

**IMPACT OF MICROBIAL LOAD ON THE  
BIOREMEDIATION OF CRUDE OIL POLLUTED SOIL**

**A PROJECT SUBMITTED TO THE DEPARTMENT OF  
CHEMICAL ENGINEERING**

**BY**

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## **ABSTRACT**

The effect of microbial load and nutrient (NPK 15:15:15) on crude oil polluted soil was investigated in this study. 10% crude oil polluted soil for the samples were impacted with *Aspergillus niger* of concentrations  $1 \times 10^6$  cfu/g,  $2 \times 10^6$  cfu/g,  $5 \times 10^6$  cfu/g,  $1 \times 10^7$  cfu/g,  $2 \times 10^7$  cfu/g respectively and NPK 15:15:15 were introduced to five samples which served as test samples, and the other as control sample. Parameters such as pH, Residual Hydrocarbon Content (RHC), Total Microbial Count (TMC) were monitored over a period of seven (7) weeks. The results obtained show an increase in pH for the samples. The Total Hydrocarbon Content (THC) decreased for all the samples. The control sample dropped from 183-27mg/kg (85% drop), sample impacted with  $1 \times 10^6$  cfu/g dropped from 179-10mg/kg (94% drop), sample impacted with  $2 \times 10^6$  cfu/g dropped from 181-9mg/kg (95% drop), sample impacted with  $5 \times 10^6$  cfu/g dropped from 181-8mg/kg (96% drop), sample impacted with  $1 \times 10^7$  cfu/g dropped from 180-6mg/kg (97% drop) and the sample impacted with  $2 \times 10^7$  cfu/g dropped from 184-4mg/kg (98% drop). From the results, bioremediation strategy was found to be very effective.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Bioremediation is a term used to describe the destruction of contaminants by biological mechanisms, including micro-organisms (eg bacteria, fungi or yeast), in contaminated soil and water. This process uses micro-organisms or their enzymes to return the environment to its original condition. Micro-organisms used to perform the function of bioremediation are known as bioremediators. Bioremediation may be employed to attack specific contaminants, such as pesticides or more commonly oil spills that are broken down using multiple techniques including the addition of fertilizers to facilitate the decomposition of crude oil bacteria (Lovely, 2000).

Micro organisms eat and digest organic substances for nutrients and energy. Certain micro organisms digest organic substances such as fuels and solvents and turn them into harmless products such as carbon dioxide, methane and water. Bioremediation may rely either on indigenous micro-organisms (those that are native to the site) or exogenous micro-organisms (those that are imported from other locations). In other cases, bioremediation technologies optimize the environmental conditions so the appropriate micro-organisms will flourish and destroy the maximum amount of contaminants. Bioremediation can take place under aerobic or anaerobic conditions. Under aerobic conditions, micro organisms consume atmospheric oxygen to function. Under anaerobic conditions, no oxygen is present. In this case, the micro organisms break down chemical compounds in the soil to release the energy they need.

Bioremediation of areas of oil contamination is of interest because of the threat that such contamination has proved to be to the natural, terrestrial ecosystem, whose effects may be reflected in animals, plants and human beings. Soil which is contaminated by hydrocarbons have

extensive damage of local ecosystem since accumulation of pollutants in animals and plants may cause death or mutation (Alvarez et al.,1991). Crude oil is physically, chemically and biologically harmful to soil because it contains many toxic compounds in relatively high concentration (Erdogan and Karaca, 2011) .The presence of high molecular weight compounds with low solubility in water prevents natural biodegradation process from working efficiently in hydrocarbon contaminated soils. These compounds also penetrate macro-and micro pores in soil and thus limit water and air transport that would be necessary for organic matter conversion (Caravaca and Roldan, 2003). Generally petroleum hydrocarbon compounds bind to soil components and are difficult to remove and degrade.

Efforts to achieve biodegradation of oil products have involved bacteria and fungi, since they are the only biological species which have the metabolic capability of utilizing petroleum carbon for cell synthesis (Jobson et al, 1980). Most of the restoration of hydrocarbon contaminated sites depends on the activities of bacteria (microbial bioremediation) like *Pseudomonas Aeruginosa* or the use of plants (Phytoremediation) or by the use of fungi (Mycoremediation) (Olusola and Anslem, 2010).

Not all contaminants , however , are easily treated by bioremediation using micro organisms .For example , heavy metals such as cadmium and lead are not readily absorbed or captured by organisms .The assimilation of metals such as mercury into food chain may worsen matters. Phytoremediation is useful in this circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above –ground parts, which are then harvested for removal.

Bioremediation is usually conducted with high selectivity under mild conditions, offering an attractive alternate to classical physical methods of burning off the spills and scheming off the

spills through mechanical means. Burning of the spills leaves the land dead and uncultivable, devoid of nutrients and soil microbes, mechanical scheming has been found to be only 60% effective.

### **1.1 AIM**

- The aim of this project is to evaluate the impact of microbial load on the bioremediation of crude oil polluted soil.

### **1.2 OBJECTIVES**

- To study the impact of microbial load on the bioremediation of crude oil contaminated soil.
- To determine the bioremediation rate in the presence of varying concentration of microbes.
- To show that bioremediation is a cost effective treatment tool, if used properly.

### **1.3 SCOPE OF STUDY**

- Treatment of contaminated samples by the addition of micro organisms.

### **1.4 RELEVANCE OF STUDY**

- To compare the microbial concentration favourable for effective bioremediation.
- To show that the addition of nutrients (NPK) aids in bioremediation

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.0 BIOREMEDIATION**

Bioremediation is defined as the use of biological processes to degrade, breakdown, transform and essentially remove contaminants or pollutants from soil and water. Bioremediation is any process that uses micro organisms, fungi, plants or their enzymes to return the natural environment altered by contaminants to its original condition.

This research involves the general processes of bioremediation within the soil environment, focusing on the biodegradation of petroleum hydrocarbons. The impact of microbial load on the rate of biodegradation of hydrocarbons. The limitations and potential of both ex-situ and in situ bioremediation as a viable alternative to conventional remediation. (Lovely, 2003)

Many substances known to have toxic properties have been introduced into the environment through human activities. These substances range in degree of toxicity and danger to human health. Most of these substances either immediately or ultimately come in contact with the soil. Conventional methods to remove, reduce, or mitigate toxic substances introduced to the soil or ground water via anthropogenic activities and processes include pump and treat system, soil vapour extraction, incineration and containment utility of each of these conventional methods of treatment of contaminated soil or water. The emerging science and technology of bioremediation offers alternative methods to detoxify contaminants. Bioremediation has been demonstrated and been used as an effective means of mitigating.

- **Hydrocarbons**

- **Metals (Lead, Mercury, Chromium)**
- **Halogenated organic compounds**
- **Halogenated organic solvent**
- **Nitrogen compounds**
- **Non chlorinated pesticides and herbicides**

Natural occurring bioremediation and phytoremediation has been used for centuries. For example desalination of agricultural land by phytoremediation has a long tradition. (Meagher, 2000).

Bioremediation technology using micro organisms was reported to be invented by George .M. Robinson. He was the assistant counting petroleum engineer for Santa-Maria , California. During the 1960's , he spent his spare time experimenting with dirty jars and various mixtures of microbes. (Meagher,2000).

## **2.1 TYPES OF BIOREMEDIATION**

### **1. AEROBIC BIOREMEDIATION**

This occurs in the presence of sufficient oxygen (aerobic conditions) and other nutrients. Micro organisms will ultimately convert many organic contaminants to carbon dioxide, methane, water and microbial cell mass.

### **2. ANAEROBIC BIOREMEDIATION**

This occurs in the absence of oxygen (anaerobic conditions).The organic contaminants will be ultimately metabolized to methane, limited amounts of carbon dioxide and trace amounts of hydrogen gas.

### 2.1.1 EXAMPLES OF BIOREMEDIATION TECHNOLOGY

Bioremediation technology involves various naturally occurring mitigation processes;

- Natural attenuation
- Bioaugmentation
- Biostimulation
- **Natural Attenuation:** This involves bioremediation which occurs without human intervention. This natural attenuation relies on natural conditions and the behaviour of organisms that are indigenous to the soil. ( Meagher, 2000 )
- **Bioaugmentation:** This involves the introduction of exogenic micro organism (sources from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered micro organisms (Biobasics , 2006).

Bioaugmentation is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of Microbial cultures, containing all necessary microorganisms .Whereas activated sludge systems are generally based on microorganisms like bacteria, protozoa, nematodes, rotifers and fungi capable to degrade bio degradable organic matter.

- **Biostimulation:** This involves utilizing indigenous microbial populations to remediate contaminated soils. Biostimulation consists of adding nutrients and other substances of the soil to catalyze natural attenuation processes.

## **2.1.2 BIOREMEDIATION TECHNIQUES**

Bioremediation techniques involve two major categories; in-situ and ex-situ (which is further divided into solid and slurry). In the in-situ techniques, the soil and associated ground water is treated in place without excavation, while it is excavated prior to treatment with *ex situ* applications. Selection of appropriate technology among the wide range of bioremediation strategies developed to treat contaminants depends on three basic principles *i.e.*, the amenability of the pollutant to biological transformation (Biochemical process), the accessibility of the contaminant to micro organisms (Bioavailability) and the opportunity for optimization of biological activity (Bioactivity).

## **2.1.3 IN-SITU BIOREMEDIATION**

### **Biosparging**

This is an in-situ remediation technology that uses indigenous micro organisms to biodegrade organic constituents in the saturated zone. In biosparging , air (or oxygen) and nutrients are injected into the saturated zone to increase the biological activity of the indigenous micro organisms. It can be used to reduce concentrations of petroleum constituents that are dissolved in groundwater or adsorbed to soil (CPEO, 2009)

### **Bioventing**

This involves biodegradation of hydrocarbons by providing the micro organisms in the soil with oxygen to degrade. Bioventing enhances the activityof indigenous bacteria and simulates the natural in-situ biodegradation of hydrocarbons by inducing air or oxygen flow into the unsaturated zone and if necessary, by adding nutrients (CPEO, 2009)

## **2.1.4 EX-SITU BIOREMEDIATION**

### **Composting**

Composting is a process by which organic wastes are degraded by micro organisms, mostly at elevated temperatures. Typical compost temperatures are in the range of 55° to 65° C. The increased temperatures result from heat produced by micro organisms during the degradation of the organic material in the waste.

### **Biopiling**

Biopile treatment is a full-scale technology in which excavated soils are mixed with soil amendments, placed on a treatment area, and bioremediated using forced aeration. The contaminants are reduced to carbon dioxide and water.

The basic biopile system includes a treatment bed, an aeration system, an irrigation/nutrient system and a leachate collection system. Moisture, heat, nutrients, oxygen, and pH are controlled to enhance biodegradation. Treatment time is typically 3 to 6 months.

### **Land Farming**

This is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The aim is to stimulate indigenous micro organisms and facilitate their aerobic degradation of contaminants. These land farming activities cultivate and enhance microbial degradation of hazardous compounds (Theodore and Reynolds, 1987)

### **2.1.5 GENETIC ENGINEERING APPROACHES TO BIOREMEDIATION**

The use of genetic engineering to create micro organisms especially designed for bioremediation has great potential (Lovely, 2003). The bacterium *Deinococcus radio Durant* (the most radio resistant organisms known) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste (Brim et al, 2000)

### **2.1.6 BIOREMEDIATION APPLICATIONS**

Bioremediation techniques have been successfully used to remediate oil, sludges and ground water contaminants with petroleum hydrocarbons, solvents, pesticides and other organic chemicals. Case studies have demonstrated the effectiveness of aerobic microbial degradation of waste water treatment.

Bioremediation (nor any other remediation technology) cannot degrade inorganic contaminants, bioremediation can be used to change the valence state of inorganic compounds and cause adsorption, immobilization (<http://en.wikipedia.org/wiki.org>)

The process of bioremediation can be monitored indirectly by measuring the oxidation reduction in ground water, the pH, temperature, oxygen content, electron acceptor/donor concentration of breakdown products e.g carbon dioxide.

### **2.1.7 ADVANTAGES OF BIOREMEDIATION**

Bioremediation is attractive because of several advantages that its application has over more conventional technology. For instance, bioremediation techniques are typically more economical than traditional methods. It is estimated that bioremediation costs one-third to one half that of incineration and for complex mixtures of waste, bioremediation typically offers savings of 60-

90% over landfill-disposal costs. Additionally, by using in situ bioremediation, the risk of exposure to cleanup personnel is greatly reduced. The risk of wide exposure due to transportation accidents is totally eliminated by using in-situ bioremediation. Furthermore, the techniques can be expected to have minimal environmental impact since bioremediation usually results in the complete degradation of the contaminants; therefore, waste products are usually not generated.

Why use *in situ* bioremediation to treat contaminated soils?

- Land filling only moves the contamination, it does not treat it.
- Many countries are running out of landfill space.
- Soil itself is a valuable resource--it should be reused, not thrown away. ([www.cee.vt.edu](http://www.cee.vt.edu))

### **2.1.8 DISADVANTAGES OF BIOREMEDIATION**

However, there are also a number of disadvantages associated with bioremediation. In order for organisms to successfully reduce pollutant levels, their growth conditions must be determined and maintained at the contaminated site. Controlling these conditions may prove difficult, particularly as conditions may vary from site to site. Even in an ideal environment, an organism may prefer to metabolize other more readily available nutrients within a contaminated area instead of the contaminant, or the contaminant may be partially or completely inaccessible to the degrading organism ([www.cee.vt.edu](http://www.cee.vt.edu))

Factors that may limit the effectiveness and applicability of the process include:

- High concentrations of heavy metals are likely to be toxic to micro organisms.
- Bioremediation is slow at low temperature.

- Preferential colonization by microbes may occur, causing clogging of nutrients and water injection well.

## **2.2 THEORY**

Soil bioremediation is the process in which most of the organic pollutants are decomposed by soil micro organisms and converted to harmless end products such as carbon dioxide, methane and water (Walter et al., 1997). As no single microbial species is capable of degradation all components of crude oil, complete oil degradation requires simultaneous action of different microbial populations. One of the factors limiting biodegradation of soil pollutants is their limited availability to micro organisms (Providenti et al., 1995).

Soil micro organisms are a fundamentally important component of terrestrial habitats. Their primary roles are governing the nutrient cycles and involving in genesis and maintenance of soil structure. A characteristic feature of soil micro organisms is their complexity, both in terms of numbers of organisms and their genetic and functional diversity. This feature is usually termed as soil microbial diversity and describes the variability of biological organization at different levels in soil. In soil microbial universe, certain microbes have a distinctive ability to degrade or to convert organic pollutants to harmless biological products. The fact of bioremediation mainly relies on the use of these talented micro organisms surviving in soil.

There are many biological techniques used in the cleanup of land and water sources (i.e., bioventing, bioslurping, hydraulic-pneumatic fracturing, soil bioinjection, air and water flushing, biopolymer shields, electrobioreclamation and phytoremediation). However, most of these techniques are highly expensive, preferred for cleanup of deep soil layers and may be limited in terms of soil properties and environmental conditions (Erdogan and Karaca, 2011). These several

technologies that biostimulations involves the addition of oxygen, water and mineral nutrients (Orzech et al., 1991; Turgay et al., 2010); bioventing technique combines conventional soil venting with biodegradation (Van Eyk, 1994; Reisinger et al., 1994).

Apart from the presence of the micro organisms capable of degrading crude oil, the rapidity with which this oil can be degraded is determined by the nature of the molecular configuration of the hydrocarbon that make up the crude oil. Generally, alkanes are attacked by microbial species more rapidly and support more growth than naphthenic compounds. Within the alkane series, straight-chained compounds are easily degraded than branched chained compounds, in other words biodegradability decreases with the chain length of the branches.

However in ring compounds, bacteria oxidizes polycyclic hydrocarbons more readily than benzene and an introduction of alkyl group into the benzene ring tends to render the resulting compound more susceptible to microbial oxidation.

Majority of the crude oil degrading bacteria require oxygen. Oxygen is often a limiting factor in oil degradation in environments where rapid microbial activity uses up oxygen much faster than it can be supplied. Oil degradation requires the presence of mineral elements such as carbon, magnesium, nitrogen, calcium, phosphorus, and potassium in addition to various trace elements. Lack of these elements may restrict degradation by limiting the growth of these organisms. Some heavy metals for example, Cu, Zn, Cr, Ni and Fe are essential for the growth of micro organisms in trace amounts but have also been shown to be toxic at high concentrations (Yamamoto et al, 1985; Baldrian, 2003) and their addition in soil has been known to inhibit soil respiration, nitrogen mineralization and nitrification (Baath, 1989; McGrath, 1994). Heavy metals have been implicated in the reduction of degradation of vegetable materials (Baath, 1989)

and can potentially limit the biodegradation of organic contaminants in the environment (Sokhn et al., 2001; Riis et al., 2002; Atagana, 2006).

### **2.3 MICROBIAL GROWTH**

Micro organisms multiply during the acceleration phase to the period of exponential growth, which continues until the food supply becomes exhausted. It then proceeds to the stationary phase during which organisms cease to grow and then further proceeds to the period of cell death (endogenous phase) as the cell population dies. Microbial growth is often restricted to cells which are fully in their environment and are growing exponentially. In the case of continuous culture, this requirement is normally fulfilled in batch culture where the lag phase assumes more importance since it represents a significant proportion of the fermentation takes place.

Exponential growth is the most extensively quantified of the phases of microbial growth and in the case of growth-associated products is very important. The Monod equation can be related to the growth of micro organism culture to the prevailing feed concentration. Though, the microbe may well require several substrates for its growth to proceed, it is assumed that all but one is in excess of the requirement and the substance to which is related is the limiting substrate component. (<http://www.co.sandfood.edu>)

### **2.4 MICROBIAL ACTIVITY IN BIOREMEDIATION**

Microbial degradation of hydrocarbon is initiated by the production of bio-surfactants from certain species of bacteria. These surfactants molecules encapsulate a small quantity of oil,

which is then transported across the bacteria cell wall and membrane. Once the oil has been internalized, microbial biodegradation occurs in two main routes;

- Straight chain compounds are first oxidized to alcohol then dehydrogenated to form an aldehyde which is then converted to carboxylic acid then transformed into acetic acid.
- Two hydroxyl groups are added to aromatic molecules via a dioxygenase in order to saturate a pair of adjacent carbons.

Both acetic acid and the adipate are then assimilated into the microbial tricarboxylic acid cycle (TCA) for the production of energy in the form of ATP. However, because biodegrading pathways for each compound are catalyzed by specific enzymes, a single strain of bacterium cannot process all the hydrocarbons present in the crude oil (US EPA Guide, 2006).

Several organisms are known, each capable of degrading usually one or, at best, a few petroleum components at a time. Therefore, effective bioremediation of petroleum contamination requires a mixture of population consisting of different genera each capable of metabolizing the respective compound. Bioremediation refers to the enhancement of this native capability of the micro organisms (Bouwer and Zehner, 1993). The table below shows some examples of microbes used in bioremediation.

**Table 2.4 Microbial genera degrading hydrocarbons in soil**

<b>Bacteria</b>	<b>Actinomycetes</b>	<b>Fungi</b>	<b>Yeast</b>
Pseudomonas	Actinomyces	Aspergillus	Candida
Bacillus	Endomyces	Cephalosporium	Rhodotorula
Bacterium	Nocardia	Cunninghamella	Torula

Clostridium		Torulopsis	
Thiobacillus		Trichoderma	
Methanobacterium		Saccharomyces	
Enterobacter			

The right microbes are those bacteria or fungi, which have the physiological and metabolic capabilities to degrade the contaminants. In many instances these organisms will already be present at the site (indigenous microbes). In other circumstances, such as bioreactors treating wastes with high concentration of toxic materials, there may be a need to add exogenous microbes to the material. In order for the microbes to degrade the contaminants they must be in close proximity to the contaminants (Baker and Diana, 1994). Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area. The term mycoremediation refers specifically to the use of fungal mycelia in bioremediation.

One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extra cellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fibre. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant (Singh, 2006).

Once the right micro organisms are present in the right place, the environmental conditions must be controlled or altered to optimize the growth and metabolic activity of the microbes. Such environmental factors as temperature, inorganic nutrients (primarily nitrogen and phosphorous),

electron acceptors (oxygen, nitrate, sulphate and pH) can be modified to optimize the environment for bioremediation (Ugochukwu et al, 2008).

## **2.5 BIOCHEMISTRY OF BIOREMEDIATION**

Crude oil is highly complex mixture, containing hundreds of thousands of hydrocarbons (Cooney, 1980). Compounds in crude oil can be divided into three general classes consisting of saturated hydrocarbons, aromatic hydrocarbons and polar organic compounds (Huesemann and Moore, 1993). The release of crude oil into the environment is subject to a variety of physical, chemical, and biological changes. The evaporation of light component will take place immediately and up to 40% of the crude oil may evaporate within a short period of time. This process may lead to an increase in the velocity of the spilled oil. Another part of the oil will be loaded into the soil since volatile hydrocarbon compounds such as solubility, which is toxic. (Barry, 2007).

Basically, crude oil is a combination of long chain hydrocarbons, hence it involves the degradation of long chain alkanes. The long chain degradation takes place through two mechanisms, which are

- The terminal oxidation pathway
- The sub terminal oxidation pathway

In the terminal oxidation pathways, one or both of the terminal methyl group are oxidized in the primary and secondary stages of the pathways can either be mono terminal or di-terminal. The primary oxidation step in alkane molecules, started with initial reactions which are catalyzed by an oxygenase are followed by

- A mixed function oxidation system without involvement of cytochrome

- A mixed function oxidation system with involvement of cytochrome
- Formation of hydro-peroxides via free radical intermediates and reduction of primary and secondary alcohol.

In the secondary oxidation step, the product from the cell liquids are biodegraded through the various microbial degradation pathways this is done through the mono terminal, di-terminal and sub-terminal oxidation pathway. (Madhu, 2002)

## **2.6 CRUDE OIL**

Crude oil is a complex liquid mixture of organic molecules, mostly hydrocarbon with varied chemical and physical properties. Crude oil is physically, chemically and biologically harmful to soil because it contains many toxic compounds in relatively high concentration (Franco et al, 2004). The presence of high molecular weight compounds with very low solubility in water prevents natural biodegradation process from working efficiently in hydrocarbon contaminated soils.

## **2.7 BIODEGRADATION OF CRUDE OIL**

Biodegradation can be defined as process by which microbes alter and breakdown petroleum hydrocarbons into other substances such as carbon dioxide, water, microbial biomass and methane. Biodegradation is highly used in the weathering and eventual removal of petroleum from the environment, particularly the non-volatile components of petroleum. Bacteria that consume hydrocarbons as “hydrocarbon oxidizers” because they oxidize compounds to bring about degradation. The higher the molecular weight of the polycyclic aromatic hydrocarbon, the slower the rate of its degradation (Owabor et al.,2009). The extent of biodegradation of

hydrocarbons by microbes is directly measured by noting the amount of total hydrocarbons that were degraded. The activity of aerobic microbes can be measured by the amount of oxygen they consume or the amount of carbon dioxide they produce.

## **2.8 CAUSES OF OIL SPILLS**

An oil spill is a release of a liquid petroleum hydrocarbon into the environment due to human activity, and is a form of pollution. Oil spills include releases of crude oil from tankers, offshore platforms, drilling rigs and wells, as well as spills of refined petroleum products (such as gasoline, diesel) and their by-products, and heavier fuels used by large ships such as bunker fuel, or the spill of any oily refuse or waste oil.

Sites contaminated by petroleum compounds range from leaking household oil tanks to areas polluted by oil tanker spills, e.g., old and new petrol stations as well as areas surrounding oil storage facilities, pipelines, terminals and refineries (Erdogan and Karaca, 2011).

Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention. Many accidents can cause soil pollution and, for this reason, many techniques are being developed to clean up petroleum contaminated soil (Millioli et al, 2009).

In Nigeria, 50% of oil spills is due to corrosion, 28% to sabotage and 21% to oil production operations, with 1% of the spills being accounted for inadequate or non-functional production equipment, inability of effectively control oil wells, failure of machines and inadequate care in loading and offloading of oil vessels (8). This loss is a result of lack of regular maintenance of the pipelines and storage tanks. The reason that corrosion accounts for a such a high percentage

of oil spills is as a result of the small size of the oilfields in the Niger Delta, there is an extensive network of flow-lines, allowing many opportunities for leaks (5). Sabotage is performed primarily through what is known as “bunkering” , in the attempts to tap the pipelines , it is mostly destroyed or damaged (10). Damaged lines may go unnoticed for days and repairs the damage pipes even longer.

## **2.9 OIL SPILL DISPERSAL**

The removal of the hydrocarbon from soil surface is an essential practice in preventing ground soil contamination. The efficiency of crude oil removal from contaminated soil requires detailed information of the soil type and composition of the crude oil. Crude oil when spilled undergoes certain process like biodegradation, evaporation, dissolution, and photochemical oxidation. Factors such as wind drift current, wind waves, sea topography and the density of the oil affect oil spill dispersal (Akpofure et al, 2000). For instance, heavy crude oil does not readily penetrate porous media. The average or medium crude oil is more toxic than the heavy crude oil and has the tendency to penetrate into porous surfaces.

## **2.10 FATE OF SPILLED OIL**

### **Biodegradation**

This can be defined as process by which microbes alter and breakdown petroleum hydrocarbons into other substances such as carbon dioxide, water, microbial biomass and methane. This occurs when micro organism such as bacteria or fungi feed on the oil (Diaz, 2008)

**Evaporation**

This occurs when the lighter or more volatile substances within the oil mixture turn to vapour and leaves the surface of the water, therefore leaving the heavier components (Nester et al, 2001).

**Emulsification**

This is the process that forms emulsion, which are mixtures of small droplets of oil and water emulsions are formed by wave action. This is actually a change in state from oil-in-water dispersion to water-in-oil emulsion with a resulting thick, sticky mixture containing up 80% water (Akpofure et al, 2000)

**Weathering**

This involves a series of chemical and physical changes that caused spilled oil to break down and become heavier than water.

**Dissolution**

Dissolution of petroleum hydrocarbons into water column poses risk to aquatic organisms because of the acute toxicity of the compound that have significant water solubility (US EPA, 2006)

## **2.11 WAYS OF PREVENTING OIL SPILLS**

The preferred method to reduce and control environmental pollution is to prevent the pollution. Preventive measures must be integrated into any planned industrial process, operation, or product as part of the cost of daily operations. Certain measures have been put in place to prevent such forms of pollution.

- Secondary containment methods to prevent releases of oil or hydrocarbons into environment.
- Oil Spill Prevention Containment and Countermeasures (SPCC) program by the United States EPA
- Double hulling builds: which involves the construction of oil carrying tankers with two layers?
- Proper security measures to protect pipelines from possible vandalization.
- Maintenance and replacement of worn out pipelines.

## **2.12 HEALTH AND ENVIRONMENTAL CONCERNS**

Crude oil is a mixture of hydrocarbons; it contains aromatics such as Benzene which is associated with various diseases. Some trace elements found in crude oil are harmful to plants, wildlife and habitats.

### **2.12.1 EFFECT OF CRUDE OIL SPILL ON MAN**

Crude oil contains Benzene, which is associated with various blood disorders, anaemia, and leukaemia in humans, and PAHs, which have been shown to produce skin tumors in laboratory

animals after prolonged and repeated contact. Other adverse effects include; eye and respiratory irritation, skin irritation and risk of impaired fertility. Contamination of marine life has negative consequences for human health from consuming contaminated seafood (Nwilo and Badejo, 2004)

### **2.12.2 EFFECT OF CRUDE OIL SPILL ON SOIL**

Oil spills has also destroyed farmland and polluted ground soil. Oil deposited on leaves of plants, penetrates the leaves causing the reduction of transpiration and photosynthesis. Oil contaminated soil become barren and are no longer fertile to aid growth. In agricultural communities, often a year's supply of food can be destroyed instantaneously. Due to the careless nature of oil operations in the Delta, the environment is growing increasingly inhabitable (Baird, 2010).

### **2.12.3 EFFECT OF CRUDE OIL SPILL ON THE ENVIRONMENT**

Oil spill has adverse effect on marine life, which in turn has negative effects on human health. Sea animals are being exposed to oil and most likely ingest large quantities of it leaving them contaminated. Oil spills has a major impact on the ecosystem into which it is released. An estimated 5 to 10 % of Nigerian mangrove ecosystems have been wiped out either by settlement or oil (Bronwen, 2007). Spills in populated areas often spread out over a wide area, destroying crops and aquacultures through contamination of groundwater and soil. The consumption of dissolved oxygen by bacteria feeding on the spilled hydrocarbons also contributes to the death of sea life.

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHOD**

#### **3.1 LIST OF MATERIALS USED AND THEIR SOURCES**

- **SOIL**

The soil sample was collected from the Chemical Engineering Department, University of Benin, Ugbowo. Large particles of stones and soil were removed.

- **CRUDE OIL**

Crude oil was obtained from Oredo flow station Ologbo (NPDC). A dark green viscous liquid with specific gravity of 0.825 was used to pollute the soil.

- **NPK FERTILIZER**

It was obtained from the Department of Crop Science, Faculty of Agriculture, University of Benin.

- **N-HEXANE**

- **ASPERGILLUS N**

### **3.2 LIST OF EQUIPMENTS USED**

- pH meter
- Beaker, 100ml
- 250 ml Separating funnel
- Spectrophotometer
- Pipette, 10ml
- Mechanical Shaker
- Cuvette
- Conical flask, 100ml
- Volumetric flask, 100ml

### **3.3 PROCEEDURE**

1kg of soil sample was weighed into six plastic buckets and was mixed with 100g of crude oil. One sample will serve as a control ,which will contain only crude oil and soil. 40g of NPK 15:15:15 was added to the remaining five samples which will serve as test samples. Aspergillus Niger of various concentrations ( $1 \times 10^6$ ,  $2 \times 10^6$ ,  $5 \times 10^6$ ,  $1 \times 10^7$  and  $2 \times 10^7$ ) was added to the test samples and labelled respectively.

The samples were allowed to stay for 1week, so as to allow the indigenous microbes to adapt to its new environment before taking the readings. Parameters such as pH, Residual Hydrocarbon Content (RHC) and Total Microbial Count (TMC) were monitored over a period of seven (7) weeks.

### **3.4. DETERMINATION OF pH**

#### **❖ APPARATUS**

- i. pH
- ii. Beaker, 100ml
- iii. Stirrer

#### **❖ PROCEEDURE**

20g of soil sample was weighed into a 100ml beaker and 20ml of distilled water was added. The mixture was stirred thoroughly and allowed to stand for 30mins. The pH of the sample was then measured using a pH meter.

### **3.5. DETERMINATION OF RESIDUAL HYDROCARBON**

#### **❖ APPARATUS**

- i. 250ml Separating funnel
- ii. Spectrophotometer
- iii. Pipette, 10ml
- iv. Mechanical Shaker
- v. N-Hexane

#### **❖ PROCEEDURE**

5g of the sample was weighed into a 100ml plastic bottle and 25ml of n-Hexane was added to it, the mixture was then shaken for 10 minutes, covered and let to stand. It was filtered out and the filtrate was read at 460nm.

### **3.6. DETERMINATION OF TOTAL MICROBIAL COUNT**

#### **❖ APPARATUS**

- i. A microscope
- ii. Counting Chamber
- iii. Wire-loop
- iv. Sensitive weighing balance
- v. Diluents for soil sample
- vi. Methylene blue (0.1%) solution

#### **❖ PROCEDURE**

Prepare dilution of the soil sample by washing soil with distilled water and diluting using the diluent already prepared (obtain  $10^{-1}$ ,  $10^{-3}$  and  $10^{-6}$  dilutions). Assemble the Colony counting chamber by applying the cover glass. Add few drops of Methylene blue solution to water sample and dilution. With a standard loop place a loop-full of water sample (including the various dilution) on the ruled area of the counting chamber. Allow the chamber to rest for 5mins. Examine under a microscope using a four mm lens (x 16 objective lens) to count the bacteria in 50-100 square selected at random, so that the total number of bacteria is about 500. For each sample obtain a triplicate count divide the number of count by the number of squares and multiply the result by the dilution factor and a constant k. This gives the number of organism in a milliliter of the given water sample.

## CHAPTER FOUR

### 4.0. RESULTS AND DISCUSSION

The results obtained for the pH of samples of varying concentrations over a period of seven weeks is given in Table 4.1

**Table 4.1 : VALUES OF pH WITH TIME**

Time (weeks)	Control	NPK + $1 \times 10^6$ cfu/g	NPK + $2 \times 10^6$ cfu/g	NPK + $5 \times 10^6$ cfu/g	NPK + $1 \times 10^7$ cfu/g	NPK + $2 \times 10^7$ cfu/g
1	5.3	5.4	5.7	5.6	5.4	5.8
2	5.3	5.5	5.6	5.4	5.5	5.8
3	5.2	5.6	5.3	5.7	5.6	5.6
4	5.5	5.6	5.6	5.6	5.4	5.7
5	5.8	5.7	5.8	5.8	5.6	5.7
6	5.7	5.8	5.7	5.8	5.8	5.6
7	5.9	5.8	5.8	5.9	5.8	5.8

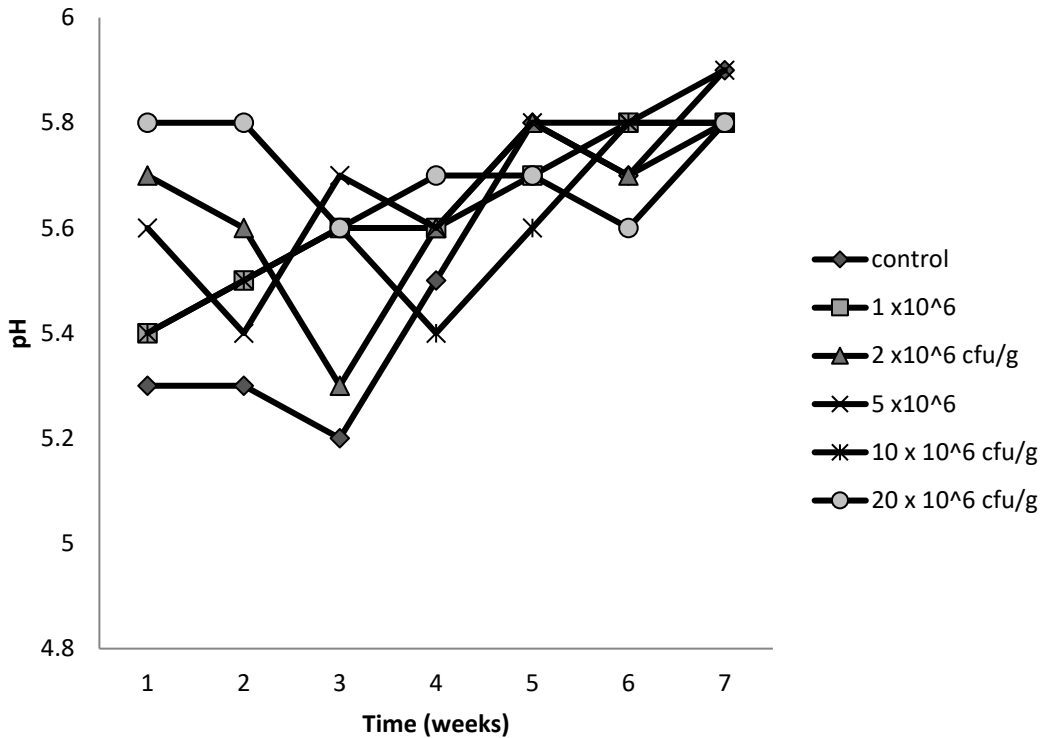


Fig 4.1 : Variation of pH with time.

The results show the amount of pH of the samples over a period of 7 weeks. The control sample containing only crude oil and soil had the lowest pH value 5.3 on the first week. There was a slight drop in pH to 5.2 on the third week as a result of the indigenous microbes low resistance to acidic toxins released by the crude oil. At this point the indigenous microbes are adjusting to the new environment and as such likely to be less resistant. Over time there was a steady increase in pH as a result of the microbes which tend to increase the pH of the soil due to their alkaline nature. At this phase the micro organisms have settled into their new environment and have increased in population therefore increasing their microbial activity. On week seven the pH had increased to 5.9.

The pH of the test samples impacted with NPK and *Aspergillus Niger* showed an increase in the pH of the soil. The sample with the least amount of *Aspergillus Niger* ( $1 \times 10^6$  cfu/g) was seen at its lowest pH of 5.4 on the first week. This value is higher than that of the control on the first week which indicates the presence of additional micro organisms and the effect of NPK which boosts the microbial activity by providing them with nutrients. Over time the pH of the samples were constantly increasing and by week seven the pH had increased to 5.8. It was also observed that the pH of the test samples on the first week kept increasing with the increase in microbial concentration. The sample impacted with least amount of micro organisms ( $1 \times 10^6$  cfu/g) had a pH of 5.3 while that impacted with the highest amount ( $2 \times 10^7$  cfu/g) had a pH of 5.8 on week one. The pH of the samples increase with time, indicating the presence of the micro organisms which have increased in population and activity, hence the pH tending towards alkalinity. On the seventh week, the results showed that the pH of the control and the test samples were relatively close ranging from 5.8-5.9. This shows the extent to which bioremediation had taken place in restoring the soil to its initial pH before contamination. The pH of soil ranges from 6.0-7.0, therefore the results of the sample on the seventh week shows the efficiency of bioremediation

**Table 4.2: VALUES OF RESIDUAL HYDROCARBON CONTENT WITH TIME (mg/kg)**

<b>Time (weeks)</b>	<b>Control</b>	<b>NPK + <math>1 \times 10^6</math> cfu/g</b>	<b>NPK + <math>2 \times 10^6</math> cfu/g</b>	<b>NPK + <math>5 \times 10^6</math> cfu/g</b>	<b>NPK + <math>1 \times 10^7</math> cfu/g</b>	<b>NPK+ <math>2 \times 10^7</math> cfu/g</b>
<b>1</b>	<b>183</b>	<b>179</b>	<b>181</b>	<b>181</b>	<b>180</b>	<b>184</b>
<b>2</b>	<b>163</b>	<b>157</b>	<b>158</b>	<b>153</b>	<b>150</b>	<b>152</b>
<b>3</b>	<b>137</b>	<b>128</b>	<b>123</b>	<b>119</b>	<b>110</b>	<b>104</b>
<b>4</b>	<b>104</b>	<b>98</b>	<b>88</b>	<b>80</b>	<b>69</b>	<b>61</b>
<b>5</b>	<b>86</b>	<b>78</b>	<b>67</b>	<b>58</b>	<b>49</b>	<b>38</b>
<b>6</b>	<b>59</b>	<b>43</b>	<b>39</b>	<b>34</b>	<b>26</b>	<b>16</b>
<b>7</b>	<b>27</b>	<b>10</b>	<b>9</b>	<b>8</b>	<b>6</b>	<b>4</b>

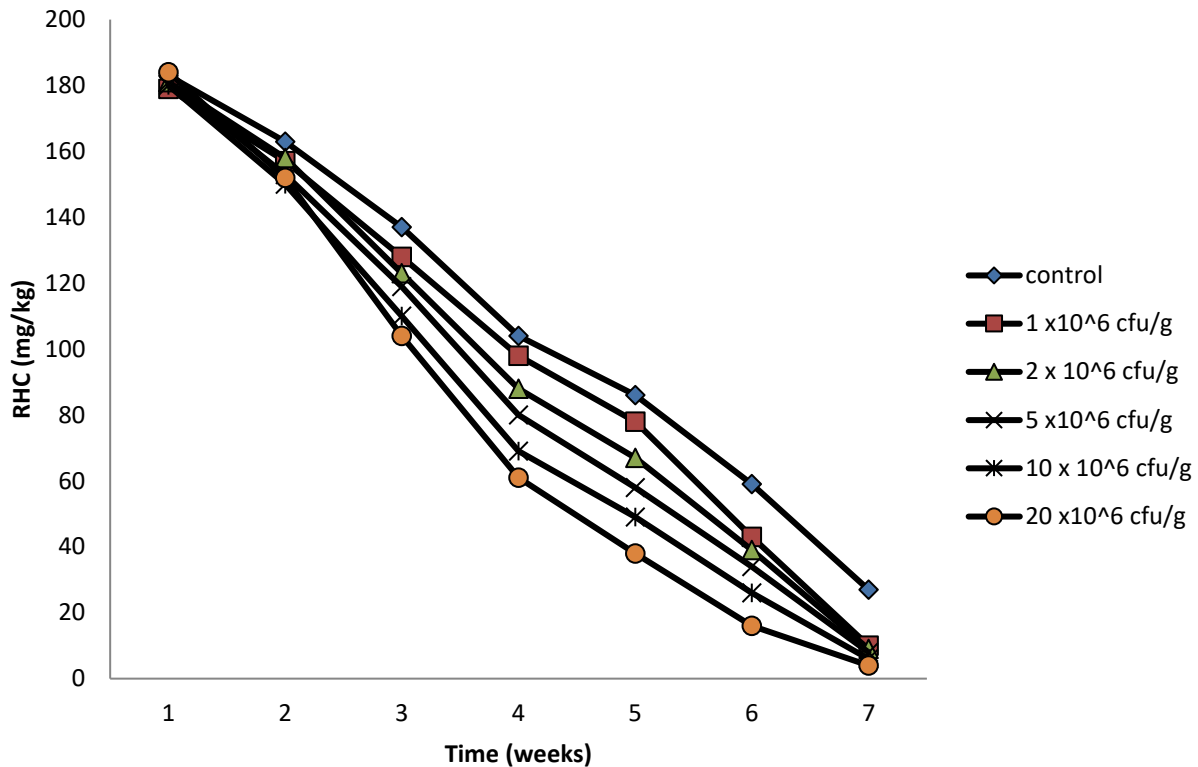


Fig 4.2 : Variation of Residual Hydrocarbon Content with time

The results show the amount of residual hydrocarbon content over a period of seven weeks. The control sample on the first week had a RHC of 183mg/kg and constantly decreased with time. It had about a 85.3% decrease from 183mg/kg on the first week to 27mg/kg on the seventh week. This proves that the indigenous microbes in the soil were able to biodegrade the soil. The test samples which were impacted with NPK and *Aspergillus Niger* also decreased at a constant rate. The amount of RHC in the sample containing NPK +  $2 \times 10^7$  ml had the lowest amount of RHC 4mg/kg while the control sample had 27mg/kg. This clearly shows the effect the concentration of micro organisms has on the degradation process. It was observed that as the weeks progressed, the THC reduces, indicating the increase in micro organisms which multiplied over time, i.e the higher the concentration of micro organisms, the higher the rate of degradation. Therefore bioremediation was seen to be highly efficient with the increase in microbial population.

**TABLE 4.3 : VALUES OF TOTAL MICROBIAL COUNT WITH TIME**

<b>Time (weeks)</b>	<b>Control</b>	<b>NPK + 1×10<sup>6</sup> cfu/g</b>	<b>NPK + 2×10<sup>6</sup> cfu/g</b>	<b>NPK + 5×10<sup>6</sup> cfu/g</b>	<b>NPK + 1×10<sup>7</sup> cfu/g</b>	<b>NPK+ 2×10<sup>7</sup> cfu/g</b>
<b>1</b>	<b>0.7×10<sup>6</sup></b>	<b>1.4×10<sup>6</sup></b>	<b>2.6×10<sup>6</sup></b>	<b>5.4×10<sup>6</sup></b>	<b>1.8×10<sup>7</sup></b>	<b>2.5×10<sup>7</sup></b>
<b>2</b>	<b>0.9×10<sup>6</sup></b>	<b>1.7×10<sup>6</sup></b>	<b>2.7×10<sup>6</sup></b>	<b>5.6×10<sup>6</sup></b>	<b>1.9×10<sup>7</sup></b>	<b>2.8×10<sup>7</sup></b>
<b>3</b>	<b>0.8×10<sup>6</sup></b>	<b>1.8×10<sup>6</sup></b>	<b>2.9×10<sup>6</sup></b>	<b>5.6×10<sup>6</sup></b>	<b>2.0×10<sup>7</sup></b>	<b>2.9×10<sup>7</sup></b>
<b>4</b>	<b>0.9×10<sup>6</sup></b>	<b>1.8×10<sup>6</sup></b>	<b>2.9×10<sup>6</sup></b>	<b>5.8×10<sup>6</sup></b>	<b>2.0×10<sup>7</sup></b>	<b>2.8×10<sup>7</sup></b>
<b>5</b>	<b>1.0×10<sup>6</sup></b>	<b>2.0×10<sup>6</sup></b>	<b>2.8×10<sup>6</sup></b>	<b>5.7×10<sup>6</sup></b>	<b>2.1×10<sup>7</sup></b>	<b>2.9×10<sup>7</sup></b>
<b>6</b>	<b>1.2×10<sup>6</sup></b>	<b>2.1×10<sup>6</sup></b>	<b>2.9×10<sup>6</sup></b>	<b>5.8×10<sup>6</sup></b>	<b>2.3×10<sup>7</sup></b>	<b>3.1×10<sup>7</sup></b>
<b>7</b>	<b>1.2×10<sup>6</sup></b>	<b>2.2×10<sup>6</sup></b>	<b>3.1×10<sup>6</sup></b>	<b>6.0×10<sup>6</sup></b>	<b>2.6×10<sup>7</sup></b>	<b>3.4×10<sup>7</sup></b>

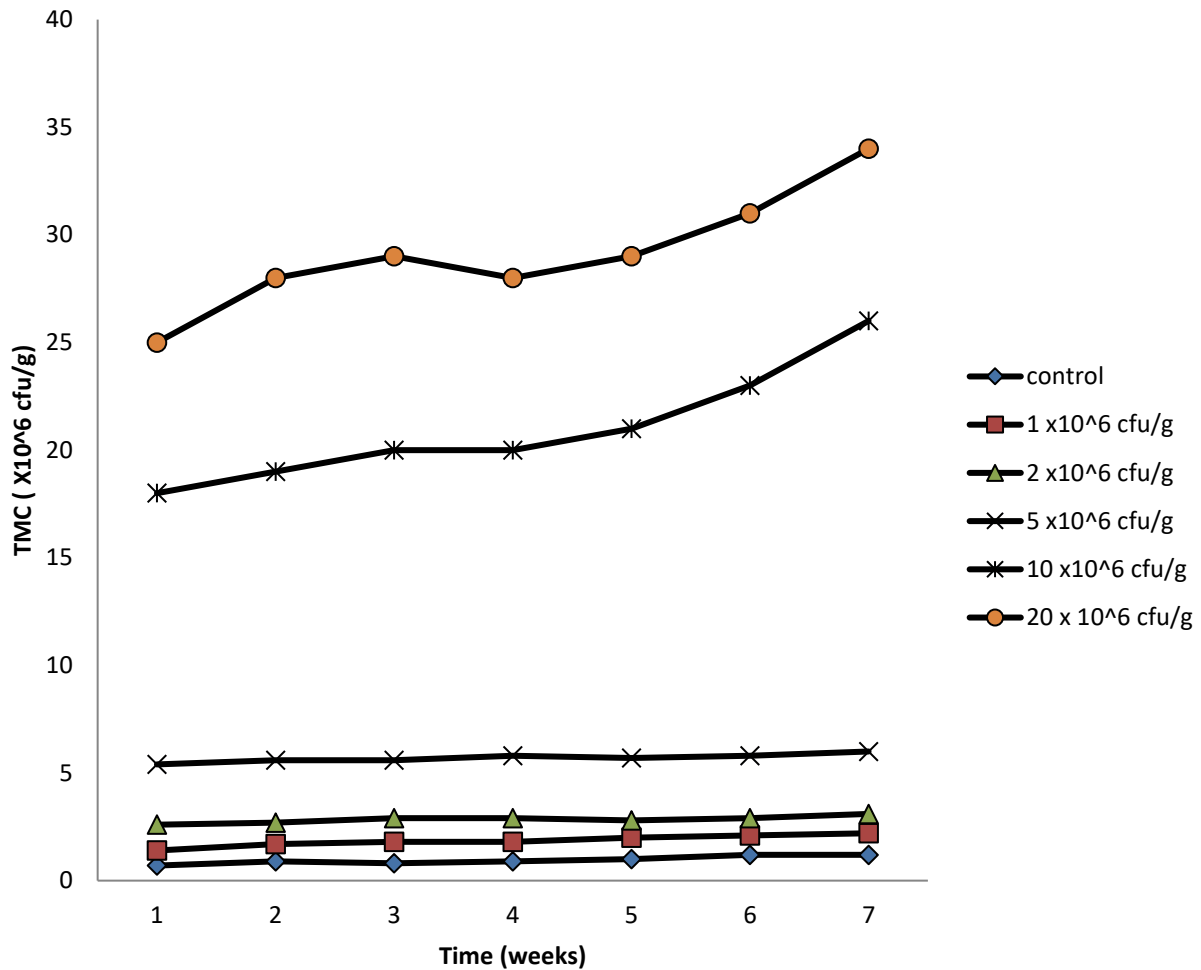


Fig 4.3: Variation of Total Microbial Count with time.

The results above show the microbial population present in the samples.

The control sample which contains only soil and crude oil is observed to contain the least amount of microbes of  $1.2 \times 10^6$  cfu/g, because the only microbes present are the indigenous microbes contained in the soil. The amount of micro organism multiply with time. The addition of the NPK aids microbial growth by providing nutrients for the micro organism. For each concentration of micro organism, there was an increase. The sample containing the highest concentration of microbes  $2 \times 10^7$  cfu/g after the seven weeks period had multiplied to  $3.4 \times 10^7$  cfu/g. This showed a steady increase in microbial population over time.

## **CHAPTER FIVE**

### **5.0 CONCLUSION AND RECOMMENDATION**

#### **5.1 CONCLUSION**

The study revealed that the rate of degradation was best and fastest in the sample with the highest microbial load i.e the higher the microbial load, the higher the rate of degradation.

Therefore the impact of microbial load on bioremediation can be observed.

It was also observed that nutrients such as NPK served as a good supplement for the growth of the degrading bacteria present in bioremediation.

In summary, the appropriate microbial load with nutrients of equivalent weight is an effective means of bioremediation.

#### **5.2 RECOMMENDATION**

- Bioremediation using equivalent nutrients is an effective means of biodegradation.
- Microbial nutrients like NPK fertilizer should be introduced into the system so as to supply Nitrogen, Phosphorous and Potassium to the oil degrading microbes.
- Bioremediation using mixed microbes and nutrients in the present in the polluted environment should be greatly encouraged and more research should be carried out in this regard.

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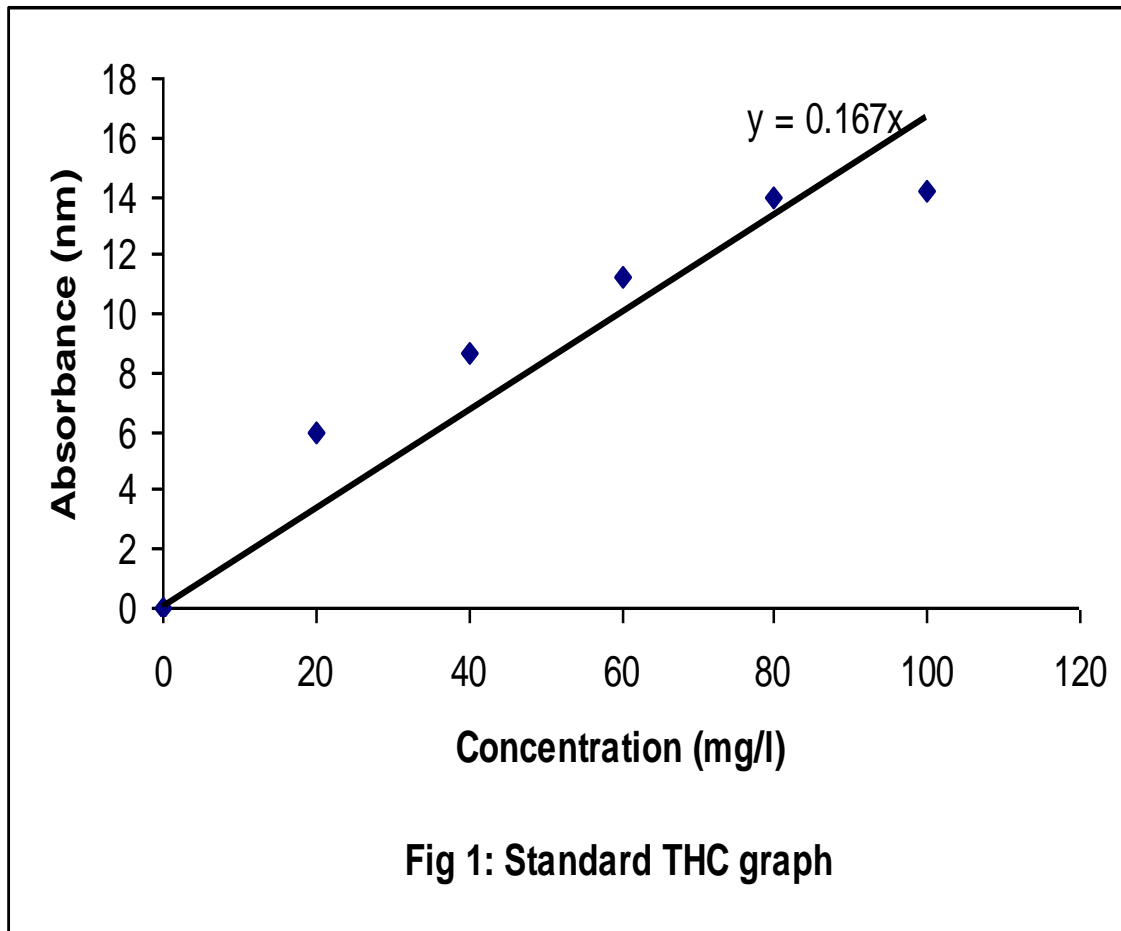
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## APPENDIX



$$\text{RHC (mg/kg soil)} = \frac{\text{Absorbance} \times \text{CF} \times \text{DF} \times \text{EV}}{\text{Weight of soil}}$$

Where

CF = conversion factor from absorbance to mg/l extract

DF = Dilution factor

EV = Extract volume of solvent (L)

