanes), ring condensations, and interclass combinations e.g., phenylalkanes, account for the high numbers of hydrocarbons that occur in crude oil. The inherent biodegradability of these individual components is a reflection of their chemical structure, but is also strongly influenced by the physical state and toxicity of the compounds. As an example, while n alkanes as a structural group are the most biodegradable petroleum hydrocarbons, the C5 – C10 homologues have been shown to be inhibitory to the majority of hydrocarbon degraders. As solvents, these homologues tend to disrupt lipid membrane structures of microorganisms. Similarly, alkanes in the C20 –C40 range, often referred to as "waxes", are hydrophobic solids at physiological temperatures. Primary attack on intact hydrocarbons always requires the action of oxygenases and therefore, requires the presence of free oxygen. In the case of alkanes, monooxygenase attack results in the production of alcohol. Most microorganisms attack alkanes terminally whereas some perform sub-terminal oxidation. The alcohol product is oxidised finally into an aldehyde and finally, to a fatty acid. The latter is degraded further by beta-oxidation (Bartha, 1986b).

Extensive methyl branching interferes with the beta-oxidation process and necessitates diterminal attack or other bypass mechanisms. Therefore, nalkanes are degraded more readily than iso alkanes. Cycloalkanes are transformed by a not fully characterized oxidase system to a corresponding cyclic alcohol, which is dehydrated to ketone. Then, a monooxygenase system lactonises the ring, which is subsequently opened by a lactone hydrolase. These two oxygenase systems usually never occur in the same organisms and hence, the frustrated attempts to isolate pure cultures that grow on cycloalkanes (Bartha, 1986b). However, synergistic actions of microbial communities are capable of dealing with degradation of various cycloalkanes quite effectively. As in the case of alkanes, the monocyclic compounds, cyclopentane, cyclohexane, and cycloheptane have a strong solvent effect on lipid membranes, and are toxic to the majority of hydrocarbon degrading microorganisms.

Highly condensed cycloalkane compounds resist biodegradation due to their structure and physical state (Bartha,1986a). Prokaryotes convert aromatic hydrocarbons by an initial dioxygenase attack, to trans-dihydrodiols that are further oxidised to dihydroxy products, e.g., catechol in the case of benzene (Atlas and Bartha, 1998). Eucaryotic microorganisms use monooxygenases, producing benzene 1, 2-oxide from benzene, followed by the addition of water, yielding dihydroxydihydrobenzene (cis-dihydrodiol). This is oxidised in turn to catechol, a key intermediate in biodegradation of aromatics, which is then opened by ortho- or meta-cleavage, yielding muconic acid or 2- hydroxymuconicsemialdehyde, respectively.

Condensed polycyclic aromatics are degraded, one ring at a time, by a similar mechanism, but biodegradability tend to decline with the increasing number of rings and degree of condensation (Atlas and Bartha,1992). Aromatics with more than four condensed rings are generally not suitable as substrates for microbial growth, though, they may undergo metabolic transformations. Biodegradation process also declines with the increasing number of alkyl substituents on the aromatic nucleus. Asphaltics tend to increase during biodegradation in relative and sometimes absolute amounts. This would suggest that they not only tend to resist biodegradation but may also be formed de novo by condensation reactions of biodegradation and photodegradation intermediates. In crude petroleum as well as in refined products, petroleum hydrocarbons occur in complex mixtures and influence each other's biodegradation. The effects may go in negative as well as positive directions. Some isoalkanes are apparently spared as long as n-alkanes are available as substrates, while some condensed aromatics are metabolised only in the presence of more easily utilisable petroleum hydrocarbons, a process referred to as co-metabolism (Wackett, 1996).

2.8 DISTRIBUTION OF PETROLEUM HYDROCARBON UTILIZING MICROORGANISMS

Hydrocarbon degrading bacteria and fungi are widely distributed in marine, freshwater, and soil habitats.

Table 2.8 A list microorganisms involved in the bioremediation of organic wastes (adapted from Savage *et al*. 1985).

Waste Description	Microorganisms
Crude oil	*Brevibacterium sp, Flavobacterium sp, Norcaadia Pseudomonas. Flavobacteria ,vibrio, Achrombacter*
Hexadecane	*Acinobacter sp, Candida petrophilium Pseudomonas aeruginosa, trichosporonpullulans*
Paraffins	*Trichosporonpullulans*
Jet Fuels	*Clasdospoirium.,Hormodendnrm*
Napthalene	*Pseudomonas sp*
Napthalene	*Pseudomonas putida*
Kerosene	*Torulopsis. Candida tropicalis Corynebacterium Hydrocarbonclastus*

Typical bacterial groups with already known capacity to degrade hydrocarbons include *Pseudomonas, Marinobacter, Alcanivorax, Microbulbifer, Sphingomonas, Micrococcus, Cellulomonas, Dietzia,* and *Gordoniagroups* (Brito *et al*., 2006). Molds belonging to the genera *Aspergillus, Penicillium, Fusarium, Amorphoteca, Neosartorya, Paecilomyces, Talaromyces*, *Graphium* and the yeasts Candida, *Yarrowia* and *Pichia* have been implicated in hydrocarbon degradation (Chaillan *et al*., 2004). However, reports in literature on the actual numbers of hydrocarbon utilisers are at variance with one another because of the methodological differences used to enumerate petroleum-degrading microorganisms. In some cases, a high correlation has been found between growth on agar and media containing hydrocarbons as the sole carbon source, and the ability to rigorously demonstrate hydrocarbon utilization by isolates from these media in liquid culture The Most Probable Number (MPN) procedure has been tried as a substitute for the plate count technique for the estimation of hydrocarbon utilising microorganisms, since it eliminates the need for a solidifying agent and permits direct assessment of the ability to actually utilize hydrocarbons. The use of liquid media for MPN also permits removal of trace organic contaminants and allows for the chemical definition of a medium with a hydrocarbon as a sole source of carbon. This technique thus incorporates the

specificity for counting only hydrocarbon utilizers and eliminates the problem of counting organisms growing on other trace organic contaminants (Braddock and Catterall, 1999).The problems of culture techniques arise from the fact that most (90–99%) of the species making up competent degrading communities do not form colonies when current laboratory-based culture techniques are used (MacNaughton *et al.*, 1999). However, the application of molecular techniques for the analysis of the microbial communities that take part in in situ hydrocarbon biodegradation activities is helping to address these problems. The measurement of lipid biomarkers, specifically, phospholipids fatty acids (PLFA), together with nucleic acid–based molecular techniques for fingerprinting the 16S ribosomal DNA (rDNA) component of microbial cells is a powerful combination of techniques for elucidating the microbial ecology of actively bioremediating communities (Stephen *et al.*, 1999). Lipid biomarkerbased techniques measure the lipid profiles of microbes in the environment irrespective of culturability, thereby avoiding culture bias (White *et al.*, 1998). These methods provide insight into several important characteristics of microbial communities, especially the viable biomass, community structure, and nutritional status or physiological stress responses of the gram-negative bacteria (White *et al.*, 1998). Despite the shortcomings of cultivation-based techniques, standard culture methods are still adequate for site evaluation to determine whether indigenous bacteria are capable of degrading the contaminants. Several new methodologies have enabled recent studies on the microbial biodegradation mechanisms of organic pollutants. Culture-independent techniques for analysis of the genetic and metabolic potential of natural and model microbial communities that degrade organic pollutants have identified new metabolic pathways and enzymes for aerobic and anaerobic degradation (Pieper *et al.*, 2004). Genetic studies have focused mainly on aerobic pathways, and many details of these metabolic routes have been documented (van der Meer *et al.*, 1992), although Widdel and Rabus. (2001), Heider *et al.* (1998) and Sporman and Widdel. (2000) presented some detailed and comprehensive reviews on anaerobic biodegradation of hydrocarbons and the mechanisms involved. A general comparison of the major pathways for catabolism of aromatic compounds in bacteria has revealed that the initial conversion steps are carried out by different enzymes but that the compounds are transformed to a limited number of central intermediates, such as protocatechuate and (substituted) catechols (Chaudry and Chapalamadugu, 1991). These dihydroxylated intermediates are channelled into one of two possible pathways, either a Meta-cleavage- type pathway or an ortho-cleavage type pathway.

Both types of pathways lead to intermediates of central metabolic routes, such as the tricarboxylic acid cycle. This generalised scheme of catabolic pathways for aromatic compounds suggests that microorganisms have extended their substrate range by developing peripheral enzymes, which are able to transform initial substrates into one of the central intermediates (van der Meer *et al.*, 1992).

Genetic factors play important roles in conferring biodegradation potentials on microorganisms. Plasmids probably play leading role in this aspect. The ability to degrade more recalcitrant components of petroleum such, as the aromatic fractions are generally plasmid mediated (Cerniglia, 1984). Exposures of a microbial community to hydrocarbons have been shown to result in an increase in the number of bacterial plasmids types (Leahy *et al.,* 1996). Catabolic plasmids are non-essential genetic elements in so far as viability and reproduction of an organism is concerned, but they do provide a metabolic versatility not normally present in the cell. Such genetic potential allows for the evolution of integrated and regulated pathways for the degradation of hydrocarbons. The observed increase in the study of the genetics of such systems has closely paralleled the development of advances in molecular biology, particularly the application of recombinant DNA technology gene probes and polymerase chain reaction (PCR) technology (Barriault and Sylvestre, 1993 and Singer and Finnerty 1984). Many bacterial catabolic pathways are specified by conjugative plasmids (Frantz and Chakrabarty, 1986). These plasmids are readily transferred laterally into new host bacteria, thereby enhancing the metabolic potential of other members of an ecosystem. Conjugative plasmids are thus important agents of genetic changes and evolution in bacteria, and could be picked up from or brought together in different organisms as groups of genes, which through mutations and recombination can specify new metabolic functions (Lessie and Gaffney, 1986).

2.9 NON-SCIENTIFIC FACTORS AFFECTING BIOREMEDIATION

Several non-scientific factors hinder the development of bioremediation technologies Regulatory factor drive and constrain the use of bioremediation. Regulation creates the bioremediation market by dictating what must be cleaned up, how clean it must be and which clean-up methods may be used (Caplan, 1993). The use of genetically engineered microorganisms (GEMs) presents

additional regulatory hurdles. There is much debate over whether to use natural or GEMs in bioremediation as concerns are raised about other potential environmental problems (Caplan, 1993). Regulation can have an impact on bioremediation in different ways:

1. Creating markets: Federal environmental programs require treatment of recurring wastes and remediation of existing wastes contaminating soils and groundwater (Day, 1993).

2. Controlling the product: Environmental laws and regulation may specify health and safety criteria for products before they can be marketed in USA. (Day, 1993). EPA regulates the use of microbes as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (Day, 1993).

2.9.1 HUMAN RESOURCE FACTOR

Because bioremediation is a new technology, there is a lack of trained human resources in this technology. A successful bioremediation program requires a multidisciplinary approach, integrating fields such as microbiology, engineering, geology, hydrogeology, soil science and project management (Boopathy, 2000).

2.9.2 ECONOMIC AND LIABILITY FACTOR

Unlike other industries, bioremediation does not result in the production of high value-added products. Thus, venture capital has been slow to invest in the technology and, as a consequence, commercial activity has lagged far behind other industrial sectors.

As bioremediation is considered innovative technology, clients and regulatory agencies often scrutinize bioremediation more closely than conventional technologies. Consequently, tighter restrictions and performance standards are frequently imposed on bioremediation than on other remediation technologies (Boopathy, 2000).

2.10 MICROBIAL DIVERSITY

The studies of ecological theories have been based on the study of aboveground ecosystems. Despite the fact that the soil biota plays a fundamental role in ecosystem functioning, through nutrient cycling, decomposition and energy flow, soil organisms have had a negligible influence on the development of contemporary ecological theories (Wardle and Giller, 1996). Microbial

diversity is usually taken as the number of individuals assigned to different taxa and their distribution among taxa (Atlas and Bartha, 1998).

These include the study of individual cells at the genomic and proteomic levels to obtain in vivo informational imaging. The global tools also apply to the study of communities with respect to the environment. The enlarged view seems to adequately fit the complexity of the soil environment pathways. Soil biota are characterized by a spatial diversity with possible differences between rhizosphere and bulk soil, macro aggregates and microaggergates, macropores and micropores, different horizons, etc. Indeed within a soil, there are several microhabitats, e.g. the rhizoplane, the rhizosphere, aggregates, decaying organic matter, or the bulk soil. Typically, soils are also largely stratified habitats, with distinct horizons; each of them may be regarded as a separate entity. How the diversity of these microhabitats can be incorporated in a general soil microbial diversity concept is not known.

Numerous factors are known to affect diversity. Among these are trophic interactions, spatial and temporal habitat heterogeneity, disturbance and eutrophication (Torsvik *et al.*, 2002). There are supposedly negative effects such as stress, or positive effects like resource diversity or biological interactions. Positive effects on diversity may be related to increased stability, resilience, resistance to stress, and even productivity (Griffiths *et al.*, 1997 and Nannipieri *et al.*, 2003).

2.11 EFFECT OF PETROLEUM HYDROCARBON CONTAMINANTS IN THE ENVIRONMENT

The effects of crude oil spill will vary from source to source however details of the potential biological damage will depend on the ecosystem where the spill occurred. The aquatic ecosystem, particularly the marines are the most vulnerable (Cairns and Buikema, 1984). Oil spills in the environment may affect organisms found therein by direct toxicity or by physical smothering (Perry, 1980). Oil spills generally, can cause various damages to the marsh vegetation.

It was found to reduce growth, photosynthetic rate, stem height, density, and above ground biomass of *Spartina alterniflora* and *S. patens* and may cause their death (Krebs and Tamer, 1981). Crude oil spill at sea forms a surface slick whose components can follow many pathways. Some may pass into the mass of seawater and evidence suggests they may persist for a long time before their degradation by microorganisms in the water. The slick usually becomes more

viscous and forms water-in-oil emulsion. Oil in water causes depletion of dissolved oxygen due to transformation of the organic component into inorganic compounds, loss of biodiversity through a decrease in amphipod population that is important in food chain, and eutrophication. Short-term toxicity in fishes includes lymphocytosis, epidermal hyperplasia, hemorrhagicsepticemia (Beeby, 1993). In mammals it possesses an anticoagulant potency (Onwurah, 2002a). It was estimated that some tens of thousands of seabirds were killed as a result of spilled oil in sea (Dunnet, 1982).

Dying mangrove trees, tarred beaches and declining fish catches, all seem to be threats to long term viability of some ecosystem such as the Niger Delta areas of Nigeria after. Apart from inherent toxicity of spilled oil in seas, enhanced toxicity has been reported due to ultra violet (U.V) radiation. Generally, crude oil is toxic to aquatic organisms, due to the presence of PAH (Heintz *et al.*, 1999). Oil spill in the environment could lead to an increased exposure of by-products of PAHs to a given human population. This may increase risk of mortality from infectious disease (Hall *et al*., 2006) and the reproductive capacity of that population (Tiido *et al.*, 2006).

Crude oil affects germination and growth of some plants (Onwurah, 1999a). It also affects soil fertility but the scale of impact depends on the quantity and type of oil spilled. Severe crude oil spill in Cross-River state, Nigeria, has forced some farmers to migrate out of their traditional home, especially those that depend solely on agriculture. This is because petroleum hydrocarbons 'sterilize 'the soil and prevent crop growth and yield for a long period of time. The negative impact of oil spillages remains the major cause of depletion of the Niger Delta of Nigeria vegetative cover and the mangrove ecosystem (Odu, 1987). Crude oil contamination of land affects certain soil parameters such as the mineral and organic matter content, the cation exchange capacity, redox properties and pH value. As crude oil creates anaerobic condition in the soil, coupled to water logging and acidic metabolites, the result is high accumulation of aluminum and manganese ions, which are toxic to plant growth.

Whereas human health is a deep field of science from time of old, the concept of 'environmental health 'can be viewed as a modern science, which is measured as the viability of the inhabitants of a given ecosystem as affected by ambient environmental factors (Shields, 1990). Practically, environmental health involves the assessment of the health of the individual organisms and

correlating observed changes in health with changes in environmental conditions. Some diseases have been diagnosed to be the consequences of crude oil pollution.

The health problems associated with oil spill may be through any or combinations of the following routes: contaminated food and / or water, emission and / or vapors. Toxic components in oil may exert their effects on man through inhibition of protein synthesis, nerve synapse function, and disruption in membrane transport system and damage to plasma membrane (Prescott *et al.*, 1996). Crude oil hydrocarbons can affect genetic integrity of many organisms, resulting in carcinogenesis, mutagenesis and impairment of reproductive capacity (Short and Heintz, 1997). The risk of drinking water contaminated by crude oil can be extrapolated from its effect on rats that developed hemorrhagic tendencies after exposure to water soluble components of crude oil (Onwurah, 2002). Volatile components of crude oil after a spill have been implicated in the aggravation of asthma, bronchitis and accelerating aging of the lungs (Kaladumo, 1996). Other possible health effects of oil spill can be extrapolated from rats exposed to contaminated sites and these include increased liver, kidney and spleen weights as well as lipid per-oxidation and protein oxidation (Anozie and Onwurah, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The Ahafo Gold Project is being developed by Newmont Ghana Gold Limited (NGGL) and is one of two Greenfield developments in Ghana being sponsored by Newmont Ghana. It is located in the Brong Ahafo Region some 300 km north west of the capital city of Accra, 107 km north west of the second largest city, Kumasi and 40 km south east of the regional capital of Sunyani (Plate 3.1).

The Project extends from the Kenyase area in the south to the Subenso area 45 km to the north (Plate 3.1). The Project combines two earlier gold projects that were being considered for development – the Yamfo-Sefwi Gold Project and the Ntotoroso Gold Project. The Ahafo Project will comprises of facilities and services for mining and processing of approximately 137 million tonnes of ore to be extracted by open-pit mining from 12 different deposits. The ore is processed in a Carbon In Leach (CIL) process plant near Kenyase with a capacity of 7.5 Mega tonnes per annum (Mtpa). Currently, mine life has been planned for 15 years excluding the initial development and commissioning period (9 months) and post-mining processing of stockpiled ore (12 months).The experiment was mounted at the Newmont Ahafo south plant site area with a an annual average temperature(°C), windspeed(m/sec), evaporation(mm) of 25.95 ,1.0416 and 3.97 respectively.

Plate 3.1 Location Map of project in Ghana

Plate 3.2 volatilization pad at Newmont Ghana Gold limited plant site

3.2 BIOREMEDIATION SET UP

Three different sources of Nitrogen was used in this study namely; topsoil, compost and fertilizer (urea).The hydrocarbon contaminated soil samples were obtained from the volatilization pad. (plate 3.2) at Newmont Ghana Gold limited.

Topsoil (0-15 cm) with no previous history of diesel and hydraulic lubricant contamination was collected from the surroundings of the plant site into paper bags. About 80 kg compost was taken from the Newmont Ghana gold limited compost plant. The fertilizer of strength 46% (urea) used for this study was bought from the open market. Three set ups involving mixings with 2 Kg hydrocarbon contaminated soil with portions of fertilizer, compost and topsoil was done. The samples were placed under wooden structure covered with plastic rubber (Plate 3.3).

The fertilizer/compost/topsoil was used to adjust the N- level to the optimum soil condition suitable for microbial growth. This was based on the N- levels of the contaminated soil. Laboratory assay of the N- level was carried out to verify whether the levels are consistent with the calculated values. The experiment was replicated three times in randomized complete block design. Each block contained 13 different treatments. The experimental samples were monitored for a period weakly basis.

Plate 3.2 bioremediation experimental set up

3.3 SAMPLE PREPARATION AND COLLECTION

Soil samples were taken from all the treatments on weekly basis. Soil samples were collected in brown paper bags. All samples were transported to the laboratory in a box for analysis

Plate 3.3 Weekly mixing (aeration) at the site of the experiment.

3.4 LABORATORY ANALYSIS

3.4.1 DETERMINATION OF pH

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

The pH of the supernatant water was then measured.

3.4.2 MOISTURE CONTENT

The container was cleaned, dried and weighed (W1)

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

The sample was dried to constant temperature at 105 0C for a period of 24 hours.

After drying the sample was removed from the oven and cooled in a desiccator for 30minutes.

The final constant weight (W3) of the container with dried soil sample was recorded. The percent moisture content in the soil is given by

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

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

3.4.3 DETERMINATION OF PERCENT TOTAL NITROGEN BY KJELDAHLS METHOD

Ten grams of air dry soil weighed into a 500 ml long – necked kjeldahl flask and followed by 10 ml distilled water. It was allowed standing for 10 minutes to moisten. One spatula full of kjeldahl catalyst [mixture of 1 part Selenium + 10 parts $CuSO_4$ + 100 parts Na_2SO_4] and 20 ml conc. H_2SO_4 was added. It was digested for a period of two hours until colourless or light greenish colour was observed. It was further allowed to cool .The fluid was decanted into a 100 ml volumetric flask and make up to the mark with distilled water.

- DISTILLATION

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

- TITRATION

100 ml of collected distillate was titrated with 0.1 N HCl till blue colour changes to grey and then suddenly flashes to pink. A blank determination was carried out without the soil sample.

- CALCULATION

Thus, the percentage of Nitrogen in the soil sample is,

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

Where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

3.4.4 EXCHANGEABLE CATION DETERMINATION (K)

Ten grams of soil into extraction bottle weighed. 100 ml of 1.0 N NH4OAc solution was added. Bottle with contents was placed in a mechanical shaker and shaken for 2 hours. The supernatant solution was filtered through No. 42 whatman filter paper.10 ml aliquot of it was taken and read for K or Na on a Flame Photometer after calibration of Photometer with prepared standards. Determine the flame photometer reading for soil. Using the meter reading standard curve, determine the concentration of K in the soil extract (FAO fertilizer and plant nutrient, 2008).

3.4.5 OIL AND GREASE ANALYSIS

Thirty grams soil sample was weighed into a 250 ml Schott bottle. 2 to 3 teaspoons of anhydrous Na_2S0_4 followed by 30 mL Solvent and 2 ml concentrated HCl to the Schott bottle, The Schott bottle is cooked and shaken vigorously to break up any aggregates. It was sonicated for 10 minutes.

The supernatant liquid poured off into a phase separator filter set in a glass funnel with approximately 10 g sodium sulphate and run into a pre-weighed beaker with 2 glass boiling chips added. 30 mL Solvent was further added to the Schott bottle. The sonication and filtering process

was repeated three times. The extracts were combined and evaporated to dryness on a hotplate at 70 °C.

Sample was cooled in a desiccator to constant weight. The weight was recorded and the Oil and grease level calculated as per formula below. (Standard Methods book, 2005).

CALCULATION

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

Where:

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

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

M= weight of sample taken (g)

F = moisture factor

3.4.6 TOTAL PETROLEUM HYDROCARBON ANALYSIS

Diesel oil extraction

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

After shaking, 25 ml of Freon was pipetted into the bottle and shake well again for 15 minutes using a paint or lateral shaker. Sample was allowed to stand to permit content of bottle to separate into distinct layers.

10 ml of Freon was Pipette from the appropriate layer and filtered through 5 grams of activated silica gel and 1 g of sodium sulphate in to a reference cells.

Infra-red spectroscopy measurement

The instrument was calibrated with working standards made of 100% hexadecane, chlorobenzene and iso-octane.

The analyzer was blanked with the extractant solvent and cell filled with sample inserted into the calibrated analyzer. The readings from the analyzer was recorded (Standard Methods book, 2005).

3.4.7 HETEROTROPHIC PLATE COUNT (HPC)

About 16 sterile test tubes were arranged in a test tube rack.1g of the soil was weighed on a calibrated Mettler Toledo balance into the first sterile test tube. The test tube was filled with 10mls of sterile distilled water and capped. It was then mixed thoroughly to ensure a homogeneous mixture.1ml of the sample (supernatant) in the first test tube was pipetted into the second test tube and topped up with 9m $_{ml}$ of sterile distilled water giving the first dilution factor of 10.The procedure is replicated for the rest of the test tubes.

The media (plate count agar) was hydrated by filling media vessel to the 100 $_{ml}$ mark with sterile diluent (deionized water), The test tube was re-caped and shaken to dissolve. It was then labeled with media name, date prepared, and initials.

Using a sterile tip, 1ml of sample was aseptically pipetted to the center of a simplate.

9ml of rehydrated media was slowly pipetted directly onto the sample into the center of the plate.

The plate was covered with lid and swirled gently to distribute sample into each well.

The plate was tilted $90^0 – 120^0$ to drain excess liquid into absorbent pad.

The Inverted plate was incubated for 48hrs at 35 $\pm$ 0.5 ^{0}C.

The steps were repeated using 10 $_{ml}$ of rehydrated media and no sample to act as a media blank.

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

Fluorescence wells may be counted on the bottom of the plate instead (Standard Methods book, 2005)

3.5 STATISTICAL ANALYSIS

A two-way randomized analysis of variance (ANOVA) was used for testing variance between variable using the Minitab software. The data were tested at 95% confidence level.

CHAPTER FOUR

4.0 RESULTS

4.1 MEAN RESULTS OF OIL AND GREASE, TPH AND HPC FOR COMPOST/HYDROCARBON CONTAMINATED SOIL BLEND

Initial characterization of the site revealed Oil and Grease (mg/kg) and TPH (mg/kg) levels of $3.43x10^4$ and $2.15x10^4$ respectively. Mean oil/grease (mg/kg) and TPH (mg/kg) soil samples taken from the sampling site shows that the 0.2% 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. 2.0 % nitrogen level recorded less than detection limit for both oil/grease and TPH after week seven (7) as shown in table 4.4. Statistically, there were differences ($p = 0.00$) in the hydrocarbon degradation rate of the 0.2%, 0.8%. 1.4% and 2.0% nitrogen levels within the compost blend as shown in Appendix D. By week five (5) there had been a percent degradation of more than 80% in the 0.2% nitrogen level for both oil/grease and TPH and a percent degradation of more than 99% for both oil/grease and TPH for 2.0%.

Table 4.1 Mean results of Oil and grease, TPH and HPC for 0.2% Nitrogen level in HC/compost blend.

Parameters / Time (wks)	Oil/Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.43×10^4	$2.2x\ 10^4$	4.653	0.00	0.00
Week 1	$1.8x10^4$	1.3×10^4	4.740	48.38	38.89
Week 2	$1.3x\ 10^4$	8.9×10^3	7.806	60.03	58.61
Week 3	7.8×10^3	5.1×10^3	8.806	77.16	76.35
Week 4	6.2×10^3	3.6×10^3	9.121	81.91	83.32
Week 5	$5.1x\ 10^3$	2.8×10^3	9.531	85.12	86.99
Week 6	$3.4x\ 10^3$	1.9×10^3	9.617	90.08	90.94
Week 7	$10x\ 10^2$	3.9×10^2	8.530	97.08	98.19

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 0.2% nitrogen level within the compost/HC blend is shown in figure 4.1, by week five (5), oil/grease and TPH was just around the 5000 mg/kg bar line on the graph.

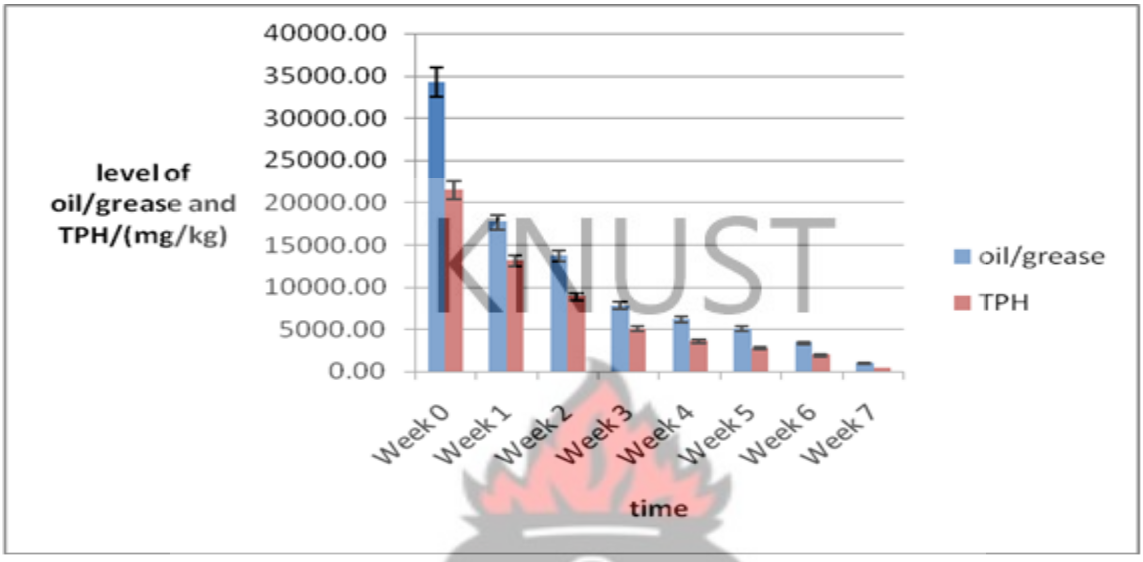


Figure 4.1 Oil/grease and TPH levels (mg/kg) of 0.2% nitrogen level in compost/HC blend.

The degradation rate of HC soil when the nitrogen level in the HC/compost blend was augmented to 0.8% is shown in Table 4.2. By week five (5) there had been a percent degradation of more than 90% for both oil/grease and TPH.

Table 4.2 Mean results of Oil and grease, TPH and HPC for 0.8% Nitrogen level in HC/compost blend.

Parameters / Time(wks)	Oil/Grease (mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.00	0.00
Week 1	1.2×10^4	7.4×10^4	4.845	64.97	65.40
Week 2	1.1×10^4	7.3×10^3	6.929	68.64	66.02
Week 3	6.5×10^3	4.5×10^3	7.708	81.02	79.14
Week 4	4950.00	3.5×10^3	8.653	85.56	83.92
Week 5	2.5×10^3	1.3×10^3	8.813	92.79	94.19
Week 6	9.8×10^2	2.7×10^3	8.881	97.14	98.76
Week 7	2.3×10^2	<10.00	7.833	99.32	100.00

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 0.8% nitrogen level within the compost/HC blend is shown in figure 4.2, by week five (5), oil/grease and TPH had dropped below 5000 mg/kg bar line.

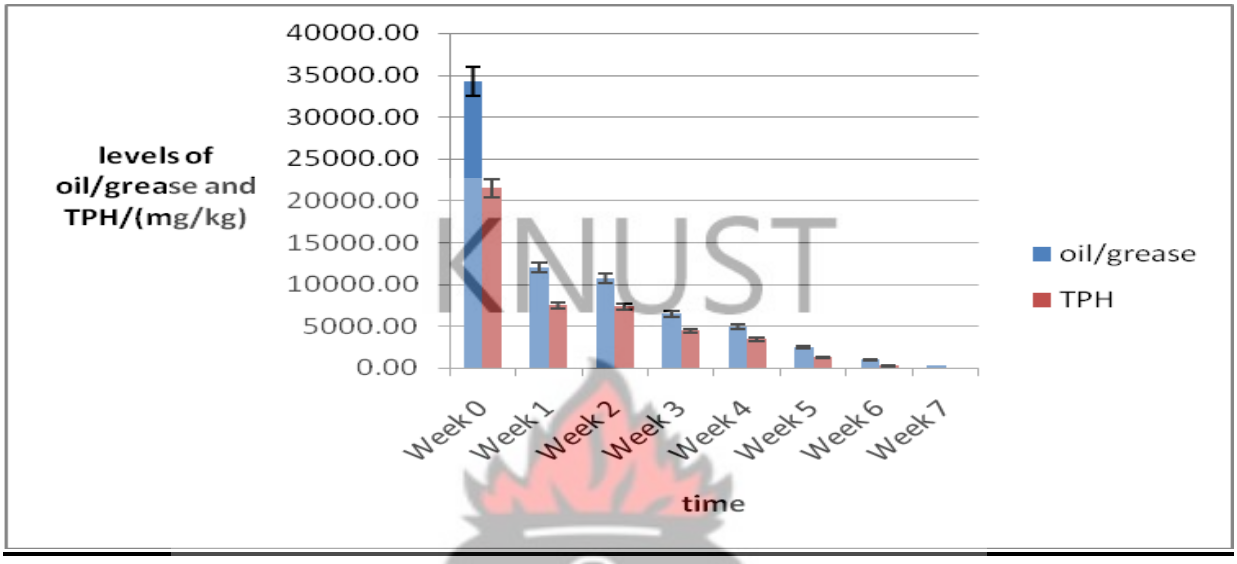


Figure 4.2 Oil/grease and TPH levels (mg/kg) in 0.8% nitrogen level compost/HC blend.

Table 4.3 shows the degradation rate of HC soil when the nitrogen level in the HC compost blend was augmented to 1.4%. By week five (5), there had been a percent degradation of more than 97% for both oil/grease and TPH

Table 4.3 Mean results Oil and grease, TPH and HPC for 1.4% Nitrogen in HC/compost blend.

Parameters / Time(wks)	Oil n Grease(mg/kg)	TPH(mg/kg)	log of count	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.00	0.00
Week 1	1.1×10^3	7.4×10^3	5.000	65.76	65.76
Week 2	8.8×10^3	5.6×10^3	7.107	74.16	74.16
Week 3	4.8×10^3	3.5×10^3	7.778	83.71	83.71
Week 4	3.5×10^3	2.3×10^3	8.653	89.27	89.27
Week 5	7.8×10^2	4.4×10^2	8.898	97.95	97.95
Week 6	1.2×10^2	<10.00	7.851	100.00	100.00

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 1.4% nitrogen level within the compost/HC blend is shown in figure 4.3, by week five (5), oil/grease and TPH had dropped significantly below 5000 mg/kg bar line.

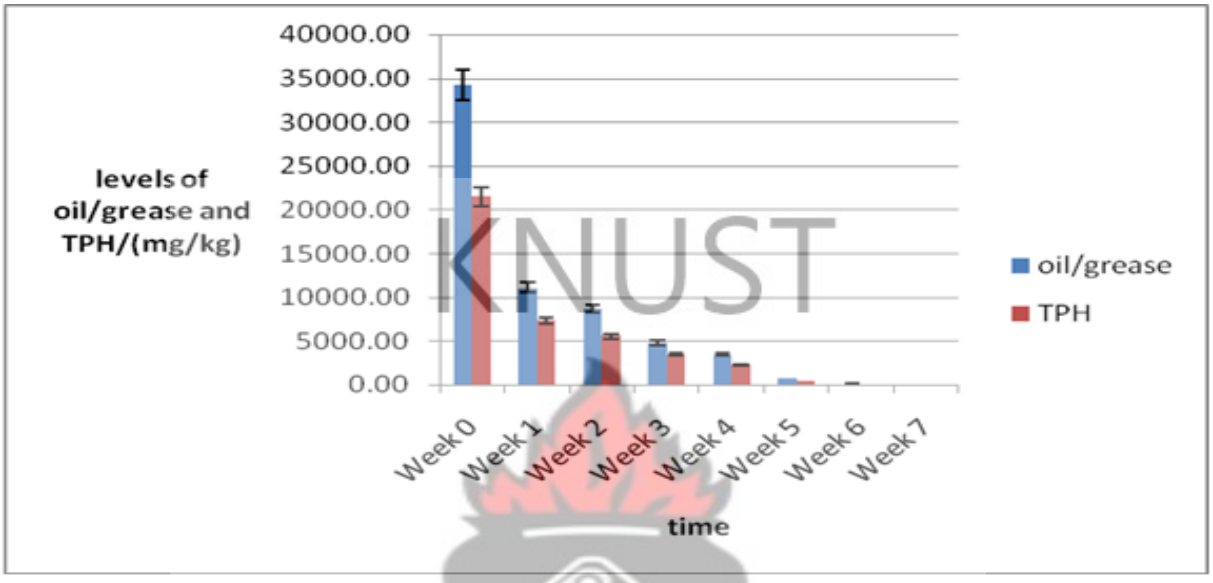


Figure 4.3 Oil/grease and TPH levels (mg/kg) in 1.4% nitrogen level compost/HC blend

Table 4.4 shows the degradation rate of HC soil when the nitrogen level in the HC compost blend was augmented to 2.0%. By week five (5) there had been a percent degradation of more than 99% for both oil/grease and TPH

Table 4.4 Mean results Oil and grease, TPH and HPC for 2.0% Nitrogen in HC/compost blend.

Parameters / Time(wks)	Oil and Grease(mg/kg)	TPH(mg/kg)	log of count	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.000	0.000
Week 1	1.1×10^4	6.4×10^3	5.362	69.071	70.486
Week 2	5.7×10^3	3.8×10^3	7.531	83.371	82.514
Week 3	3.1×10^3	2.2×10^3	8.079	91.102	89.682
Week 4	1.6×10^3	8.8×10^2	8.987	95.464	95.924
Week 5	1.1×10^2	10×10^1	9.079	99.679	99.535
Week 6	<100.00	<10.00	7.914	100.000	100.000

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 2.0 % nitrogen level within the compost/HC blend is shown in figure 4.4, by week five (5), oil/grease and TPH had dropped significantly below 5000 mg/kg bar line.

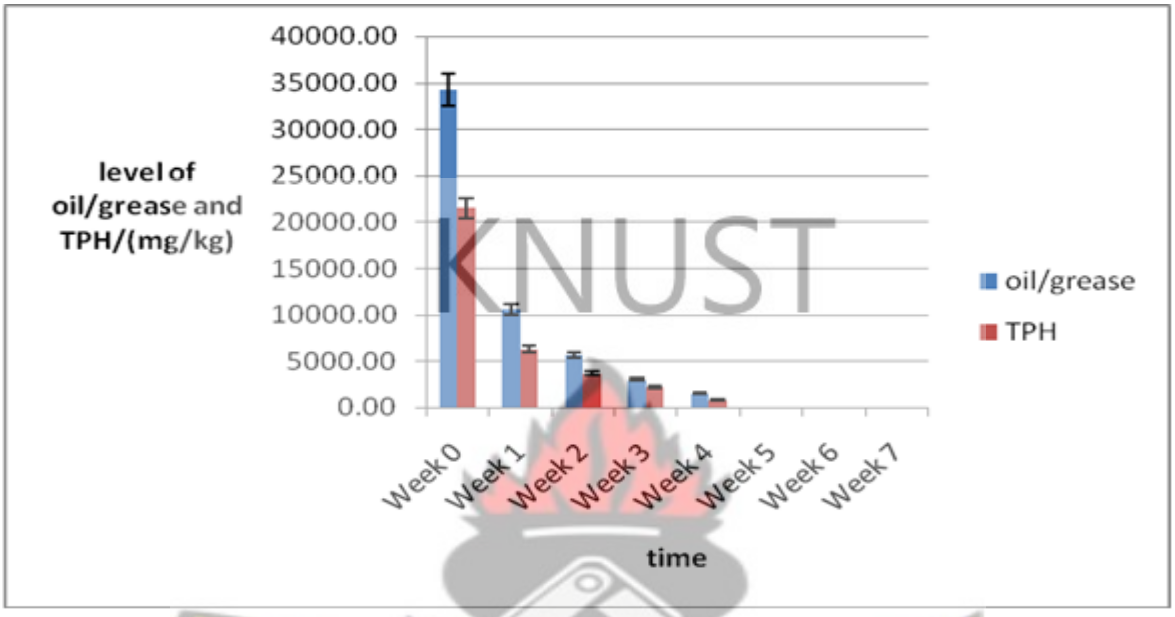


Figure 4.4 Oil/grease and TPH levels (mg/kg) in 2.0% nitrogen level compost/HC blend

Figure 4.5 shows a box plots representation of oil/grease (mg/kg) and TPH (mg/kg) degradation with time for 0.2%, 0.8%, 1.4%, 2.0% nitrogen level within the compost/HC blend. The positions of the mean for 0.2%, 0.8% nitrogen level in the graph are different height. That of 1.4% and 2.0% are almost at different height. The 25th and 75th quartile for the respective nitrogen levels are indicated by the upper and lower portions of the graph respectively.

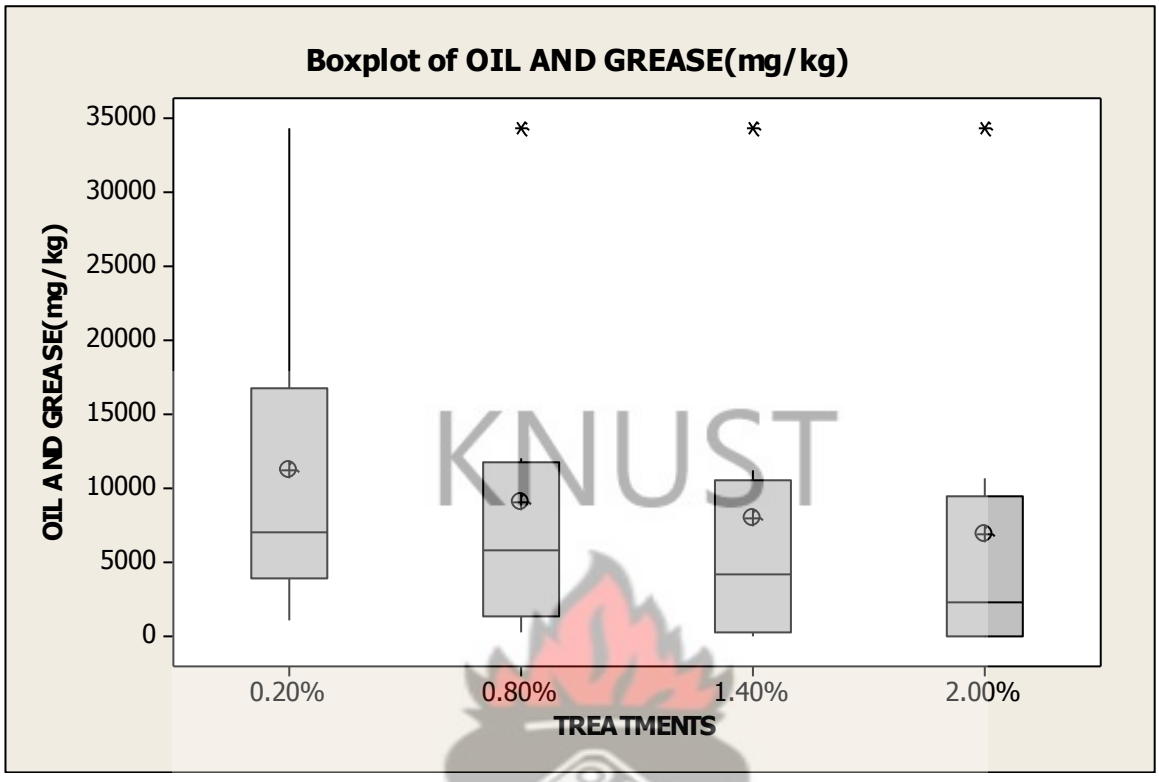


Figure 4.5 Box plots giving a pictorial view about the differences in the effects of the treatments for oil/grease

Figure 4.6 shows a box plots representation of oil/grease (mg/kg) degradation with time for 0.2%, 0.8%, 1.4% and 2.0% nitrogen level within the compost/HC blend. The position of mean in the 0.2% and 0.8% nitrogen levels in the graph are almost at the same height. The 25th and 75th quartile values are indicated by the upper and lower portions of the graph.

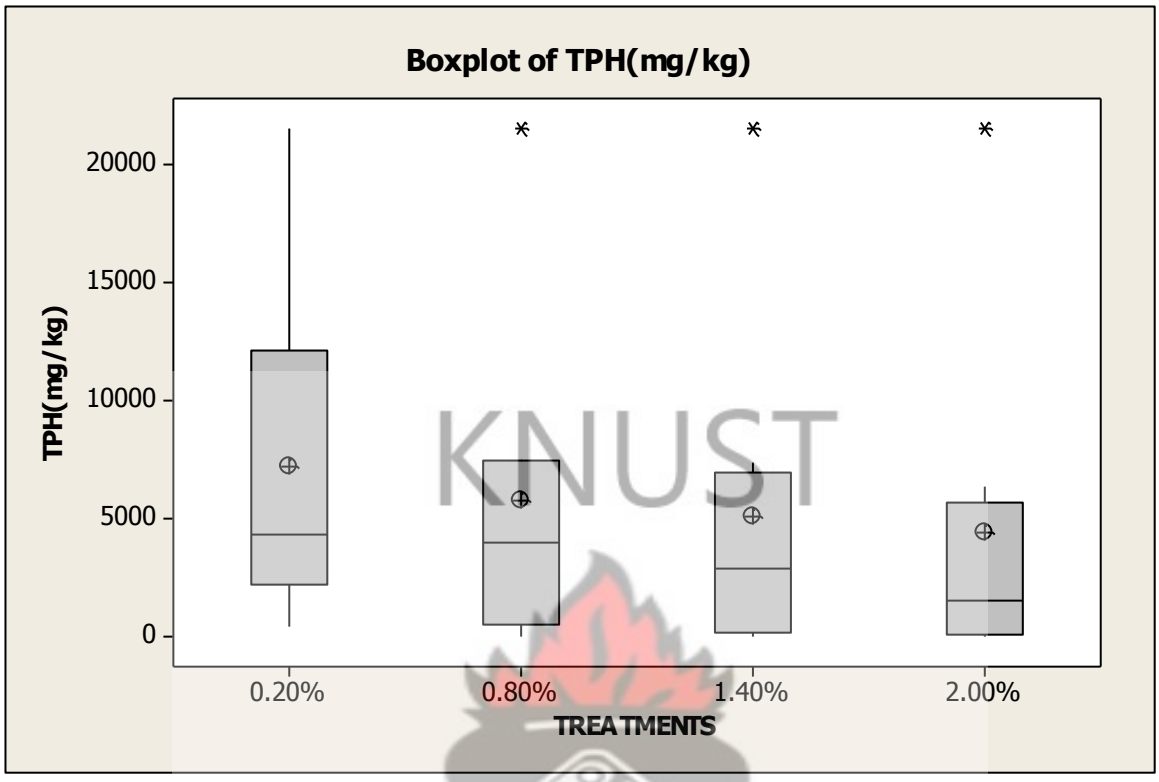


Figure 4.6 Box plots giving a pictorial view about the differences in the effects of the treatments for TPH

4.2 MEAN RESULTS OF OIL AND GREASE, TPH AND HPC FOR TOPSOIL/HYDROCARBON CONTAMINATED SOIL BLEND

Statistically, there were differences ($P<0.05$) in the degradation rate of the hydrocarbon by 0.2%, 0.8%, 1.4% and 2.0% nitrogen levels within the topsoil blend as shown in appendix D. At the end of the seven week period, 0.2% nitrogen level recorded the highest mean oil/grease (mg/kg) and TPH (mg/kg) of 2.3×10^3 and 1.2×10^3 respectively of samples taken from the site respectively as shown in Table 4.5. 2.0% nitrogen levels recorded 198 for oil/grease (mg/kg) and less than detection for TPH (mg/kg).

Table 4.5 shows the degradation rate of HC soil when the nitrogen level in the HC/ topsoil blend was augmented to 0.2%. By week five (5), 0.2% had recorded more than 75% percent degradation for both oil/grease and TPH.

Table 4.5 Mean results Oil and grease, TPH and HPC for 0.2% Nitrogen in HC/topsoil blend.

Parameters / Time(wks)	Oil /Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.000	0.000
Week 1	2.1×10^4	1.7×10^4	6.394	37.981	19.781
Week 2	1.8×10^4	1.3×10^4	6.954	47.034	40.348
Week 3	1.3×10^4	8.9×10^3	7.705	62.562	58.847
Week 4	9.4×10^3	6.0×10^3	8.672	72.685	72.149
Week 5	7.8×10^3	5.3×10^3	8.672	77.245	75.347
Week 6	4.9×10^3	2.7×10^3	7.978	85.705	87.376
Week 7	2.3×10^3	1.2×10^3	8.079	93.144	94.334

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 0.2 % nitrogen level within the topsoil/HC blend. From figure 4.7, by week five (5), oil/grease and TPH had dropped significantly below 5000 mg/kg bar line

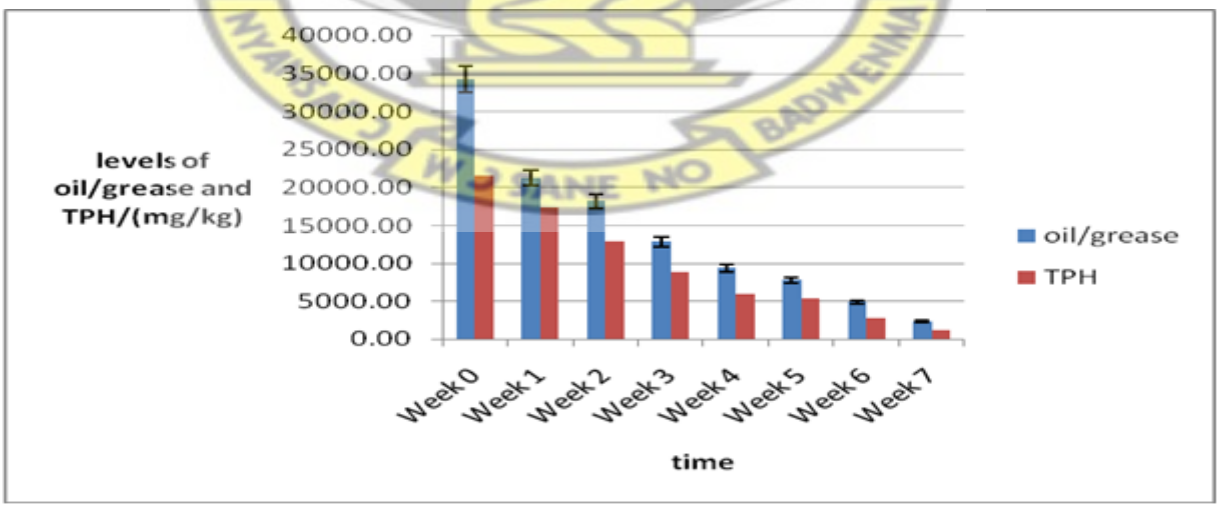


Figure 4.7 Oil/grease and TPH levels (mg/kg) in 0.2% nitrogen level for topsoil/HC blend

Table 4.6 shows the degradation rate of HC soil when the nitrogen level in the HC topsoil blend was augmented to 0.8%.By week five (5) there had been a percent degradation of more than 75% for both oil/grease and TPH**.**

Table 4.6 Mean results Oil and grease, TPH and HPC for 0.8% Nitrogen in HC/topsoil blend.

Parameters / Time(wks)	Oil/ Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.000	0.000
Week 1	2.1×10^4	1.7×10^4	6.394	37.981	19.781
Week 2	1.8×10^4	1.3×10^4	6.954	47.034	40.348
Week 3	1.3×10^4	8.9×10^3	7.705	62.562	58.847
Week 4	9.4×10^3	$6. \times 10^3$	8.672	72.685	72.149
Week 5	7.8×10^3	5.3×10^3	8.672	77.245	75.347
Week 6	3.9×10^3	2.0×10^3	8.617	88.622	90.630
Week 7	900.00	390.00	8.550	97.374	98.187

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 0.8% nitrogen level within the topsoil HC blend is shown in figure 4.8, by week five (5), oil/grease and TPH had dropped below 5000 mg/kg bar line.

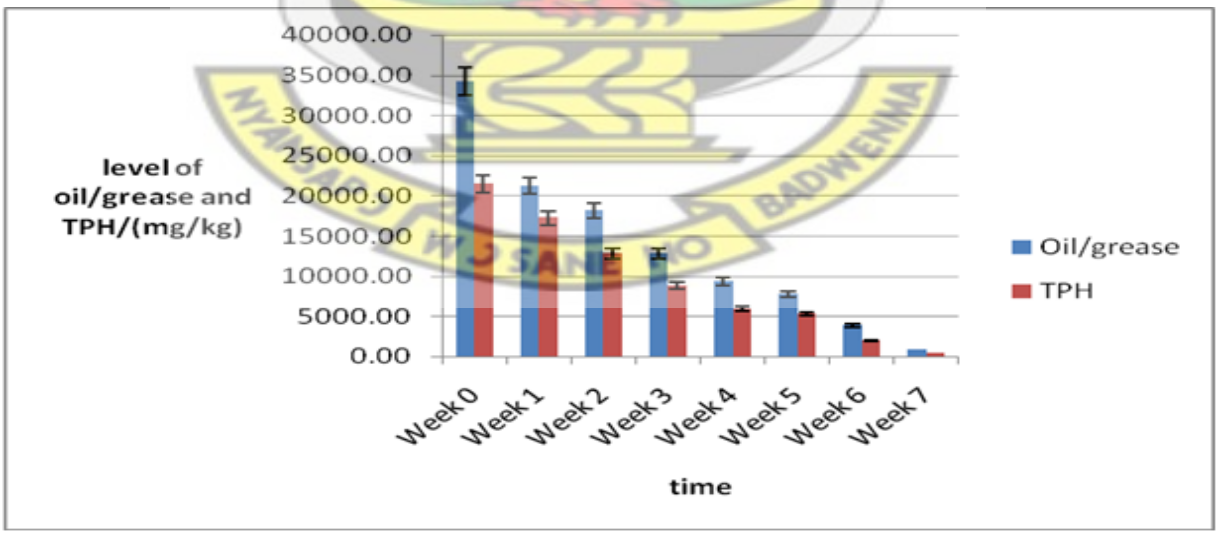


Figure 4.8 Oil/grease and TPH levels (mg/kg) in 0.8% nitrogen level of topsoil/HC blend.

The degradation rate of HC soil when the nitrogen level in the HC/topsoil blend was augmented to 1.4% is as shown in Table 4.7.By week five (5) there had been a percent degradation of more than 90% for both oil/grease and TPH.

Table 4.7 Mean results Oil and grease, TPH and HPC for 1.4% Nitrogen level in HC/Topsoil blend.

Parameters / Time(wks)	Oil/ Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.1×10^4	4.653	0.000	0.000
Week 1	2.0×10^4	1.6×10^4	6.672	39.185	26.349
Week 2	1.0×10^4	6.8×10^3	6.602	69.698	68.143
Week 3	5.4×10^3	3.7×10^3	7.279	84.156	82.582
Week 4	3.2×10^3	1.8×10^3	8.794	90.587	91.452
Week 5	2.5×10^3	1.2×10^3	8.868	92.590	94.017
Week 6	1.2×10^3	4.0×10^2	8.705	96.470	97.885
Week 7	3.0×10^2	<10.00	8.610	99.123	100.000

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 1.4% nitrogen level within the topsoil/HC blend is shown in figure 4.9, by week five (5), oil/grease and TPH had dropped below 5000 mg/kg bar line.

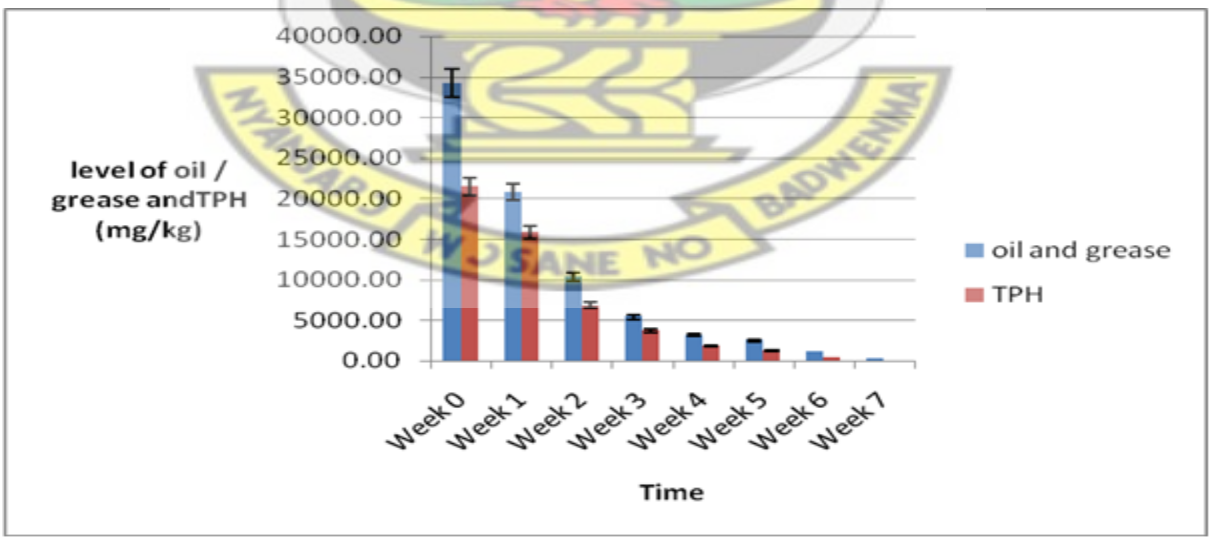


Figure 4.9 Oil/grease and TPH levels (mg/kg) in 1.4% nitrogen level of topsoil/HC blend.

The degradation rate of HC soil when the nitrogen level in the HC/topsoil blend was augmented to 2.0% is as shown in Table 4.8.By week five (5) there had been a percent degradation of more than 92% for both oil/grease and TPH.

Table 4.8 Mean results Oil and grease, TPH and HPC for 2.0% Nitrogen level in topsoil / HC blend.

Parameters / Time(wks)	Oil/ Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	% degradation TPH
Week 0	3.4×10^4	2.2×10^4	4.653	0.000	0.000
Week 1	1.4×10^4	9.6×10^3	6.794	59.741	55.433
Week 2	7.6×10^3	6.0×10^3	7.279	77.942	72.177
Week 3	3.8×10^3	2.4×10^3	7.732	88.987	88.679
Week 4	3.5×10^3	2.1×10^3	8.705	89.821	90.270
Week 5	2.1×10^3	1.4×10^3	8.794	93.874	93.286
Week 6	1.0×10^3	1.1×10^3	8.756	97.059	99.503
Week 7	2.0×10^3	<10.00	8.672	99.422	100.000

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 2.0% nitrogen level within the topsoil/HC blend is shown in figure 4.10, by week five (5), oil/grease and TPH had dropped below 5000 mg/kg bar line.

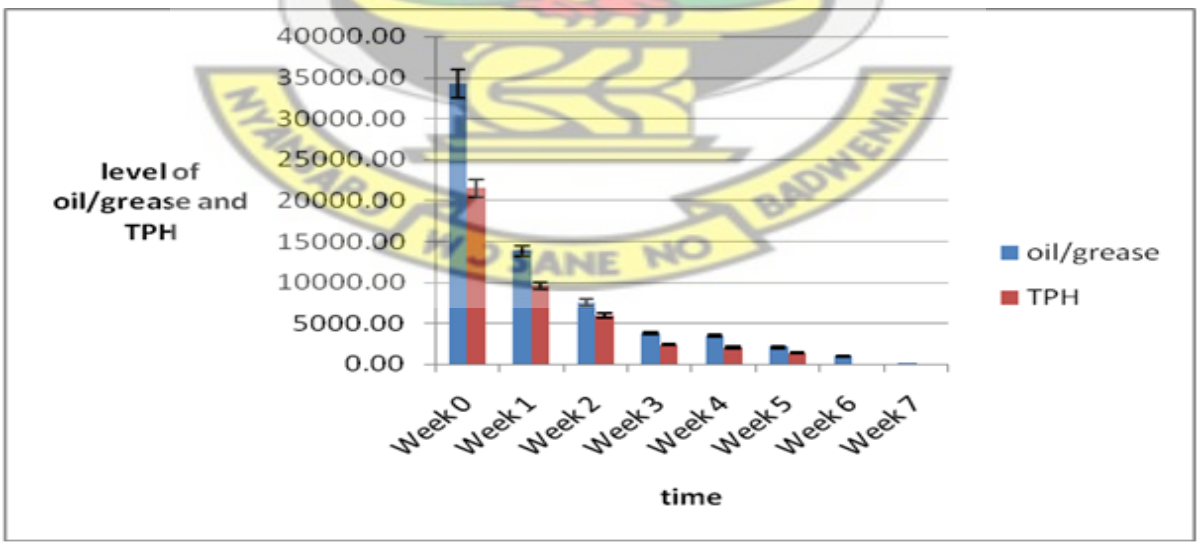


Figure 4.10 Oil/grease and TPH levels (mg/kg) in 2.0% nitrogen level topsoil/HC blend.

Figure 4.11 shows a box plots representation of oil/grease (mg/kg) degradation with time for 0.2%, 0.8%, 1.4%, 2.0% nitrogen level within the compost/HC blend. The positions of the mean for 0.2%, 0.8% nitrogen level in the graph are at almost the same height. The 1.4% and 2.0% means positions are almost the same. The 25th and 75th quartile of the respective nitrogen levels are indicated by the upper and lower portions of the graph respectively.

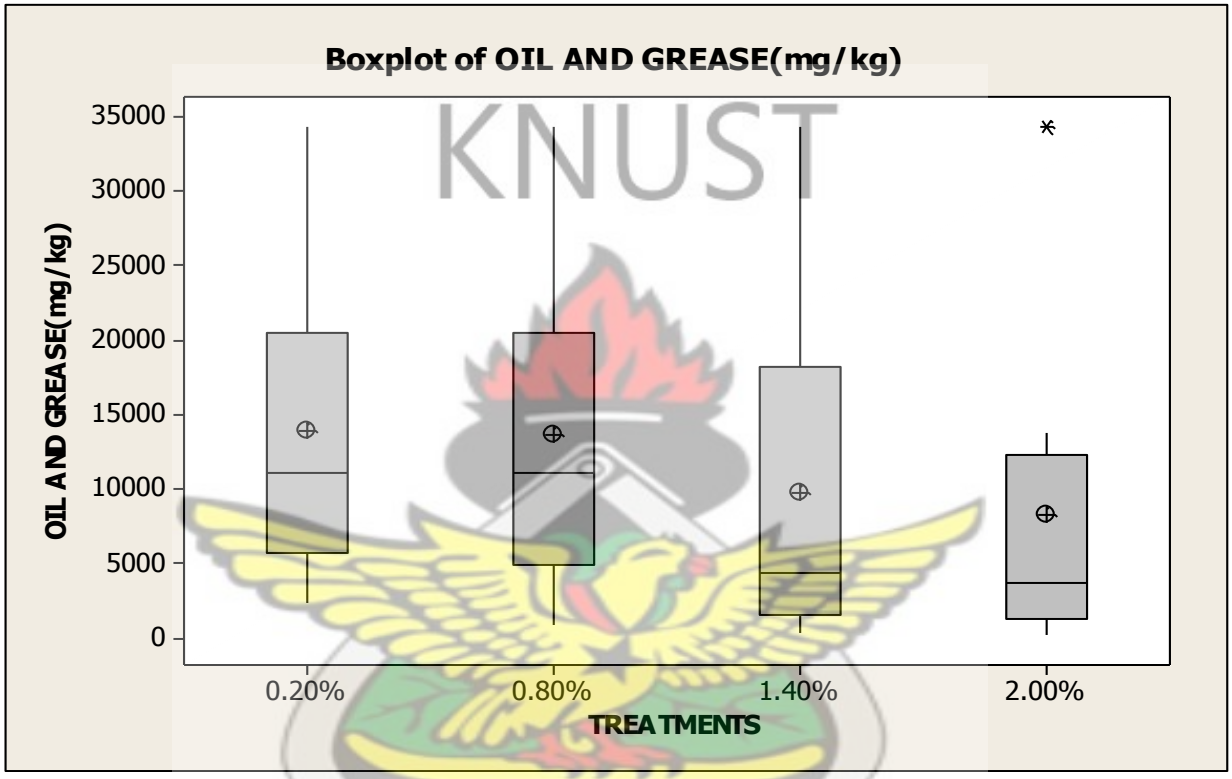


Figure 4.11 Box plots showing the differences in the effects of the treatments for oil and grease in the topsoil/HC blend.

Figure 4.12 shows a box plots representation of TPH (mg/kg) degradation with time for 0.2%, 0.8%, 1.4%, 2.0% nitrogen level within the compost/HC blend. The positions of the mean for 0.2%, 0.8% nitrogen level in the graph are at almost the same height. The 1.4% and 2.0% means almost the same. The 25th and 75th quartile of the respective nitrogen levels are indicated by the upper and lower portions of the graph respectively

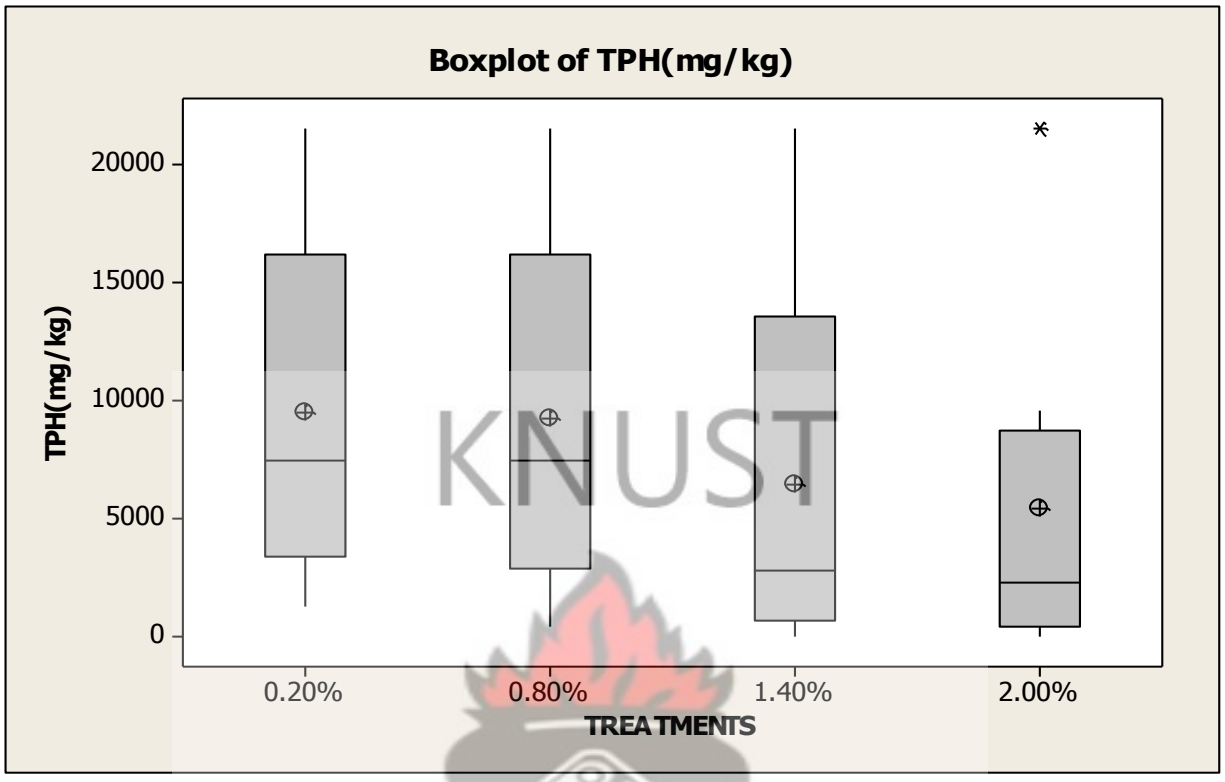


Figure 4.12 Box plots showing the differences in the effects of the treatments for TPH in the topsoil/HC blend.

4.3 MEAN RESULTS OF OIL AND GREASE, TPH AND HPC FOR FERTILIZER/HYDROCARBON CONTAMINATED SOIL BLEND

Mean oil/grease and TPH (mg/kg) of the fertilizer blend followed similar pattern as compost and topsoil.0.2% nitrogen level recorded 2.8 x 10^3 and 1.8 x 10^3 for oil/grease (mg/kg) and TPH (mg/kg) respectively at the end of the study and this represented the highest oil/grease and TPH levels within the various adopted levels in the fertilizer/HC blend.2.0% Nitrogen level recorded oil/grease and TPH (mg/kg) of 8 x 10^2 and 100 respectively.Statistically, there were differences ($p \leq 0.050$) in the degradation of hydrocarbon by 0.2%, 0.8%, 1.4% and 2.0% nitrogen levels within the fertilizer blend (Appendix D).

Table 4.9 Mean results Oil and grease, TPH and HPC for 0.2% nitrogen level in HC/ Fertilizer blend.

Parameters / Time	Oil/Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.000	0.00
Week 1	2.3×10^4	2×10^4	4.740	32.902	2.742
Week 2	2.2×10^4	2.0×10^4	4.505	36.554	9.109
Week 3	1.9×10^4	1.3×10^4	4.903	45.344	41.668
Week 4	1.8×10^4	1.2×10^4	7.617	48.410	44.225
Week 5	1.1×10^4	7.7×10^3	8.672	67.551	64.257
Week 6	8.2×10^3	4.8×10^3	8.705	75.967	77.490
Week 7	5.5×10^3	2.7×10^3	8.794	83.990	87.272
Week 8	2.8×10^3	1.8×10^3	8.617	91.767	91.620

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 0.2% nitrogen level within the fertilizer/HC blend is shown in figure 4.13. By week five (5), oil/grease level was just above the 10×10^3 mg/kg and TPH had dropped below 10×10^3 mg/kg bar line.

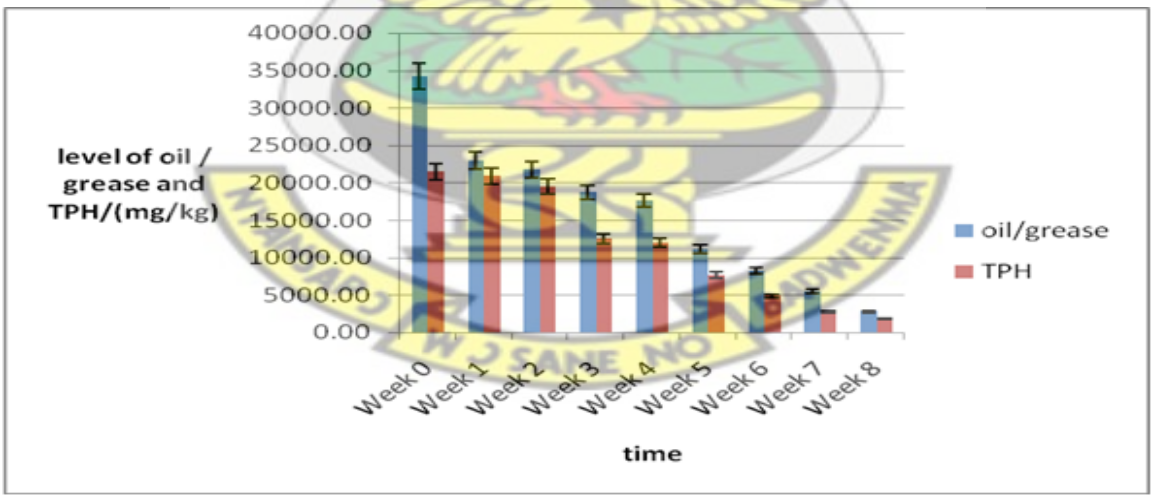


Figure 4.13 Oil/grease and TPH levels (mg/kg) in 0.2% nitrogen level fertilizer/HC blend.

The degradation rate of HC soil when the nitrogen level in the HC fertilizer blend was augmented to 0.8% is as shown in Table 4.10. By week five (5), oil/grease (mg/kg) and TPH (mg/kg) recorded 9.8×10^3 and 6.7×10^3 respectively representing a percent degradation of more than 69% for both oil/grease and TPH.

Table 4.10 Mean results Oil and grease, TPH and HPC for 0.8% nitrogen level in HC/ Fertilizer blend.

Parameters / Time	Oil/ Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.000	0.000
Week 1	2.2×10^4	2.0×10^4	4.881	35.321	7.924
Week 2	2.0×10^4	1.9×10^4	5.143	40.662	9.128
Week 3	1.5×10^4	1.2×10^4	6.362	57.203	45.359
Week 4	1.1×10^4	7.2×10^3	7.868	66.743	66.618
Week 5	9.8×10^3	6.7×10^3	8.617	71.410	69.026
Week 6	6.3×10^3	3.9×10^3	8.643	81.621	81.552
Week 7	3.5×10^3	2.0×10^3	8.672	89.935	90.541
Week 8	1.9×10^3	6×10^2	8.593	94.457	96.853

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) degradation with time for 0.8% nitrogen level within the fertilizer/HC blend is shown in figure 4.14. By week five (5), oil/grease and TPH had dropped below the 10×10^3 mg/kg bar line.

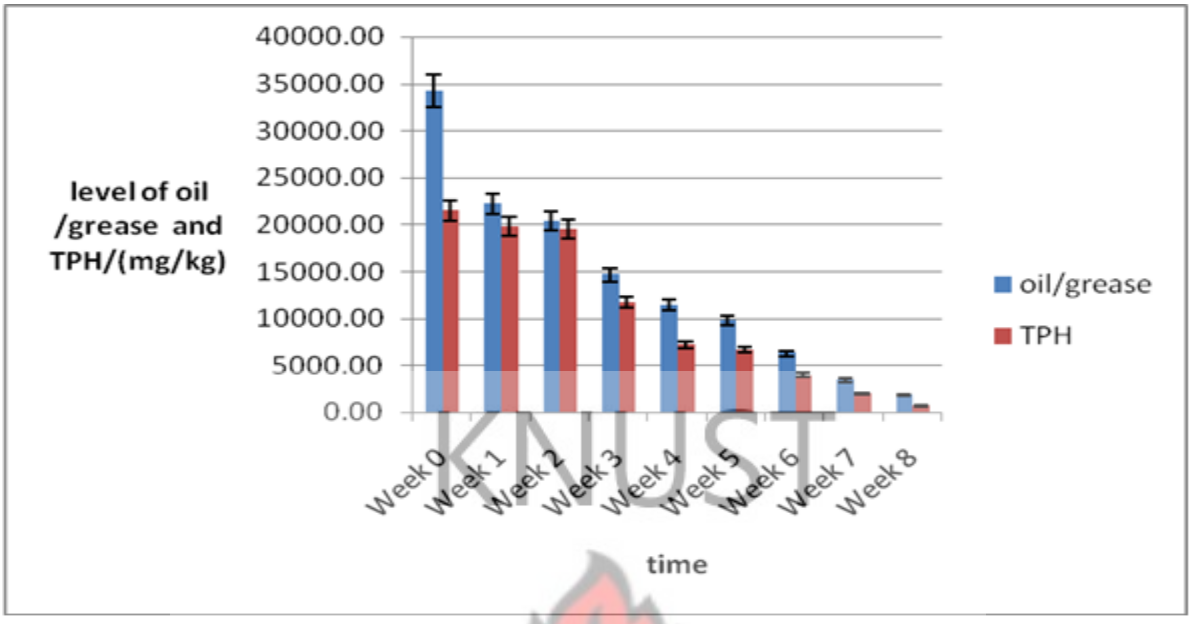


Figure 4.14 Oil/grease and TPH levels (mg/kg) in 0.8% nitrogen level fertilizer/HC blend.

The degradation rate of HC soil when the nitrogen level in the HC fertilizer blend was augmented to 1.4 % is as shown in Table 4.11. By week five (5), oil/grease (mg/kg) and TPH (mg/kg) recorded 8.9×10^3 and 6.5×10^3 respectively representing a percent degradation of more than 69% for both oil/grease and TPH.

Table 4.11. Mean results Oil and grease, TPH and HPC for 1.4% nitrogen level in HC Fertilizer blend.

Parameters / Time	Oil / Grease(mg/kg)	TPH(mg/kg)	log of HPC	oil/grease degradation (%)	TPH degradation (%)
Week 0	3.4×10^4	2.2×10^4	4.653	0.000	0.000
Week 1	2.1×10^4	2.0×10^4	5.037	38.491	8.510
Week 2	1.9×10^4	1.5×10^4	5.483	42.908	25.642
Week 3	1.4×10^4	9.8×10^3	6.305	59.665	54.241
Week 4	9.7×10^3	5.3×10^3	6.794	71.702	75.203
Week 5	8.9×10^3	6.5×10^3	8.593	74.123	69.788
Week 6	6.2×10^3	3.9×10^3	8.617	81.869	81.515
Week 7	4.0×10^3	2.4×10^3	8.493	88.258	88.891

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) degradation with time for 1.4% nitrogen level within the fertilizer/HC blend is shown in figure 4.15. By week five (5), oil/grease and TPH had dropped below the 10 x 10^3 mg/kg bar line.

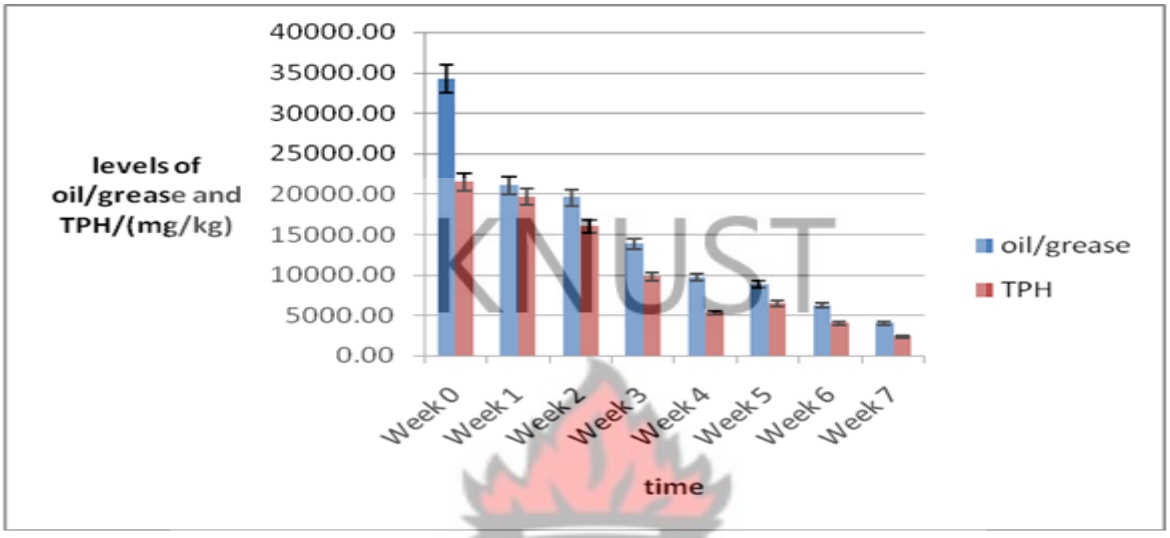


Figure 4.15 Oil/grease and TPH levels (mg/kg) in 1.4% nitrogen level fertilizer/HC blend

The degradation rate of HC soil when the nitrogen level in the HC fertilizer blend was augmented to 2.0 % is as shown in Table 4.12. By week five (5), oil/grease (mg/kg) and TPH (mg/kg) recorded 8.9 x 10^3 and 6.5 x 10^3 respectively representing a percent degradation of more than 88% for both oil/grease and TPH.

Table 4.12 Mean results Oil and grease, TPH and HPC for 2.0% nitrogen level in HC/ Fertilizer blend.

Parameters / Time(wks)	Oil/Grease (mg/kg)	TPH(mg/kg)	log of count	% degradation oil/grease	TPH degradation (%)
Week 0	3.4 x 10^4	2.2 x 10^4	4.653	0.000	0.000
Week 1	1.9 x 10^4	1.7 x 10^4	5.276	42.295	22.983
Week 2	1.5 x 10^4	1.3 x 10^4	5.702	57.477	41.134
Week 3	9.8 x 10^3	5.9 x 10^3	7.079	71.294	72.558
Week 4	7.5 x 10^3	4.9 x 10^4	7.868	78.120	77.341
Week 5	4.1 x 10^3	2.5 x 10^3	8.000	88.039	88.380
Week 6	1.9 x 10^3	9. 9x 10^2	8.121	94.224	95.357
Week 7	8 .2x 10^2	1.0 x 10^2	7.851	97.666	99.535

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) with error bars degradation with time for 2.0% nitrogen level within the fertilizer/HC blend is shown in figure 4.16. By week five (5), oil/grease and TPH had dropped below the 5.0 x 10^3 mg/kg bar line.

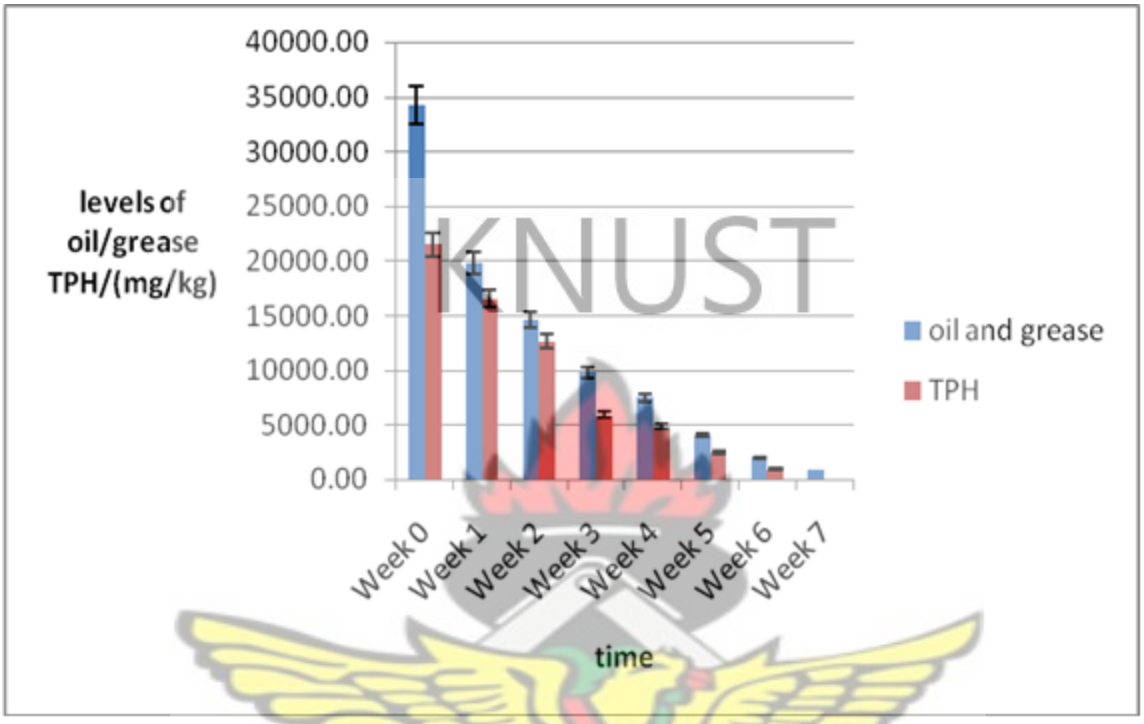


Figure 4.16 Oil/grease and TPH levels (mg/kg) in 2.0% nitrogen level fertilizer/HC blend

Figure 4.17 shows a box plots representation of oil/grease (mg/kg) and TPH (mg/kg) degradation with time for 0.2%, 0.8%, 1.4%, 2.0% nitrogen level within the fertilizer/HC blend. The positions of the mean for 0.8% and 1.4% nitrogen levels are at same heights. 0.2% and 2.0% nitrogen levels are different heights. The 25th and 75th quartile of the respective nitrogen levels are indicated by the upper and lower portions of the graph respectively.

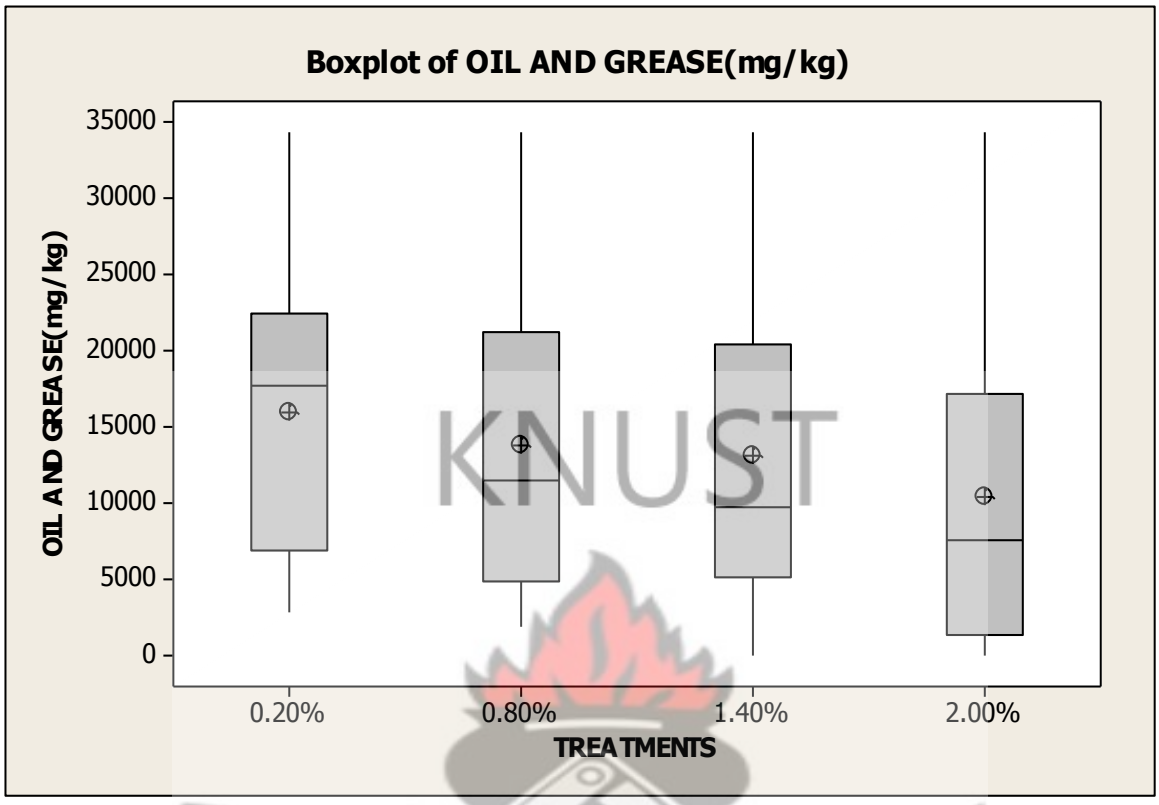


Figure 4.17 Box plots showing the differences in the effects of the of the treatments for oil/grease in the fertilizer/HC blend.

Figure 4.18 shows a box plots representation of oil/grease (mg/kg) and TPH (mg/kg) degradation with time for 0.2%, 0.8%, 1.4%, 2.0% nitrogen level within the fertilizer/HC blend. The positions of the mean for 0.8% and 1.4% nitrogen levels are at same heights. The 25th and 75th quartile of the respective nitrogen levels are indicated by the upper and lower portions of the graph respectively.

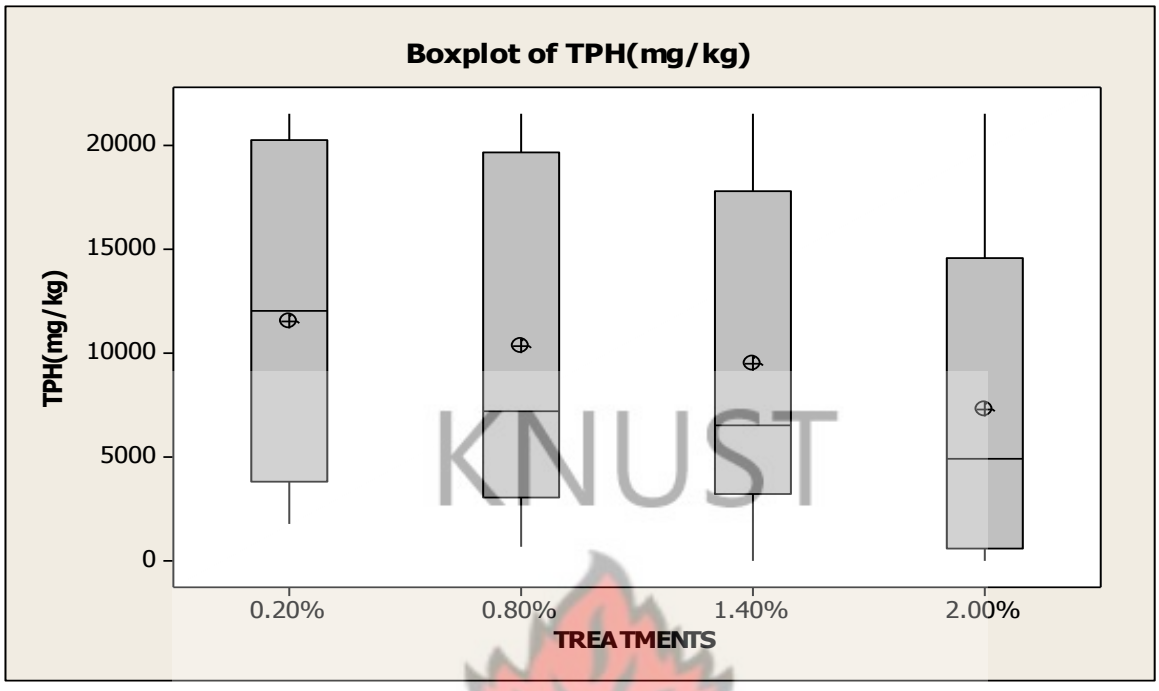


Figure 4.18 Box plots showing the differences in the effects of the treatments for TPH in the fertilizer/HC blend.

4.4 MEAN RESULTS OF OIL AND GREASE, TPH AND HPC FOR CONTROL SAMPLES.

The non-treated (NA) or control soils revealed lower degradation rates over time. At the end of the seven weeks period, there was marginal percent degradation of 8.09 and 8.27 for oil and grease and TPH respectively.

Table 4.13 Mean results for Oil and grease, TPH levels (mg/kg) of control sample.

Parameters / Time (wks)	Oil/ grease/(mg/kg)	TPH/(mg/kg)	oil/grease degradation (%)	TPH degradation (%)
Week 1	3.4×10^4	2.2×10^4	0.00	0.00
Week 2	3.3×10^4	2.1×10^4	3.18	0.65
Week 3	3.4×10^4	2.1×10^4	0.51	0.58
Week 4	3.3×10^4	2.0×10^4	4.13	4.12
Week 5	3.2×10^4	2.0×10^4	5.48	6.63
Week 6	3.2×10^4	1.9×10^4	7.19	7.23
Week 7	3.1×10^4	19×10^4	8.09	8.27

A graphical representation of oil/grease (mg/kg) and TPH (mg/kg) error bars degradation with time for the control sample is shown in figure 4.19.

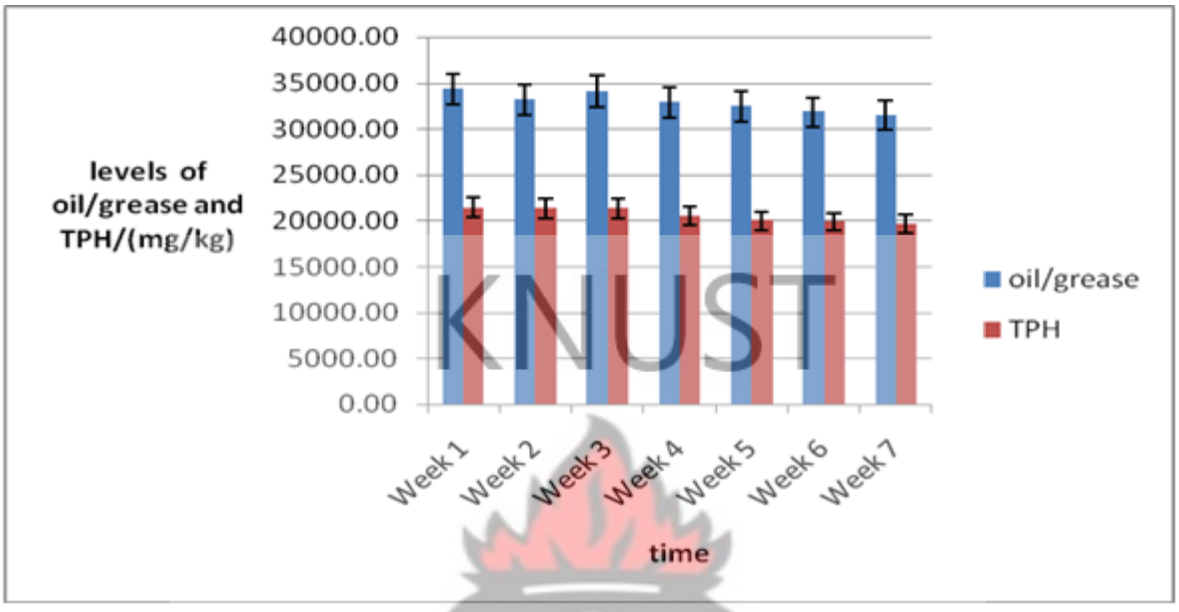


Figure 4.19 Oil/grease and TPH levels (mg/kg) in control samples

4.5 COMPARISON OF DIFFERENT LEVELS OF COMPOST, TOPSOIL AND FERTILIZER BLENDS

The 0.2% nitrogen level (mg/kg) recorded the highest residual oil and grease and TPH in all four different blends (0.2%, 0.8%, 1.4% and 2.0%). At 0.2% nitrogen level, Compost and fertilizer recorded the highest and lowest residual oil and grease (mg/kg) and TPH of $1.1 \times 10^4$and 1.6×10^4 respectively. 2.0% nitrogen level recorded the least residual oil and grease (mg/kg) and TPH (mg/kg) in all the three different blends. For 2.0% nitrogen level, compost, topsoil and fertilizer recorded residual oil and grease (mg/kg) of 6.9×10^3 , 8.3×10^3 and 1.1×10^4. The residual TPH for compost, topsoil, fertilizer 4.4×10^3, 5.4×10^3, 8.1×10^3 respectively.

Table 4.14 Mean Residual Oil/grease (mg/kg) for compost, fertilizer and topsoil.

Levels of nitrogen/ (%)	Compost	Fertilizer	Topsoil
0.2%	1.1×10^4	1.6×10^4	13.9×10^3
0.8%	9.0×10^4	1.4×10^4	13.6×10^3
1.4%	7.9×10^3	1.2×10^4	9.7×10^3
2.0%	6.9×10^3	1.1×10^4	8.3×10^3

Table 4.15 Mean Residual TPH (mg/kg) for compost, fertilizer and topsoil.

Levels of nitrogen/(%)	Compost	Fertilizer	Topsoil
0.2	7.2×10^3	1.2×10^4	9.5×10^3
0.8	5.7×10^3	1.0×10^4	9.3×10^3
1.4	5.1×10^3	1.1×10^4	6.4×10^3
2.0	4.4×10^3	8.1×10^3	5.4×10^3

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 TOPSOIL AND HYDROCARBON CONTAMINATED SOIL BLEND

The heterotrophic plate count (HPC) levels of the topsoil blend for the entire seven weeks are presented in Appendix D. The HPC (CFU) of the topsoil decreased from its original count of 89 x 10^8 to 248 x 10^4, 248 x 10^4, 470 x 10^4 , 623 x 10^4 for 0.2%, 0.8% , 1.4% and 2.0% nitrogen level respectively after one week in response to oil contamination. The HPC increased again from week 2 up to week 5 before reducing again in weeks 6 and 7. The drop in the heterotrophic plate counts level in the topsoil soil in the first week can be attributed to selective inhibition of members of the microbial community as a result of the toxic components of petroleum and also as a result of reduce degradation and upsets in carbon/Inorganic nutrient balance for the indigenous population caused by the presence of petroleum. By week seven (7), 0.2% , 0.8% , 1.4%, 2.0% nitrogen level recorded percent degradation (%) of 99.422 , 99.123 , 97.374 , 93.144.

The corresponding residual means(mg/kg) for oil and grease after week seven for 0.2% , 0.8% , 1.4% and 2.0% nitrogen levels were 13.9 x 10^3, 13.6 x 10^3 , 9.7 x 10^3 , 8.3 x 10^3 respectively. TPH also recorded 9.5 x 10^3, 9.3 x 10^3 , 6.4 x 10^3 , 5.4 x 10^3 for 0.2 % , 0.8% , 1.4% and 2.0% nitrogen levels respectively. Percent degradation for TPH followed the order of 0.2 % < 0.8 %,< 1.4 %< 2.0% nitrogen level as shown in table 4.14 to 4.15 In both oil and grease and TPH, the lowest residual mean was recorded by 0.2% nitrogen level whereas 2.0% nitrogen level recorded the highest. Statistically ($p<0.05$, Tukey Method) the 0.2% nitrogen level and 0.8% nitrogen level were significantly different from 1.4% and 2.0%.nitrogen level.The nitrogen level pairs (0.2%, 0.8%) and (1.4%, 2.0%) was not significantly different. Moreover, since the residual means for nitrogen level pair of (1.4%, 2.0%) were smaller than (0.2%, 0.8%) we conclude that the nitrogen level pair (1.4%, 2.0%) is more effective in the breakdown of oil/grease than (0.2%, 0.8%) nitrogen level.

5.2 FERTILIZER/HYDROCARBON CONTAMINATED SOIL BLEND

The HPC (CFU) of the fertilizer increased from 45 x 10^3 originally in the hydrocarbon contaminated soil to 55 x 10^3, 76 x 10^3, 109 x 10^3 and 189 x 10^3 for 0.2% , 0.8% , 1.4% and 2.0% nitrogen levels respectively in week one. This study supports 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 1.2 x10^4, 1.0 x 10^4 , 1.1 x 10^4 and 8.1 x 10^3 representing 0.2% , 0.8% , 1.4% and 2.0% nitrogen level respectively. This correlated with the percent degradation at the end of the entire duration of the study for the various levels of nitrogen within the fertilizer hydrocarbon contaminated soil blend. Statistically ($P<0.05$,Tukey Methods) for oil and grease, 0.2% and 2.0% levels of nitrogen in the fertilizer performed significantly different from 0.8% and 1.4% in the breakdown of oil/grease. The treatment pair (0.8%, 1.4%) was not significantly different in breaking down oil/grease levels. Moreover, since the 2.0% had the least mean residual oil/grease level, we conclude that it is the most effective in the breakdown of oil/grease.

The order of increasing mean residual TPH (mg/kg) was similar to Oil/grease (mg/kg) as shown in table 4.15. In the case of TPH (Tukey Methods). 2.0% nitrogen level of nitrogen in the fertilizer performed significantly different from 0.2% , 0.8% and 1.4% nitrogen level in the breakdown of TPH. There was also a significant difference between 0.2% and 1.4% nitrogen level. The nitrogen level pairs of (0.2%, 0.8%) and (0.8%, 1.4%) was not significantly different. Moreover, since the mean residual TPH (mg/kg) of 2.0% nitrogen level was smaller than the others as shown in table 4.15, we conclude that it performed better than the others.

5.3 COMPOST/HYDROCARBON CONTAMINATED SOIL BLEND

Residual oil and grease (mg/kg) after the seven weeks period for 2.0% , 1.4% , 0.8% , and 0.2% nitrogen levels were 1.1 x 10^4 , 9.0 x 10^4 , 7.9 x 10^3 and 6.9 x 10^3 respectively.

Within the compost/hydrocarbon blend for oil and grease level, 0.2% recorded the least percent degradation (mg/kg) of 90.08% whiles 2.0 % recorded the highest percent degradation of 100% by week 6. For TPH (mg/kg), 0.2% nitrogen level recorded a percent degradation of 90.94% and 2.0% nitrogen level recorded 100% degradation.

During the second week of the experiment, counts of heterotrophic micro-organisms (CFU) increased from 45 x 10^3 in the hydrocarbon contaminated soil to 55 x 10^3, 70 x 10^3 ,
100 x $10^{3,}$ 230 x 10^3 in the 0.2%, 0.8%, 1.4%, and 2.0% nitrogen levels in the compost-hydrocarbon blend, probably due to the ready supply of available nutrients in the blend. The rapid degradation of hydrocarbons in the compost system was expected since compost has the potential improving soil structure, texture, and aeration capacity. There was a general decrease in microbial population for all the four levels (0.2%, 0.8%, 1.4%, and 2.0%) of nitrogen by week six as shown in Appendix D. During this period, there had been significant degradation in the concentration of hydrocarbon in the various blends. The same observation was made in topsoil/contaminated soil, fertilizer contaminated soil blends.

Statistically ($P<0.05$,Tukey Methods) for oil and grease, 0.2% nitrogen level in the compost is significantly different from the 0.8%, 1.4%, and 2.0% nitrogen levels in the breakdown of oil and grease. The performances of the following treatment nitrogen level pairs (0.8%, 1.4%) and (1.4%, 2.0%) were not significantly different. Since the mean residual Oil/grease (mg/kg) of 2.0% nitrogen level is less than 0.2% 0.8%, 1.4% nitrogen level, we conclude that the 2.0% performed effectively than all the other levels.

In the case of TPH, the 0.2% nitrogen level in the compost is significantly different from 1.4% and 2.0% nitrogen level in the breakdown of TPH. Treatment pair (1.4%, 2.0%) nitrogen level were not significantly different but was also not significantly different from 0.8% nitrogen level. The 1.4% and 2.0% nitrogen levels were the most effective levels with and recorded the least residual means as compared to the other nitrogen levels within the compost/hydrocarbon blend.

5.4 COMPARING DIFFERENT BLENDS: COMPOST, TOPSOIL FERTILIZER HYDROCARBON BLENDS OIL AND GREASE

In all the four (4) nitrogen levels (0.2%, 0.8%, 1.4% and 2.0%), compost-hydrocarbon blend recorded the least residual Oil and grease and TPH values with fertilizer blend recording the highest as shown in table Table 4.14 to 4.15. The rapid degradation of hydrocarbons in the compost-hydrocarbon blend was expected since compost is rich in nutrients and has additional qualities such as improving soil structure, texture, and aeration capacity.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

There was significant degradation of hydrocarbon contaminated soil with the nutrient addition. Compost gave the best results with respect to hydrocarbon removal, followed by topsoil and fertilizer. Generally treatment within all the blends followed a pattern with the order of increasing performance as 0.2% < 0.8% < 1.4% and < 2.0% nitrogen level of the three nitrogen sources. From the discussion of results, the treatment pairs that were not significantly different could be replaced for each other to produce similar results. Similarly, the pairs that were significantly different can't be replaced for each other since they will achieve degradation at different period of times.

6.2 RECOMMENDATION

Since compost is generated on site, it is recommended that it is used for the bioremediation technique. It is recommended that 1.4% nitrogen level which performed closely to the 2.0% nitrogen level with a relatively smaller quantity of nitrogen added be used. It is also recommended that further studies should be carried out on the degradation rate of the hydrocarbon contaminated soil using higher levels of nitrogen (i.e. beyond 2%).

REFERENCE

Abu-Hilal, A. H. and Khordagui, H. K. (1994). Petroleum hydrocarbons in the nearshore marine sediments of the United Arab Emirates. Environ. Pollut.,85, 315–319.

Alloway, B.J and Ayres, D.C. (1993). Organic Pollutants. In: Chemical Principles of Environmental Pollution.1st edition.Chapman and Hall, India, Publishers, p. 201

Anderson, T.A., Guthrie, E.A., and Walton, B.T. (1993). Bioremediation in the rhizosphere: plant roots and associated microbes clean contaminated soil. Environ. Sci. Technol. 27, 2630–2636.

Atlas, R.M., Philp, J. (2005). Bioremediation: applied microbial solutions for real-world environmental cleanup. ASM Press, Washington D.C., pp. 1-292

Anozie, O., Onwurah,I. N. E. (2001). Toxic effects of Bonny light crude oil in rats after ingestion of contaminated diet. Nigerian J. Biochemistry and Molecular Biology (Proceedings Supplement). 16(3), 1035-1085

Atlas, R. M., A. Sexstone, Gustin, P, 0., Miller, P. Linkins, and Everett. K. (1980). Biodegradation of crude oil by tundra soil microorganisms, p. 21-28. In T. A. Oxley, G. Becker, and D. Allsop, Proceedings of the 4th International Biodeterioration Symposium.*Pitman Publishing Ltd., London.*

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

Atlas, R. M., and Bartha. R. (1992) .Hydrocarbon biodegradation and oil spill bioremediation. Adv. Microb. Ecol. 12:287–338.Hinchee, R. E. 1998. In situ bioremediation: practices and challenges, p.

Atlas, R. M., (1988). Microbiology-fundamentals and applications, 2nd ed., p. 352-353. *Macmillan Publishing Co., New York*

Atlas, R.M. and Bartha,R. (1993) Microbial Ecology: Fundmentals and Applications, 3rded., Benjamin/Cumrnings Publ. Co.. CA.

Avian Demography Unit. (2000). Department of Statistical Sciences, University of Cape Town, South Africa Press Release: Rescue of Seabirds in Western Cape

Babu, T. S., Huang, X. D., Greenberg, B. M. (2002). Reactive oxygen species mediated toxicity of environmental contaminants In: Learned Discourses: Timely Scientific Opinions SETAC Globe, 3(3), 26-28.

Barkay, T., and H. Pritchard. (1988). Adaptation of aquatic microbial communities to pollutant stress. Microbiol. Sci.5:165-169

Barth H, J. (2003). The influence of cyanobacteria on oil polluted intertidal soils at the Saudi Arabian gulf shores. Mar. Pollut. Bull. 46:1245-52.

Bartha, R. (1986b). Biotechnology of Petroleum pollutant biodegradation. Microb. Ecol. 12:155-172.

Bartha, R. and Bossert, I. (1984). The treatment and disposal of petroleum wastes, p. 553-578. In R. M. Atlas (ed.), Petroleum microbiology. *Macmillan Publishing Co., New* York. Cairns, J., Buikema, A. L. (1984). Restoration of habitats impacted by oil spills. (Ann Arbor Science) Butterworth, Boston.

Barriault, D. and Sylvestre, M. (1993). Factors affecting PCB degradation by an implanted bacterial strain in soil microcosms. Can. J. Microb.39:594-602.

Barkay, T. and Pritchard, H. (1988). Adaptation of aquatic microbial communities to pollutant stress. Microbiol. Sci.5:165-169.

Bartha. R. (1986). Microbial ecology: fundamentals and applications. *Addisson_Wesley Publ., Reading, Mass*.

Bauer, J. E. and Capone, D, G. (1988). Effects of co-occurring aromatic hydrocarbons on degradation of individual polycyclic aromatic hydrocarbons in marine sediment slurries. Appl. Environ. Microbiol. 54:1649-1655.

Beeby, A. (1993). Measuring the effect of Pollution In:Applying Ecology.Chapman and Hall, London, New York.

Bertrand, J. C., E. Rambeloarisoa, J. F.,Rontani, G. Giusti, and G. Mattei. (1983). Microbial degradation of crude oil in sea water in continuous culture. Biotechnol. Lett. 5:567-572.

Biobasics: The Science and the Issues. 9 Feb (2006). 24 Nov (2006)Biotechnol. Bioeng. 14(3): 309-318.

Boopathy, R., Manning, J. (1999). Surfactant-enhanced bioremediation of soil contaminated with 2,4,6-trinitrotoluene in soil slurry reactors. Water Environ. Res. 71, 119±124.

Bossert, I. and R. Bartha. (1984). The fate of petroleum in soil ecosystems, p. 434-476. In R. M. Atlas (ed.), Petroleum microbiology. *Macmillan Publishing Co., New York.*

Boyd, S. A. and D. R. Shelton. (1984). Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Appl. Environ.Microbiol. 47:272-277.

Borneman, J, Skroch, P.W., O'Sullivan, K.M., Palus, S.Rumjanek,N.G.,Jansen J.L. (1996). Molecular microbial diversity of an agricultural soil in Wisconsin. Appl. Environ. Microb. 62:1935-1943.

Broderick, L. S. and J. J. Cooney. (1982). Emulsification of hydrocarbons by bacteria from freshwater ecosystems. Dev.Ind. Microbiol. 23:425-434.

Button, D. K., and B. R. Robertson. (1986). Dissolved hydrocarbon metabolism: the concentration-dependent kinetics of toluene oxidation in some North American estuaries. Limnol. Oceanogr. 31:101-111

Braddock, J. F, Catterall, P. H. (1999). A Simple Method for Enumerating Gasoline- and Diesel-Degrading Microorganisms. Bioremed. J. 3(2):81-84.

Brazil, G. M., Kenefick, L., Callanan, M., Haro, A., de Lorenzo, V., Dowling, D. N., and O'Gara, F. (1995). Construction of a rhizosphere Pseudomonad with potential to degrade polychlorinated biphenyls and detection of *bph*gene expression in the rhizosphere. Appl. Environ.Microbiol. 61:1946-1952

Breedveld, G.D. and Sparrevik, M. (2000) Nutrient-limited Biodegradation of PAH in Various Soil Strata at aCreosote Contaminated Site. Biodegradation.,11, 391.

Brito, E .M., S, Guyoneaud R, Goñi-Urriza M, Ranchou-Peyruse A, Verbaere A, Crapez MAC, Wasserman JCA, Duran R (2006). Brito EMS, Guyoneaud R, Goñi-Urriza M, Ranchou-Peyruse A, Verbaere A, Crapez MAC, Wasserman JCA, Duran R. (2006). Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. Research inMicrobiology. In press. Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. Research in Microbiology. In press.

Brown, E. D., Norcross, B. L and Short, J. W. (1996). An introduction to studies on the effects of Exxenvaldez oil spill in early life history stages of Pacific herring, *clupaepallasi*, in Prince.Williian Sound, Alaska. Can J Fish Aquat, Sci., 53, 2337-2342.

Cairns, J., Buikema, A. L. (1984). Restoration of habitats impacted by oil spills. (Ann Arbor Science) Butterworth, Boston.

Cerniglia, C. E and Heitkamp, M. A. (1987). Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. Pages 41-68 in: Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. U. Varanasi, ed. CRC Press, Inc., Boca Raton, FL, U.S. A.

Cerniglia, C. E. (1993). Biodegradation of polycyclic aromatic hydrocarbons. Curr. Opin. Biotechnol. 4:331-338.

Cerniglia, C. E. (1984). Microbial transformation of aromatic hydrocarbons, p. 99-128. In R. M. Atlas (ed.), Petroleum microbiology. *Macmillan Publishing Co., New York.*

Carcinogenic Risk of Some Industrial Chemicals to Humans, Lyon, France, (2000).

Chaillana, F.Flècheb A, Burya E, Phantavonga Y-hui, Saliot A, Oudot, J. (2004). Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Res. Microb. 155(7):587-595.

Caplan, J.A. (1993). The worldwide bioremediation industry: prospectsfor pro®t. TIB Tech. 11, 320±323.

Chakrabarty, A. M. (1996). Microbial degradation of toxic chemicals: Evolutionaryinsights and practical considerations. ASM News 62:130-137

Chaıneau, C.H., Yepremian, C. Vidalie J.F., Ducreux, J, Ballerini D. (2003). Bioremediation of a crude oil-polluted soil: biodegradation, leaching and toxicity assessments. Wat. Air. Soil. Poll. 144: 419-440.

Chen, M., C. S. Hong, B. Bush, and G.-Y. Rhee. (1988). Anaerobic biodegradation of polychlorinated biphenyls by bacteria from Hudson River sediments. Ecotoxicol. Environ. Saf. 16:95-105

Cookson, J.T. (1995). Bioremediation Engineering: Design and Application. McGraw Hill.

Cooney, J. J. (1984). The fate of petroleum pollutants in freshwater ecosystems, p. 399-434. In R. M. Atlas (ed.), Petroleum microbiology. Macmillan Publishing Co., New York.

Cooney, J. J., S. A. Silver, and E. A. Beck. (1985). Factors influencing hydrocarbon degradation in three freshwater lakes. Microb. Ecol. 11:127-137.

Day, S.M. (1993). US environmental regulations and policies: their impact on the commercial development of bioremediation. TIB Tech. 11, 324±328.

Dean-Ross, D. (1987). Biodegradation of toxic wastes in soil. ASM News 53:490-492

Dixon, B. (1996). Bioremediation is here to stay. ASM News 62:527-528. Doty, S. L., Shang, T. Q., Wilson, A. M., Tangen, J., Westergreen, A. D., Newman, L. A., Strand, S. E., and Gordon, M. P. 2000. Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2e1. Proc. Natl. Acad. Sci. U.S.A. 97:6287-6291.

Dunnet, G. M. (1982). Oil pollution and seabird populations. Philosophical Transactions of the Royal Society of London, B 297, 413- 427.

Dzantor, E.K. (1999). Bioremediation of contaminated soils - what it is and how to do it. University of Agriculture and Natural Resources, pp.1007-1019.

Eberhart B., L, King D, Holder E.,L. (1996). Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. Environ. Sci.Technol. 30(5): 1764-1775

Environmental Sciences Group (ESG), (1999). Experimental Bioremediation Technologies for Hydrocarbon and PCB Contarninated Soils at Arctic Sites. MC-CCE-ES-99- 19

Erickson, L. E. (1997). An overview of research on the beneficial effects of vegetation in contaminated soil. Ann. N. Y. Acad. Sci. 829:30-35.

Fimerty, W.R. (1994)Biosurfactantsinenvironmental biotechnology. *Curr*.Opion. Biotechnol. 5,29 1-295.

Flathman. P.E., jerger. D.E.andExner, J.H. (1994) Bioremediation Field *Experience.Lewis Publishers., Boca Raton.*

Floodgate, G. (1984). The fate of petroleum in marine ecosystems, p. 355-398. In R. M. Atlas (ed.), *Petroleum microbiology. Macmillan Publishing Co., New York.*

Fogel, S., R. Lancione, A. Sewall, and R. S. Boethling. (1985). Application of biodegradability screening tests to insoluble chemicals: hexadecane. Chemosphere 14:375-382.

Foght J.M., Westlake D, Johnson W.M., Ridgway, H. F. (1996). Environmental gasoline-utilising isolates and clinical isolates of *Pseudomonas aeruginosa* are taxonomically indistinguishable bychemotaxonomic and molecular techniques. Microbiol. 142: 1333

Ford, C. Z., Sayler, G. S., and Burlage, R. S. (1999). Containment of a genetically engineered microorganism during a field bioremediation application. Appl. Microbiol. Biotechnol. 51:397-400.

Food Agricultural Organization, fertilizer and plant nutrient. (2008)

Fusey, P., and J. Oudot. (1984). Relative influence of physical removal and biodegradation in the depuration of petroleum contaminated seashore sediments. Mar. Pollut. Bull. 15:136-141.

Ghana Environmental Protection Agency (EPA), (2008). AKOBEN Environmental Rating Methodology. Draft 19. June.

Frantz B, Chakrabarty, A.M. (1986). Degradative plasmids in Pseudomonas aeruginosa. In: The Bacteria. Sokatch JR and Ornston LN (editors), *Academic Press, New York, USA*, 10: 295-323

Goldstein, R. M., L. M. Mallory, and M. Alexander. (1985). Reasons for possible failure of inoculation to enhance biodegradation. Appl. Environ. Microbiol. 50:977-983

Gogoi, B.K., Dutta, N.N., Goswami P, Mohan TRK. (2003). A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site. Adv. Environ. Res. 7(4): 767-782

Goldstein, R. M., L. M. Mallory, and M. Alexander. (1985). Reasons for possible failure of inoculation to enhance biodegradation. Appl. Environ. Microbiol. 50:977-983.

Grbic-Galic, D., and T. M. Vogel. (1987). Transformation of toluene and benzene by mixed methanogenic cultures. Appl. Environ. Microbiol. 53:254-260.

Griffiths BS, Ritz K, Weatley RE (1997) Relationship between functional diversity and genetic diversity in complex microbial communities. In: Insam H, Rangger A (eds) Microbial communities, functional versus structural approaches. Springer, Berlin Heidelberg New York, pp 1–18

Hall, A.J, Mc Connell, B.J., Rowles, T.K., Aguilar, A., Borrell, A., Schwacke, L., Reijnders, P.J.H., and Wells, R.S. (2006). Population consequences of polychlorinated biphenyl exposure in

bottlenose dolphins - an individual based model approach. *Environ. Health Perspect*. 114, Suppl. 1:60-64.

Heidelberg, J. F., Paulsen, I. T., Nelson, K. E., Gaidos, E. J., Nelson, W. C., Read, T. D., and Eisen, J. A. (2002). Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanellaoneidensis*. Nature Biotechnol. 1:1-6.

Heitkamp, M. A., and Cerniglia, C. E. (1989). Polycyclic aromatic hydrocarbon degrading by a *mycobacterium* sp. in microcosms containing sediment and water from a pristine ecosystem. Appl. Environ. Microbiol. 55:1968-1973

Heintz, R. A., Short, J. W. and Rice, S. D. (1999). Sensitivity of fish embryos to weathered crude oil Part 11.Increased mortality of puck salmon (*onchorpynchusgorbusca*) embryo incubating downstream from weathered Exxen Valdez crude oil. Environ. Toxicol. Chem., 18 , 494-503.

Holben, W. E., J. K. Jansson, B. K. Chelm, and J. M. Tiedje. (1988). DNA probe method for the detection of specific microorganisms in the soil bacterial community. Appl. Environ. Microbiol. 54:703-711

Holba, A.G., Dzou, I.L., Hickey, J.J., Franks, S.G., May, S.J., Lenney, T. (1996). Reservoir Geochemistry of South Pass 61 Field, Gulf of Mexico: Compositional Heterogeneities Reflecting Filling History and.

Horowitz, A., and R. M. Atlas. (1980). Microbial seeding to enhance petroleum hydrocarbon biodegradation in aquaticArctic ecosystems, p. 15-20. In T. A. Oxley, G. Becker, and D. Allsopp (ed.), Proceedings of the 4th International Biodeterioration Symposium. Pitman Publishing Ltd., London.

Hollaway, S. L., G. M. Faw, and R. K. Sizemore. (1980). The bacterial community composition of an active oil field in the Northwestern Gulf of Mexico. Mar. Pollut. Bull. 11:153-156.

Hambrick, G. A., III, R. D. DeLaune, and W. H. Patrick, Jr. (1980). Effect of estuarine sediment pH and oxidation-reduction potential on microbial hydrocarbon degradation. Appl. Environ. Microbiol. 40:365-369.

Hamer, G. (1993). Bioremediation: a response to gross environmental abuse. Tibtech 11:317-319.

Heider J, Spormann A.M., Beller HR, Widdel.F. (1998). Anaerobic bacterial metabolism of hydrocarbons. FEMS Microb. Rev. 22(5):459-.465.

Hinchee, R.E.; Anderson, D.B.; Metting, F.B. and Sales, G.D., eds. (1994). *Applied biotechnology for site remediation. Lewis Publishers, Boca Raton, Florida.*

Hollaway, S. L., G. M. Faw, and R. K. Sizemore. (1980). Thebacterial community composition of an active oil field in the Northwestern Gulf of Mexico. Mar. Pollut. Bull. 11:153-156.

Hoagland, R. E., Zablotowicz, R. M., and Locke, M. A. (1994). Propanil metabolism by rhizospheremicroflora. Pages 160-183 in: Bioremediation through Rhizosphere Technology. T. A. Anderson and J. R. Coats, eds. American Chemical Society, Washington, D.C.

Horowitz, A., and R. M. Atlas. (1980). Microbial seeding to enhance petroleum hydrocarbon biodegradation in aquatic Arctic ecosystems, p. 15-20. In T. A. Oxley, G. Becker, and D. Allsopp (ed.), Proceedings of the 4th International BiodeteriorationnSymposium. *Pitman Publishing Ltd., London*

Hutchinson, T.C..Hellebust.I. and Telford, M. (1994) Oil Spill effects on vegetation and soil microfauna at Norman Wells and Tuktoyaktuk, N. W.T.. Environmental-Social Program Northem Pipelines, TaskForce on Northern Oil Development, Report # 74-

International Agency for Research on Cancer (IARC), Monographs on the Evaluation of the Hutchinson, T.C..Hellebust. I. and Telford, M. (1994) Oil Spill effects on vegetation and soil micro fauna at Norman Wells and Tuktoyaktuk, N. W.T.. Environmental-Social Program Northem Pipelines, TaskForce on Northern Oil Development, Report # 74-14.

"In Situ Oxidation", Technology Status Review, performed by ThermoRetec Consulting Corporation with assistance from HydroGeoLogic, Inc. and Coleman Research Corporation – Energy &Environmental Group. (1999) Environmental Security Certification Program, USA, November 42 p

Jacobsen, C. S. (1997). Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading Burkholderia (Pseudomonas)cepaciaDBO1(pRO101) in 2,4-D contaminated soil. Plant Soil 189:139-144.

Kastner, M., and Mahro, B. (1996). Microbial degradation of polycyclic aromatic hydrocarbons in soils affected by the organic matrix of compost. Appl. Microbiol. Biotechnol. 44:668-675.

Kerr, R. P., and D. G. Capone. (1988). The effect of salinity on the microbial mineralization of two polycyclic aromatic hydrocarbons in estuarine sediments. Mar. Environ. Res. 26:181- 198.

Kilbane, J. J., D. K. Chatterjee, and A. M. Chakrabarty. (1983). Detoxification of 2,4,5-trichlorophenoxyacetic acid from contaminated soil by Pseudomonas cepacia. Appl. Environ. Microbiol. 45:1697-1700.

King, J. M. H., DiGrazia, P. M., Applegate, B., Larimer, F., and Sayler, G. S. (1990). Rapid, sensitive bioluminescent reporter technology for naphthalene exposure and biodegradation. Science 249:778-781.

Kostecki, P.T. and Calabrese. E.J. (1990) Petroleum Contminated Soils-Vol. 1 & 3.*Lewis Publishers*.

Law, R. J. and Biscaya, J. L. (2005).Polycyclic aromatic hydrocarbons (PAH) – Problems and progress in sampling, analysis and interpretation. Mar. Pollut. Bull., 1994,29, 235–241.

Leahy, J. G., C. C. Somerville, K. A. Cunningham, G. A. Adamantiades, J. J. Byrd, and R. R. Colwell. (1990). Hydrocarbon mineralization in sediments and plasmid incidence in sediment bacteria from the Campeche Bank. Appl. Environ. Microbiol. 56:1565-1570.

Lee K, Tremblay, G.H., Gauthier J, Cobanli SE, Griffin M. (1997).International Oil Spill Conference, American Petroleum Institute, Washington, DC. pp. 697-704

Lee K, Merlin, F.X. (1999). Bioremediation of oil on shoreline environments: development of techniques and guidelines. Pure Appl. Chem. 71(1): 161-171.

Lee K, Levy, E.M. (1989). International Oil Spill Conference, American Petroleum Institute, Washington, DC. pp. 479-486.

Leahy. M.C. and Brown, R.A. (1 994) Bioremediation: Optirnizing Results. Chemical Engineering May. 108- 1 1 5.

Lessie T.G., Gaffney T. (1986). Catabolic potential of *Pseudomonas cepacia*. In: The Bacteria. Sokatch JR, Ornston LN (editors), Academic Press, New York, USA. 10: 439- 481.

Kaladumo, C. O. K., (1996). The implications of gas flaring in the Niger Delta Environment. Proceedings of the 8th Biennial International NNPC Seminar . In: The Petroleum Industry and the Nigerian Environment ,Port Harcourt, Nigeria, 277-290.

Krebs, C. T., Tanner, C. E., (1981). Restoration of oiled marshes through sediment stripping and Spartina propagation. Proceeding of the 1981 oil spill conference, American petroleum institute, WashingtonDC ., 375-385.

Kosaic. **N.** (Ed) (1993) Biosurfactants - Production. Properties and applications. Marcel DekkerInc..NewYork, NY.

Kong, S, H., R.J. Watts. and Choi, J, H. (1998) "Treatment of petroleumcontaminated soils using iron mineral catalyzed hydrogen peroxide",Chemosphere, vol. 37, no. 8, pp. 1473-1482,

MacNaughton, S.J., Stephen JR, Venosa AD, Davis GA, Chang C-J, White DC (1999). Microbial population changes during bioremediation of an experimental oil spill. Appl. Environ. Microb. 65(8):3566-3574.

McElroy, E., J. W., Farrington, and J. M., Teal. (1989). In: U. Varanasi (ed.) Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment, *CRC Press Inc., Boca Raton, Florida,*

Megharaj, M., Jovcic, A., Boul, H. L., and Thiele, J. H. (1997). Recalcitrance of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) to cometabolic degradation by pure cultures of aerobic and anaerobic bacteria. Arch. Environ. Contam. Toxicol. 33:141-146.

Medeiros, P.M., Bicego, M.C., Castelao R.M., Rosso, C.D., Fillmann, G, Zamboni A.J. Natural and anthropogenic hydrocarbon inputs to sediments of Patos Lagoon Estuary, Brazil. Environ Int. (2005) ;31:77-87.

Mihelcic, J. R., and R. G. Luthy. (1988a). Degradation of polycyclic aromatic hydrocarbons under various redox conditions in soil-water systems. Appl. Environ. Microbiol. 54:1182-1187.*

Mihelcic, J. R., and R. G. Luthy. (1988b). Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. Appl. Environ. Microbiol. 54:1188-1198.

Microbial degradation of crude oil in sea water in continuous culture. Biotechnol. Lett. 5:567-572.17–20. *In* R. Serra (ed.), Biotechnology for soil remediation. CIPA, Milan, Italy. Morgan, P., and R. J. Watkinson. (1989). Hydrocarbon degradation in soils and methods for soil biotreatment. Crit. Rev. Biotechnol. **8:**305–333.

Minas, W., & Gunkel, W. (1995). Oil pollution in the North SeaÐa microbiological point of view. Helgolaender Meeresuntersuchungen, 49, 143±158

Heitkamp, M. A. and Cerniglia, C. E. (1988) Appl. Environ. Microbiol., 54. 1612.

Malroz Engineering Inc. (1994). Evaluation of Remediation Options for Petroleum Contaminated Site.

Margesin R, Schinner, F. (2001). Bioremediation (natural attenuation and biostimulation) of diesel-oil contaminated soil in an Alpine glacier skiing area. Appl. Environ. Microbiol. 67(7): 3127-3133.

Margesin, R, Walder, G. and Schinner F. (2000)The impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activities and microbial properties of soil. Acta Biotechnol;20:313–333.

Mohn, W.M**.** (1998 &1999**).**University of British Columbia. Personai Conversation

Morgan P, Watkinson R J. Hydrocarbon degradation in soils and methods for soil biotreatment. Crit Rev Biotechnol. (1989);**8**:305–333.

Mortelmans, K, Harworth, S, Lawlor, T, Speck, W, Tainer, B. and Zeiger, E. (1986) Environ. Mutagen.

Mueller, J. G., Cerniglia, C. E., P. H. Pritchard. (1996).Bioremediation of Environments contaminated by Polycyclic Aromatic Hydrocarbons. In Bioremediation: Principles and Applications, pp.125–194, *Cambridge University Press, Cambridge*

NationalAcademy of Sciences. (1985). Oil in the sea-inputs, fates, and effects. NationalAcademy Press, Washington, D.C.

Newmont Waste management plan. (2009)

NRC, Oil in the Sea: Inputs, Fates and Effects, NationalAcademy Press, National Research Council, WashingtonDC, (2000) p. 806.

Odu, C. T. (1983). Degradation and Weathering of Crude Oil Under Tropical Conditions', In : The Petroleum Industry and the Nigerian Environment, Lagos: Ogoni Bill of Right (1991)Port Harcourt: Saros Internationals Oil Times, NNPC Lagos ; June 2000.

Ogram, A., G. S. Sayler, and T. Barkay. (1987). The extraction and purification of microbial DNA from sediments. J. Microbiol. Methods 7:57-66. 99. Ogunseitan, 0. A., E. T

Olsen, K. D., and R. K. Sizemore. (1981). Effects of an established offshore oil platform on the autochthonous bacterial community. Dev.Ind.Microbiol. 22:685-694.

Onwurah, I. N. E., (2002a). Anticoagulant potency of water-soluble fractions of Bonny light oil and enzyme induction in rats. Biomed. Res., 13(1), 33-37.

Onwurah, I. N. E., (1999a). Restoring the crop sustaining potential of crude oil polluted soil by means of *Azotobacter*inoculation. Plant Prod. Res, J., 4, 6-16.

Onwurah, I. N. E. (1999b). Role of diazotrophic bacteria in bioremediation of crude oil polluted environment J.Chem. Technol. Biotechnol., 957-964.

Onwurah, I. N. E., (2002). Quantitative modelling of crude oil toxicity using the approach of cybernetics and structured mechanisms of microbial processes. Environ. Monit. Assess.,76, 157-166.

Paerl, H., Piehler, M., Swistak, J. (1996). Coastal dieselfuel pollution effects on the native microbial community.Poster presented at the Meeting of the American Societyof Microbiology, New Orleans May 19-23.

Perry, J. J., (1980). Oil in the biosphere. In "Introduction to Environmental Toxicology (F.E.Guthrie and J.J. PerryEds) Elsevier, New York, 198-209.

Pieper, H.D, Martins dos Santos VAP, Golyshin P.N. (2004). Genomic and mechanistic insights into the biodegradation of organic pollutants. Curr. Opin. Biotechn. 15 :215-224.

Perry, J. J. (1984). Microbial metabolism of cyclic alkanes, p. 61-98. In R. M. Atlas (ed.), Petroleum microbiology. *Macmillan Publishing Co., New York.*

Prescott, M. L., Harley, J. P., Klan, A. D., (1996). Industrial Microbiology and Biotechnology. In: Microbiology. 3rd. Ed. *Wim C Brown Publishers, Chicago, 923-927.*

Perry, J. J. (1984). Microbial metabolism of cyclic alkanes, p. 61-98. In R. M. Atlas (ed.), Petroleum microbiology. *Macmillan Publishing Co., New York.*

Pfaender, F. K., and G. W. Bartholomew. (1982). Measurementof aquatic biodegradation rates by determining heterotrophic uptake of radiolabeled pollutants. Appl. Environ. Microbiol. 44:159-164.

Pradham S.P., Conrad,J.R., Paterek,J. R.,Srivastava,V. J. (1998) Potential of phytoremediation for treatment for PAHs in soil at MGP sites.J.soil contam.7,467-480

Watts, R.J., Udell, M.D. and Leung. S.W. (1992). Treatment of contaminated soils using catalysed hydrogen peroxide", Chemical Oxidation Technologies for the Nineties, W.W. Eckenfeld, A.R. Bowers, J.A. Roth (eds.),Lancaster, USA: *A Technomic Publishing Company, INC, pp. 37-50*

Radwan, S., Sorkhoh, N., and El-Nemr, I.(1995). Oil biodegradation around roots. Nature 376:302.

Rahman, K.S.,M, Rahman, T.J, Kourkoutas, Y, Petsas I, Marchant R, Banat, I.M. (2003).

Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. BioresourTechnol 90:159–168

Rambeloarisoa, E., J. F. Rontani, G. Giusti, Z. Duvnjak, and J. C. Bertrand. (1984). Degradation of crude oil by a mixed population of bacteria isolated from sea-surface foams. Mar.Biol.(Berlin) 83:69-81.

Watts R.J., M.D. Udell, S.W. Leung, "Treatment of contaminated soils using catalysed hydrogen peroxide", Chemical Oxidation Technologies for the Nineties, W.W. Eckenfeld, A.R. Bowers, J.A. Roth (eds.), Lancaster, USA: *A Technomic Publishing Company, INC. (1992)*, pp. 37-50.

Robertson, B. R., and D. K. Button. (1987). Toluene induction and uptake kinetics and their inclusion in the specific-affinity relationship for describing rates of hydrocarbon metabolism.Appl. Environ. Microbiol. 53:2193-2205

Roszak, D. B., and R. R. Colwell. (1987). Survival strategies of bacteria in the natural environment. Microbiol. Rev. 51:365- 379.

Sarkar D, Ferguson M, Datta R, Birnbaum S. (2005). Growth of a thermophilic bacterium on hydrocarbons: a new source of single cell protein. Science 157:1322-1323

Sayler, G. S., M. S. Shields, E. T. Tedford, A. Breen, S. W. Hooper, K. M. Sirotkin, and J. W. Davis. (1985). Application of DNA-DNA colony hybridization to the detection of catabolic genotypes in environmental samples. Appl. Environ. Microbiol. 49:1295-1303.

Sayler, G. S., R. E. Perkins, T. W. Sherrill, B. K. Perkins, M. C. Reid, M. S. Shields, H. L. Kong, and J. W. Davis. (1983). Microcosm and experimental pond evaluation of microbial community response to synthetic oil contamination in freshwater sediments. Appl. Environ. Microbiol. 46:211-219.

Savage. G.M.. Diaz. L.F. and Golueke, C.G. (1985) Disposing of organic hazardous wastes by composting. *Biocylr* 26.3 1-34.

Schwab, A. P., and Banks, M. K. (1994). Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. Pages 132- 141 in: Bioremediation ThroughRhizosphere Technology. T. A. Anderson and J. R. Coats, eds. American Chemical Society,Washington, DC.

Short, J. W., Heintz, R. A., (1997). Identification of Exxon Valdez oil in sediments and tissue from Prince William Sound and the North Western Gulf of William based in a PAH weathering model. Environ. Sci. Technol., 31,2375-2384

Spain, J. C., P. H. Pritchard, and A. W. Bourquin. (1980). Effects of adaptation on biodegradation rates in sediment/ water cores from estuarine and freshwater environments Appl. Environ. Microbiol. 40:726-734.

Sayler, G. S., R. E. Perkins, T. W. Sherrill, B. K. Perkins,M. C. Reid, M. S. Shields, H. L. Kong, and J. W. Davis. (1983).Microcosm and experimental pond evaluation of microbialcommunity response to synthetic oil contamination in freshwater sediments. Appl. Environ. Microbiol. 46:211-219.

Sherrill, T. W., and G. S. Sayler. (1980). Phenanthrene biodegradation in freshwater environments. Appl. Environ. Microbiol.39:172-178.

Sherrill, T. W., and G. S. Sayler. (1980). Phenanthrene biodegradation in freshwater environments. Appl. Environ. Microbiol. 39:172-178.

Singer, M. E., and W. R. Finnerty. (1984). Microbial metabolism of straight-chain and branched alkanes, p, 1-60. In R. M. Atlas (ed.), *Petroleum microbiology. Macmillan Publishing Co.,New York.*

State of Mississippi. Department of Environmental Quality. (1998). Fundamental Principles of Bioremediation. April 1998. 27 Nov 2006 <http://www.deq.state.ms.us/MDEQ.nsf/pdf/GARD_Bioremediation/$File/Bioremediation.pdf? Verified 12/15/2006

Smets, B. F., Rittmann, B. E., Stahl, D. A. (1990). Genetic capabilities of biological processes, Part II. Environ. Sci. Technol., 24, 162-170.

Standard methods book for examination of water and waste water 21th edition, (2005)

Stephen, J.R., Chang C-J, Gan D, Peacock A, Pfiffner SM, Barcelona MJ,White D C, MacNaughton, S.J. (1999). Microbial characterisation of JP- 4 fuel contaminated site using a combined lipid biomarker/PCRDGGE based approach. Environ. Microb. Biotech. 1: 231-243.

Steffan, R. J., and R. M. Atlas. (1988). DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. Appl. Environ. Microbiol. 54:2185-2191.

Spain, J. C., and P. A. van Veld. (1983). Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure time, inoculum, and chemical structure. Appl. Environ. Microbiol. 45:428-435.

Spain, J. C., P. H. Pritchard, and A. W. Bourquin. (1980). Effects of adaptation on biodegradation rates in sediment/water cores from estuarine and freshwater environments. Appl. Environ. Microbiol. 40:726-734.

Suflita, J. M., A. Horowitz, D. R. Shelton, and J. M. Tiedje. (1982). Dehalogenation: a novel pathway for the anaerobic biodegradation of haloaromatic compounds. Science 218:1115-1117.

Sussman, M., C. H. Collins, F. A. Skinner, and D. E. Stewart- Tull (ed.). (1988). Release of genetically-engineered micro-organisms. Academic Press, Inc. (London), Ltd., London.

Tagger, S., A. Bianchi, M. Julliard, J. LePetit, and B. Roux. (1983). Effect of microbial seeding of crude oil in seawater in a model system. Mar. Biol. (Berlin) 78:13-20.

Technical and Regulatory Guidance for In Situ Chemical Oxidation of Contaminated Soil and Groundwater", Technical/Regulatory Guidelines, prepared by Interstate Technology and Regulatory Cooperation Work Group, In Situ Chemical Oxidation Work Team. (2001), 25 p

Thangamani, S. and Shreve. G.S. (1994) Effect of anionic biosurfactant on hexadecane partitioning in multi-phase systems. *Environ. Sci Techno!. 28(* 12). 1993-2000.

Thomas, J. M., J. R. Yordy, J. A. Amador, and M. Alexander. (1986). Rates of dissolution and biodegradation of water-insoluble organic compounds. Appl. Environ. Microbiol. 52:290-296.

Timmis, K. N and Pieper, D. H. (1999). Bacteria designed for bioremediation. Tibtech 17:201-204

Tiido, T., Rignell-Hydbom, A., Jönsson, B. A. G., Giwercman, Y. L., Pederson, H. S., Wojtyniak, B., Ludwicki, J. K., Lesovoy, V., Zvyezday, V., and 10 others. (2006). Impact of PCB and p,p´-DDE contaminants on human sperm Y:X chromosome ratio: studies on three European populations and the Inuit population in Greenland. Environ. Health Perspect.,114, 718-724.

Torrna. A.E. (1994). The basics ofbioremediation. Pollution Engineering June. 4647.

Trevors, J. T. (1985). DNA probes for the detection of specific genes in bacteria isolated from the environment. Trends Biotechnol. 3:291-293.

Torsvik V, Øvreås, L, Thingstad, T.F. (2002). Prokaryotic diversity magnitude, dynamics and controlling factors. Science 296:1064–1066

U.S. Environmental Protection Agency. (1999). Use of monitored natural attenuation at Superfund RCRA corrective action, and underground storage tank sites. OSWER Directive 9200.4–17. U.S. Environmental Protection Agency, Washington, D.C

United States . Environmental Protection Agency. Landfarming. 9 March 2006 . 24 Nov 2006

Venosa.D and X. Zhu. (2003). "Biodegradation of crude oilcontaminating marine shorelines and freshwater wetlands,"Spill Science and Technology Bulletin, vol. 8, no. 2, pp. 163–178,.

Van Hamme J. D., A. Singh, and O. P. Ward. (2003). Recent advances in petroleum microbiology. Microbiology and Molecular Biology Reviews **67**:503

vanDeuren, J., Wang, Z. and Ledbetter, J. (1997) Remediation Technologies Screening Matrix and Reference Guide, 3rd ed. SFIM-AEC-ET-CR-97053. US Army Environmental Center. MD 21010-5401.

Vidali, M. (2001). Bioremediation. An overview. *Pure Appl. Chem.* 73 (7): 1163-1172

Von Wedel, R. J., J. F. Mosquera, C. D. Goldsmith, G. R. Hater, A. Wong, T. A. Fox, W. T. Hunt, M. S. Paules, J. M. Quiros, and J. W. Wiegand. (1988). Bacterial biodegradation of petroleum hydrocarbons in groundwater: in situ augmented bioreclamation with enrichment isolates in California. Water Sci. Technol. 20:501-503.

Wackett, L.P. (1996). Co-metabolism: is the emperor wearing any clothes? Curr. Opin. Biotechn. 7:321-325

Walton, B. T., and Anderson, T. A. (1990). Microbial degradation of trichloroethylene in the rhizosphere: Potential application to biological remediation of waste sites. Appl. Environ. Microbiol. 56:1012-1016

Waste management plan for Newmont Ghana Gold Limited. (2009)

Wardle, D.A. and Giller, K.E. (1996). The quest for a contemporary ecological dimension to soil biology. *Soil Biology and Biochemistry*, **28**: 1549-1554.

White, D. C., Flemming,C.A., Leung, K.T., MacNaughton, S.J. (1998). *In situ* microbial ecology for quantitative assessment, monitoring and riskassessment of pollution remediation in soils, the subsurface, therhizosphere and in biofilms. J. Microbiol. Meth. 32:93-105.

Whyte, L. G., J. Hawari, E. Zhou, L. Bourbonnie`re, W. E. Inniss, and C. W.Greer**.** **(**1998). Biodegradation of variable-chain-length alkanes at low temperaturesby a psychrotrophic*Rhodococcus*sp. Appl. Environ. Microbiol.64 2578–2584.

Widdel. F, Rabus R. (2001). Anaerobic biodegradation of saturated and aromatic hydrocarbons. Curr. Opin.Biotech. 12:259-276.

Wong, A. D., and C. D. Goldsmith. (1988). The impact of achemostat discharge containing oil degrading bacteria on the biological kinetics of a refinery activated sludge process. Water Sci. Technol. 20:131-136.

Wyndham, R. C., and J. W. Costerton. (1981). Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment microorganisms within the Athabasca oil sands deposit. Appl. Environ. Microbiol. 41:783-790.

Yee, D. C., Maynard, J. A., and Wood, T. K. (1998). Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonasfluorescens*strain expressing toluene *ortho*-monooxygenase constitutively. Appl. Environ. Microbiol. 64:112-118.

Zablotowicz, R. M., Hoagland, R. E., and Locke, M. A. (1994). Glutathione S-transferase activity in rhizosphere bacteria and the potential for herbicide detoxification. Pages 184-198 in: Bioremediation Through Rhizosphere Technology. T. A. Anderson and J. R. Coats, eds. American Chemical Society, Washington, DC.

Zeyer, J., E. P. Kuhn, and R. P. Schwarzenbach. (1986). Rapid microbial mineralization of toluene and 1, 3-dimethylbenzene in the absence of molecular oxygen. Appl. Environ. Microbiol.52:944-947.

Zengler K, Richnow, H.H., Rossello-Mora, R, Michaelis W, Widdel. F. (1999). Methane formation from long-chain alkanes by anaerobic microorganisms: Natr. (401):266-269.

APPENDIX (A)

Levels of Oil/grease, TPH and HPC in HC, Topsoil and Compost.

	Before fertilizer/compost/topsoil blend		
Sample ID	Oil n Grease(mg/kg)	TPH(mg/kg)	Heterotrophic plate count/(mpn)
HC	34278.00	21514.85	$45*10^{3}$
Topsoil	<100	<10	$89*10^{18}$
Compost	<100	<10	4.00

Levels of moisture content, pH, Temperature, Available phosphorous, Potassium

Sample ID	Moisture content (%)	pH	Temp(OC)	Total Nitrogen (%)	Available Phosphorus(mg/kg)	Potassium (mol/Kg)
HC	19.00	7.60	24.80	0.08	5.52	0.20
Topsoil	24.00	7.40	25.20	0.35	58.4	0.27
Compost	38.90	7.78	26.20	0.80	850.00	0.45

APPENDIX (B)

Calculations for soil amendment

Amount (g) of topsoil, compost, fertilizer added to hydrocarbon contaminated soil.

Nitrogen levels	(0.2%, 0.8%, 1.4%, and 2.0%)
Nitrogen level in topsoil (%)	0.35
weight of HC cont. soil used/g	2000
Nitrogen level in HC(%)	0.08
weight of N in contaminated soil/g	1.60
level of nitrogen(%)	0.20
expected weight of 0.2% nitrogen in HC	4.00
Nitrogen deficit(g)	2.40
amount of topsoil that contains 2.4g of N	**685.71**
level of nitrogen(%)	0.80
expected weight of 0.8% nitrogen in HC	16.00
Nitrogen deficit	14.40
amount of topsoil that contains 14.4g of N	**4114.29**
Level of nitrogen (%)	1.40
expected weight of 1.4% nitrogen in HC	28.00
Nitrogen deficit	26.40
amount of topsoil that contains 26.4g of N	**7542.86**
level of nitrogen(%)	2.00
expected weight of 2.0% nitrogen in HC	40.00
Nitrogen deficit	38.40
amount of topsoil that contains 38.4g of N	**10971.43**

nitrogen levels	**(0.2%, 0.8%, 1.4%, and 2.0%)**
Nitrogen level in compost(%)	0.90
weight of HC cont. soil used	2000.00
Nitrogen level in HC	0.08
Percent	100.00
weight of N in contaminated soil	1.60
level of nitrogen	0.20
expected weight of 0.2% nitrogen in HC	4.00
Nitrogen deficit	2.40
amount of compost that contains 2.4 of N	**266.67**
Level of nitrogen (%)	0.80
expected weight of 0.8% nitrogen in HC	16.00
Nitrogen deficit	14.40
amount of topsoil that contains 14.4g of N	**1600**
Level of nitrogen	1.40
expected weight of 1.4% nitrogen in HC	28.00
Nitrogen deficit	26.40
amount of topsoil that contains 26.4g of N	**2933.33**
Level of nitrogen (%)	2.00
expected weight of 2.0% nitrogen in HC	40.00
Nitrogen deficit	38.4
amount of compost that contains 38.4g of N	**4266.67**

Nitrogen levels	(0.2%, 0.8%, 1.4%, and 2.0%)
Nitrogen level in fertilizer (%)	46.00
weight of HC cont. soil used	2000.00
Nitrogen level in HC	0.08
weight of N in contaminated soil	1.60
level of nitrogen	0.20
expected weight of 0.2% nitrogen in HC	4.00
Nitrogen deficit	2.40
amount of fertilizer that contains of 2.4g of N	**5.22**
Level of nitrogen (%)	0.8
expected weight of 0.8% nitrogen in HC	16
Nitrogen deficit	14.4
amount of fertilizer that contains 14.4g of N	**31.30**
Level of nitrogen	1.4
expected weight of 1.4% nitrogen in HC	28
Nitrogen deficit	26.4
amount of fertilizer that contains 26.4g of N	**57.39**
Level of nitrogen (%)	2
expected weight of 2.0% nitrogen in HC	40
Nitrogen deficit	38.4
amount of fertilizer that contains 38.4g of N	**83.48**

Weight of compost/topsoil/fertilizer added to 2kg contaminated Soil.

Level of nitrogen (%)	Compost(g) added to HC)	Topsoil(g) added to HC)	Fertilizer(g) HC added)
0.2	266.67	685.71	5.20
0.8	1600.00	4114.29	31.30
1.4	2933.33	7542.86	57.40
2.0	4266.67	10971.43	83.48

APPENDIX (C)

Levels of 0.2%, 0.8%, 1.4%, 2.0% Nitrogen after blending.

After topsoil blend		
Sample ID/ (%)	Total Nitrogen (%)	% Recovery(75-125)
0.20	0.16	80.00
0.80	0.88	110.00
1.40	1.7	121.43
2.00	2.2	110.00

Levels of 0.2%, 0.8%, 1.4%, 2.0% Nitrogen after blending.

After compost blend		
Sample ID	Total Nitrogen (%)	% Recovery(75-125)
0.20	0.24	120.00
0.80	0.90	112.50
1.40	1.70	121.43
2.00	1.70	85.00

Levels of 0.2%, 0.8%, 1.4%, 2.0% Nitrogen after blending.

After fertilizer blend		
Sample ID	Total Nitrogen (%)	% Recovery(75-125)
0.20	0.15	75.00
0.80	0.78	97.50
1.40	1.70	121.43
2.00	2.40	120.00

APPENDIX (D)

STATISTICAL ANALYSISFOR HYDROCARBON CONTAMINATED TOPSOIL BLEND

Variable	TREATMENTS	Mean	StDev
OIL AND GREASE	0.20%	13867.00	10426.00
	0.80%	13561.00	10787.00
	1.40%	9777.00	11956.00
	2.00%	8276.00	11380.00
TPH	0.20%	9462.00	7177.00
	0.80%	9270.00	7412.00
	1.40%	6443.00	8001.00
	2.00%	5396.00	7271.00

ANALYSIS OF VARIANCE

Analysis of Variance for OIL AND GREASE					
Source	DF	SS	MS	F	P
TREATMENTS	3	185167623.00	61722541.00	13.93	0.000
WEEKS	7	3389411088.00	484201584.00	109.25	0.000
ERROR	21	93071393.00	4431971.00		
Analysis of Variance for TPH					
TREATMENTS	3	99551947	33183982	12.99	0.000
WEEKS	7	1509813815	215687688	84.44	0.000
ERROR	21	52628407	2554210		
TOTAL	31	1663004169			

Grouping Information Using Tukey Method and 95.0% Confidence for OIL AND GREASE

TREATMENT	N	MEAN	GROUPING
0.20%	8	13867.30	A
0.80%	8	13561.10	A
1.40%	8	9777.40	B
2.00%	8	8276.10	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for TPH

TREATMENT	N	MEAN	GROUPING
0.20%	8	9461.60	A
0.80%	8	9270.50	A
1.40%	8	6442.90	B
2.00%	8	5396.30	B

Means that do not share a letter are significantly different.

STATISTICAL ANALYSISFOR HYDROCARBON CONTAMINATED SOIL - COMPOST BLEND

Variable	TREATMENTS	Mean	StDev
OIL AND GREASE	0.20%	11150.00	10820.00
	0.80%	9022.00	11068.00
	1.40%	7919.00	11399.00
	2.00%	6912.00	11646.00
TPH	0.20%	7173.00	7111.00
	0.80%	5717.00	7011.00
	1.40%	5087.00	7160.00
	2.00%	4353.00	7285.00

ANALYSIS OF VARIANCE

Analysis of Variance for OIL AND GREASE					
Source	DF	SS	MS	F	P
TREATMENTS	3	79230947.00	26410316.00	15.92	0.000
WEEKS	7	3501088905.00	500155558.00	301.48	0.000
Error	21	34839464.00	1659022.00		
Analysis of Variance for TPH					
TREATMENTS	3	34442039.00	11480680.00	9.46	0.000
WEEKS	7	1402884386.00	200412055.00	165.05	0.000
Error	21	25498987.00	1214237.00		

Grouping Information Using Tukey Method and 95.0% Confidence for OIL AND GREASE

TREATMENT	N	MEAN	GROUPING
0.20%	8	11150.30	A
0.80%	8	9021.80	B
1.40%	8	7919.30	BC
2.00%	8	6911.90	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for TPH

TREATMENT	N	MEAN	GROUPING
0.20%	8	7173.00	A
0.80%	8	5716.80	AB
1.40%	8	5086.70	B
2.00%	8	4353.00	B

Means that do not share a letter are significantly different.

STATISTICAL ANALYSISFOR HYDROCARBON CONTAMINATED SOIL- FERTILIZER

Variable	TREATMENTS	Mean	StDev
OIL AND GREASE	0.20%	15902.00	9966.00
	0.80%	13812.00	10366.00
	1.40%	14696.00	9953.00
	2.00%	11607.00	11183.00
TPH	0.20%	11513.00	7786.00
	0.80%	10351.00	8123.00
	1.40%	10655.00	7437.00
	2.00%	8141.00	7870.00

ANALYSIS OF VARIANCE

Analysis of Variance for OIL AND GREASE					
Source	DF	SS	MS	F	P
TREATMENTS	3	143239525.00	47746508.00	20.96	0.000
WEEKS	8	3173003329.00	396625416.00	174.13	0.000
Error	22	50111007.00	2277773.00		
Analysis of Variance for TPH					
TREATMENTS	3	91667888.00	30555963.00	17.30	0.000
WEEKS	8	1794625380.00	224328173.00	127.01	0.000
Error	22	38858264.00	1766285.00		

Grouping Information Using Tukey Method and 95.0% Confidence for OIL AND GREASE

TREATMENT	N	MEAN	GROUPING
0.20%	9	15901.80	A
0.80%	9	13812.10	B
1.40%	8	13134.00	B
2.00%	8	10044.80	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for TPH

TREATMENT	N	MEAN	GROUPING
0.20%	9	11513.30	A
0.80%	9	10351.00	AB
1.40%	8	9444.00	B
2.00%	8	6929.50	C

Means that do not share a letter are significantly different.

Descriptive Statistics for Topsoil, compost and fertilizer hydrocarbon blends

TOPSOIL/ HC

Variable	Level of Nitrogen (%)	Mean	StDev
Oil/Grease	0.2	13561.00	10787.00
	0.8	12096.00	11600.00
	1.4	12785.00	12515.00
	2.0	9430.00	11775.00
TPH	0.2	9270.00	7412.00
	0.8	8249.00	7820.00
	1.4	8515.00	8307.00
	2.0	6167.00	7493.00

HC COMPOST

Variable	Level of Nitrogen (%)	Mean	StDev
Oil/Grease	0.2	11150.00	10820.00
	0.8	10277.00	11323.00
	1.4	10539.00	12205.00
	2.0	9216.00	12822.00
TPH	0.2	7173.00	7111.00
	0.8	6533.00	7150.00
	1.4	6782.00	7614.00
	2.0	5804.00	8012.00

FERT/ HC

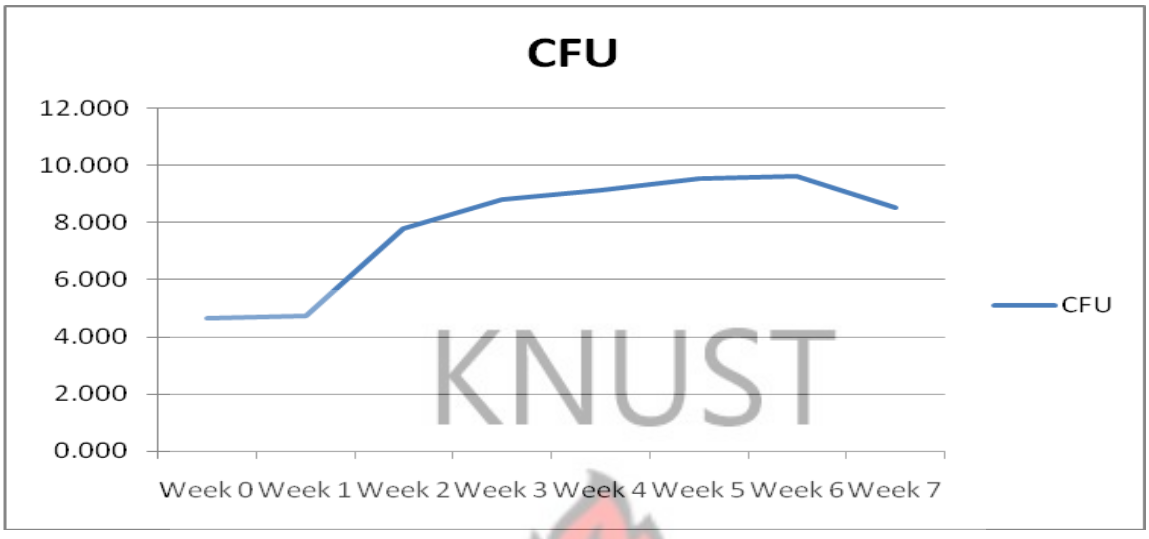
Variable	Level of Nitrogen (%)	Mean	StDev
Oil/Grease	0.2	15902.00	9966.00
	0.8	13812.00	10366.00
	1.4	14696.00	9953.00
	2.0	11607.00	11183.00
TPH	0.2	11513.00	7786.00
	0.8	10351.00	8123.00
	1.4	10655.00	7437.00
	2.0	8141.00	7870.00

Compost/Hydrocarbon blend

Mean results of, HPC in compost /Hydrocarbon contaminated soil blend for 0.2% N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^3	4.653
Week 1	55×10^3	4.740
Week 2	64×106	7.806
Week 3	64×107	8.806
Week 4	132×107	9.121
Week 5	340×107	9.531
Week 6	414×10^7	9.617
Week 7	339×10^6	8.530

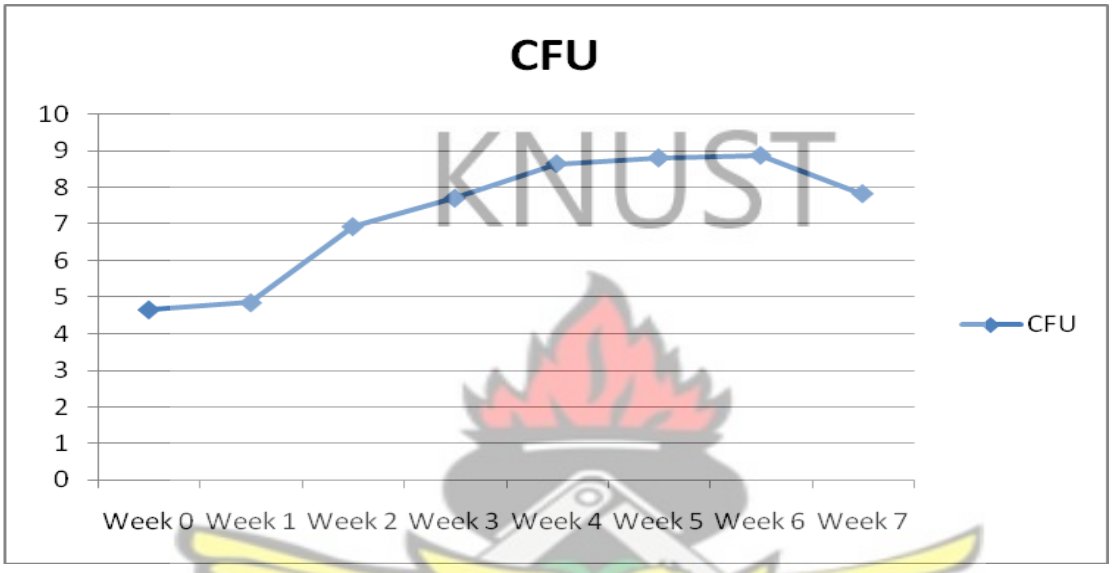
Graphical representation of HPC against time for 0.2% nitrogen level in the compost/HC blend.



Mean results of HPC in compost /Hydrocarbon contaminated soil blend for 0.8% N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.6532
Week 1	70×10^{3}	4.8451
Week 2	85×10^{5}	6.9294
Week 3	51×10^{6}	7.7076
Week 4	45×10^{7}	8.6532
Week 5	65×10^{7}	8.8129
Week 6	76×10^{7}	8.8808
Week 7	68×106	7.8325

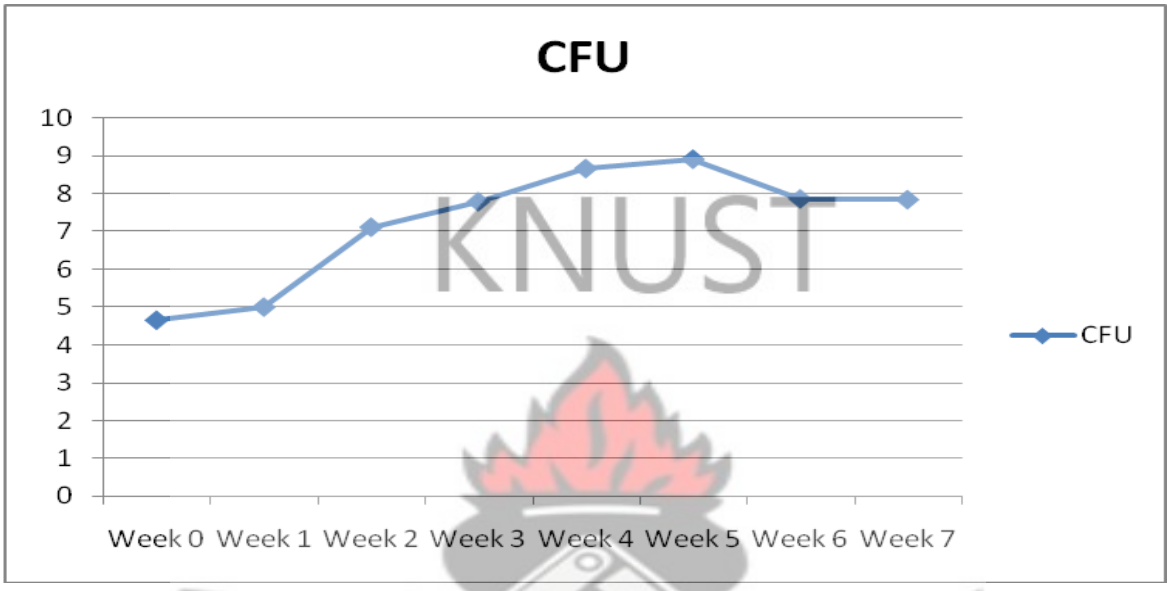
Graphical representation of HPC against time for 0.8% nitrogen level in the compost/HC blend.



Mean results of HPC in compost /Hydrocarbon contaminated soil blend for 1.4% N-Level.

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	100×10^{3}	5.000
Week 2	128×10^{5}	7.107
Week 3	6×10^{6}	7.778
Week 4	45×10^{7}	8.653
Week 5	79×10^{7}	8.898
Week 6	71×10^{6}	7.851
Week 7	68×10^{6}	7.833

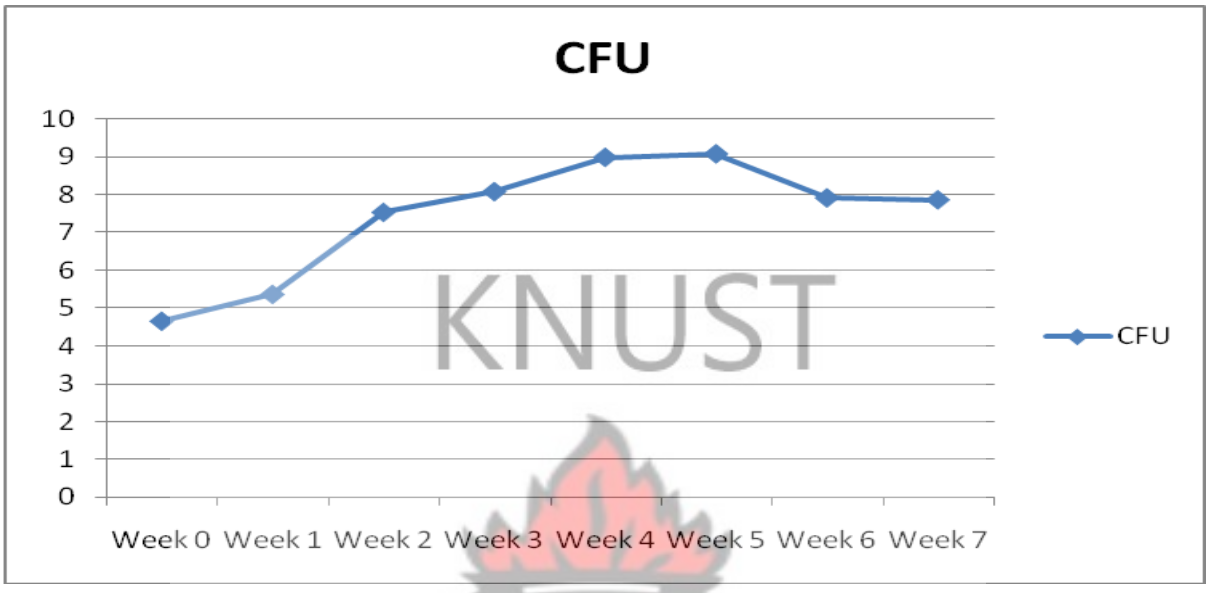
Graphical representation of HPC against time for the 1.4% nitrogen level in the compost/HC blend.



Means results of HPC in compost /Hydrocarbon contaminated soil blend for 2.0% N-Level.

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	230×10^{3}	5.362
Week 2	340×10^{5}	7.531
Week 3	120×10^{6}	8.079
Week 4	97×10^{7}	8.987
Week 5	120×10^{7}	9.079
Week 6	82×10^{6}	7.914
Week 7	73×10^{6}	7.863

Graphical representation of HPC against time for the 2.0% nitrogen level in the compost/HC blend.

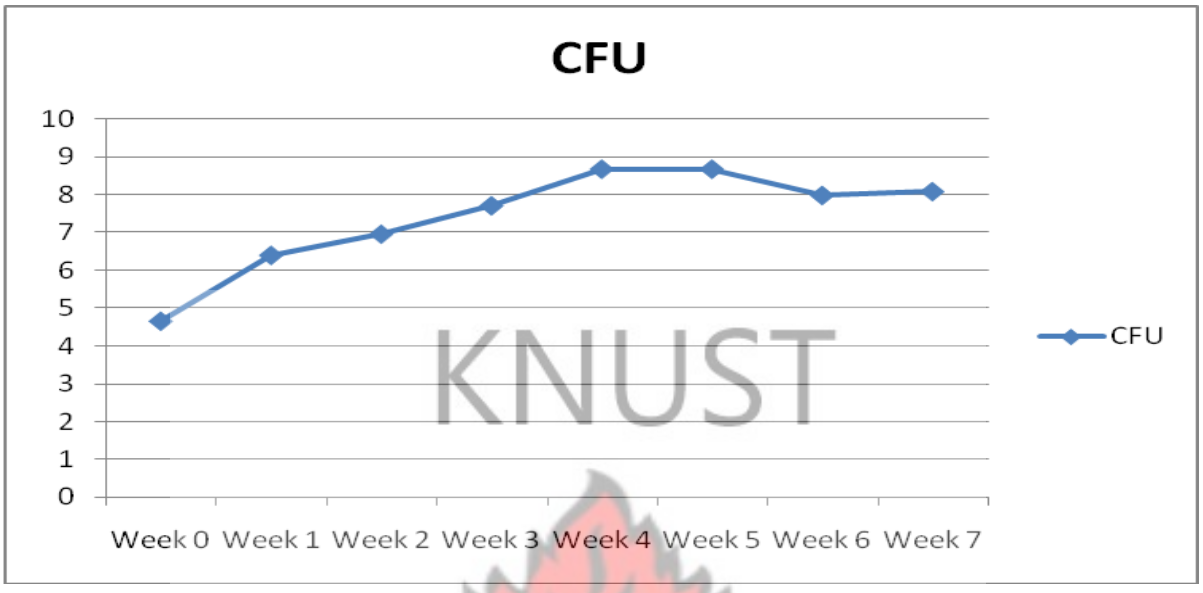


Topsoil/Hydrocarbon blend

Mean results of HPC in Topsoil /Hydrocarbon contaminated soil blend for 0.2% N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	248×10^{4}	6.394
Week 2	90×10^{5}	6.954
Week 3	507×10^{5}	7.705
Week 4	555×10^{5}	8.672
Week 5	470×10^{6}	8.672
Week 6	95×10^{6}	7.978
Week 7	120×10^{6}	8.079

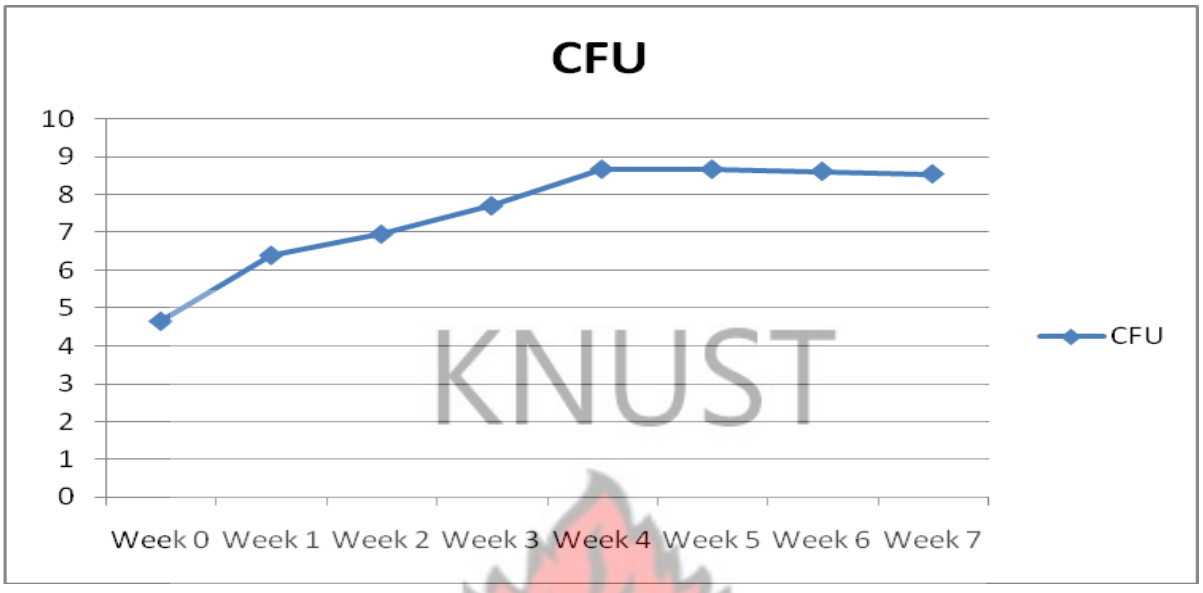
Graphical representation of HPC against time of 0.2% nitrogen level for the topsoil/HC blend.



Mean results of HPC in Topsoil /Hydrocarbon contaminated soil blend for 0.8% N-Level

Time	HPC(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	248×10^{4}	6.394
Week 2	90×10^{5}	6.954
Week 3	507×10^{5}	7.705
Week 4	555×10^{5}	8.672
Week 5	470×10^{6}	8.672
Week 6	414×10^{6}	8.617
Week 7	355×10^{6}	8.550

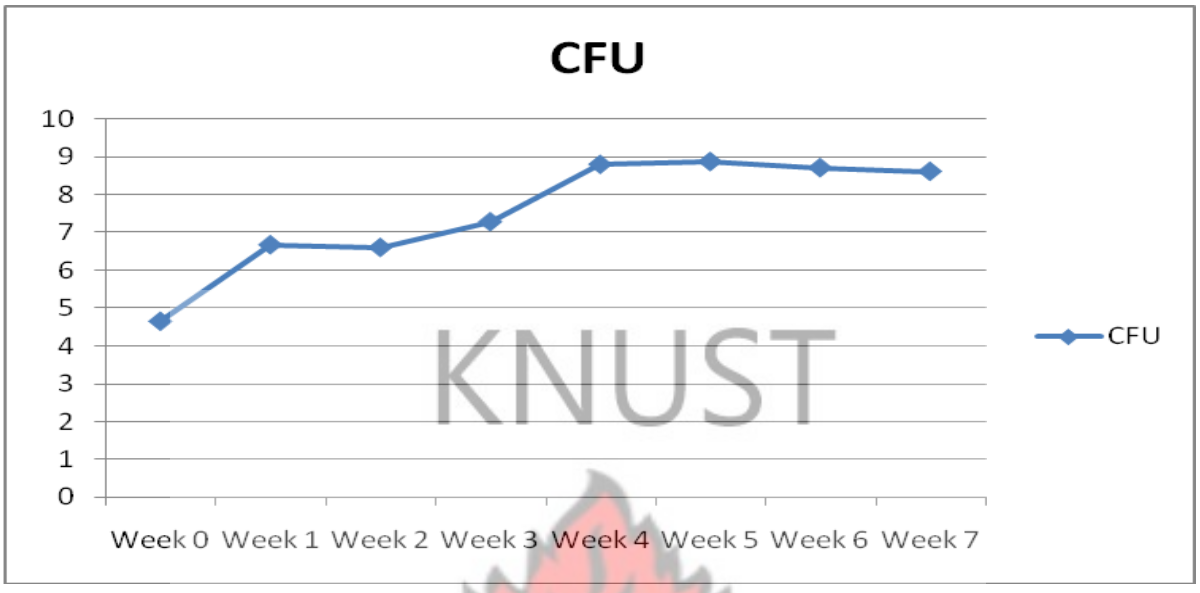
Graphical representation of HPC against time of 0.8% nitrogen level for topsoil/HC blend.



Mean results of HPC in Topsoil /Hydrocarbon contaminated soil blend for 1.4% N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	470×10^{4}	6.672
Week 2	4×10^{6}	6.602
Week 3	19×10^{6}	7.279
Week 4	623×10^{6}	8.794
Week 5	738×10^{6}	8.868
Week 6	507×10^{6}	8.705
Week 7	407×106	8.610

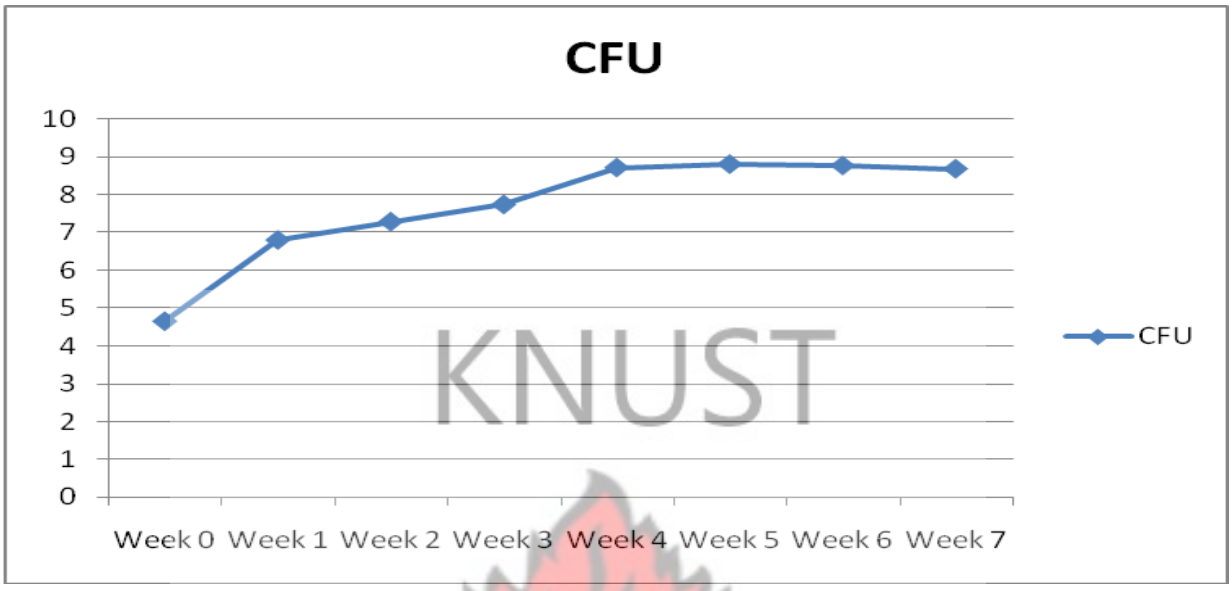
Graphical representation of HPC against time of 1.4% nitrogen level for topsoil/HC blend



Mean results of HPC in Topsoil /Hydrocarbon contaminated soil blend for 2.0% N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	623×10^{4}	6.794
Week 2	19×10^{6}	7.279
Week 3	54×10^{6}	7.732
Week 4	507×10^{6}	8.705
Week 5	623×10^{6}	8.794
Week 6	570×106	8.756
Week 7	470×10^{6}	8.672

Graphical representation of HPC against time of 2.0% nitrogen level for topsoil/HC blend

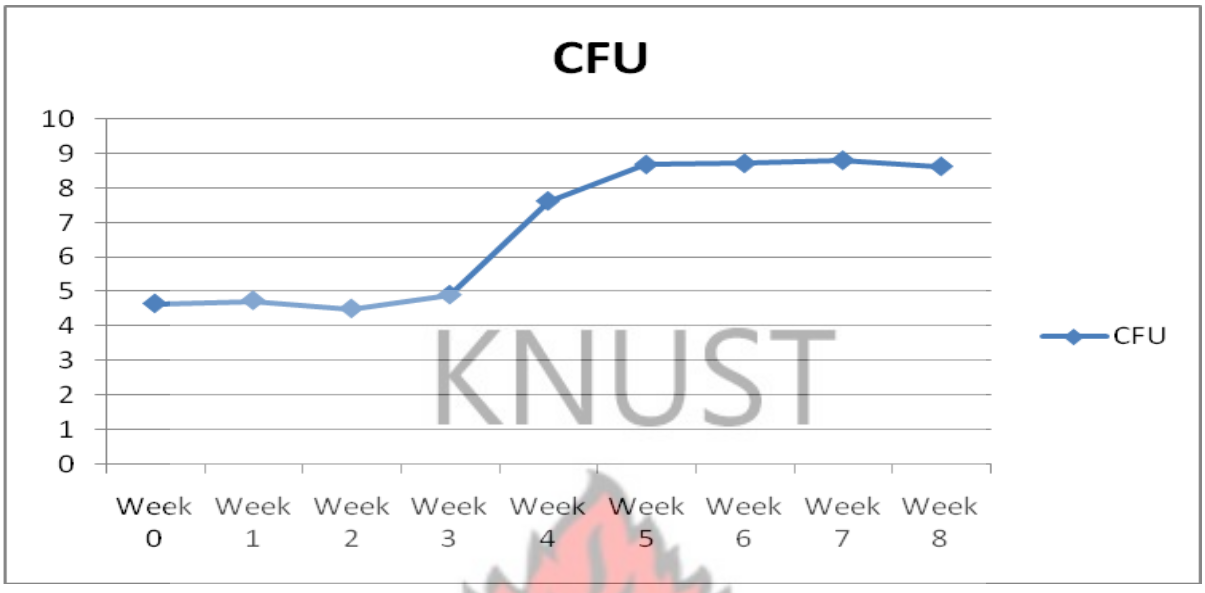


Fertilizer/Hydrocarbon blend

Mean results of HPC in fertilizer /Hydrocarbon contaminated soil blend for 0.2% N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^3	4.653
Week 1	55×10^3	4.740
Week 2	32×10^3	4.505
Week 3	8×10^4	4.903
Week 4	414×10^5	7.617
Week 5	470×10^6	8.672
Week 6	507×10^6	8.705
Week 7	623×10^6	8.794
Week 8	414×10^6	8.617

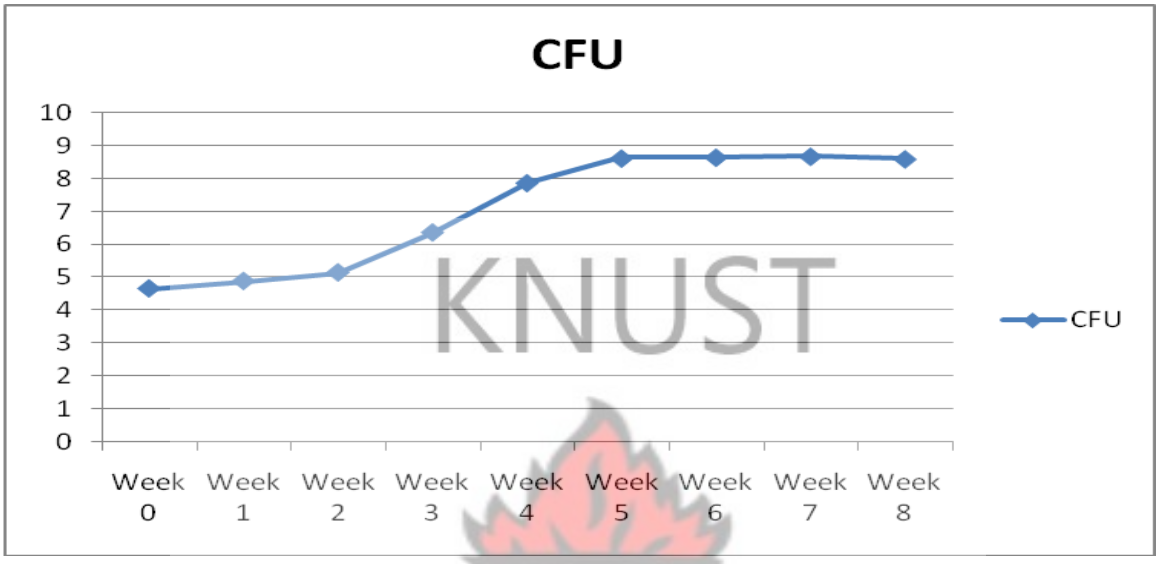
Graphical representation of HPC against time of 0.2% nitrogen level for fertilizer/HC blend.



Mean results of HPC in fertilizer /Hydrocarbon contaminated soil blend for 0.8 % N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	76×10^{3}	4.881
Week 2	139×10^{3}	5.143
Week 3	230×10^{4}	6.362
Week 4	738×10^{5}	7.868
Week 5	414×10^{6}	8.617
Week 6	440×10^{6}	8.643
Week 7	470×10^{6}	8.672
Week 8	393×10^{6}	8.593

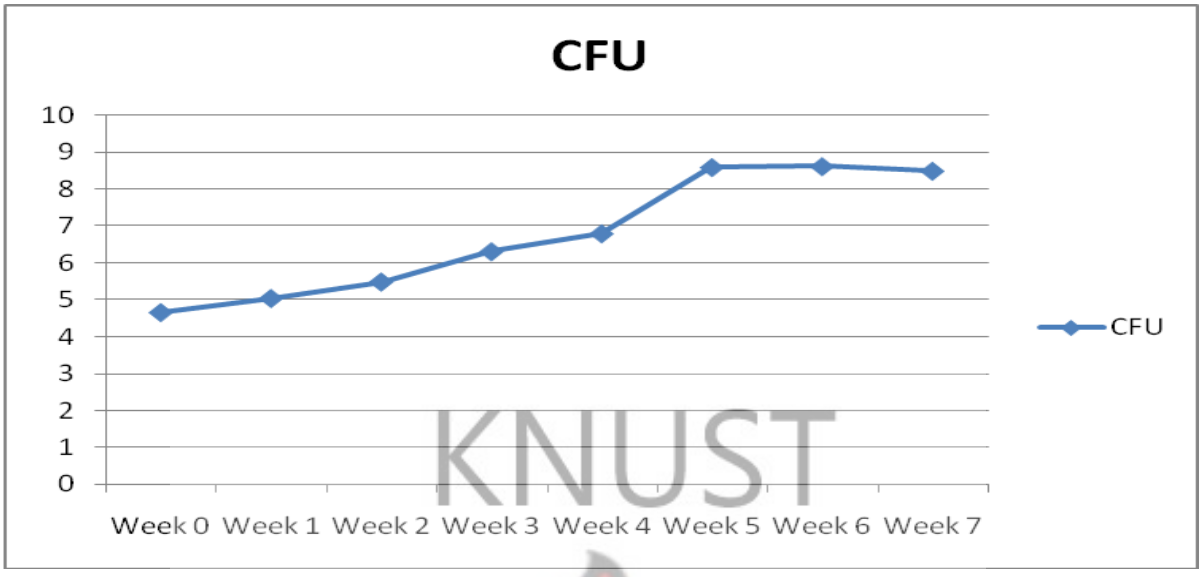
Graphical representation of HPC against time of 0.8% nitrogen level for fertilizer/HC blend**.**



Mean results of HPC in fertilizer/Hydrocarbon contaminated soil blend for 1.4 % N-Level.

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	109×10^{3}	5.037
Week 2	304×10^{3}	5.483
Week 3	202×10^{4}	6.305
Week 4	623×10^{4}	6.794
Week 5	392×10^{6}	8.593
Week 6	414×10^{6}	8.617
Week 7	311×10^{6}	8.493

Graphical representation of HPC against time of 1.4% nitrogen level for fertilizer/HC blend.



Mean results of HPC in fertilizer/Hydrocarbon contaminated soil blend for 2.0% N-Level

Time	plate count/(CFU)	log of count
Week 0	45×10^{3}	4.653
Week 1	189×10^{3}	5.276
Week 2	504×10^{3}	5.702
Week 3	120×10^{4}	7.079
Week 4	738×10^{5}	7.868
Week 5	100×10^{6}	8.000
Week 6	132×10^{6}	8.121
Week 7	71×10^{6}	7.851

Graphical representation of HPC against time of 2.0% nitrogen level for fertilizer/HC blend**.**