UNIVERSITY OF BENIN

FACULTY OF ENGINEERING

DEPARTMENT OF PETROLEUM ENGINEERING

A

PROJECT WORK

ON

BIOREMEDIATION OF USED ENGINE OIL POLLUTED SOIL USING GOAT MANURE

BY

ISAAC GODWIN UTIP

ENG1503990

JULY 2021.

BIOREMEDIATION OF USED ENGINE OIL POLLUTED SOIL USING GOAT MANURE

BY

ISAAC GODWIN UTIP

ENG1503990

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PETROLEUM ENGINEERING, FACULTY OF ENGINEERING, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF ENGINEERING (B.ENG) DEGREE IN PETROLEUM ENGINEERING.

JULY 2021.

CERTIFICATION

This is to certify that this project titled "Bioremediation of used engine oil polluted soil using goat manure" was carried out by ISAAC GODWIN UTIP of the Department of Petroleum Engineering with matriculation number ENG1503990 in partial fulfilment of the requirements for the Award of the Degree, Bachelor of Engineering (B.ENG).

ENGR. (DR) ONAIWU ODUWA	DATE
(PROJECT SUPERVISOR)	
ENGR. TAIWO OLUWASEUN	DATE
(PROJECT COORDINATOR)	
DR. IKPONMWOSA OHENHEN	DATE
(HEAD OF DEPARTMENT)	
PROF. S. O. ISEHUNWA	DATE
(EXTERNAL SUPERVISOR)	

DEDICATION

This project is dedicated to God Almighty for his grace and strength accorded to me to be able to complete this study without any misfortune. And also to my parents Mr. and Mrs. Utip Isaac for their support.

ACKNOWLEDGEMENT

I am grateful to the Almighty God for his faithfulness throughout this project. I am also grateful to my Supervisor Dr. Onaiwu Oduwa who has been the perfect supervisor of which his advice, insightful criticisms and patient encouragements aided the successful completion of this project which led to the writing of this comprehensive.

I am also grateful to Dr. Ilaboya for his support, supervision and for not giving up on me, I want to thank him for his advice, patience and for being a good instructor and teacher.

I am thankful to the Department of Petroleum Engineering, University of Benin, for all the knowledge they have impacted in me.

My profound gratitude also extends to Mr. and Mrs. Utip Isaac for their financial support, advice and prayers and to my project colleagues and also to my friends Success Oghogho, Kennedy Oloma, God's power Saturday and Abigail Asiri for their moral support and contribution into my life.

ABSTRACT

Hydrocarbon contamination of land, water, air, vegetation and human is a widespread global environmental concern. The aim of this study was to evaluate the performance of goat manure for the bioremediation of used engine oil polluted soil. 10kg soil sample was collected from a site free of used engine oil contamination (from an agricultural land in The Department of Petroleum Engineering, Faculty of engineering, University of Benin, Ugbowo campus, Benin City, Edo State in Nigeria) using a 22-cm hand-dug soil auger and stored in labeled black polythene bag. The sample was air dried, grinded and sieved through 2mm mesh before use. Before contamination, the soil sample was subjected to chemical digestion using 1:1 ratio of 0.25M hydrochloric acid and Nitric acid. Thereafter, it was characterize to determine the physio-chemical properties. The physio-chemical properties determined include; Total Heterotrophic bacterial, Moisture content Soil, pH, Electrical conductivity, Total hydrocarbon content (THC), Total organic carbon, Total nitrogen content in addition to the soil composition including percent sand, Total Phosphorus, Lead (Pb) and Iron (Fe). The used engine oil was added gradually into the bowl containing the unpolluted sieved soil sample and was properly mixed. The used engine oil was to serve as the pollutant. The soil samples were left for 4days for stabilization before the commencement of treatment process. The experiment was monitored for a period of eight (8) weeks under which appreciable level of remediation had been obtained. Result obtained shows that there was a gradual increase in pH, Electrical conductivity(EC) and Total Heterotrophic bacterial(THB), and also a gradual decrease in total nitrogen content(TNC), total organic carbon(TOC), total phosphorus(TP), lead(Pb), Iron(Fe) and total hydrocarbon content(THC). The result explicitly showed that goat manure is a good substrate for bioremediation of used engine oil polluted site with calculated engine oil removal efficiency of 62.67%. The kinetic

modeling shows that the experimental data fitted well with pseudo-second order kinetic model. On predicting the rate of hydrocarbon loss with time the non-linear regression model gave higher coefficient of determination of 0.9874 compared to the linear regression model that gave 0.9665.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Bioremediation is a treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances (EPA).It is a process to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate growth of micro-organisms and degrade the target pollutant (EPA, 2011). Bioremediation can also be defined as the use of micro-organisms to detoxify or remove pollutants owing to their diverse metabolic capabilities, it is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry(Medina-Bellver *et al.,*2005). Generally, bioremediation technologies can be classified as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site while ex situ involves the removal of the contaminated material to be treated elsewhere (Gavrilescu 2010).Different techniques are employed depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. Ex situ techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation for soil or pumping for water (Vidali 2001). It mainly involved bio-stimulation where organic or inorganic components were introduced to enhance indigenous microbial growth that directly degrades the contaminants.

Environmental degradation is the deterioration of the environment through depletion of resources such as air, water and soil; the destruction of ecosystems; habitat destruction; the extinction of

wildlife; and pollution (Johnson *et al.,* 1997). Environmental degradation is occasioned by consistent flow of industrial waste, oil spills, gas flares, fire disasters, acid rain,etc., which has led to the pollution of farmlands and fishponds. It has also led to the destruction of properties and human life, including aquatic and bio-diversity (Ugboma, 2015).Petroleumhas many uses, and the environmental impact of the petroleum industry is correspondingly extensive and expansive. Crude oil and natural gas are primary energy and raw material sources that enable numerous aspects of modern daily life and the world economy. Their supply has grown quickly over the last 150 years to meet the demands of rapidly increasing human population, creativity, and consumerism (The Library of Congress, 2016). Substantial quantities of toxic and non-toxic waste are generated during the extraction, refinement, and transportation stages of oil and gas. Some industry by-products, such as volatile organic compounds, nitrogen and Sulphur compounds, and spilled oil can pollute air, water, and soil at levels that are harmful to the human life where improperly managed (EPA 2000 and 2012, Baustita, *et al.,*2016). Climate warming, ocean acidification, and sea level rise are global changes enhanced by the industry's emissions of greenhouse gases like methane and micro-particulate aerosols like black carbon (Eggleton, 2013., Stohl, *et al*., 2013, Michael, 2018).

Petroleum hydrocarbon contamination of land, water, air, vegetation and human is a widespread global environmental concern. Used engine oil and fuel spills in soil are among the most extensive and environmentally damaging pollution problems as it is threatening to human health and eco-systems. Biochemical and physiochemical properties of soil is deteriorated by hydrocarbon and it also limit the growth and the development of the plants. Water and oxygen deficits as well as to shortage of available forms of nitrogen and phosphorus are the main changes of soil properties due to contamination with petroleum derived substances (Fowzia *et al.,*

2018). Used Engine oil contaminated soil causes organic pollution of underground water which restrict it use and causes economic loss, environmental problems and decreases the agricultural productivity of the soil. Micro-organisms, plants, animals and humans are facing vulnerable situation because of the toxicity of petroleum hydrocarbons. Soil enzymes are of the important biotic component which are responsible for soil biochemical reactions. Petroleum hydrocarbon products has adverse effect on soil enzymes activities. Engine oil spills affect plants by creating conditions which make essential nutrient like nitrogen and oxygen needed for the plant growth unavailable to them. Used Engine oil contamination at different levels caused significant reduction in the growth of the plant using plant height, fresh weight and leaf area and the effect is proportional to the levels of contamination. It could reduce or stop plant growth leading to death as a result of forming a physical barrier and coating the roots (Fowzia *et al.,* 2018).Some diseases have been diagnosed to be the consequences of oil spills (Onwurah, *et al.,* 2007). The health problems associated with oil spill may be through any or combination of the following routes: contaminated food and / or water, emission of 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). Used engine oil 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 hydrocarbon can be extrapolated from its effect on rats that developed hemorrhagic tendencies after exposure to watersoluble components of crude oil (Onwurah, 2002). Volatile component of hydrocarbons after a spill have been implicated in the aggravation of asthma, bronchitis and accelerating aging of the lungs (Kaladumo, 1996).

Contamination due to is widespread in the environment and contaminates surface and groundwater (Balasubramaniam, *et al.,* 2007). Contamination causes threat to human health and safety and can affect nature by contaminating surface and groundwater (Balasubramaniam, *et al.,* 2007). Efforts are made both nationally and internationally in order to remediate the pollution caused by hydrocarbon contamination which can cause environmental and health risk. There are three methods involved in the remediation of sites contaminated due to hydrocarbon (Abha, *et al.,*2012, Dave, *et al.,*2011):

- Phytoremediation
- Bioremediation
- Chemical remediation

Phytoremediation is the process which involves the use of plants for the biodegradation, extraction, and elimination of the contaminants from the air, water, and soil (Mbhele, 2007, Peer, *et al.,* 2006). It includes various mechanisms which can lead to degradation of contaminants, dissipation, immobilization, and accumulation (Pivetz, 2001, Kamath, *et al*., 2007).

Bioremediation is the technique which involves the productive use of the bio-degradative process for the elimination or detoxification of pollutants from the environment. It is the management of suitable levels of nutrients fertilizer addition, moisture control to optimize soil degradation by micro-organisms, aeration and mixing, and pH amendment are required for the process of land treatment (Salanitro, *et al.,* 1997). This process converts pollutants into useful or nontoxic substances by using bacteria, fungi, and yeast which are the naturally occurring microorganism (Mbhele, 2007). This is also a process in which micro-organisms restore the quality of the environment by degrading and metabolizing the chemical substances (Dave, *et al.,*2011).

Chemical Remediation is the process that requires the use of chemicals. Contaminants can be treated by using various chemicals. Chemicals usually have the capability of altering the contaminant's chemical and physical properties (Vergetis, 2002). Dispersants, solidifier, and chemical oxidants are the three categories in which the chemical remediation are grouped (Abha, *et al.,* 2012, Dave, *et al.,* 2011).

In this work, the method that is adopted is the Bioremediation method because it uses nature to fix nature. some advantages of these method are listed below:

- Completely natural process with almost no harmful side effects
- Carried out in situ for most applications with no dangerous transport
- Quick turn around time to make soil and water useful
- Minimal equipment needed except for specialized pieces
- Positive public acceptance due to organic process and little disturbance
- Cost effective to maintain and economical to input
- Lowers liability, as contaminants are less likely escape
- Little energy consumed compared to incineration and landfilling
- High acceptance from regulatory authorities

Bioremediation involves two processes: Bioaugmentation and Bio-stimulation

Bioaugmentation process involves the degradation of the harmful hydrocarbons by the addition of microorganisms in order to achieve the pollutant reduction (Sharma, 2012). It is also the injection of polluted water with micro-organisms capable of hydrocarbon degradation (Dave, *et al.,*2011). This process sometimes involves biodegradation of the hydrocarbon pollutants by adding the genetically engineered microorganisms into the polluted water (Gentry, *et al*., 2004).

But this process is not often used for the hydrocarbon degradation because microorganisms responsible for hydrocarbon degradation naturally exist in the environment. Bioaugmentation is not so much effective to be used in oil spill remediation sites, and nonindigenous microorganisms used in this process can cause competition with the microbes already present in the environment (Swannell *et al.,* 2004).

Biostimulation is the process which involves degradation of the harmful compounds by adding the nutrients required by indigenous hydrocarbon degrading microbes. The maximum bio-stimulation is achieved by obtaining the ideal nutrient concentration which is required for the utmost growth of the micro-organisms and maintaining concentration as long as possible for micro-organisms (Lee, *et al.,* 2007).

Some selected studies and what they found out:

1. Assessing the Use of Local Indigenous Microorganisms for the Bioremediation of Sites Contaminated With Petroleum Hydrocarbons by Feras Mohammad Salamah.

   In conclusion, two bacterial strains were isolated from already contaminated soils; these two starins were chacracterized by sequencing of the 16S rRNA gene. They were: Pseudoxanthomonas sp. and Bacillus sp.The potential of the isolated strains to degrade crude oil were investigated under three environmental factors: pH, temperature and nutrition with different concentrations of nutrition. The efficiency of biodegradation on removal of TPH was good, which reach to 70% at optimum conditions which were with 5mM of ammonium chloride at pH=7 and temperature degree at 35 degree celcius.
2. Assessment of a Bioremediation Process for Hydrocarbons Polluted Soil J.C. Setier, TOTAL; S. Ouvrard and C. Schwartz, Laboratoire Sols et Environnement; P. Faure, G2R; S. Guimont and J.C. Renat, TVD; and J.L. Pornain and F. Perié, TOTAL

In conclusion, the biological treatment used in this experiment proved very efficient and a 72% total hydrocarbons removal yield was achieved. Pollution characterization showed that this was the result of a differential degradation between organic compounds with preferential degradation of lower molecular compounds. Some of the bigger and less biodegradable molecules remained in the soil and were responsible for the residual measured concentration

3. Bioremediation of Polluted Soil Sites with Crude Oil Hydrocarbons Using Carrot Peel Waste

   Latifa Hamoudi-Belarbi , Safia Hamoudi , Khaled Belkacemi , L'Hadi Nouri , Leila Bendifallah and Mohamed Khodja.

   In conclusion, this study demonstrated that bio-stimulation of biodegrading crude oil microbiota with carrot peel waste enhanced degradation of crude oil under laboratory conditions. The TPH degradation in the crude oil contaminated soil was enhanced by bio-stimulation with nutrients present in the carrot peel waste in comparison to carob kibbles and control. Degradation of TPH increased after 45 days of incubation during bioremediation. The biodegrading crude oil microbiota in the crude oil polluted soil was positively related to TPH degradation efficiency during bio-remediation. Carrot peel waste, containing high amounts of phosphorus, enhanced bioremediation of crude oil polluted soil by increasing microbial activities of biodegrading bacteria.

1.2 Statement of the problem

Engine oil is a product of crude oil which is one of the most important source of energy worldwide; however, routine operations of extraction and drilling of this fossil energy resource

cause serious environmental problems. Used engine oil contains a wide range of compounds that pose a significant risk for the environment and human health.

Recent epidemiological surveys have revealed that there is increasing incidence of degenerative diseases in the regions where oil spill is increasing such that life expectancy is on the downward trend (Nwankwoala and Gorgewill, 2006).

The major impact of used engine oil spills are destruction of farmlands, soil fauna and flora , poisoning of both surface and ground water , destruction of aquatic life and other economic livelihood (Agunobi, *et al.,* 2014).

Used engine oil spill can pollute streams and rivers and can have a devastating effect on the water environment by spreading over the surface in a thin layer that stops oxygen getting to the plants and animals that live in the water. It harms insect and animals such as periwinkles, crabs and crayfish; prevents photosynthesis in plants, disrupts the food chain and takes a long time to recover or remediate. Most animals are particularly vulnerable (Nwilo and Badejo, 2007). Moreso, fishing which are the traditional occupation of most citizens that live on riverine areas source of income is being eroded as a result of engine oil spills. Many affected residents especially in the worst affected areas have not recovered from the loss of their livelihood and income from the effect of engine oil spill.

Also unused engine oil does not contain aromatic component but used engine oil contains several poly-nuclear aromatic hydrocarbons(such as alkylbenzenes, toluene,benzene,xylene,ethyl-benzene, methyl-naphthalenes, polycyclic aromatic hydrocarbons and naphthalenes.), many of which are also classified as known human carcinogens. These materials mostly have very limited volatility, but they can penetrate human and animal skin

resulting in significant exposure to those contacting the used engine oil. They can adhere also to airborne dust particles and be inhaled. These polynuclear aromatics hydrocarbons (PNAs) tend to concentrate in fatty tissue and are slowly released into the body producing a long-term exposure scenario. Animals and aquatic life exposed to used engine oil also would be expected to concentrate these polynuclear aromatic hydrocarbons (PNAs) in fatty tissue which could later be consumed by humans resulting in additional exposure to polynuclear aromatic hydrocarbon (WHO. IARC,1989).

Reducing the petroleum hydrocarbon compounds in a polluted environment becomes a significant challenge for oil. companies are forced to conduct an adequate and effective treatment of these pollutant emissions. Thermal treatment, soil washing, soil vapor extraction, solidification, and stabilization are physical and chemical techniques used to treat petroleum hydrocarbon-polluted soil. However, they are often expensive, ineffective, and rarely neutral. Bioremediation of hydrocarbons in polluted soils is a promising treatment method. Based on the principle of complete mineralization or transformation of petroleum products into less toxic forms by different groups of microorganisms, bioremediation is the most effective, non-invasive, the least expensive and eco-friendly technique.

1.3 Aim and Objective

The aim of the study is to evaluate the performance of goat manure for the bioremediation of used engine oil polluted soil.

The specific Objectives are as follows:

- Determine the physiochemical parameter of the polluted soil.
- To monitor Bio-remediation process and determine the process of it removal.

- Study the kinectics of bio-remediation, knowing the order of its reaction.
- Identify the optimum bio-treatment conditions of hydrocarbon contaminated soil.

1.4 Scope of work

The overall Scope of the study include:

- Collection and analysis of unpolluted soil to determine the physiochemical and biological properties.
- Pollution of the soil and determining the effect of the hydrocarbon (used engine oil) pollution.
- Treatment of the soil using goat manure.
- Sampling and analysis of the polluted soil to determine the extent at which the microorganisms has degraded the polluted used engine oil soil.
- Determine the kinetics of the remediation process

1.5 Relevance of Study

The increase in population growth and industrialization places a heavy demand on petrochemical products and this great demand on fossils fuels has resulted in serious environment issues over the recent decades. The eco-toxicity and the potential health implications that petroleum pose for both environmental and human health have led to increased on how to detoxify environment impacted by petrogenic compounds. This study is embarked on, in order to bring up more optimal ways to tackle hydrocarbon polluted site and bring back the environment to it original state.

Bioremediation technology makes it possible to clean up the environment afterused engine oil spills and other unfortunate environmental disasters. By using naturally occurring bacteria to

eliminate contaminants in the sea, we protect and encourage aqua-culturists and their attempts to solve the problem of global food production.

Bioremediation is Ecofriendly and Sustainable thereby it has almost no side effect to human health because it does not use any dangerous chemicals, bioremediation micro-organisms destroys harmful chemicals and thereby clean up contaminated site bringing it back to its original state.

The contaminants in soil are destroyed and not simply transferred to different environmental media, thereby bringing back the soil and bringing back the growth of plant and making the vegetation greener. Bioremediation techniques accelerates the natural occurring biodegradation by optimizing conditions for biodegradation through aeration, addition of nutrients and control of pH and temperature (Norris, *et al.,* 1994; Atlas and Bartha, 1992).Physical and chemical properties of the soil, such as aeration, pH, water-holding capacity, and ion exchange capacity are also improved after bioremediation.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fundamental principles of Bioremediation

Bioremediation process involves the utilization of natural microorganisms for the decontamination of atmosphere (Sharma S, 2012). This process converts pollutants into useful or nontoxic substances by using bacteria, fungi, and yeast which are the naturally occurring microorganisms (Mbhele PP, 2007). This is also a process in which microorganisms restore the quality of the environment by degrading and metabolizing the chemical substances (Dave and Ghaly, 2011).

The engine oil constituents vary particularly in susceptibility, volatility, and volubilityto biodegradation. A number of substances are easily degraded, some are non-biodegradable, and some oppose degradation. Diverse species of microbes preferentially attack diverse compounds due to this biodegradation of petroleum that occurs at different rates but concurrently. Enzymes produced by microorganisms in the presence of sources of carbon are accountable for attacking the hydrocarbon molecules. Hydrocarbon present in the petroleum is degraded by different enzymes and metabolic pathways. Hydrocarbon degradation is prevented by the lack of suitable enzyme (Thapa *et al.,* 2012).

The microorganisms act against the contaminants only when they have access to a variety of materials compounds to help them generate energy and nutrients to build more cells. In a few cases the natural conditions at the contaminated site provide all the essential materials in large enough quantities that bioremediation can occur without human intervention a process called intrinsic bioremediation. More often, bioremediation requires the construction of engineered

systems to supply microbe-stimulating materials a process called engineered bioremediation. Engineered bioremediation relies on accelerating the desired biodegradation reactions by encouraging the growth of more organisms, as well as by optimizing the environment in which the organisms must carry out the detoxification reactions.

2.2 Types of Bioremediation

2.2.1 Mycoremediation

This is a form of bioremediation in which fungi-based technology is used to decontaminate the environment. Fungi has been proven to be a very cheap, effective and an environmental sound way for removing toxic materials from the environment. The toxins include heavy metals, persistent organic pollutant, textile dyes, petroleum fuels, pesticides and herbicides (Deshmukh *et al.,*2016). Pharmaceuticals and personal care products in land, fresh water and marine environments.

2.2.2 Biostimulation

Is a remediation technique that is highly efficient, cost effective and eco-friendly in nature. Biostimulation refers to the addition of rate limiting nutrients like phosphorus, nitrogen, oxygen, electron donors to severely polluted sites to stimulate the existing bacteria to degrade the hazardous and toxic contaminants (Tyagi *et al.,*2010 , Elektorowicz M, 1994 , Piehler *et al*., 1999, Rhykerd *et al.,*1999).

The addition of rate limiting nutrients improves the degradation potential of the inhabitant micro-organisms efficiently as it significantly accelerates the decontamination rate (Adams *et al.,*2015 , Nikolopoulou and Kalogerakis, 2009). Existing works of literature have established biostimulatin

as an important remediation tool for the degradation of hydrocarbons especially petroleum products and its derivatives (Abid *et al.,* 2014).

2.2.3 Bioventing

Bioventing is a process of stimulating the natural in situ biodegradation of contaminants in soil by providing air or oxygen to existing soil micro-organisms. Bioventing uses low air flowrates to provide only enough oxygen to sustain microbial activity in the vadose zone. Oxygen is most commonly supplied through direct air injection into residual contamination in soil. In addition to degradation of absorbed fuel residuals, volatile compounds are biodegraded as vapors more slowly through biologically active soil (Hinchee R.E,1993, U.S Airforce Environics Directorate of the Armstrong laboratory, 1995). Bioventing is applicable to any chemical that can be aerobically biodegraded. Factors that may limit the applicability and effectiveness of the process include: low permeability soils, air near the structure of concern has to be extracted in order to avoid vapor build up in basements within the radius of influence of air injection wells, low soil moisture content, which may be caused by bioventing, limits biodegradation (Hinchee R.E, 1993, EPA).

2.2.4 Bioleaching

This is the extraction of metals from their ores through the use of living organisms. This is much cleaner than the traditional heap leaching using cyanide. Bioleaching is regarded as a Green Technology that will become even more important in future years, as it avoids much of the costs and carbon foot print associated with conventional mining. Although extracting metals using bacteria is much slower than roasting ores, the bacteria work for nothing as they actually use the energy present in the minerals themselves. As an added bonus, they take carbon dioxide from the

air(like green plants) and therefore help to counter the problem that is acknowledged to be behind climate change (Barrie Johnson, 2011). Bioleaching consist of two related microbial process which are bacterial leaching and bio-oxidation.

2.2.5 Landfarming

Landfarming is a well proven ex-situ bioremediation technology that has been successfully used since the 1980s for treating petroleum impacted soils/sediments, drill cuttings, low brine drilling fluids, oily sludges, tank buttoms and pit sludges. The material to be treated is incorporated into surface soil. Naturally occuring microbes in the soil and waste material transform the organic contaminants to carbon dioxide, water and biomass (USEPA 1993 , USEPA 2003, USEPA 2017.) . Maintainig optimum soil conditions for rapid biodegradation of organic contaminants can help meet clean up goals within a reasonable timeframe.

2.2.6 Bioreactors

A bioreactor refers to any manufactured device or system that supports a biologically active environment (IUPAC 1997). In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel. These devices are being developed for use in tissue engineering or biochemical/bio process engineering.

2.2.7 Composting

Is an aerobic method (meaning that it requires the presence of air) of decomposing organic solid wastes. It can therefore be used to recycle organic material. The process involves decomposition

of organic material into a humus-like material, known as compost, which is a good fertilizer for plants.

2.2.8 Bioaugmentation

Bioaugmentation is the addition of bacterial cultures required to speed up the rate of degradation of a contaminant (Morganwalp and David W, 2001) . Organisms that originate from contaminated areas may already be able to breakdown waste, but perhaps inefficiently and slowly. Bioaugmentation is more commonly and successfully used on contaminants removed from the original site, such as in municipal wastewater treatment facilities. To date, this method has not been very successful because of it difficulty to control site conditions for the optimal growth of the micro-organisms added. Scientists are yet to completely understand all the mechanisms involved in bioremediation, and organisms introduced into a foreign environment may have a hard time surviving (Margesin and Schinner, 2001).

2.2.9 Rhizofiltration

Rhizofiltration refers to the use of plant roots to absorb, concentrate, and precipate toxic metals from contaminated groundwater. Initially, suitable plants with stable root systems are supplied with contaminated water to acclimate the plants. These plants are then transferred to the contaminated site to collect the contaminants, and once the roots are saturated, they are harvested. Rhizofiltration allows in-situ treatment, minimizing disturbance to the environment.

2.2.10 Biopiles

This is a method of excavating soil contaminated with aerobically remediable hydrocarbons. Biopiles also known as biocells, bioheaps, biomounds and compost Piles) are used to reduce concentrations of petroleum pollutants in excavated soils during the time of biodegradation.

During this process, air is supplied to the biopile system during a system of piping and pumps that either forces air into the pile under positive pressure or draws air through the pile under negative pressure (Delille *et al.,*2008). The microbial activity is enhanced through microbial respiration then the result in degradation of absorbed petroleum pollutant becomes high (Emami *et al.,*2012).

2.2.11 Phytoremediation

Phytoremediation technologies use living plants to clean up soils, air, and water contaminated with hazardous contaminants (Reichenauer and Germida, 2008). It is defined as the use of green plants and the associated micro-organisms, along with proper soil amendments and agronomic techniques to either contain, remove or render toxic environmental contaminants harmless (Das and Pratyush, 2018). The term is an amalgam of the Greek *phyto* (plant) and latin *remedium* (restoring balance). Although attractive for its cost, phytoremediation has not been demonstrated to redress any significant environmental challenge to the extent that contaminated space has been reclaimed. Phytoremediation is proposed as a cost-effective plant-based approach of environmental remediation that takes advantage of the ability of the plants to concentrate elements and compounds from the environment and to detoxify various compounds. Toxic heavy metals cannot be degraded, but organic pollutants can be and are generally the major targets for phytoremediation. several fields trials has confirmed the feasibility of using plants for environmental cleanup (Salt et.al., 1998).

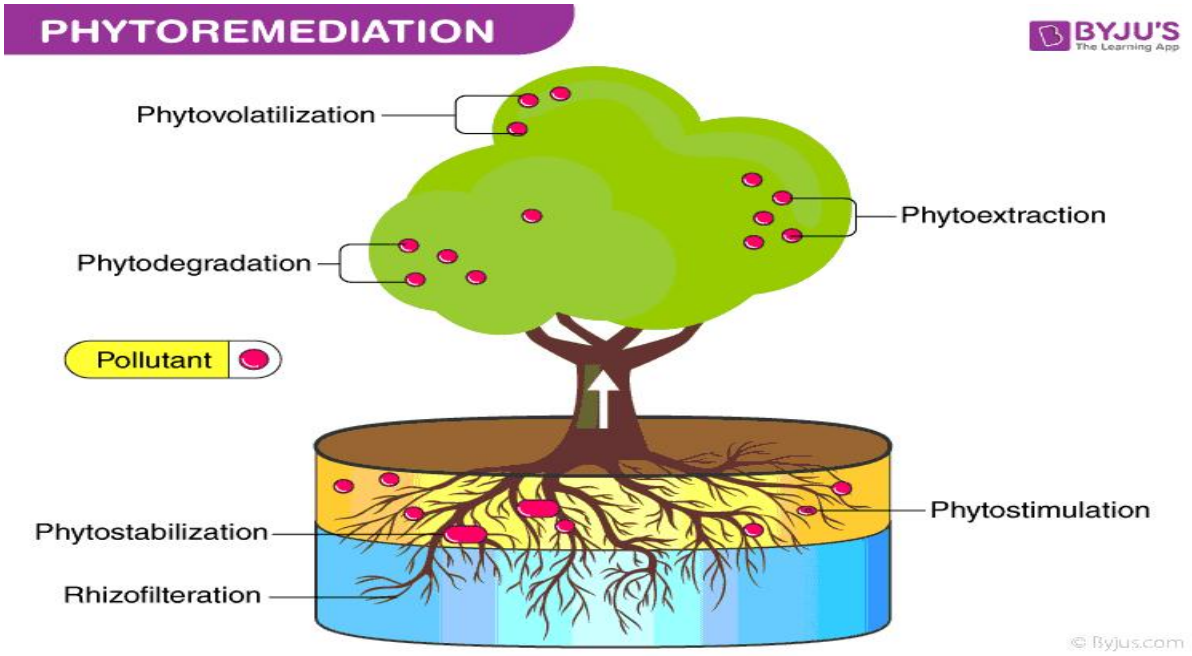

Figure 2. 1 Phytoremediation Process

2.3 Factors Affecting Bioremediation

2.3.1 Biological factors

Biotic factors affect the degradation of organic compounds through competition between microorganisms for limited carbon sources, antagonistic interactions between microorganisms or the predation of microorganisms by protozoa and bacteriophages. The rate of contaminant degradation is often dependent on the concentration of the contaminant and the amount of "catalyst" present. In this context, the amount of "catalyst" represents the number of organisms able to metabolize the contaminant as well as the amount of enzymes(s) produced by each cell. The expression of specific enzymes by the cells can increase or decrease the rate of contaminant degradation. The major biological factors are included here: mutation, horizontal gene transfer, enzyme activity, interaction (competition, succession, and predation), its own growth until

critical biomass is reached, population size and composition (Madhavi and Mohini, 2012, Boopathy R, 2000).

2.3.2 Environmental factors

Microorganism growth and activity are affected by pH, temperature, moisture, soil structure, solubility in water, nutrients, site characteristics, redox potential and oxygen content, lack of trained human resources in this field and Physico-chemical bioavailability of pollutants (contaminant concentration, type, solubility, chemical structure and toxicity). These above listed factors are determining kinetics of degradation (Madhavi and Mohini, 2012, Adams *et al.,* 2015). Biodegradation can occur under a wide-range of pH; however, a pH of 6.5 to 8.5 is generally optimal for biodegradation in most aquatic and terrestrial systems. Moisture influences the rate of contaminant metabolism because it influences the kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems (Cases and de Lorenzo, 2005). Most environmental factors are listed below.

- **Availability of nutrients**

  The addition of nutrients adjusts the essential nutrient balance for microbial growth and reproduction as well as having impact on the biodegradation rate and effectiveness. Nutrient balancing especially the supply of essential nutrients such as N and P can improve the biodegradation efficiency by optimizing the bacterial C: N: P ratio. To survive and continue their microbial activities microorganisms need a number of nutrients such as carbon, nitrogen, and phosphorous. In small concentrations the extent of hydrocarbon degradation also limits. The addition of an appropriate quantity of nutrients is a favourable strategy for increasing the metabolic activity of microorganisms and thus the biodegradation rate in cold environments (Couto *et al.,*2014, Phulia *et al.,*2013).

Biodegradation in aquatic environment is limited by the availability of nutrients (Thavas *et al.,*2011). Similar to the nutritional needs of other organisms, oil-eating microbes also require nutrients for optimal growth and development. These nutrients are available in the natural environment but occur in low quantities (Macaulay BM, 2014).

- **Temperature**

Among the physical factors temperature is the most important one to determining the survival of microorganisms and composition of the hydrocarbons (Das and Chandran, 2011). In cold environments such as the Arctic, oil degradation via natural processes is very slow and puts the microbes under more pressure to clean up the spilled petroleum. The sub-zero temperature of water in this region causes the transport channels within the microbial cells to shut down or may even freeze the entire cytoplasm, thus, rendering most oleophilic microbes metabolically inactive (Yang *et al.,*2009, Macaulay BM, 2014). Biological enzymes are participated in the degradation pathway have an optimum temperature and will not have the same metabolic turnover for every temperature. Moreover, the degradation process for specific compound needs specific temperature. Temperature also speed up or slow down bioremediation process because highly influence microbial physiological properties. The rate of microbial activities increases with temperature, and reaches to its maximum level at an optimum temperature. It became decline suddenly with further increase or decrease in temperature and eventually stop after reaching a specific temperature.

- **Oxygen limitations**

The initial steps in the catabolism of aliphatic, cyclic and aromatic hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases for which molecular oxygen is required. Although anaerobic biodegradation has been shown to occur in different ecosystems including marine environments, its ecological significance has been generally considered to be minor and the biodegradation rate is rather low (Ghosal *et al.,*2016). Conditions of oxygen limitation normally exist in aquatic sediments and soils. Oxygen depletion can occur in the presence of easily utilizable substrates that increase microbial oxygen consumption. In several instances, the concentration of dissolved oxygen can be close to zero leading to practically zero aerobic biodegradation rates. Although oxygen can be successfully delivered (in various forms) to hydrocarbon-contaminated soils and groundwater, enhancing biodegradation rates, this is not the case in marine environments as it is very difficult to implement such technologies in the field. Tilling is essentially the only option in aerating the top layers of contaminated sediments during low tide (Boopathy R 2000, Bamforth and Singleton 2005). Thus, oxygen represents a very significant and potentially factor which limit the rate of hydrocarbon degradation.

- **Moisture content**

Microorganisms require adequate water to accomplish their growth. The soil moisture content has adverse effect in biodegradation agents.

- **pH**

pH is a scale used to specify the acidity or basicity of an aqueous solution. The measurement of pH in soil could indicate the potential for microbial growth (Asira and

Enim, 2013). Higher or lower pH values showed inferior results; metabolic processes are highly susceptible to even slight changes in pH (Wang *et al.,*2011).pH varies less in aquatic environments and most bacteria and fungi capable of degrading hydrocarbon require a neutral pH (Margesin and Schinner, 2001, Leahy and Colwell, 1990). In general, microbial activity is influenced by extremely low or high pH (Leahy and Colwell, 1990). According to Bamforth and Singleton (Bamforth and Singleton 2005), 40% of phenan- threne degradation was observed for Burkholderia cocovenenans at pH 5.5. However, the deg- radation at neutral pH in the same conditions was 80%. Additionally, Leahy and Colwell (1990) reported that microbial degradation of naphthalene decreased at pH 5.0 compared to the highest rate of degradation observed at pH 7. Moreover, other reports described the efficiency of some microorganisms, such as Pseudomonas, to degrade hydrocarbon at alkaline pH (Bamforth and Singleton 2005). A degradation of PAH in acidic contaminated environment (pH 2) by indigenous microor- ganisms was reported (Shallu *et al.,*2014). The adequate pH depends on microorganisms to be used for the bioremediation.

- **Bioavailability of hydrocarbon**

The bioavailability is defined as the rate of substrate mass transfer into the microbial cells. It is considered as one of the most determinant parameters regarding hydrocarbon degradation rate. PAHs are characterized by a low bioavailability due to their low aqueous solubility. That is the reason why they are reported to be resistant to the degradation process and persistent in the environment (Ghosal *et al.,*2016). Unsuccessful remediation of PAH-contaminated sites was reported due to the low bioavailability of

PAHs (Ghosal *et al.,*2016). It has been reported that bioavailability of hydro- carbon decreases with time (Ghosal *et al.,*2016). Although the photo-oxidation increased the biodegradation of petroleum hydrocarbon by increasing its bioavailability which promote microbial activities (Maki *et al.,* 2012). Hydrocarbon and particularly PAHs become more bioavailable when they are dissolved or evaporated (Abdel-Shafy and Mansour, 2016). The bioavailability of pollutant in contaminated environment may be increased by the application of surfactants.

2.4 Special Features of Bioremediation

- Ecologically sound, natural process; there is an increase in the number of the existing microorganisms when the contaminants are present, and the microbial population decreases naturally when the contaminants are degraded. The residues such as water, carbon dioxide, and fatty acids obtained as a result of the biological treatment are usually nonhazardous product, and the obtained CO2 can be used for the photosynthesis process by the plants.
- Bioremediation is responsible for destroying the target chemicals in place of transferring the contaminants from one place to another.
- Other techniques which are used for the cleanup of harmful waste are more costly than bioremediation. For example, through the cleanup of the Exxon Valdez spill, the cost of 1-day physical washing is more than bioremediating 120 km of shoreline.
- Eco-friendly and sustainable (Dell *et al.,* 2012).
- Relative ease of implementation (Kumar *et al.,* 2011).

- Bioremediation deals with in situ treatment and does not involve the transfer of a large amount of the polluted wastes off-site, and the risk due to the transportation can be overcome.
- Microbe efficiency can be enhanced by using nutrient formulation in the bioremediation process.
- The residues such as CO2, fatty acids, water, etc. obtained from the biological treatment are generally non-hazardous.
- It is a less costly technique than other techniques which are used for cleaning up of the toxic waste. (Patel and Shah, 2014).
- It does not use any dangerous chemicals. Nutrients especially fertilizers added to quicken the process of microbial growth converts the harmful chemicals into water , harmless gas and substances, the harmful chemicals are completely destroyed (Shilpi Sharma, 2012).

2.5 Limitations of Bioremediation

- Bioremediation takes longer time compare to other treatment options, such as excavation and removal of soil from contaminated soil.
- Technological Advancement: Research is needed to develop and engineer bioremediation technologies that are suitable for sites with complex mixtures of contaminants that are not evenly distributed in the environment. It may be present as solid liquids, and gases.
- Specificity: Biological process are highly specific. Important factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- Products of biodegradation may be more toxic than the parent compound.

- Bioremediation is limited to those compound that are biodegradable.
- May not Reduce concentration of contaminants to required levels.

2.6 Literature Review on Bioremediation

A Bioremediation plan on contaminated soil shiraz oil refinery site: Identification of heavy hydrocarbon degrading micro-organisms was carried out by Koohestani *et al.,* 2015, In this study, After sampling the refinery contaminated soil at three points from three stations in spring and summer, determining the distribution of toluene-degrading bacteria was performed through microbial count test by variable plate count method as an indicator of microbial activity. Toulene -degrading bacteria in MSM saline medium with toluene as a carbon source were isolated. After screening and identification of superior strains through biochemical and molecular detection method (16SrNA), the growth curve of various concentrations of toluene was studied during eight days. The numbers of bacteria per cfu/ml per gram of soil were 6.4×10^6, 3.4×10^6, 3.2×10^6, in spring and 3.3×10^8, 5.4×10^8, 6.4×10^9 in summer, respectively. Identified strains belonged to the klebsiella, Kluyvera, Staphylococcus and Pseudomonas genus. Klebsiella had the highest growth in concentrations of 1,2,3,4 mol per liter over 8 days. Statistical analysis between strains by ANOVA and DUNCAN tests indicated a meaningful difference at the 0.05 level. The results of this study indicated the capability of the local mentioned strains as the first aim in degrading heavy hydrocarbons. In addition identification of diversity and efficiency of the local micro-organism for the next stage of bioremediation plan was considered.

Olukunle (2013), studied the characterization of indigenous microorganism associated with crude oil polluted soils and water using traditional techniques, This study was conducted to

isolate and identify bacteria and fungi associated with crude oil-polluted sites using traditional techniques, Environmental samples were collected from Awoye, Mese and Oluwa villages in Ondo State and three different flow stations (Agbada-Aluu shell, Obite and Bonny) in Rivers state. Pour plate technique was used for the analyses of microbial population. The bacterial isolates were identified by morphological and biochemical characterization using the taxonomy scheme of Bergey's Manual of determinative bacteriology while identification of fungi was based on the microscopic and macroscopic features of the hyphal mass, nature of the fruiting bodies and the morphology of cells and spores. The bacterial load of the Obite water was highest (33.00 ± 1.0 SFU mL^{-1}) and that of the Oluwa polluted water was lowest (10.00 ± 0 CFU mL^{-1}). Soil samples collected from Mese, Oluwa and Awoye had fungal counts of 15 ± 3, 42 and 16 ± 3 SFU g^{-1}, respectively while the fungal population of the water samples varied from 8.87 ± 2.1 SFU mL^{-1} (Oluwa) to 15.00 ± 1.0 SFU mL^{-1} (Awoye). Seven bacteria each and fourteen fungi were obtained from ondo and Rivers states. The advent of molecular biology in 1980's has no doubt provided new set of tools to identifying microorganisms to the specie as well as the strain of individual microorganisms. However, traditional techniques are useful especially in laboratories where there are no molecular biology facilities. The results obtained in this study have revealed that oil degrading bacteria and fungi can be isolated from oil polluted sites. These bacteria and fungi are indigenous in the polluted sites and they are responsible for the degradation of oil. The traditional technique of identification, however, enables microbiologists to understand the basic principle of identification of microorganisms which are based on morphological, metabolic and physiologic traits.

Ghulam Shabir et al. 2008, investigated the biodegradation of kerosene in soil using a mixed bacterial culture under two levels of nutrients: (C1) low nutrient concentration as compared to

(C2), both of them contained 4 % (w/w) kerosene in soil as a sole carbon source. After 6 weeks of incubation: (C1) and (C2) exhibited 27±3% and 65±7% kerosene degradation, respectively. The highest bacterial growth was observed in (C2) with a significant reduction in nutrient content of soil over 2–3 weeks of incubation. Overall, 46±12% and 54±24% of nitrogen, 36±3% and 43±3% of phosphorus and 24±2% and 35±2% of potassium content of the soil were depleted under (C1) and (C2) respectively. Briefly, their work has defined nutrient requirements for kerosene oil degradation and its remediation from contaminated soil.

Lacotte et al. 1995, improved the biotreatment of Arabian Light crude oil in synthetic sea water by a mixed culture of bacteria. The evaluated the effects of fish meal, meat meal 80% proteins and sophorolipids (with concentration at 0, 5 mgml) on the biodegradation during a three-week incubation using GC/FID and GC/MS analysis of the hydrocarbons in remaining oil. They had involved no nitrogen and phosphorous limits when used them as source of nutrients. Biodegradation processes in the first four days of the incubation supplemented with sophorolipids are accelerated two fold over controls. They demonstrated the natural limitation of the microbial oxidation of crude oi1 such as the limiting conditions in nitrogen and phosphorous assailable by microorganisms. Also, they had assessed the potential usefulness of bioremediation using N and P fertilizers to cleanup coastal environment contaminated with petroleum hydrocarbons.

Rahman et al. 2002, assessed the optimum conditions for biodegradation of Bombay High BH crude oil. They isolated 130 oil degrading bacterial cultures from contaminated soil samples, but they made a consortium of (Micrococcus sp. GS2-22, Corynebacterium sp. GS5-66, Flavobacterium sp. DS5-73, Bacillus sp. DS6-86 and Pseudomonas sp. DS10-129) for studying the efficiency of crude oil biodegradation. They noticed that using Individual bacterial cultures

had showed less growth and biodegradation than the bacterial consortium. They showed that at 1% crude oil concentration, the consortium degraded 78% of BH crude oil, followed by 66% by Pseudomonas sp. DS10-129, 59% by Bacillus sp. DS6-86, 49% by Micrococcus sp. GS222, 43% by Corynebacterium sp. GS5-66 and 41% by Flavobacterium sp. DS5-73. Furthermore, The rate of biodegradation by bacterial consortium decreased from 78% to 52% as the concentration of crude oil was increased from 1% to 10%. Also, they had found that the optimum Temperature was at 30°C and the optimum pH was at 7.5 for maximum biodegradation. This study used a consortia of two types of bacteria (Pseudoxanthomonas sp. and Bacillus sp.) as indigenous bacteria which isolated from contaminated sandy soil with petroleum hydrocarbons, and assessed the effects of three environmental factors on removal of total petroleum hydrocarbons (TPH), they were (nutrition , pH and temperature) at three degrees for each.

Jiin-Shuh Jean et al. 2008, demonstrated that the morphological adaptation of Pseudomonas spp. is strongly influenced by temperature and nutrient levels in an environment. They studied the effects of inorganic nutrient (sulfate, phosphate, and ammonium chloride) levels on the aerobic biodegradation of benzene, toluene, and xylene (BTX) by Pseudomonas spp. in a laboratory using a glass sand tank. They found that the increase of nutrient levels resulted in enhanced bacterial growth and BTX degradation. Sulfate and phosphate serve as electron acceptors in the microbiological Processes degrading BTX. Also, they assessed the influence of the different environmental parameters (temperature, nutrient levels, and incubation time) on microbial morphology. They noticed that bacterial morphological changes during BTX degradation reveal that the filamentous bacteria were the dominant species at low temperatures about (20°C) while the spherical and rod-shaped cells became dominant at higher temperatures ranging from (25-28°C) and pH ranging from (7–7.5). The morphological adaptation appears to be controlled by

the temperature and nutrient levels in the sandy medium where Pseudomonas spp. thrives. That they found the optimal concentrations of phosphates (650–1250 mg/l), ammonia chloride (10–50 mg/l), and sulfates (10–20 mg/l) were amended into the similar aquifer.

Abii and Nwosu (2009) studied the effect of oil spillage on the soil of Eleme in Rivers State of Nigeria on two sides. While another area (Aleto) served as the controlled. The results indicated that oil spillage adversely affected the nutrient level and fertility status of the Eleme soil. Idodo-Umeh and Ogbeibu (2010) investigated the values of Total Petroleum Hydrocarbon (TPH) and heavy metals in soils, plantain fruits and cassava tubes harvested from farms impacted with petroleum and non-petroleum activities in Delta State, Nigeria. The results revealed that the values of heavy metals were higher in cassava tubers, epicap and mesocap of plantation fruits harvested from petroleum impacted soil than from non-petroleum impacted soils. Ojimba (2011) evaluated the social-economic variables associated with poverty in crude oil polluted crops farms in Rivers State. The study used a primary data (questionnaires) and employing tobit censored regression found that extent of income diversification reduced poverty drastically by 9.8 times in crude oil polluted farm-households and 12.7 times in non-polluted farm-households. Other variables identified in reducing poverty in crude oil polluted farms include land ownership by inheritance, years of farming experience, access to extension services and farm labour (Ojimba, 2011).

Daniel Delille et al. 2009, assessed the effects of nutrient and temperature on biodegradation of petroleum hydrocarbons in sub-Antarctic coastal seawater. They provided strong evidence of the presence of indigenous hydrocarbon degrading bacteria in Antarctic seawater and their high potential for hydrocarbon bioremediation. They studied three incubation temperatures (4°C, 10°C and 20°C) with two different concentrations of oil. Their research indicated that

temperature had only a rather limited influence on petroleum degradation in the studied Antarctic seawater, especially when considering bioremediation as an efficient mean to remedy contaminated soils there. Also, the rate of oil degradation could be increased by the addition of a commercial fertilizer to a larger extent than elevated seawater temperature and global warming should not significantly increase oil biodegradation in Antarctica waters in the future.

Makut and Majekodunmi (2019) carried out an experiment on the bioremediation potentials of chicken droppings on crude oil polluted soil from automobile workshop. Two experimental treatment (T1 and T2) with 10% and 30% chicken dropping addition to the contaminated soil were set up. A control (T3) was also set up consisting of contaminated soil only. The set-up design was left for a period of 4 weeks during which the total heterotrophic bacteria(THB) count, total petroleum hydrocarbon degraded and gas chromatography and mass spectroscopy (GCMS) identification of the residue hydrocarbon after remediation were carried out. Soil samples remediated with 30% CD had significantly ($P<0.05$) the highest THB in the range of 6.45×10^7 – 11.23×10^7 when compared with those remediated with 10% CD [5.34×10^7 – 8.36×10^7]. The non-remediated control had the least THB count of 3.45×10^7 cfu/g. There was a rapid and progressive reduction in the total hydrocarbon during remediation in all the soil bio-remediated with 30% chicken droppings compared to that of un-remediated soil. This study showed that the contaminated soil with 30% chicken droppings had the highest TPH degraded of 57.04% at the end of remediation, and this may be due to the high nutrient availability from the samples in the location and poultry droppings resulting in high percentage of nutrient (N and C) which are needed for optimum growth and performances of HUB thus facilitating the synthesize of necessary enzymes needed to break down the petroleum hydrocarbon contaminants. This

research shows that chicken droppings enhanced bioremediation of crude oil contaminated soil from automobile workshops.

Studies comparing the performance of bio-augmentation and bio-stimulation have suggested that nutrient addition alone had a greater effect on oil biodegradation than did with the addition of microbial products when oxygen supply was not limited (Jobson et al. 1974; Lee et al., 1997; Venosa et al., 1996). This is probably because the hydrocarbon degrading population is rarely a limiting factor as compared to the nutrients since the size of the hydrocarbon-degrading bacterial population usually increases rapidly in response to oil contamination.

One of the first comprehensive field tests evaluating various bioremediation approaches to enhance oil degradation was carried out in a soil environment in north west area of Canada in early 1970s (Jobson et al., 1974). A randomized block design was used to examine the effects of four treatments (control, inorganic fertilizer application, addition of a microbial culture alone, and combined fertilizer and microbial culture addition) over a 308-day time period. The microbial culture was grown in the laboratory and consisting of several genera of oil-degrading bacteria (Flavobacterium and cytophoga sp., Pseudomonas sp., Xanthomonas sp., Alcaligenes sp., and Arthrobacter sp.) . The study showed that the nutrient application resulted in a significant stimulation of bacterial numbers and in the degradation rate of n-alkane components of the crude oil. The application of the microbial agent, however, resulted in only a slightly enhanced degradation rate of n-alkane components of chain lengths $C_{20} – C_{25}$.

A field study conducted on a sandy beach in Delaware also showed that addition of a microbial inoculum did not enhance oil biodegradation more than addition of inorganic nutrients alone (Venosa et al., 1996). A randomized block design was used in this study to assess the effects of three treatments: a no nutrients control (natural attenuation), addition of water-soluble nutrients,

and addition of water-soluble nutrients supplemented with a natural microbial inoculums from the site. No significant differences were observed between plots treated with nutrients alone and plots treated with nutrients and the indigenous inoculums, suggesting that supplementation of the natural population with indigenous cultures from the same site still did not result in further enhancement over simple nutrient addition on marine shorelines. The authors also indicated that this conclusion could be extended to include exogenous microbial bacteria inocula or commercial microbial agents because "if indigenous cultures do not accelerate the degradation rates, organisms enriched from different environments, grown in the laboratory, and not acclimated to a particular climate or geographic location should be even less able to compete with the natural population."

Lee et al., (1997) conducted a 129-day field trial to compare the effect of four treatments on biodegradation of weathered venture condensate on a sandy beach in Nova Scotia, Canada. The four treatments (control, inorganic nutrient addition, a commercial bioremediation product, and addition of inorganic nutrients along with bioremediation product) as well as an un-oiled control were replicated in a complete block design using 20 enclosures or plots. C_2 – Chrysene was used as the normalizing biomarker due to the low concentration of hopane in the condensate. PRP (petrolRem, Inc.) was selected to be the representative commercial bioremediation agent in this study. This product is no longer listed in the current NCP product schedule. According to Lee et al., (1997), PRP contains mineral nutrients and non-pathogenic bacteria within spherical particles made from plant derived natural products (beeswax) and exhibits both bio-augmentation and bio-stimulation properties. The agricultural fertilizer used in this study was a mixture of granular forms of ammonium nitrate (N:P:K: 30 – 0 – 0) and triple super phosphate (N:P:K: 0 – 46 – 0). The study showed that an average of 11.0% of the n-alkanes remained in the oiled control plots,

and only 0.1% of the oil remained in the enclosures treated with inorganic nutrients alone; 5.4% of the alkanes were found in the plots treated with inorganic nutrients and PRP, and 25.3% remained in the plots treated with PRP alone. The results indicate that periodic addition of inorganic nutrients was the most effective strategy for enhancing oil degradation and that the full potential of the bioremediation product was limited by nutrient availability. This field trial demonstrated that adding the bioremediation product did not perform better in terms of enhancing alkane degradation than applying inorganic agricultural fertilizers alone.

A few field trials did claim success in demonstrating the effectiveness of oil bio-augmentation, such as using Alpha Biosea ^{TM} (Alpha Environmental,Inc) to treat the Angolan Palanca crude oil spilled from Mega Borg off Texas coast (Mauro and Wynne, 1990; Swannell et al.,1996) and using Terrazyme ^{TM} (Oppenheimer Biotechnology) in enhancing bio-degradation of a heavy oil spilled from Nakhodka in Japan (Tsutsumi et al., 2000). However, the success of these studies was based on either visual observation (i.e. the Mega Borg study) or digital photographic image analysis (i.e. the Nakhodka study). No comprehensive monitoring program was used to verify the oil was indeed removed through enhanced bio-degradation. The two products basically contain the same bacterial cultures and nutrients (Hozumi et al., 2000). The observed visual effects might have been due to physical or chemical processes such as surfactant action associated with the products (Swannell et al., 1996) or sinking.

One of the first field trials on oil bioremediation using a microbial product in a marine environment was reported by Lee and Levy (1987). The study involved seeding a mixed culture of marine oil degrading bacteria (strains of pseudomonas aeruginosa, Pseudomonas stutzeri, and bacillus subtilis grown on bran) in a scotian shelf condensate (SSC) contaminated sandy beach. The extent of bio-degradation was measured by the decline in the n-C_{17}/pristine ratio in this

study. The results showed that the n-C_{17}/pristine ratio in the seeded plots did decrease slightly. However, due to high inter and intra-plot variability, no significant difference in the rate of oil loss was observed among the treatments. This study also observed that the number of oil degrading bacteria did not increase until 10 to 15 days after the addition of oil. However, the addition of the microbial product did not reduce this lag period, suggesting that the toxic volatile components in the oil, which evaporated mostly during the first week, was the main cause of the lag period.

To evaluate the effectiveness of two commercial bio-augmentation products in an estuarine environment, a field trial was carried out in a Texas coastal wetland by a research group from Texas A&M University (simon et al., 1999; Townsend et al., 1999). The two products were selected based on a previous laboratory efficacy test, in which four out of twelve products showed an enhancement of oil bio-degradation with significantly higher degradation rates of alkanes and aromatics when compared to a nutrient control (Aldrett et al., 1997). The 21 – plot site named San Jacinto wetland research facility (SJWRF) has been used for a series of studies on oil spills and their counter measures. In this study, four treatment strategies were examined: an oil control bio-stimulation with inorganic nutrient addition (diammonium phosphate), and commercial bio-augmentation with two different product. Arabian medium crude oil was selected in this test and the 21-plots each measuring 5x5m were arrange in a balanced, incomplete block experimental design oil constituents were determined using gas chromatography/mass spectrometry (GC/MS) and were normalized to 17α(H), 21β(H) – hopane to reduce the effect of sample heterogeneity and physical losses. The results showed that the addition of microbial product could not significantly enhance oil biodegradation rates. No difference were observed between treatment when comparing the first order degradation rate

coefficient for the total saturates, total target aromatics and individual hydrocarbon target analyte. The authors also pointed out that one of the product (BP8) "did show consistently higher biodegradation rates, though the rates were not significantly different from the control". Because this microbial product was applied with vendor supplied inorganic nutrient (Townsend et al., 1999), it is difficult to conclude whether the "consistently but insignificantly" higher rates resulted from the additions of microbial components or the nutrient components. The fact that neither addition of bio-augmentation agent nor application of inorganic nutrients, significantly enhance oil biodegradation suggested that other factors, such as oxygen, could have limited oil degradation in that environment.

Ajoy et al., (2012) carried out a case study on a large scale bioremediation of petroleum hydrocarbon contaminated waste at Indian oil refineries. An indigenous microbial consortium was developed by assemble of four species of bacteria, isolated from various all contaminated site of India, which could bio-degrade different fraction of total petroleum hydrocarbon (TPH) of the oily waste to environment friendly products. The scud consortium was applied on field scale at different oil refineries in India and successfully bio-remediated 48,914 tons of different types of oily waste. In 44 field case studies of different batch size of ex-situ bioremediation process, the initial TPH content varying from 83.50 to 531.30gm/kg of oily waste, has been biodegraded to <10gm/kg of oily waste in major case in two 2-12 months. In one refinery due to coastal climate, the bioremediation time was > 20months. The bio-remediated soil was non-toxic and natural vegetation was found to be grown on the same. Bioremediation technology has been of great help to the oil industries for the management of hazardous waste in our environment in a friendly manner.

Bacterial degradation of petroleum hydrocarbons in crude oil polluted soil amended with cassava peels by Akpe et al., (2015). Effort has been put into bioremediation of the environment and this has led to concerted effort in studying the feasibility of detoxifying oil contaminants using organic and inorganic waste. Standard physiochemical and micro-biological procedures were used in this study for eight weeks. The result showed that CP contain appreciable amounts of some biodegradation enhancing elements/ nutrients such as nitrogen (2.37%), potassium(7.13meq/100g), phosphorus(0.78mg/kg) and organic carbon (2.37%). The soil samples used for the study were composed of 81.6% clay ,16.4% sand and 2% silt. The PH of the amended samples during the period of study range from 6.54 to 8.16. The hydrocarbon degrading bacteria types and numbers were found to be lesser that their heterotrophic counterpart. Also, samples amended with CP were found to have more types and higher number of heterotrophic and hydrocarbon degrading bacteria than the samples without amendment (controls). The hydrocarbon degrading bacteria count for the amended samples ranged from $3.8\pm0.01 \times 10^5$cfu/g to $16.50\pm0.01 \times 10^5$cfu/g with the 5% crude oil polluted samples having the highest count, for the nonamended samples the count of hydrocarbon degrading bacteria were in range $2.30\pm0.01 \times 10^5$cfu/g to $4.90\pm0.01 \times 10^5$cfu/g.The total petroleum hydrocarbon (TPH) in the samples decrease from day zero to day 56 at the various pollution levels(5%, 10%, 15%). The highest reduction in TPH was in the 5% crude oil polluted soil sample with amendment(89.82%) while the least TPH reduction was in the 15% polluted control sample (without amendment)(27.38%). These findings showed that lower percentage of crude oil was degraded as concentration of crude oil increased and that cassava peels which is an agro waste can enhance biodegradation of crude oil in polluted soil. Therefore, cassava peels instead of being disposed of a waste can be harnessed and used as a bioremediation agent in polluted sites.

In order to evaluate the efficiency of bio-stimulation of soil contaminated with oil hydrocarbons under sub-antarctic conditions, a mesocosm study was initiated in may 2001 in the kerguelen Archipelago (49 degrees 21'S, 70degrees 13'E) by F.coulon and D.Delille. The effects of temperature and fertilizer addition (Inipol EAP-22, Elf Atochem) on soil bacterial assemblages contaminated with hydrocarbons were studied in 6-1 batches of sub Antarctic soil incubated in the dark. Six different conditions were used at three temperatures (4,10 and 20 degree celcius): control, fertilizer (50ml), diesel oil (100ml), diesel oil (100ml) + fertilizer (50ml), "Arabian light" crude oil (100ml) and crude oil (100ml) + Fertilizer (50ml). Mesocosms were sampled on a regular basis over a seven month period. All samples were analyzed for total bacteria, viable heterotrophic assemblages and hydrocarbon utilizing microflora. The results clearly showed a significant response of sub-Antarctic microbial soil communities to hydrocarbon contamination. Large increases in total, heterotrophic and hydrocarbon-utilizing bacteria were observed (from less than 5 x 10^5 $MPNg^{-1}$ to more than 10^8 $MPNg^{-1}$ for hydrocarbon degrading bacteria). Temperature elevation had no significant impact on the total or heterotrophic assemblages but induced a one order of magnitude increase in hydrocarbon-utilizing bacteria in contaminated mesocosms. In contrast, fertilizers addition had no clear effect on hydrocarbon-degrading specific bacteria but stimulated heterotrophic growth in diesel oil-contaminated soils.

Cai et al., (2020) conducted a study on diagnosing bioremediation of crude oil contaminated soil and related geochemical processes at the field scale through microbial community and functional genes. In this study the microbial community and functional genes related to hydrocarbon and nitrogen metabolism, combined with the soil physio-chemical properties were used to diagnose a set of bioremediation experiments, including bio-augmentation, bio-stimulation, phyto-remediation at the field scale. The field that was remediated was part of an abandoned oil well

site located in Puyang county, Henan province, China. The oil well was abandoned in 2000 after a short period of operation due to low productivity. A blowout occurred when the well was drilled, and as a result, the dispersed oil heavily contaminated 3000m2 of farmland soil. The study area was divided into four blocks: blank control, Bio-augmentation(BA), Bio-stimulation and Phyto-remediation. Each block was a soil cuboid that was 80long, 80cm wide, and 30 cm deep. A five point sampling, coning, and quartering method was used to collect samples; thus, a mixed sample was collected in each block at each time. The results showed that the added nutrients stimulated a variety of micro-organisms, including hydrocarbon degradation bacteria and nitrogen metabolism micro-organism. The functional genes reflected the possibility of aerobic denitrification in the field, which may be helpful in biodegradation. Bio-stimulation was found to be the most suitable of the studied bioremediation methods in the field. The study used a feasible approach to obtain useful bioremediation information and assist with the development of appropriate remediation procedures which has improve our knowledge of the interactions between micro-organisms and edaphic parameters.

Adedokun and Ataga(2007) reported a significant improvement in the growth of cowpea (Vignauguiculata) when a soil polluted with crude oil, automotive gasoline oil and spent engine oil was bio-stimulated (with saw dust and waste cotton) and bio-augmented (with pleurotuspulmonarium). They reported that bioremediation of polluted soil reduced the time for seed germination from 8 to 3 days, increased seed germination from 60% to 96%, plant height from 10.3cm to 22cm, leaf number from 3 to 5 and biomass from 0.5g to 1.5g dry weight. A similar study by kyungHwa et al.,(2004) showed that the length of corn (Zeamays) and red beans (Phaseolusnipponesis) grown on a polycyclic aromatic hydrocarbon (PAH) polluted soil inoculated with Norcardia spp. for bioremediation purposes increased by 77% and 56%

respectively with respect to the control (unpolluted soil). However, on the polluted soil that were not bio-remediated, length of corn and red beans were 16% and 49% respectively. The authors also reported that bioremediation reduced phytotoxicity in the test crops.

Njoku et al.,(2008) reported a general improvement in the chlorophyll content, leaf area, growth, pod production and dry weight of soy bean (Glycine max) grown on a crude oil polluted soil bio-remediated with cow dung. Significant increase in the germination, growth and productivity of soy-beans were also recorded on a crude oil polluted soil subjected to bio-augmentation and bio-stimulation using Pseudomonas, bacillus and poultry manure (Nwadinigwe and Onyeidu, 2012). The study also showed that bio-augmentation performed better than bio-stimulation with respect to crop growth and development.

Child (2009) used five kinds of PAHs-degrading bacterium to impregnate the barley seeds. He discovered that the mycobacterium directly attached to the root system by seed germination, improving the degradation rate of PAHs in the soil. Joner (2003) grew rye grass and white clover in two different concentrations of PAHs, after 28weeks it was found that the root of arbuscular mycorrhizal fungi (AMF) system was 1.65times than before. The larger root biomass and surface area are beneficial to the adsorption and absorption of organic matter. Domestically, Gao (2013) made use of the combination of high efficient petroleum degradation bacteria and plants to repair th oil –heavy metal pollution in Gudao oil province. This research had shown that the petroleum hydrocarbon degradation rate of joint repair technology is significantly higher than that of single repair technology. Li (2013) used suaeda, the local salt raw plant, and dominant mycorrhizal fungi in Dongying region to establish the AMF-plant nitrogen addition joint repair system. Through container experiment she found out that the oil degradation rates of AMF-Suaeda and

Carbamide, AMF-Suaeda increased significantly. To sum up, the microbe-plant combination system can obviously improve the removal efficiency of petroleum pollutant in the soil.

CHAPTER THREE

RESEARCH METHODOLOGY

This study aimed at the evaluation of goat manure performance for the bioremediation of used engine oil polluted soil, was carried out in the following steps listed below.

- Collection/storage of soil samples
- Characterization of soil sample
- Preparation of used engine oil polluted soil
- Characterization of used engine oil polluted soil
- Experimentation and soil treatment procedures
- Sampling/Analysis of treated soil samples
- Determination of the amount of petroleum degradation
- Reaction kinetic studies
- Statistical analysis of data

3.1 Collection/Storage of Soil Samples

The procedures adopted by Isitekhale et al., (2013) was employed with slight modifications as follows; 10kg soil sample was collected from a site free of used engine oil contamination (from an agricultural land in The Department of Petroleum Engineering, Faculty of engineering, University of Benin, Ugbowo campus, Benin City, Edo State in Nigeria)using a 22-cm hand-dug soil auger and stored in labeled black polythene bag. The sample was air dried, grinded and sieved through 2mm mesh before use (Agamuthu and Dadrasnia, 2013).

3.2 Characterization of soil samples

Before contamination, the soil sample was subjected to chemical digestion using 1:1 ratio of 0.25M hydrochloric acid and Nitric acid. Thereafter it was characterized to determine the physio-chemical properties (Ayotamuno et al., 2006). The physio-chemical properties determined include; Total Heterotrophic bacterial, Moisture content Soil, pH, Electrical conductivity, Total hydrocarbon content (THC),Total organic carbon, Total nitrogen content in addition to the soil composition including percent sand, Total Phosphorus, Lead (Pb) and Iron (Fe).

3.2.1 Standard Method for Soil Parameters Analysis

3.2.1.1 Determination of Soil pH

A. Reagents

- pH Buffers, 4.00, 7.00 and 10.00, commercially available
- Potassium chloride (KCl) solution, saturated, used for filling the combination electrode. If separate glass and reference electrodes are used, the reference electrode is filled with saturated aqueous KCl.
- Reagent Water distilled or deionized water, pH 6.5 to 7.5. Deionized or distilled water should be used for rinsing the probe between samples.
- Calcium Chloride Solution, 0.01M - Dilute 20.0 millilitres (ml) of stock 1.0 M calcium chloride solution to 2 litres (L) with deionized water. The pH of this solution should be between 5 and 7. Adjust pH of this solution if necessary (SERAS, 2002).

B. Sample Preparation with Reagent Water

- Air dries the soil sample to be tested.

- Sieve the soil sample through a No. 10 sieve (2 mm mesh) to remove the coarser soil fraction.
- Weigh out approximately 10 g of the air-dried and sieved soil sample.
- Place the soil into a glass container and add approximately 10 mL of distilled or deionized water.
- Mix thoroughly and let stand for 1 hour. (SERAS, 2002).

C. Sample Measurement

- Samples should be analyzed immediately (within 15 minutes after preparing sample in Section 7.4 or 7.5).
- Measure the temperature of the suspended soil sample. Set the temperature dial on the pH meter to match the measured temperature in C.
- Rinse the probes with distilled or deionized water. Blot dry.
- With the meter on, place the electrode in the partially settled sample suspension to be measured.
- If the meter is calibrated using pH 4.00 and pH 7.00 buffers and the sample reading is >7.00, the meter must be recalibrated using pH 7.00 and 10.00 buffers. Likewise, if the meter is calibrated using 7.00 and 10.00 buffers and the pH reading is <7.00, the meter must be recalibrated using the 4.00 and 7.00 buffers. The sample pH will be displayed. Record the reading once the meter has stabilized. (SERAS, 2002).

3.2.1.2 Determination of Soil Organic Carbon

A. Apparatus

- 250ml conical flask
- 50ml Burette
- 10ml Pipette

B. Reagents Required

- N potassium dichromate
- 0.4N Ferrous Ammonium Sulphate
- Conc. Sulphuric acid
- Phosphoric acid
- 1% Diphenylamine in conc. H_2SO_4 (Miroslav and Vladimir, 1999)

C. Preparation of Sample

About 50g of air-dried fine Topsoil, which has been passed through the 2mm sieve, should be ground to a fine consistency for carbon and nitrogen determinations. The ground sample should pass the 0.5mm sieve. Before grinding, it should be checked for roots and organic debris. (Miroslav and Vladimir, 1999)

D. Procedure

- Weigh out a sample of soil containing about 0.2g of carbon (0.1-0.5g) into a 250ml conical flask.
- From an automatic pipette or a burette, add 10ml of N $k_2Cr_2O_7$, and then 10ml of Conc. H_2SO_4.
- Shake for 1min.and allows the mixture to cool on an asbestos sheet.

- To the cooled solution, add 60ml of distilled water and make the volume up to 150ml. shake and allow cooling further.
- After adding 5ml of Phosphoric acid and 8-10 drops of 1% Diphenylamine solution the solution assumes a dark violet colour.
- Titrate with 0.4N ferron Ammonium Sulphur solution until the colour changes to green.
- Weigh 0.2g of Glucose in triplicate and treat as above (Miroslav and Vladimir, 1999).

E. Calculation : (% Carbon = Titre x 0.24) (3.5)

3.2.1.3 Digestion and Determination of Soil Nitrogen

A: Introduction

Nitrogen in the soil is determined by Kjeldahl digestion, and the resulting Ammonium ion measured calorimetrically.Elements such as Iron and Manganese, which may interfere in the alkaline medium during colorimetric determination, are first complexed with Sodium Potassium Tartrate. The Ammonia is determined calorimetrically as the indophenol blue complex by reaction with alkaline Sodium phenate and Sodium Hypochlorite(Miroslav and Vladimir, 1999).

B: Micro-Kjeldahl Digestion Apparatus

- 30ml Kjeldahl flasks
- Micro Kjeldahl heaters

C: Reagents

- Concentrated sulphuric acid
- Kjeldahl catalyst (one tablet of sodium sulphate containing 0.05g selenium), each tablet is 1g. (Miroslav and Vladimir, 1999)

D: Procedure

- Weigh 0.2g of finely ground soil into 30ml Kjeldahl digestion flask; add one tablet of catalyst and 4.0ml of conc. H_2SO_4. Shake well to ensure complete mixing of the soil and catalyst mixture.
- Place the flask on the heater and digest for about 45 minutes.
- On completion of the digestion, the mixture will be clear. Remove from the heater. Cool until just warm to touch, and then add 10ml of distilled water. It is important that the mixture in the Kjeldahl flask does not solidify before the addition of water, as it is time consuming re-dissolving the solids.
- Decant solution through a Whatman filter paper No. 42 into 100ml volumetric flask. Wash the Kjeldahl flask with 2 or 3 small aliquots of distilled water adding all the washings into the volumetric flask via the filter paper and make up to volume. Nitrogen is determined in the filtrate as follows; (Miroslav and Vladimir, 1999)
  i. Pipette 5ml of the filtrate from the digest into a 25ml flask.
  ii. Add 2.5ml of the Alkaline Phenol. Shake well.
  iii. Add 1ml of Sodium Potassium Tartrate and shake well.
  iv. Add 2.5ml of Sodium Hypochlorite or Bleach; shake well and let colour develop, and then make to the mark.
  v. Read calorimetrically at 630nm.

E: Calculation

$$\%N = \frac{Instr.\,\text{Reading} \times \text{Reciprocal of slope} \times \text{Colour Vol.} \times \text{Digest Vol.} \times 10^{-6} \times 100 \times Cf}{Weight\ \text{of Sample} \times \text{Aliquot taken}} \qquad (3.6)$$

Where: CF = correction factor

3.2.1.4 Determination of microbial population

For total heterotrophic bacteria (THB) 1g of soil was mixed with 9ml phosphate buffered saline (PBS) solution and diluted with 0.85% NaCl until 10-9 dilution. 0.1 ml of each dilution was then plated on nutrient agar and Petri plates were incubated at 37^0C for 48 hr.

After incubation period colonies were counted. The resultant colony forming unit (cfu/g) was calculated for both the untreated and treated soil samples (Martin et al., 2012).

3.2.1.5 Determination of Total Hydrocarbons

A: Apparatus

- 250ml separating glass funnels.
- Spectrophotometer
- Pipette, 10ml
- Mechanical Shaker (Miroslav and Vladimir, 1999)

B: Reagents

- n- Hexane

C: Procedure

- Dry the soil properly
- Weigh 5g into a 100ml plastic bottle.
- Add 25ml of n-Hexane.
- Shake for 10minutes, cover it and allow standing for some time.
- Filter and read filtrate at 460nm. (Miroslav and Vladimir, 1999)

D: Calculation

$$THC\,(\text{PPM}) = \frac{\text{Instrument Reading} \times \text{Slope Reciprocal} \times 25}{\textit{Weight}\ \text{of sample}(5\text{g})} \tag{3.7}$$

3.3 Preparation of used engine oil polluted soil

10kg of unpolluted soil wasweighed into a plastic bowl in addition to 2.5kg of concentrated used engine oil (Agarry et al., 2010). The used engine oil was added gradually into the bowl containing the unpolluted sieved soil sample and was properly mixed. The used engine oil was to serve as the pollutant. The moisture content of the used engine oil contaminated soil sample was determined immediately after adequate mixing. The soil samples were left for 4days for stabilization before the commencement of treatment process.

3.4 Characterization of used engine oil polluted soil

After contamination, the used engine oil polluted soil sample was again subjected to chemical digestion using 1:1 ratio of 0.25M hydrochloric acid and Nitric acid. Thereafter, the polluted soil was characterized to determine the physio-chemical properties (Ayotamuno et al., 2006). The physio-chemical properties determined include; soil pH, electrical conductivity, total hydrocarbon content (THC), total organic carbon and total nitrogen content. The aim was to determine the effects of used engine oil pollution on the intrinsic properties of the soil. More also, characterization of the polluted soil was to help determine the initial conditions of the soil before substrate addition.

3.5 Experimentation and Soil Treatment Procedures

The contaminated soil was in a clean dried plastic container perforated at the bottom. The plastic container and its content was open throughout the period of experimentation to allow for the

influence of atmospheric oxygen. For effective treatment, a 2:1 mixture (w/w) of the polluted soil and the substrate in addition to 25ml of nutrient agar was utilized (Agamuthu and Dadrasnia, 2013).

3.6 Sampling/Analysis of treated soil sample

The experiment was monitoredfor a period of eight (8) weeks under which appreciable level of remediation had been obtained. Samples werecollected for analysis on weekly basiswith the sample mean and standard deviation of the parameters computed to ascertain the rate of used engine oil degradation with treatment time. Analyses of the treated soil was done to ascertain the effects of treatment on the initial conditions of the soil after pollution. The properties or parameters of interest were; pH, electrical conductivity, total hydrocarbon content (THC), total organic carbon and total nitrogen content

3.7 Determination of the amount of hydrocarbon degradation

The amount of petroleum hydrocarbon removed during the series of batch investigation was determined using the mass balance equation of the form (Raghuvanshi et al., 2004):

$$q = \frac{v}{m}\left[C_0 - C_e\right] \tag{3.1}$$

Where: q, defines the petroleum hydrocarbon uptake (mg/g); C_0 and C_e: are the initial and equilibrium petroleum hydrocarbon concentrations in the digested soil solution [mg/l] respectively; V: is the weight of soil sample taken (g) and M: is the mass of substrate used (g).

The efficiency of petroleum hydrocarbon removal (%) was calculated using the mass balance equation of the form (Badmus et al., 2007;Eba et al., 2010).

$$\text{Removal Efficiency (\%)} = \left(\frac{C_0 - C_e}{C_0} \times 100\right) \tag{3.2}$$

Where: C_0 and C_e are total hydrocarbon content (THC) (mg/l) in digested soil solution before and after treatment respectively.

3.8 Kinetics of Used Engine Oil Degradation

3.8.1 Pseudo First Order Kinetic Model

The pseudo first-order rate expression of Lagergren based on the solid capacity is generally expressed as follows:

$$\frac{dq_t}{dt} = K_1(q_e - q_t) \tag{3.3}$$

Where:

q_e and q_t are the amount of engine oil removed at equilibrium and at time*t*, respectively (mg·g-1),$K_1$is the rate constant of pseudo first-order adsorption (Lagergren and Svenska, 1998). The linear plots of Log [$q_e - q_t$] versus time (t) show the appropriateness of the above equation and subsequently the first order nature of the process involved (Lagergren and Svenska, 1998).

3.8.2 Pseudo Second Order Kinetic Model

The pseudo-second- order equation is also based on the sorption capacity of the solid phase.

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2 \tag{3.4}$$

where :

K_2 = The rate constant of pseudo - second order ($gmg^{-1}\,min^{-1}$)

The plot of $(\frac{t}{q_t})$ against (t) should give a linear relationship from which q_e and K_2 can be determined from the slope and intercept of the plot (Blanchard et al., 1984; HO and McKay, 1999; Yuh-shan, 2006; Shamik and Papita, 2010).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

This chapter contains the results of various investigation carried out in chapter three as follows:

4.1 Analysis of Un-polluted and Polluted soil

Table 4. 1 Physio-chemical properties of Experimental soil

S/n	Parameters	Unit	Unpolluted Soil
1	Moisture Content (%)	%	0.54
2	pH	Nil	8.4
3	Electrical Conductivity	(µs/cm)	43.27
4	Total Organic Carbon (TOC)	(g/kg)	6.82
5	Total Nitrogen (TN)	(g/kg)	7.33
6	Total Phosphorous (TP)	(g/kg)	6.75
7	Total Hydrocarbon Content (THC)	(mg/kg)	0.00
8	Lead (Pb)	mg/kg	0.00
9	Iron (Fe)	mg/kg	1.74
10	Total Heterotrophic Bacterial	(cfu/g)	$7.6*10^5$

Results of Table 4.1 shows that the experimental soil is an alkaline soil with a pH of 8.4, and with a moisture content of 0.54 percent and fertile with total organic carbon, total nitrogen, and total Phosphorous of 6.82g/kg, 7.33g/kg, and 6.75g/kg respectively. The soil electrical conductivity which is also an important indicator of soil health was measured to be 43.27µs/cm. It was also discovered from the physio-chemical analysis that the soil was completely free from crude petroleum hydrocarbon pollution as evident from the result of the total hydrocarbon content. The iron content and the total heterotrophic bacterial was also gotten to be 1.74mg/kg and $7.6*10^5$.

The physio-chemical properties of the used engine oil that was used for this study are presented as shown in Table 4.2

Table 4. 2 Physio-chemical properties of polluted soil

S/n	Parameters	Unit	Unpolluted Soil	Polluted Soil(100g:20ml used engine oil)
1	Moisture Content (%)	%	0.54	22.66
2	pH	Nil	8.4	2.89
3	Electrical Conductivity	(µs/cm)	43.27	79.93
4	Total Organic Carbon (TOC)	(g/kg)	6.82	4.22
5	Total Nitrogen (TN)	(g/kg)	7.33	4.04
6	Total Phosphorous (TP)	(g/kg)	6.75	3.51
7	Total Hydrocarbon Content(THC)	(mg/kg)	0.00	8.09
8	Lead (Pb)	mg/kg	0.00	0.059
9	Iron (Fe)	mg/kg	1.74	1.73
10	Total Heterotrophic Bacterial	(cfu/g)	$7.6*10^5$	$2.16*10^5$

The addition of used engine oil to the experimental soil resulted in the contamination of the soil which altered the physio-chemical properties of the soil. The results of changes in the soil physio-chemical properties occasioned by the addition of used engine oil are presented in Table 4.2 above and the following effect are observed:

- The pH value of the soil decrease from 8.4 to 2.89 and this shows that the presence of the used engine oil made the soil to become highly acidic and this will result in poor plant growth or no growth at all around the area of contamination.
- The high level of electrical conductivity (from 43.27 µs/cm to 79.93 µs/cm) occasioned by the presence of high concentration of dissolved solids as seen from the results of conductivity, this higher electrical conductivity hinders the nutrient uptake by increasing the osmotic pressure of the nutrient solution, wastes nutrients, and increases discharged of nutrients into the environment, resulting in environmental pollution.

- A drastic reduction in total nitrogen, total phosphorus and total organic carbon concentration occasioned by a sudden increase in the hydrocarbon content of the soil due to contamination.

- It was also observed from the result that the addition of used engine oil to the soil resulted in a drastic reduction in the total heterotrophic bacterial count from $7.6x10^5$cfu/g to $2.16x10^5$cfu/g due to contamination.
- There was also a slight decrease in Iron(Fe) and a slight increase in Lead(Pb)

4.2 Results of Bioremediation Treatment

4.2.1 Effects of Treatment on Moisture Content

When the effects of substrate addition (Bioremediation) on the moisture content of used engine oil contaminated soil was studied for a period of 8 weeks, the following results as presented in table 4.3 were obtained.

Table 4. 3 Effect of treatment on moisture content of used engine oil polluted soil

S/N	Time (Weeks)	Parameter (%)	Initial Moisture content	During Treatment
1	Week 1	Moisture content	22.66	40
2	Week 2	Moisture content	22.66	40
3	Week 3	Moisture content	22.66	38
4	Week 4	Moisture content	22.66	38.5
5	Week 5	Moisture content	22.66	38.5
6	Week 6	Moisture content	22.66	38.5
7	Week 7	Moisture content	22.66	38.5
8	Week 8	Moisture content	22.66	38.5

The graphical relationship between the moisture content and the remediation time with respects to the substrate used is presented as shown in Figure 4.1 below:

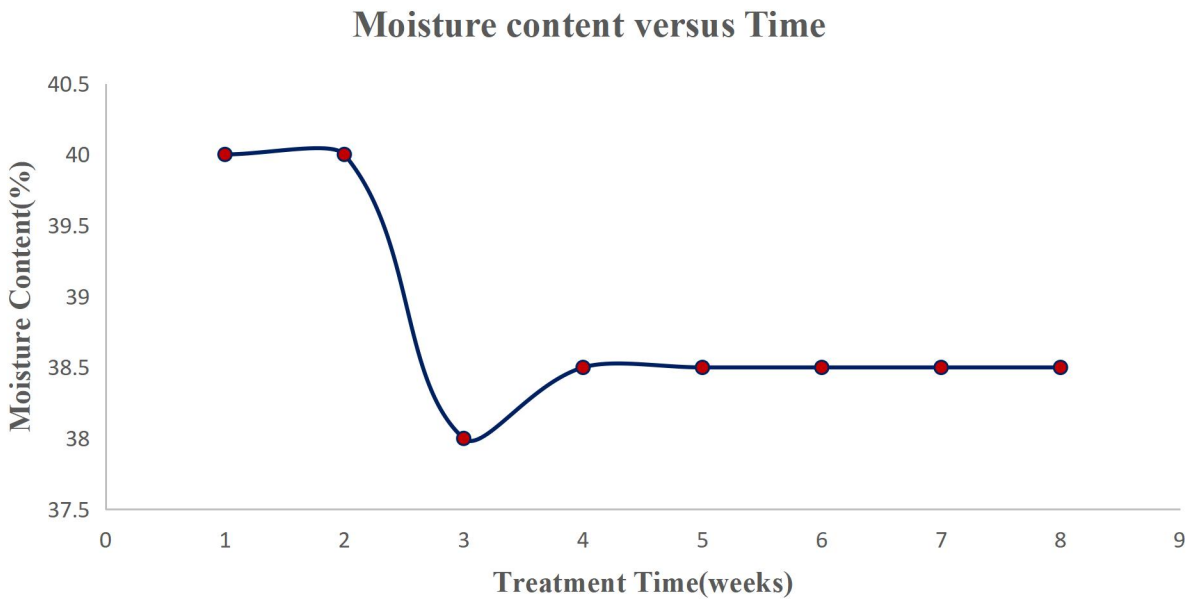

Figure 4. 1 Variation of moisture content with treatment time

Soil moisture content is quantity of water it contains, this moisture content may have been affected by temperature, soil characteristics, the input of addition water to the experimental soil. From the graph a gradual increase was observed between week one and week two, then a decrease in the moisture content between week two and week three, followed by an increase and a constant moisture content was observed between week four to week eight which shows stability.

4.2.2 Effects of Treatment on pH

When the effects of substrate addition (Bioremediation) on the total pH of used engine oil contaminated soil was studied for a period of 8 weeks, the following results as presented in table 4.4 were obtained.

Table 4. 4 Effect of treatment on pH of used engine oil polluted soil

S/N	Time (Weeks)	Parameter	Initial pH	During Treatment
1	Week 1	pH	2.89	4.78
2	Week 2	pH	2.89	5.20
3	Week 3	pH	2.89	5.40
4	Week 4	pH	2.89	5.67
5	Week 5	pH	2.89	5.80
6	Week 6	pH	2.89	5.95
7	Week 7	pH	2.89	6.10
8	Week 8	pH	2.89	6.10

The graphical relationship between the pH and the remediation time with respects to the substrate used is presented as shown in Figure 4.2 below:

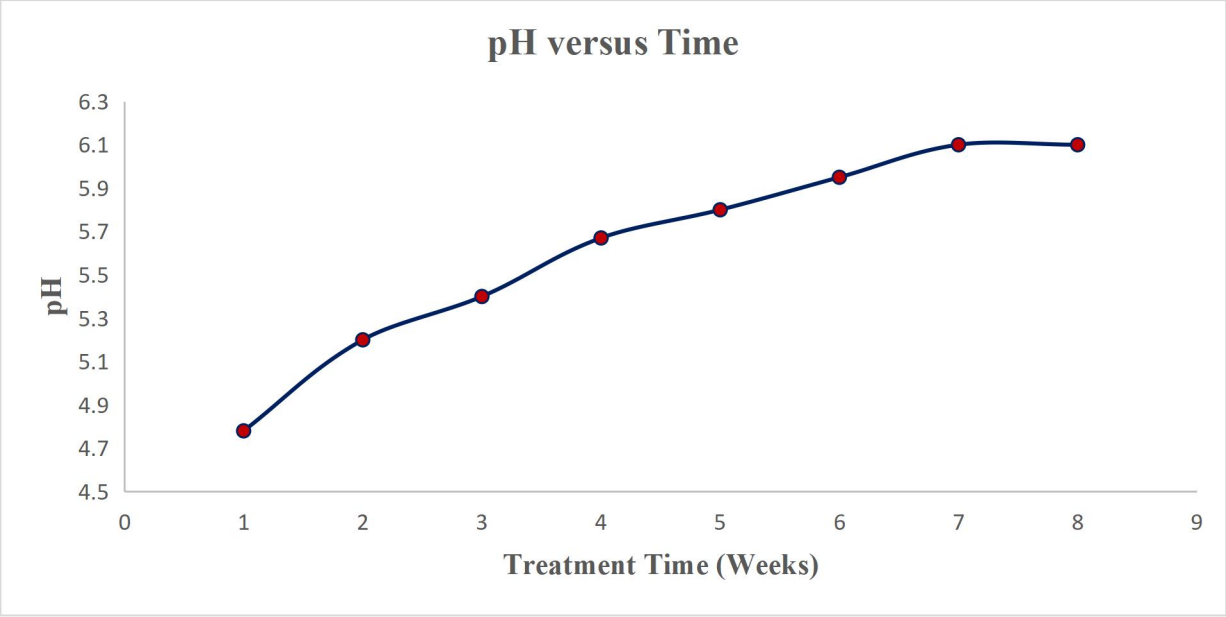

Figure 4. 2 Variation of pH with treatment time

From the above graph it can be deduced that the pH of the soil increases upon treatment, the graph depicts a gradual increase in pH. The percentage of remediation is gotten from (6.1/8.4)*100 which is equal to 72.6%.

Awari *et al.,* carried out a bioremediation on crude oil contaminated soil using fish waste and goat manure, it was observed from the experimental results of goat manure that there was a gradual increase in the pH of the soil over a period of 7 to 56 days of treatment, and this aligns with the result gotten from this experiment. Agarry *et al.,*(2010) , also had a similar result, that is an increase in pH due to treatment which lasted for 4 weeks. Nwogu *et al.,* (2015), carried out an enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with goat manure the pH was observed to increase from 6.11 to 6.20 within 42 days. Stanley (2013) stimulated biodegradation of spent lubricating motor oil in the soil using animal droppings, his experimental result also shows an increasing value of pH during treatment. Ilaboya and Otuaro (2019) in their comparative studies on the bioremediation of used engine oil contaminated soil using urea fertilizer, goat manure, pig manure and brewery spent grain, the experimental result shows that the pH increase during treatment and the trend of the result gotten is consistent with the result in this experiment.

The effect of pH on a soil is to remove from the soil or to make available certain ions. Soils with high acidity tend to have toxic amounts of aluminium and manganese. Plants which need calcium need moderate alkalinity, but most minerals are more soluble in acid soils. The acidity (pH) of the soil is an important soil parameter. Soil pH can be highly variable, ranging from 2.5 in mine spoils to 11 in alkaline deserts. Most heterotrophic bacteria favour a pH 7.0 but fungi being more tolerant to acidic conditions. Therefore, extremes pH of soils would have a negative influence on the ability of microbial populations to degrade hydrocarbons.

The pH value of a soil sample is the most frequently determined parameter in soil analysis. It is the characteristic value of what is known as “soil reaction,” and allows soils to be classified according to their acidity and alkalinity.

4.2.3 Effects of Treatment on Electrical Conductivity

When the effects of substrate addition (Bioremediation) on the electrical conductivity of used engine oil contaminated soil was studied for a period of 8 weeks, the following results as presented in table 4.5 were obtained.

Table 4. 5 Effect of treatment on electrical conductivity of used engine oil polluted soil

S/N	Time (Weeks)	Parameter (µs/cm)	Initial EC	During Treatment
1	Week 1	Electrical conductivity	79.93	95.06
2	Week 2	Electrical conductivity	79.93	118.3
3	Week 3	Electrical conductivity	79.93	124.5
4	Week 4	Electrical conductivity	79.93	133.4
5	Week 5	Electrical conductivity	79.93	138.7
6	Week 6	Electrical conductivity	79.93	141.2
7	Week 7	Electrical conductivity	79.93	148.7
8	Week 8	Electrical conductivity	79.93	149.9

The graphical relationship between the total organic carbon and the remediation time with respects to the substrate used is presented as shown in Figure 4.3 below:

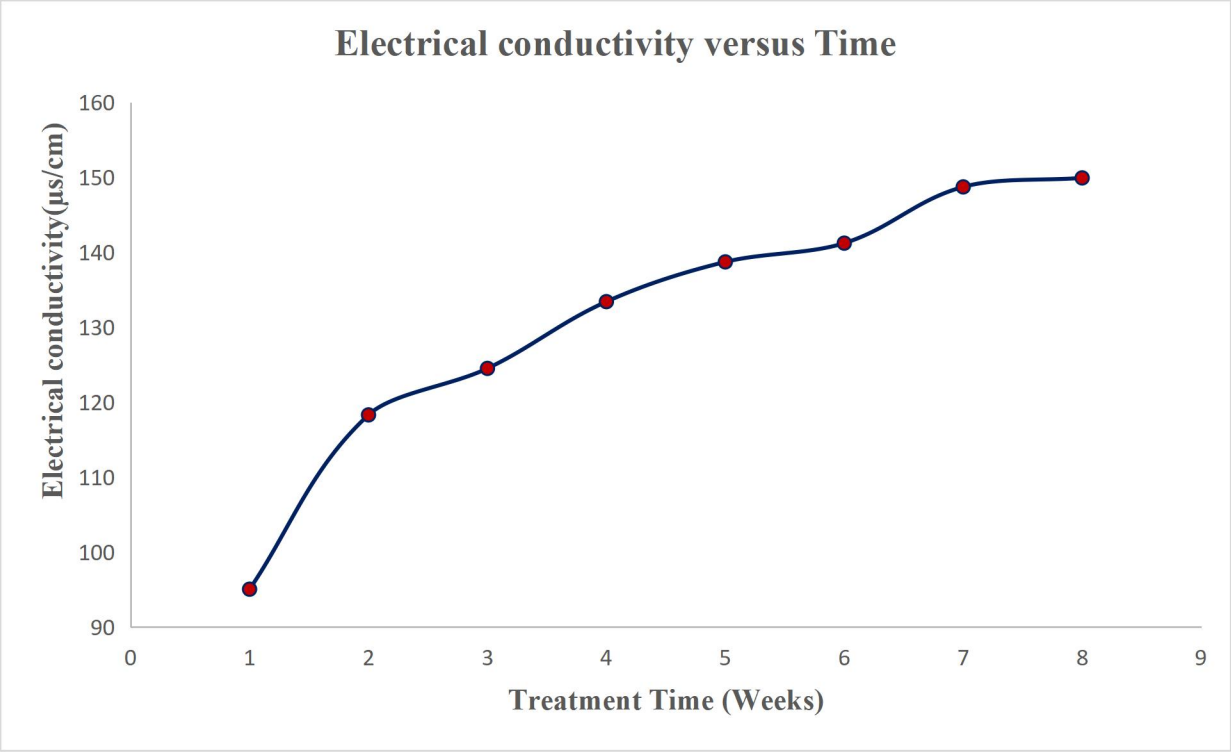

Figure 4. 3 Variation of electrical conductivity with treatment time

The above graph shows a gradual increase in the conductivity of the polluted soil with increasing time of remediation. This trend could be attributed to the presence of high level of dissolved ions occasioned by the breakdown of the hydrocarbon by the heterotrophic bacterial present in the substrate used (Ilaboya and Otuaro, 2019). Nwogu *et al.,*(2015) during a bioremediation of petroleum hydrocarbon polluted soil using goat manure, observed a slight decrease in electrical conductivity which can be attributed to the presence of low level of dissolved ions occasioned by breakdown of the hydrocarbon and can also be attributed by some other factors like soil minerals, climate, soil texture,bulk density, water potential and soil aggregation (USDA, NRCS, 2014).

4.2.4 Effects of Treatment on Total Organic Carbon (TOC)

The trend that applies to total organic carbon content due to substrate addition also applies to total nitrogen content since the nutrient level of soil is measured based on the concentration of total nitrogen, total organic carbon and total phosphorous content. The variation of total organic carbon with remediation time is presented as shown in Table 4.6 below;

Table 4. 6 Effect of treatment on Total organic carbon of used engine oil polluted soil

S/N	Time (Weeks)	Parameter (g/kg)	Initial TOC	During Treatment
1	Week 1	TOC	4.22	4.01
2	Week 2	TOC	4.22	3.56
3	Week 3	TOC	4.22	3.37
4	Week 4	TOC	4.22	3.28
5	Week 5	TOC	4.22	3.19
6	Week 6	TOC	4.22	3.06
7	Week 7	TOC	4.22	2.77
8	Week 8	TOC	4.22	2.65

The graphical relationship between the total organic carbon and the remediation time with respects to the substrate used is presented as shown in Figure 4.5 below:

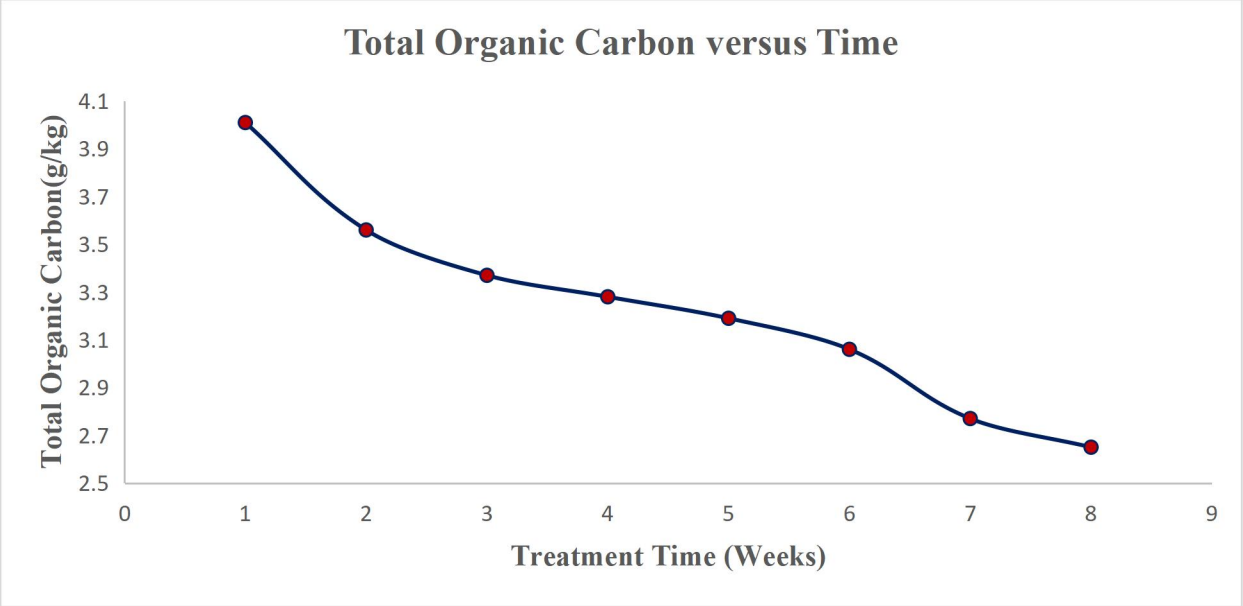

Figure 4. 4 Variation of Total organic carbon with treatment time

The gradual decrease in the total organic carbon with increase in remediation time could be traced to the increase in the population of total heterotrophic bacterial count occasioned by nutrient utilization. This reduction can also be attributed to the steady and continuous consumption of this nutrient by the microorganism for growth and development. Chinenye *et al.,*(2014), carried out an experiment on the biodegradation of crude oil polluted soil by co-composting with agricultural wastes and inorganic fertilizer, the result of the total organic carbon content was observed that there was a gradual decrease in the TOC content during the study period of 112days, and this result is consistent with the result obtained in this work. Nwogu *et al.,*(2015) in his work on enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons using goat manure, a gradual decrease was observed in the total organic carbon content during the treatment period of 28days and this result is consistent with this experimental result. The result of Ilaboya and Otuaro (2019) also corroborate with this experimental result.

4.2.5 Effects of Treatment on Total Nitrogen (TN)

When the effects of substrate addition (Bioremediation) on the total nitrogen content was studied for a period of 8 weeks, the following results as presented in table 4.7 were obtained

Table 4. 7 Effect of treatment on total nitrogen of used engine oil polluted soil

S/N	Time (Weeks)	Parameter(g/kg)	Initial TN	During Treatment
1	Week 1	Total Nitrogen	4.04	3.99
2	Week 2	Total Nitrogen	4.04	3.42
3	Week 3	Total Nitrogen	4.04	3.18
4	Week 4	Total Nitrogen	4.04	3.08
5	Week 5	Total Nitrogen	4.04	3.01
6	Week 6	Total Nitrogen	4.04	2.88
7	Week 7	Total Nitrogen	4.04	2.54
8	Week 8	Total Nitrogen	4.04	2.37

The graphical relationship between the residual total nitrogen concentration and the remediation time with respects to the substrate used is presented as shown in Figure 4.5 below:

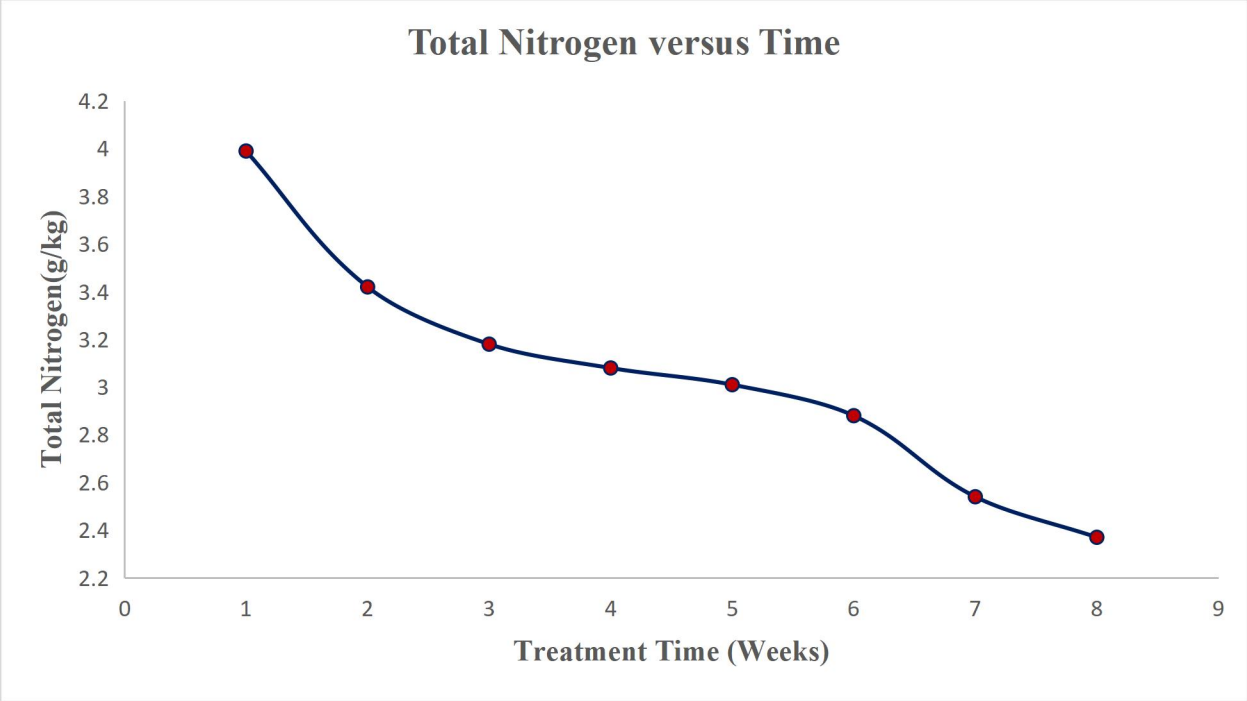

Figure 4. 5 Variation of total nitrogen with treatment time

The gradual decrease in the total nitrogen content with increase in remediation time could be traced to the increase in the population of total heterotrophic bacterial count occasioned by nutrient utilization. Heterotrophic bacterial normally utilized the available nutrient in the form of total nitrogen, total organic carbon and total phosphorous for their growth and cell development; consequently, leading to a drastic reduction in the nutrient level. Therefore, as the remediation time increases, there is a corresponding increase in the population of total heterotrophic bacterial present in the treatment substrate due to utilization of available nutrient resulting to decline in the concentration of these nutrients. More also, increase in bacterial population may lead to unhealthy competition for the available nutrients since the bacterial would need the nutrient for survival.

The results gotten by Chinenye *et al.,*(2014), is contrary to this experimental result, Chinenye *et al.,* observed a gradual increase in nitrogen content instead of a decrease, this can be attributed to the fact that the soil we work on had a lower nitrogen content and goat manure which has almost

the lowest nitrogen content in comparison to other types of animal manure did not have the capacity to increase the nitrogen content ,instead the little nitrogen in the composition was used to feed the heterotrophic bacterial for their growth and cell development, also since it was an artificial contamination and much time was not given for the micro-organisms in the soil to degrade the hydrocarbon to some extent before applying the goat manure, so the effect of the engine oil was very high that the heterotrophic bacterial needed to feed from the available nitrogen in other to degrade the effect of the engine oil. Awari *et al.,*(2020), obtained a slight increase in the nitrogen content gotten from the experimental result.

Nwogu *et al.,*(2015) carried out an enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons using goat manure, the result obtained from this experiment corroborate with the results gotten from this experiment , that is, a gradual decrease in nitrogen content was observed. Ilaboya and Otuaro (2019) also observed a gradual decrease in nitrogen content in their comparative studies on bioremediation of used engine oil contaminated soil using urea fertilizer, goat manure, pig manure and brewery spent grain.

4.2.6 Effects of Treatment on Total Phosphorous (TP)

When the effects of substrate addition (Bioremediation) on the total phosphorous content was studied for a period of 8 weeks, the following results as presented in table 4.8 were obtained:

Table 4. 8 Effect of treatment on total phosphorous of used engine oil polluted soil

S/N	Time (Weeks)	Parameter(g/kg)	Initial TP	During Treatment
1	Week 1	Total Phosphorous	3.51	3.11
2	Week 2	Total Phosphorous	3.51	3.07
3	Week 3	Total Phosphorous	3.51	3.02
4	Week 4	Total Phosphorous	3.51	3.00
5	Week 5	Total Phosphorous	3.51	2.76
6	Week 6	Total Phosphorous	3.51	2.45
7	Week 7	Total Phosphorous	3.51	2.38
8	Week 8	Total Phosphorous	3.51	2.26

The graphical relationship between the residual total phosphorous concentration and the remediation time with respects to the substrate used is presented as shown in Figure 4.5 below:

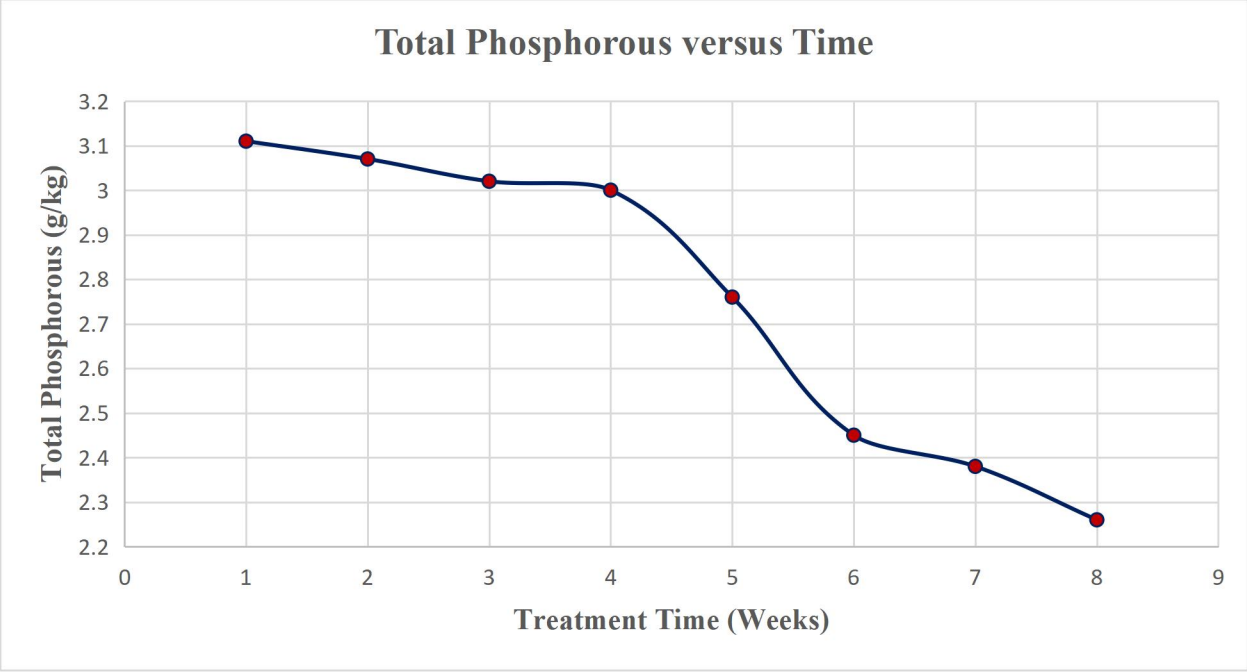

Figure 4. 6 Variation of total phosphorous with treatment time

From the above graph it can be said that there is a gradual decrease in phosphorous which may be attributed to the increase in the population of total heterotrophic bacterial count occasioned by nutrient utilization. Heterotrophic bacterial normally utilized the available nutrient in the form of total nitrogen, total organic carbon and total phosphorous for their growth and cell development. Nwogu *et al.,*(2015) observed a gradual decrease in the total phosphorus from experimental results when using goat manure to remediate artificially contaminated petroleum hydrocarbon soil, and Nwogu *et al.,* result corroborate with this experimental result. Awari *et al.,*(2020) observed a very slight increase in phosphate during remediation. This slight increase is highly due to the composition of the soil that was treated and also the composition of the goat manure, it shows that the available phosphorous content was a little bit high.

4.2.7 Effects of Treatment on Total Hydrocarbon Content (THC)

When the effects of substrate addition (Bioremediation) on the total hydrocarbon content of used engine oil contaminated soil was studied for a period of 8 weeks, the following results as presented in table 4.9 were obtained:

Table 4. 9 Effect of treatment on total hydrocarbon content of used engine oil polluted soil

S/N	Time (Weeks)	Parameter(mg/kg)	Initial THC	During Treatment
1	Week 1	THC	8.09	6.02
2	Week 2	THC	8.09	5.33
3	Week 3	THC	8.09	5.01
4	Week 4	THC	8.09	4.67
5	Week 5	THC	8.09	4.32
6	Week 6	THC	8.09	3.29
7	Week 7	THC	8.09	3.17
8	Week 8	THC	8.09	3.02

According to Lee et al. (1995); organic manures have effect in stimulating hydrocarbon degradation by increasing the total heterotrophic microbial growth and activity.

The graphical relationship between the total hydrocarbon content and the remediation time with respects to the substrate used is presented as shown in Figure 4.7 below:

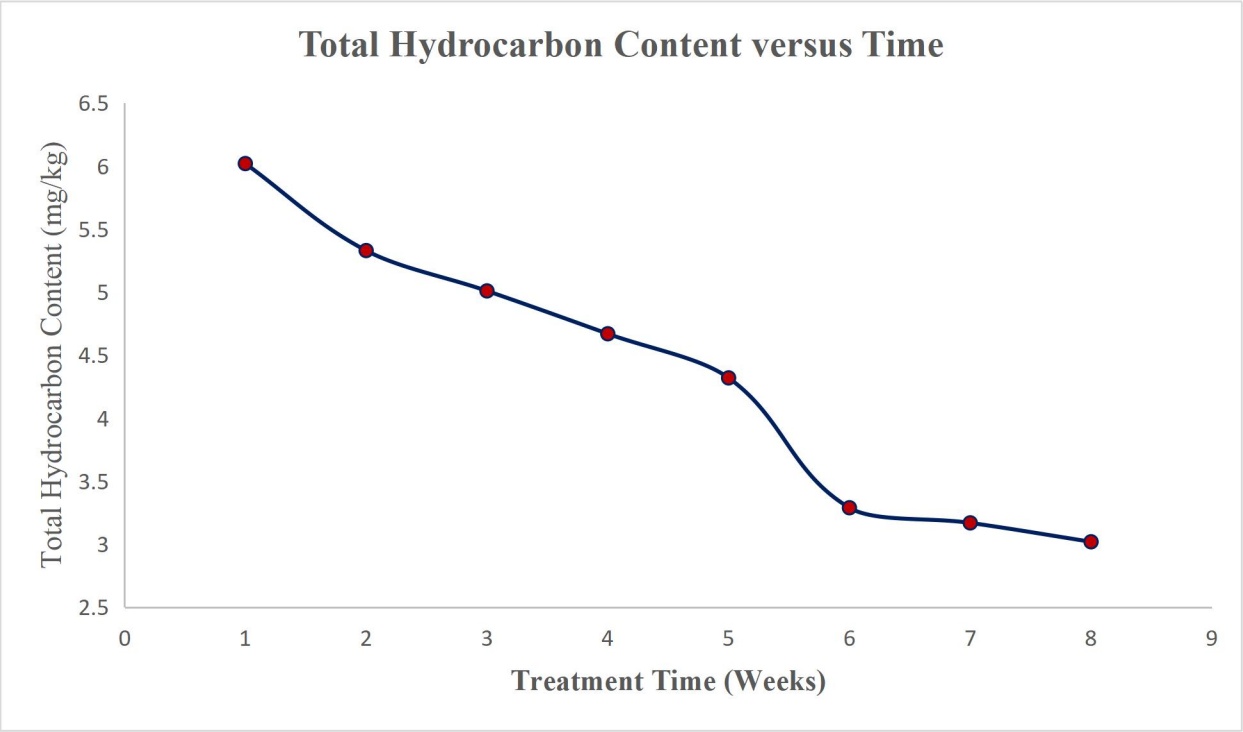

Figure 4. 7 Variation of total hydrocarbon content with treatment time

From the above graph, the degradation rate of the total hydrocarbon content with respect to the substrate used from week one to week five shows a constant degradation rate but with week five and week six there was a change , a rapid increase in degradation which may be as a result of the microorganisms stabilizing and became well adapted to the environment , they tend to grow and developed based on the available nutrient while also eating up the hydrocarbon thus bringing about possible cleanup.

Ogoanah *et al.,*(2020) carried out experiment title enhancement of the soil quality of an oil polluted ultisol using livestock wastes, a gradual decrease of the total hydrocarbon content was observed and this result is consistent with what is gotten from this experiment, the reduction in total hydrocarbon content indicates that the micro-organism have metabolized and the harmful toxicant has been converted to non-toxic substance, thereby making the environmental friendly to both plants, animals and humans. The result gotten by Awari *et al.,*(2020) revealed a decrease in total hydrocarbon content and this aligns with this experimental result. Agarry *et al.,*(2010) also observed a decrease in the total hydrocarbon content while carrying out a study on bioremediation of artificially contaminated soil using animal manure and chemical fertilizer. Nwogu *et al.,*(2015) observe a 62.08% decrease in total hydrocarbon content. Stanley (2013) also observe a decrease in total hydrocarbon content during the biodegradation of spent lubricating motor oil in soil using animal droppings. Ilaboya and Otuaro, (2019) observed that the total hydrocarbon content reduce from 9.76mg/l to 5.87mg/l for goat manure. Result gotten by Ogoanah SO *et al.,*(2020) shows a significant decrease in the total hydrocarbon content. Chinenye *et al.,*(2014) also observed a significant decrease in total hydrocarbon content.

All the literatures stated above thereby validate the authencity of the results gotten from this experiment.

4.2.8 Effects of Treatment on Lead (Pb)

When the effects of substrate addition (Bioremediation) on Lead (Pb) of used engine oil contaminated soil was studied for a period of 8 weeks, the following results as presented in table 4.10 were obtained:

Table 4. 10 Effect of treatment on lead(Pb) of used engine oil polluted soil

S/N	Time (Weeks)	Parameter(mg/kg)	Initial Lead(Pb)	During Treatment
1	Week 1	Lead (Pb)	0.059	0.023
2	Week 2	Lead (Pb)	0.059	0.008
3	Week 3	Lead (Pb)	0.059	0.004
4	Week 4	Lead (Pb)	0.059	0.003
5	Week 5	Lead (Pb)	0.059	0.002
6	Week 6	Lead (Pb)	0.059	0.002
7	Week 7	Lead (Pb)	0.059	0.001
8	Week 8	Lead (Pb)	0.059	0.001

The graphical relationship between Lead(Pb) and the remediation time with respects to the substrate used is presented as shown in Figure 4.8 below:

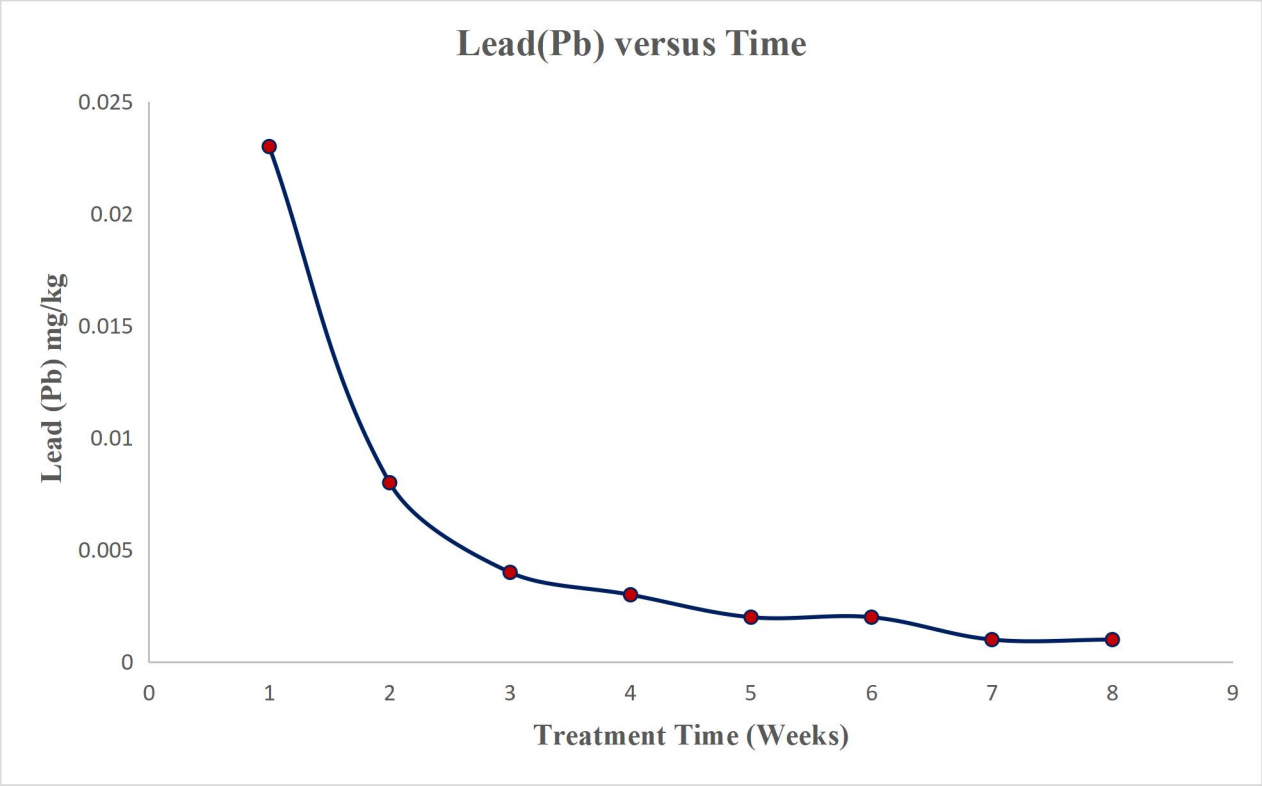

Figure 4. 8 Variation of Lead(Pb) with Treatment Time

Lead(Pb) is one of the most widespread heavy metal contaminants in soils. It is highly toxic to living organisms. Lead(Pb) has no biological function but can cause morphological, psychological, and biochemical dysfunctions in plants (Mouna *et al.,*2013).

The graph above shows a gradual degradation of Lead (Pb) with remediation time. At the end of eight weeks there was a large reduction in Lead which makes the soil less toxic.

4.2.9 Effects of Treatment on Iron (Fe)

When the effects of substrate addition (Bioremediation) on Iron (Fe) of used engine oil contaminated soil was studied for a period of 8 weeks, the following results as presented in table 4.11 were obtained:

Table 4. 11 Effect of treatment on Iron(Fe) of used engine oil polluted soil

S/N	Time (Weeks)	Parameter(mg/kg)	Initial Iron(Fe)	During Treatment
1	Week 1	Iron (Fe)	1.73	1.07
2	Week 2	Iron (Fe)	1.73	1.02
3	Week 3	Iron (Fe)	1.73	0.96
4	Week 4	Iron (Fe)	1.73	0.67
5	Week 5	Iron (Fe)	1.73	0.55
6	Week 6	Iron (Fe)	1.73	0.46
7	Week 7	Iron (Fe)	1.73	0.41
8	Week 8	Iron (Fe)	1.73	0.31

The graphical relationship between Iron (Fe) and the remediation time with respects to the substrate used is presented as shown in Figure 4.9 below:

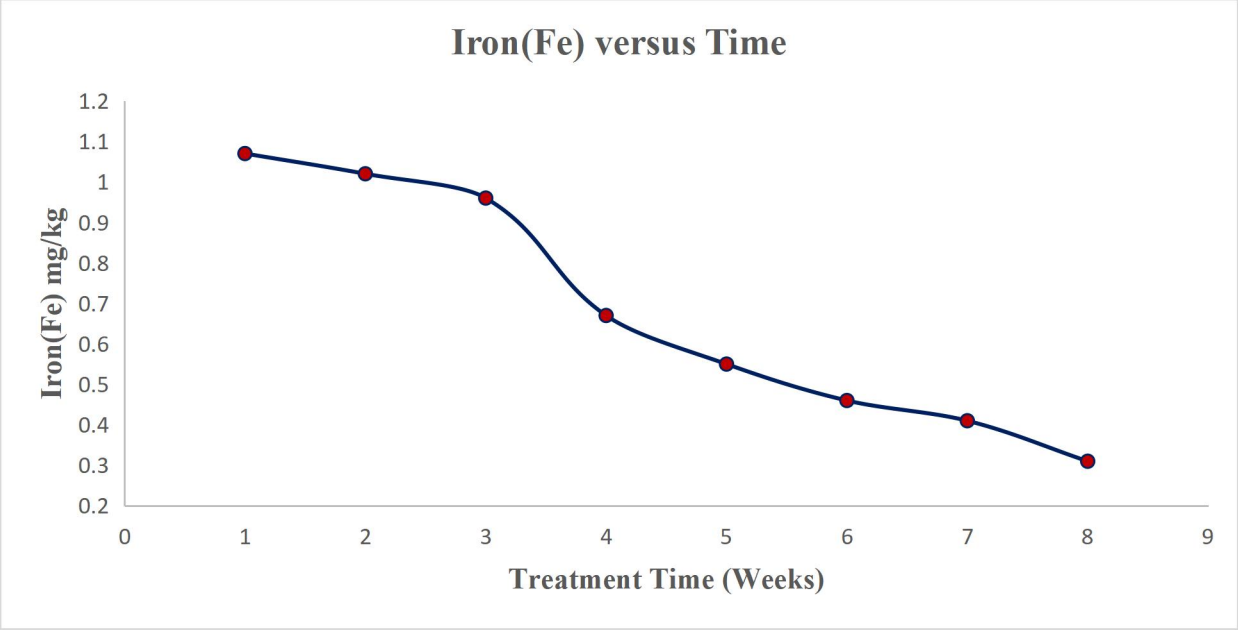

Figure 4. 9 Variation of Iron(Fe) with Treatment Time

From the figure above it can be observed that there was a gradual reduction in the amount of Iron content with remediation time, from literature it is stated that the amount of Iron and its availability in soil is influenced by the following: pH – high pH reduces iron availability which can be seen from this studies that as pH increases, iron availability decreases. The amount of iron and its availability are also influenced by moisture, phosphorus, that is, excess phosphorus inhibits the uptake of iron, compacted and/or poorly aerated soils have an increased iron availability, particularly if the soil is acidic, Organic matter also provides iron and also make it more readily available (K.M. Wade, 2019). In this studies the soil moved from acidic to alkaline gradually this lead to the reduction of Iron.

4.2.10 Effects of Treatment on Total Heterotrophic Bacterial (THB)

When the effects of substrate addition (Bioremediation) on the total heterotrophic bacterial growth was studied for a period of 8 weeks, the following results as presented in table 4.12 were obtained

Table 4. 12 Effect of treatment on total heterotrophic bacterial of used engine oil polluted soil

S/N	Time (Weeks)	Parameter(cfu/g)	Initial THB	During Treatment
1	Week 1	THB	$2.16*10^5$	$4.01*10^5$
2	Week 2	THB	$2.16*10^5$	$4.12*10^5$
3	Week 3	THB	$2.16*10^5$	$4.33*10^5$
4	Week 4	THB	$2.16*10^5$	$4.48*10^5$
5	Week 5	THB	$2.16*10^5$	$4.56*10^5$
6	Week 6	THB	$2.16*10^5$	$4.62*10^5$
7	Week 7	THB	$2.16*10^5$	$4.69*10^5$
8	Week 8	THB	$2.16*10^5$	$4.71*10^5$

Table 4.12 shows the effect of remediation on the total heterotrophic bacterial count for the substrate (Goat manure) used. It was observed from result of Table 4.1 that the initial total heterotrophic bacterial present in the experimental soil was in the range of $7.6*10^5$cfu/g, but was drastically reduced to $2.16*10^5$cfu/g due to contamination caused by the addition of used engine oil. The graphical relationship between the total heterotrophic bacterial population and the remediation time for the different treatment substrate used is presented as shown in Figure 4.10 below;

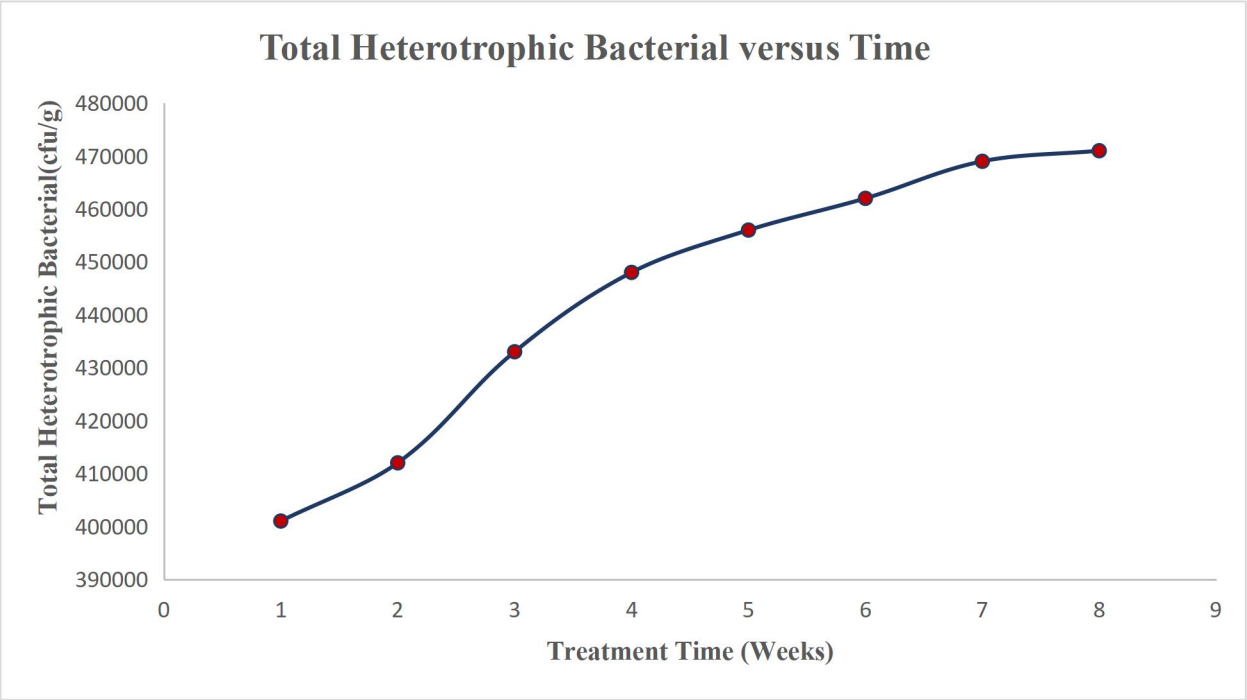

Figure 4. 10 Variation of total heterotrophic bacterial with Treatment Time

The initial decrease in the bacterial count due to contamination from the used engine oil revealed the toxic effect of the used engine oil and probably proved that some of the microorganisms present in the soil cannot survive in the used engine oil polluted environment (Ilaboya and Otuaro, 2019).

However, Table 4.12 revealed a continuous increase in the population of hydrocarbon utilizing microorganisms especially with the addition of treatment substrates, hence the higher reduction in residual used engine oil observed throughout the period of experimentation.

Ogoanah SO *et al.,*(2020) observed an increase in the total heterotrophic bacterial with time , and this resulted in a corresponding removal of hydrocarbon and thus this agrees with the inference drawn from the present findings. Stanley (2013) observed an increase in the total heterotrophic bacterial in his studies. Nwogu *et al.,*(2015) observed that the culturable hydrocarbon-utilizing bacteria increase steadily from 8.5 x 10^5 cfu/g to 2.7 x 10^6. Chinenye *et al.,*(2014) observed that the total heterotrophic bacterial count in the control was observed to be low on the first day which is the period of adaptation and there was a rapid multiplication in bacteria population, reaching a peak at day 84 with a little decline at day 112. The increase in the total heterotrophic bacterial counts was as a result of utilization of the hydrocarbon in the engine oil as the source of carbon and energy by these bacteria. Agarry *et al* (2014) also observed an increase in the total heterotrophic bacterial with time.

The above literatures result all aligns with the result gotten from this experiment, this simply shows that the growth of heterotrophic bacterial leads to the reduction in the hydrocarbon content , that is, Total heterotrophic bacterial is inversely proportional to the total hydrocarbon content.

4.3 Determination of the Extent of Bioremediation

The amount of used engine oil hydrocarbon removed during the series of batch investigation was determined using the mass balance equation of the form (Raghuvanshi et al, 2004):

$$q = \frac{v}{m}\left[C_0 - C_e\right] \qquad (4.1)$$

Where: q, defines the used engine oil hydrocarbon uptake (mg/g); C_0 and C_e: are the initial and equilibrium hydrocarbon concentrations [mg/l] respectively; V: is the volume of used engine oil contaminated soil sample taken (cm^3) and M: is the mass of substrate used (g).

For the batch remediation a study, (V/M) was taken as 2, that is 2:1w/w ratio of polluted soil and the treatment substrate was used throughout the period of experimentation. In which case (v) was taken as 1000w/w and (m) was taken as 500w/w. This value consequently reduces equation 4.1 to the linear form of;

$$q = 2(C_0 - C_e) \quad (4.2)$$

Where C_0 = 8.09mg/l and C_e was taken as the reduced values of total hydrocarbon content (THC) due to treatment occasioned by the use of goat manure. Based on equation 4.2, the amount of used engine oil degradation resulting from the different substrate was then computed as follows.

For the substrate (goat manure), at the end of the remediation time of 8 weeks C_0 = 8.09mg/l and C_e = 3.02mg/l, then equation 4.2 reduces to [q = 2(5.07) = 10.14mg/g while the efficiency of used engine oil removal becomes [(5.07)/10.14)*100 = 62.67%. The table below shows all the differents data calculation from week one of treatment.

Table 4. 13 Amount of used engine oil removed within time of treatment

S/N	Time(weeks)	Co	Ce	Co - Ce	V/m	q=V/m(Co-Ce)
1	1	8.09	6.02	2.07	2.00	4.14
2	2	8.09	5.33	2.76	2.00	5.52
3	3	8.09	5.01	3.08	2.00	6.16
4	4	8.09	4.67	3.42	2.00	6.84
5	5	8.09	4.32	3.77	2.00	7.54
6	6	8.09	3.29	4.80	2.00	9.60
7	7	8.09	3.17	4.92	2.00	9.84
8	8	8.09	3.02	5.07	2.00	10.14

4.4 Efficiency of Removal

The efficiency of used engine oil removal (%) was calculated using the mass balance equation of the form;

$$\text{Removal efficiency (\%)} = \left(\frac{C_0 - C_e}{C_0} \times 100 \right) \quad (4.3)$$

Where: C_0 and C_e are total hydrocarbon content (THC) (mg/l) of used engine oil polluted soil before and after treatment respectively.

Efficiency of remediation obtained from the series of batch remediation process is presented in the Table below;

Table 4. 14 Efficiency of used engine oil removal (%)

Time(weeks)	Co	Ce	Co - Ce	V/m	q=V/m(Co-Ce)	%
1	8.09	6.02	2.07	2.00	4.14	25.59
2	8.09	5.33	2.76	2.00	5.52	34.12
3	8.09	5.01	3.08	2.00	6.16	38.07
4	8.09	4.67	3.42	2.00	6.84	42.27
5	8.09	4.32	3.77	2.00	7.54	46.60
6	8.09	3.29	4.80	2.00	9.60	59.33
7	8.09	3.17	4.92	2.00	9.84	60.82
8	8.09	3.02	5.07	2.00	10.14	62.67

The graphical variation between the amount of used engine oil degradation and the efficiency of bioremediation with treatment time for the substrate used are presented as shown in Figure 4.11 and 4.12 respectively

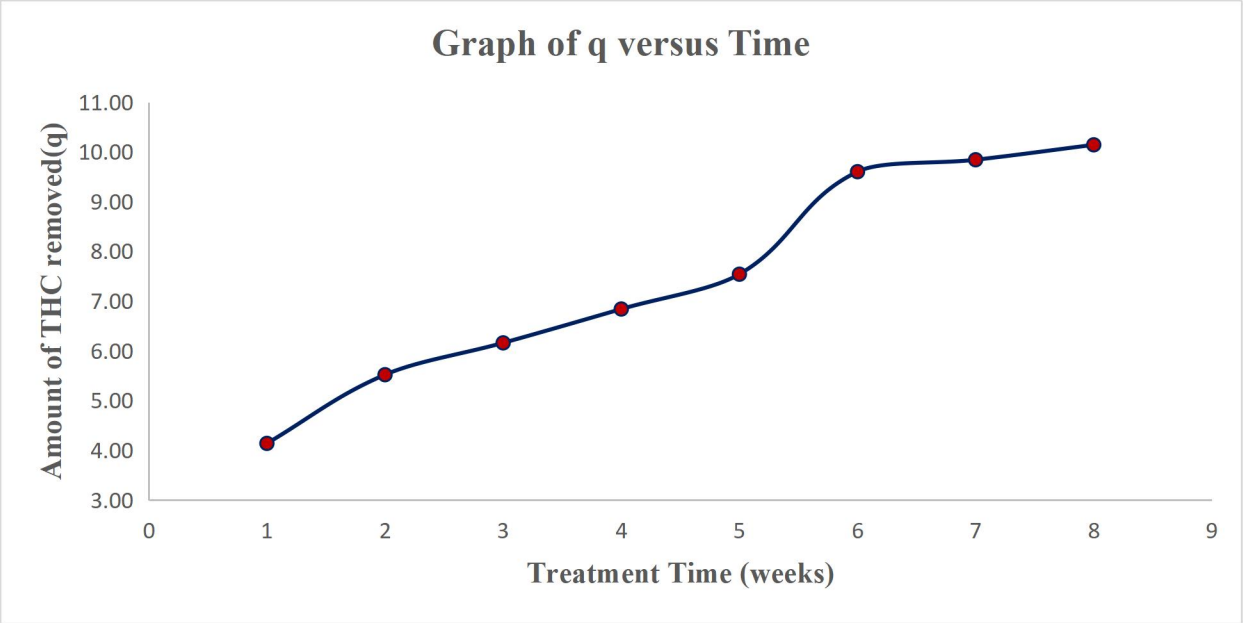

Figure 4. 11 Amount of used engine oil removed with time

It can be deduced from Figure 4.11 that, the amount of engine oil removed increases with Treatment time. Increase in the amount of total hydrocarbon content removed with treatment time can be traced to the increase in the population of the heterotrophic bacteria resulting from the gradual depletion in the available nutrients such as total organic carbon and total nitrogen.

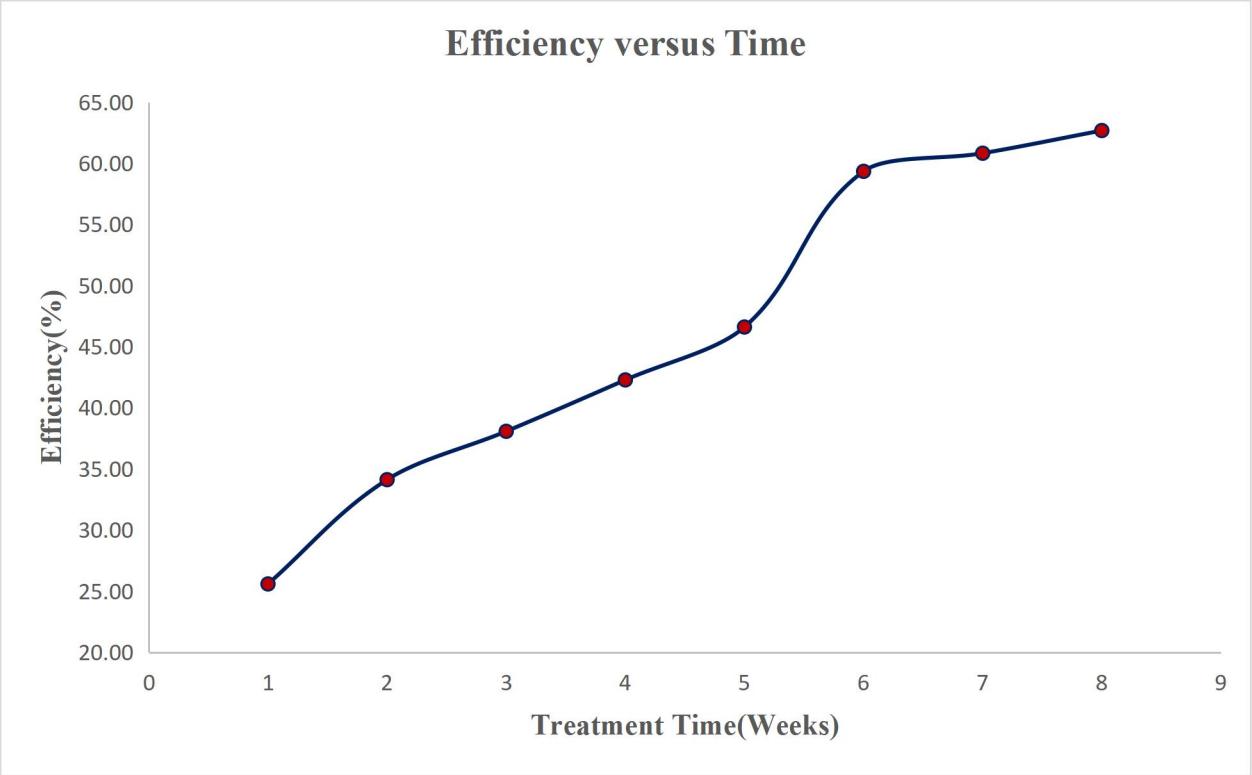

Figure 4. 12 Efficiency of used engine oil removal with time

It can be deduced from the result of Figure 4.12 that, the efficiency of remediation increases with treatment time. Increase in the efficiency of remediation with treatment time can be traced to the increase in the population of the heterotrophic bacteria resulting from the gradual depletion in the available nutrients such as total organic carbon and total nitrogen.

4.5 Predicting the Efficiency of Remediation using Linear and Non-linear Regression.

4.5.1 Linear Regression Analysis

The input parameters for the linear regression analysis are the percentage removal of used engine oil and the remediation time as presented in Table 4.14. The input data were prepared in Microsoft excel spread sheet. The regression analysis was done and the output of the analysis is presented as shown below;

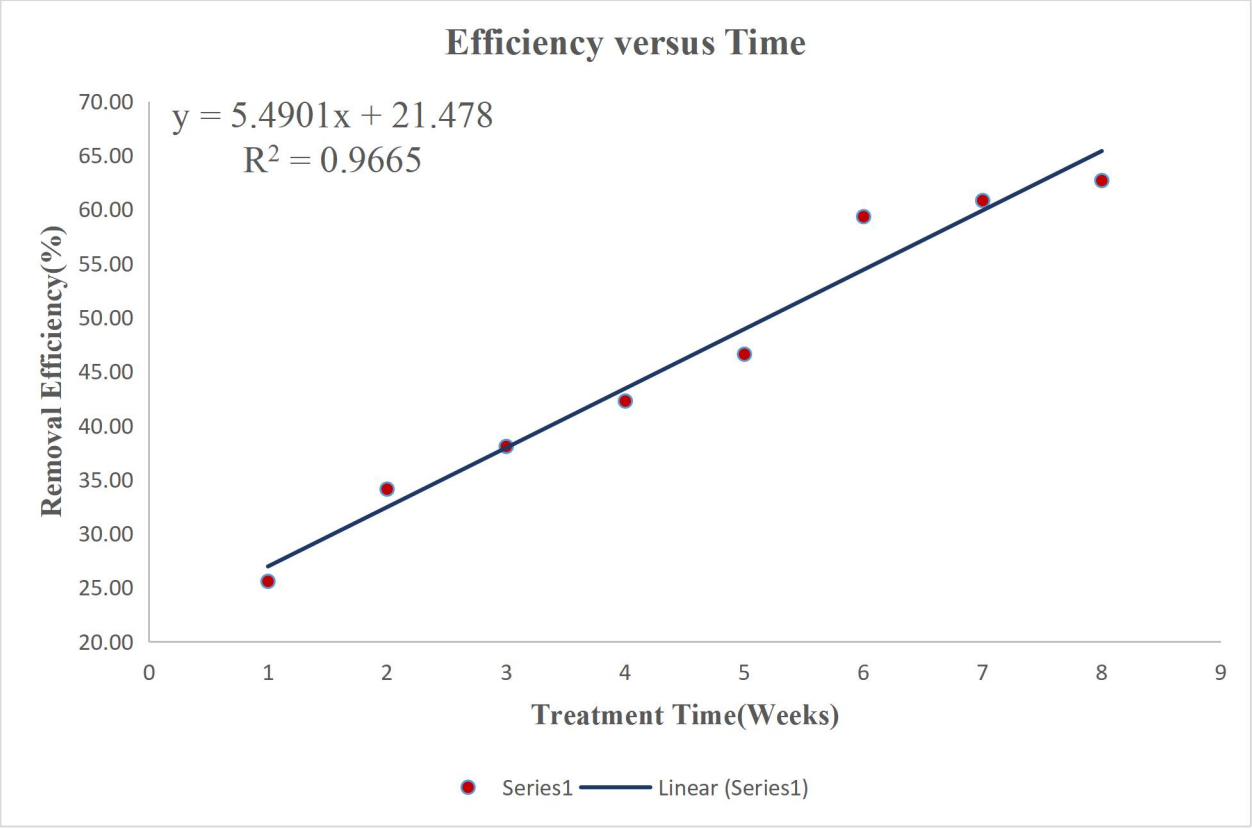

Figure 4. 13 Plot of Remediation Time versus removal efficiency for goat manure

From the result of Figure 4.13, it was observed that the coefficient of linear correlation (the R squared value) is 0.9665 representing 96.65% reliability.

The mathematical relationship between the remediation time and used engine oil removal efficiency for goat manure as the substrate was developed as follows;

Efficiency of Removal (%) = 21.478 + 5.4901(Remediation Time)

In terms of the coded variable; Y = 21.478 + 5.4901X

4.5.2 Non-Linear Regression Analysis

The input parameters for the non-linear regression analysis are the percentage removal of used engine oil and the remediation time as presented in Figure. The input data were prepared in Microsoft excel spread sheet and thereafter a curve fitting tool in excel was employed. Different mathematical equations were tested and the adequacy of the equation in defining the exact relationship between the percentage removal of used engine oil and the remediation time was validated using the goodness of fit statistics.

The non-linear regression analysis was performed by using the mathematical relationship that possesses the highest (R-squared) value. The output of the non-linear regressions analysis is presented as shown below;

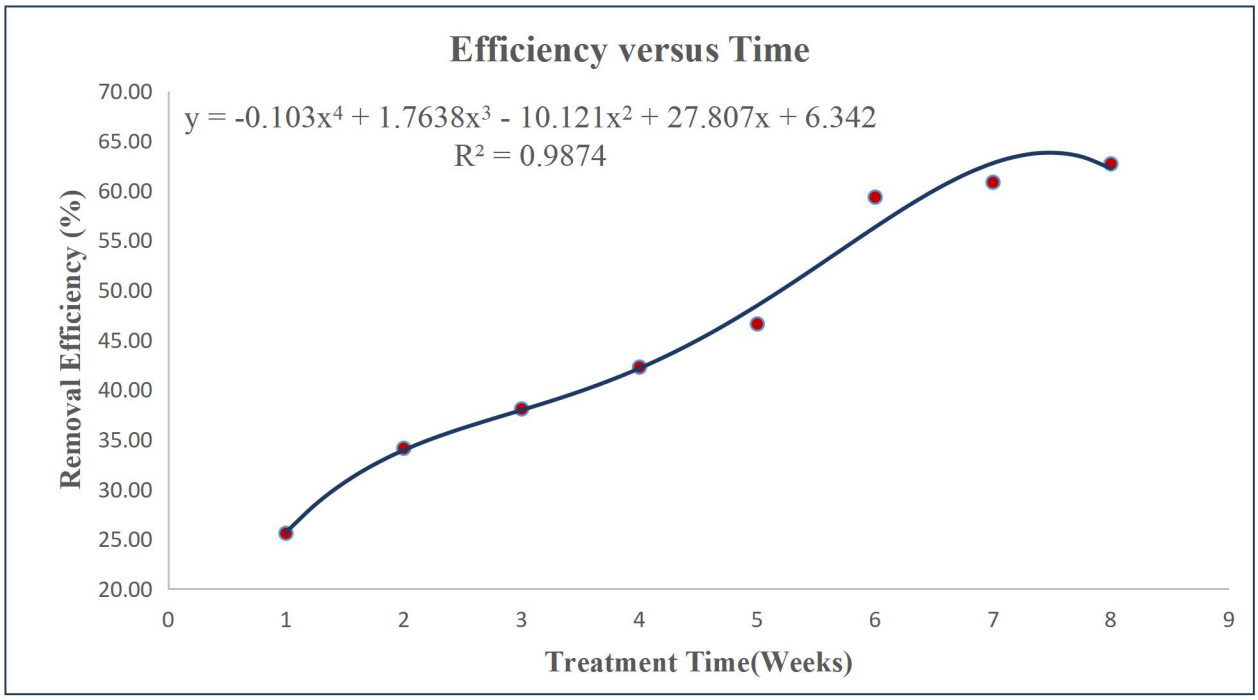

Figure 4. 14 Plot of Remediation Time versus removal efficiency for goat manure

From the result of Figure 4.14, it was observed that the coefficient of linear correlation (the R squared value) is 0.9874 representing 98.74% reliability.

Based on the higher (R^2) value of the non-linear regression analysis compared to the linear regression analysis, prediction of the efficiency of removal with projected values of remediation time was done using the quartic polynomial (Fourth degree polynomials).

The mathematical relationship between the remediation time and used engine oil removal efficiency for goat manure as the substrate was developed as follows;

Efficiency of Removal (%) = $6.342 + 27.807x – 10.121x^2 + 1.7638x^3 – 0.103x^4$

In terms of the coded variable; $Y = 6.342 + 27.807x – 10.121x^2 + 1.7638x^3 – 0.103x^4$

Where Y= Dependent variable (removal efficiency %), x = Independent variable (Treatment time)

4.6 Kinetics of Bioremediation

The kinetic model was employed to study how the remediation process depends on time. In order to study the kinetics of bioremediation, the data gotten from the experiment were fitted into the

Time (weeks)	q_e	q_t	$q_e - q_t$	$\log(q_e - q_t)$

first and second order kinetic model. The main focus is to monitor the change in the concentration of hydrocarbon with treatment time in other to understand the nature of chemical reaction involved in the biodegradation of petroleum hydrocarbon.

4.6.1 Pseudo First Order Kinetic Model

The pseudo first-order rate expression of Lagergren based on the solid capacity is generally expressed as follows:

$$\frac{dq_t}{dt} = K_1(q_e - q_t) \quad (4.4)$$

where: q_e and q_t are the amount of used engine oil removed at equilibrium and at time *t*, respectively (mg·g-1), $K_1$is the rate constant of pseudo first-order adsorption (Lagergren and Svenska, 1998). The linear plots of Log [q_e – q_t] versus time (t) show the appropriateness of the above equation and subsequently the first order nature of the process involved (Lagergren and Svenska, 1998). For the first order kinetic computation, the value of q_e was taken as the amount of used engine oil removed in week eight as shown in Table 4.13 while q_t represent amount adsorbed with time (see Table 4.13). Result of the first order computation is presented as shown in Table 4.15.

Table 4. 15 Pseudo-First order kinetics of bioremediation using goat manure as treatment substrate

1	10.1400	4.1400	6.0000	0.7782
2	10.1400	5.5200	4.6200	0.6646
3	10.1400	6.1600	3.9800	0.5999
4	10.1400	6.8400	3.3000	0.5185
5	10.1400	7.5400	2.6000	0.4150
6	10.1400	9.6000	0.5400	-0.2676
7	10.1400	9.8400	0.3000	-0.5229

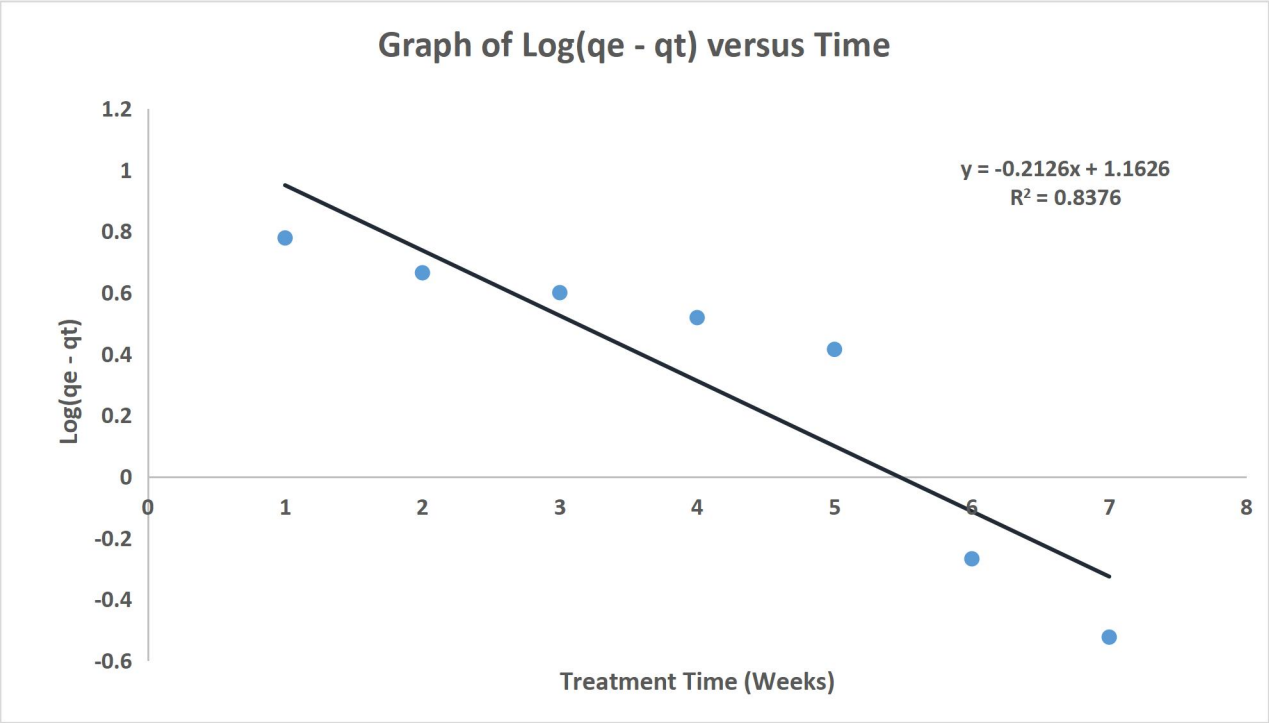

Figure 4. 15 Pseudo-First order kinetic model for the bioremediation of used engine oil

4.6.2 Pseudo Second Order Kinetic Model

The pseudo-second- order equation is also based on the remediation capacity of the solid phase.

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2 \quad (4.5)$$

where:

K_2 = The rate constant of pseudo - second order ($gmg^{-1}\ min^{-1}$)

The plot of $(\frac{t}{q_t})$ against (t) should give a linear relationship from which q_e and K_2 can be determined from the slope and intercept of the plot (Ho et al., 2001, Blanchard et al., 1984, Shamik and Papita, 2010, Yuh-shan, 2006). For the second order kinetic computation, the value of q_t was taken as the amount of used engine oil removed with time (see Table 4.13). Results of the second order kinetic computations are presented as shown in Table 4.16.

Table 4. 16 Pseudo-Second order kinetics of bioremediation using goat manure as treatment substrate

S/N	Time(weeks)	q_t	t/q_t
1	1	4.1400	0.2415
2	2	5.5200	0.3623
3	3	6.1600	0.4870
4	4	6.8400	0.5848
5	5	7.5400	0.6631
6	6	9.6000	0.6250
7	7	9.8400	0.7114
8	8	10.1400	0.7890

The graphical variation between the remediation time (t) and (t/qt) based on the second order kinetic modeling is presented as shown in Figure 4.16.

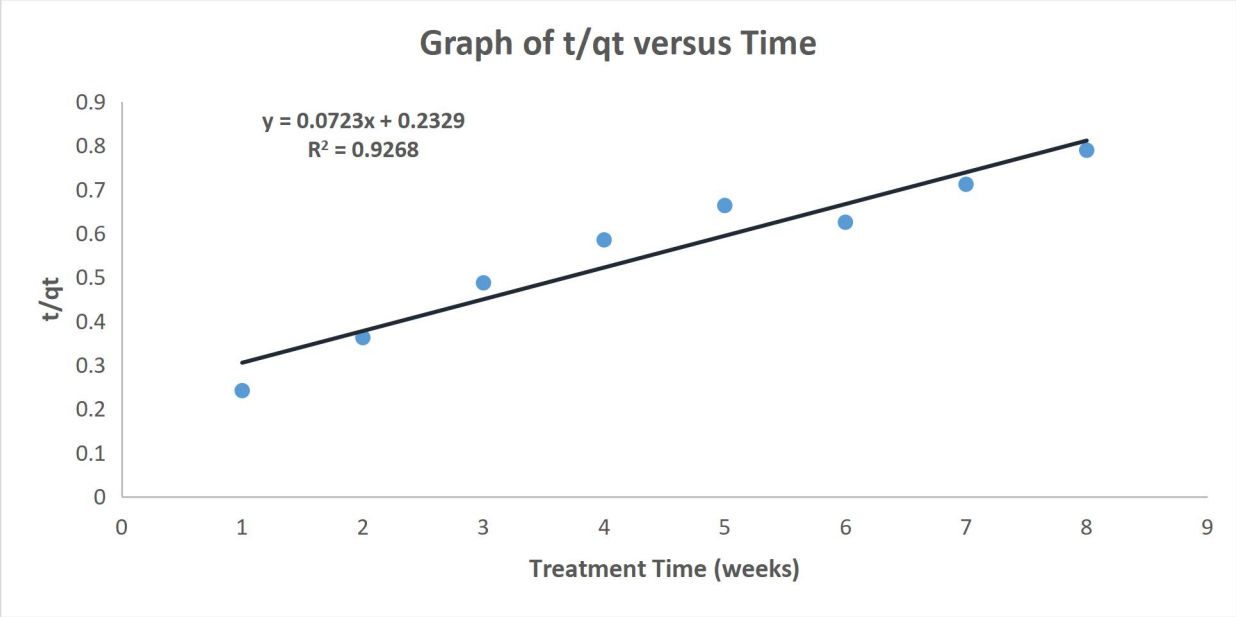

Figure 4. 16 Pseudo-Second order kinetic model for the bioremediation of used engine oil

The kinetics that best explains the experimental data, the values of the linear coefficient of determination was employed as bases for judgment and result of Table 4.17 shows that the second order kinetic model best explains the experimental data for all the treatment substrate used.

Table 4. 17 R-squared values for the kinectics

PseudoFirst Order Kinetic Parameters	**Goat Manure**
R^2	0.8376
PseudoSecond Order Kinetic Parameters	**Goat Manure**
R^2	0.9268

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Bioremediation is a treatment that uses naturally occurring organism to break down hazardous substance into less toxic or non-toxic substance. Bioremediation is emerging as the most ideal alternative technology for removing pollutants from the environment, restoring contaminated sites and preventing further pollution from soil. From the experimental results obtained coupled with the analysis of the experimental data sets, it could be seen that goat manure is an effective substrates for the biodegradation of used engine oil since they can facilitate the rate of breakdown of the hydrocarbon component of the used engine oil.

The extent of remediation was judged based on change in the concentration of some selected parameters with time. The selected parameters include; pH, electrical conductivity, total organic carbon, total nitrogen, total phosphorus, lead(Pb), Iron(Fe), total heterotrophic bacterial and the total hydrocarbon content. Based on the research studies, the following conclusion were drawn.

- It was observed that for the entire period of experimentation (8 weeks), there occur a gradual increase in pH, Electrical conductivity and Total Heterotrophic bacterial.
- It was also observed that for the entire period of experimentation (8 weeks), there occur a gradual decrease in the total nitrogen content (TNC), total organic carbon (TOC), total phosphorus(TP), Lead(Pb), Iron(Fe) and total hydrocarbon content (THC).
- As the microorganism utilizes the available nitrogen, phosphorus and organic carbon present in the soil and increase in population, they again react with and break down the agent that causes the pollution which is the used engine oil.

- The second order kinetic model was observed to have a better fitting of the experimental data sets.
- The non-linear regression model performs better than the linear regression model in predicting the rate of hydrocarbon loss with time for the substrate and was applied to predict the efficiency of used engine oil degradation as a function of remediation time..

5.2 Contributions to Knowledge

In this research work, the following contribtions to knowledge have been made

- The research study have provided additional information on the performance of goat manure as treatment substrate in the bioremediation of used engine oil contaminated soil.
- First and second order kinetic modeling on the application of goat manure in the bioremediation of used engine oil contaminated soil have been investgated.
- Linear and non linear regression modeling using Microsoft excel on the application of goat manure as treatment substrate in the bioremediation of used engine oil contaminated soil have been developed.

5.3 Recommendations

Based on the overall results and discussion, the following recommendations were drawn:

- It is recommended that the rate of diffusion of used engine oil within the soil be adequately studied to understand the mechanism.
- It is also recommended that the population and activity of the existing microorganism be adequately monitored as the experiment progresses.

- A comparative investigation of the performance of living cell microorganism, dead cell microorganism and selected organic and inorganic substrate is also strongly recommended for further studies.

REFERENCES

Abha S, Singh CS. (2012),Hydrocarbon pollution: Effects on living organisms, remediation of contaminated environment and effects of heavy metals co-concentration on bioremediation. In:Romero-zeron L, editor. Introduction to Enhanced oil on Recovery (EOR) processes and Bioremediation of oil contaminated sites. China: Intech publisher;.pp.186-206. ISBN:978-953-51-0629-6.

Abid A, Zaafouri K, Aydi A, et al. Feasibility of a bioremediation process using bio-stimulation with inorganic nutrient NPK for hydrocarbon contaminated soil in Tunisia Journal of Bioremediation and Biodegration 2014; 5(4):224.

Abid A, Zaafouri K, Aydi A, et al.(2014) Feasibility of a bioremediation process using bio-stimulation with inorganic nutrient NPK for hydrocarbon contaminated soil in Tunisia Journal of Bioremediation and Biodegration; 5(4):224.

Abii TA, Nwosu PC. The effect of oil-spillage on the soil of eleme in Rivers state of the Niger-Delta area of Nigeria. Research Journal of Environmental Sciences. 2009;3:316-320.

Adams GO, Fufeyin PT, Okoro SE, Ehinomen I (2015) Bioremedition, Biostimulation and Bioaugmentation: A Review. International Journal of Environmental Bioremediation and Biodegradation: 28-29.

Adams GO, Fufeyin PT, Okoro SE, et al.(2015) Bioremediation, bio-stimulation and Bio-augmentation: a review. International journal of Environmental Bioremediation and Bio-degradation; 3(1):28-39.

Adedokun, O. M., and Ataga, A. E., (2007), Effects of amendments and bioaugmentationof soil polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of cowpea (Vignaunguiculata L. Walp), Scientific Research and Essays 2(5), pp 147-149.

Agamuthu, P., and Dadrasnia, A., (2013), Potential of biowastes to remediate diesel, fuel contaminated soil, Global NEST Journal, Vol. 15, No 4, pp 474-484,

Agunobi KN, Obienausi F.A, Onuoha DC, (2014),An investigation of the pattern and Environmental impact of oil spillage in Etche Local Government Area of Rivers-State, Nigeria, Journal of national Resources.; 4(16):124-137.

Ajoy Kumar Mandal , Priyangshu Manab Sarma , C Paul Jeyaseelan , Veeranna A Channashettar , Bina Singh , Banwari Lal and Jayati Datta,(2012), Large Scale Bioremediation Of Petroleum Hydrocarbon Contaminated Waste At Indian Oil Refineries: Case Studies, Vol 2/Issue 4/Oct-Dec 2012, ISSN 2250-0480.

Akpe, Azuka Romanus; Ekundayo, Afe Omolola; Aigere, Sandra Patrick; Okwu Grace Ifeoma. Bacterial degradation of petroleum hydrocarbons in crude oil polluted soil amended with cassava peels. American Journal of Research Communication, 2015, 3(7): 99118} www.usa-journals.com, ISSN: 2325-4076.

Akpoveta, O. V., Egharevba, F., Medjor, O. W., Osaro, K. I. and Enyemike, E. D., (2011), Microbial degradation and its kinetics on crude oil polluted soil, Research Journal of Chemical Sciences, 1(6), pp 8-14.

Aldrett, S., Bonner, J.S., McDonalds, T.J., Mills, M.A., Autenrieth, R.L. (1997) Degradation of crude oil enhanced by commercial microbial cultures. Proceedings of 1997 International Oil Spill Conference. American Petroleum Institute, Washington DC, pp995-996.

Asira, Enim Enim (2013) Factors that determine bioremediation of organic compounds in the soil. Academy Journal of Interdisciplinary studies 2:125-128.

Awari et al.,(2020), Bio-stimulation approach in bioremediation of crude oil contaminated soil using fish waste and goat manure. Microbiology research journal international, 30(1):33-46,2020; Artcle no. MRJI.54247, ISSN:2456-7043.

Ayotamuno, M.J.; Kogbara, R.B.; Ogaji, S.O.T., and Prober, S.D., (2006), Bioremediation of a crude-oil polluted agricultural-soil at Port Harcourt, Nigeria, Applied Energy, 83, pp 1249 – 1257.

Ayotamuno, M.J.;Kogbara, R.B., and Agunwamba, J.C., (2006),Bioremediation of a petroleumhydrocarbon polluted agricultural soil at various levels of soil tillage in Port Harcourt, Nigeria; Nigerian Journal of Technology, Vol. 25, No. 1, pp 45 – 51.

Balasubramanium A, Boyle AR, Voulvoulis N. Improving petroleum contaminated land remediation decision making through the MCA weighting process. Chemosphere 2007;66:791-798.

Barrie Johnson,(2011). Bangor's expertise in "world-challenging" technology; school of Biological sciences Bangor university, Deinioal Road, Bangor Gwynedd, LL572UW, www.bangor.ac.uk/biology/news/banger-s-expertise-in-world-changing-technology-6273.

Baustita H. and Rahman K. M. M. (2016). "Efects of Crude oil pollution in the tropical Rainforest Biodiversity of Ecuadorian Amazon Region". Journal of Biodiversity and Environmental Sciences. 8 (2): 2499-254.

Baustita H. and Rahman K. M. M. (2016). Review on the Sundarbans Delta oil spill: Effects on Wildlife and Habitats. International research Journal, 1(43), Part 2, pp: 93-96.

Blanchard, G.; Maunaye, M., and Martin, G., (1984), Removal of heavy metals from water by means of natural zeolites, Water resources journal, Vol. 18, pp 1501-1507.

Boopathy R(2000) Factors Limiting Bioremediation technologies. Bioresource Technologies 74:63-67

Cases I, de Lorenzo V (2005), Genetically modified organisms for the environment: stories of success and failure ad what we have learned from them. International microbiology 8:213-222.

Child R, Miller C, Liang Y, et al. Polycyclic aromatic hydrocarbon-degrading mycobacterium isolates: their association with plant roots. Applied Microbiology and Biotechnology, 2007, 75 (3): 655-663.

Chinenye C.C, Peace N.I, Ebenezer J.D. (2014), Biodegradation of crude oil polluted soil by co-composting with Agricultural wastes and inorganic fertilizer.Journal of Natural science research, ISSN 2224-3186(paper) ,ISSN 22250921 (Online), Vol 4, No.6.2014.

Couto N, Fritt_Rasmussen J, Jesen PE, Hoj rup M, Rodrigo AP, et at.(2014) Suitability of oil bioremediation in an artic soil using suplus heating from an incineration Facility. Environmental science and pollution Research 21:6221-6227.

D.J. Lacotte, G. Mille, M. Acquaviva, J.C. Bertrand, In vitro biodegradation of Arabian light 250 by a marine mixed culture using fertilizers as Nitrogen and phosphorus sources, chemosphere, Vol.32, Nos 11112, pp. 4351 - 4358, 1995.

Daniel Delille Emilien Pelletier, Arturo Rodriquez – Blanco, Jean-francois Ghiglione, Effects of nutrient and temperature on degradation of petroleum hydrocarbons in sub-Antarctic coastal seawater polar Biol 32:1521-1528, 2009.

Das N, chandran P(2011) Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnol Res Int 2011:1-13

Dave D, Ghaly AE. Remediation technologies for marine oil spills: A critical review and comparative analysis. American Journal of Environmental Sciences. 2011;7:423-440

Delille D, Duval A, Pelletier E (2008), Highly efficient pilot biopiles for on-site fertilization treatment of diesel oil-contaminated sub-antarctic soil. Cold reg sci technol 54: 7-18.

Dell Anno A, Beolchini F, Rocchetti L, Luna G M, Danovaro R(2012) High bacterial bio-diversity increases degradation performance of hydrocarbons during bioremediation of contaminated harbor marine sediments. Environpollut 167:85-92.

Deshmukh Radhika, Khardenavis Anshuman A, Purohit Hemant J.(2016). Diverse metabolic capacities of fungi for bioremediation. Indian Journal of microbiology 56(3): 247-264.

Eggleton, Tony (2013). A short Introduction to climate change. Cambridge University Press. P. 52. ISBN 9781107618763.

Elektorowicz M. Bioremediation of petroleum contaminated clayey soil with pretreatment. Environ Technol. 1994; 15(4): 373-380.

Emami S, Pourbabaee AA, Alikhani HA (2012), Bioremediation principles and techniques on petroleum hydrocarbon contaminated soil. Technical Journal of Engineering and Applied sciences 2: 320-323.

EPA ‘enforcement targets flaring efficiency violations’’ (PDF). U.S. Environmental Protection Agency. 2012- 08-01. Retreived 2020-02-08.

F. Coulon and D. Delille (2003), Effects of Biostimulation on Growth of Indigenous Bacteria in Sub-Antarctic Soil Contaminated with Oil Hydrocarbons, Oil & Gas Science and Technology – Rev. IFP, Vol. 58 (2003), No. 4, pp. 469-479.

Fowzia A, ANM Fakhruddin. A Review on Environmental contamination of petroleum hydrocarbon and its Biodegradation. Int J Environ Sci Nat Res.2018; 11(3):555811.

Frequent, routine flaring may cause excessive, uncontrolled sulfur dioxide releases (PDF). U.S. Environmental Protection Agency. 2000-10-01. Retrieved 2020-02-08.

Gavrilescu M. Environmental Biotechnology: Achievements, Opportunities and Challenges. Dynamic Biochemistry, Process Biotechnology and Molecular Biology, 2010; 4(1): 1-36.

Gentry TJ, Rensing C, Pepper IL. New approaches for bioaugmentation as a remediation technology. Critical Reviews in Environmental Science and Technology. 2004;34:447-494.

Ghulamshabira, Muhammad Afzala, FarooqAnwarb, RaziaTahseena, Zafar Mahmood Khalida, Biodegradation of kerosene in soil by a mixed bacterial culture under different nutrients conditions, international Biodeterioration and Biodegradation 61, 161-166,2008.

Green Remediation Best Management Practices: Sites with Leaking Underground Storage Tank Systems. EPA 542-F-11-008” (PDF). EPA. June 2011.

Guo C L, Zhao Y H, Wang Y F, et al. Research on repairing oil pollution by hydrocarbon degradation microbes. Applied Chemical Industry, 2016, 45(6): 1156 (in Chinese).

Hinchee, R.E., 1993, Bioventing of Petroleum hydrocarbons, in Handbook of bioremediation, CRC Press, Boca Raton, FL.

HO, Y.S and McKay, G (1999), The kinetics of sorption of divalent metal ions onto sphagnum moss peat, Water Resource Journal, vol. 34(3), pp; 735-742.

Hozumi, T., Tsutsumi, H. and Kono, M. (2000) Bioremediation on the shore after an oil spill from the Nakhodka in the Sea of Japan. I. Chemistry and characteristics of the heavy oil loaded on the Nakhodka and biodegradation tests on oil by a bioremediation agent with microbial cultures in the laboratory. Marine Pollution Bulletin, 40, 308-314.

Ilaboya I.R. and Otuaro E.A,(2019), Comparative Studies on the Bioremediation of Used Engine oil Contaminated Soil Using Urea Fertilizer(UF), Goat Manure (GM), Pig Manure(PM) and Brewery spent Grain (BSG), Nigerian Journal of Environmental sciences and Technology, ISSN (print): 2616-051X, ISSN (electronic): 2616-0501, Vol 3, No. 1 March 2019, pp. 186-199.

Isitekhale, H.H.E,; Aboh, S.; Edion, R. I., and Abhanzioya, M. I., (2013), Remediation of Crude Oil Contaminated Soil with Inorganic and Organic Fertilizer Using Sweet Potato as a Test Crop, Journal of Environment and Earth Science, 3(7), pp 116 – 121.

IUPAC, compendium of chemical Terminology, 2nd ed.(the “Gold Book”) (1997). Online corrected version: (2006)”bioreactor”.

Jiin-shuh Jean a, Ming-Kuo Leeb, Shih-Ming Wang a, Pabitrachattopadhyaya, dyotiprakashmaitya, Effects of inorganic nutrient levels on the biodegradation of benzene, toluene, and xylene (BTX) by pseudomonas spp. In a laboratory porous media sand aquifer model.Bioresource Technology 99,78077815, 2008.

Jobson, A.M., Cook, F.D., and Westlake, D.W.S. (1974) Effect of amendments on the microbial utilization of oil applied to soil. Appl. Microbiol. 27, 166-171.

Johnson, D.L., S.H. Ambrose, T.J. Bassett, M.L. Bowen, D.E. Crummey, J.S. Isaacson, D.N. Johnson, P. Lamb, M.Saul, and A.E. Winter-Nelson.1997. Meanings of envorinmental terms. Journal of Environmental Quality 26: 581-589.

Joner E J, Leyval C. Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environ SciTechnol, 2003, 37: 2371-2375.

K.S.M Rahman, J. Thahira-Rahman, P.Lakshmananaperumalsamy and I.M. Banat, Towards efficient crude oil degradation by mixed bacterial consortium, Bioresource Technology, Volume 85, Issue 3, Pages 257261, 2002.

Kamath R, Rentz JA, Schnoor JL, Alvarez PJJ. Chapter 16: Phytoremediation of hydrocarboncontaminated soils: Principles and applications. In: Vazquez-Duhalt R, Quintero-Ramirez R, editors. Petroleum Biotechnology: Developments and Perspectives (Studies in Surface Science and Catalysis). Vol. 151. New York, USA: Elsevier; 2007. pp. 447-478. ISBN-13: 9780080473710.

Koohestani et al., (2015), A Bioremediation plan on contaminated soil shiraz oil refinery site: Identification of heavy hydrocarbon degrading micro-organisms.

Kumar A, Bisht B S, Joshi V D, Dhewa T (2011), Review on bioremediation of polluted environment. A management Tool. International Journal of environmental sciences 1: 1079-1093.

Kyung-Hwa, B., Hee-Sik, K., Hee-Mock, O., Byung-Dae, Y. Jaisoo, K., and In-Sook, L., (2004), Effects of crude oil, oil components and bioremediation on plant growth,Journal of Environmental Science and Health, 39(9), pp 2465-2472.

Lagergren, S. and Svenska, B. K. (1998). Zur theorie der sogenannten adsorption geloester stoffe. Veternskapsakad Handlingar, 24 (4), pp. 1–39.

Leahy, J.G.; Colwell, R.R. (1990) Microbial Degradation of hydrocarbons in the environment. Microbial Reviews,53(3), pp 305-315.

Lee SH, Lee S, Kim DY, Kim JG. Degradation characteristics of waste lubricants under different nutrient conditions. Journal of Hazardous Materials. 2007;143:65-72.

Lee, K., and Levy, E.M. (1987) Enhanced biodegradation of a light crude oil in sandy beaches. Proceedings of 1987 Oil Spill Conference. American Petroleum Institute, Washington, DC, pp411-416.

Lee, K., Tremblay, G.H., and Gauthier, J., Cobanli, S.E., Griffin, M. (1997) Bioaugmentation and biostimulation: a paradox between laboratory and field results. Proceedings of 1997 International Oil Spill Conference. American Petroleum Institute, Washington DC, pp697-705.

Li D. Bioremediation of petroleum contaminated saline soil with arbuscular mycorrhizal fungi, plant and nitrogen. Shandong: Shandong Normal University, 2013 (in Chinese).

Macaulay BM (2014) understanding behavior of oil-degrading micro-organisms to enhance the microbial remediation of spilled petroleum. Appl Ecol Environ Res 13:247-262.

Madhavi GN, Mohini DD (2012) Review paper on parameters affecting bioremediation. International Journal of Life Science and Pharma research 2:77-80.

Makut DM and Majekodunmi OO. (2019). Bioremediation potentials of chicken droppings on crude oil polluted soil from automobile workshop. GSC Biological and Pharmaceutical Sciences, 8(2), 45-52.

Margesin, R. and Schinner, F.(2001), Biodegradation and Bioremediation of hydrocarbons in extreme environments, Applied microbiology biotechnology, 56:650-663.

Mauro, G. and Wynne, III, B.J. (1990) Mega Borg oil spill: an open water bioremediation test. Texas General Land Office, Austin, Texas.

Mbhele PP. Remediation of Soil and Water Contaminated by Heavy Metals and Hydrocarbons Using Silica Encapsulation. Johannesburg: University of Witwatersrand; 2007

Medina-Bellver J I, Marín P, Delgado A, Rodríguez-Sánchez A, Reyes E, Ramos J L and Marqués 2005 Evidence for in situ crude oil biodegradation after the Prestige oil spill Environ. Microbiol. 7(6) 773-779

Michael Stanley (2018-12-10). " Gas flaring: An industry practice faces increasing global attention" (PDF).World bank. Retrieved 2020-02-08.

Michael Stanley (2018-12-10). " Gas flaring: An industry practice faces increasing global attention" (PDF).World bank. Retrieved 2020-02-08.

Morganwalp, David W."scientists Discover Analog For Extraterrestial life in Idaho Hot spring". Toxics.wgs.gov.Retrieved 2015-09-11.

Nikolopoulou M, Kalogerakis N. Bio-stimulation strategies for fresh and chronically polluted polluted marine environments with petroleum hydrocarbons. J chem Technol Biotechnol.2009; 84(6): 802-807.

Njoku, K. L., Akinola, M. O., and Oboh, B. O., (2008), Growth and performance of Glycine max L. (Merrill) grown in crude oil contaminated soil augmented with cow dung, Nature and Science, 6(1), pp 48-56.

Nwadinigwe, A. O., and Onyeidu, E. G., (2012), Bioremediation of crude oil polluted soil using bacteria and poultry manure monitored through soybean productivity, Polish Journal of Environmental Studies, 21(1), pp 171-176.

Nwilo PC, Badejo OT, Impacts and management of oil spill pollution along the Nigerian coastal areas. International Federation of surveyors, 2007.

Nwogu et al.,(2015), Enhanced Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbons after Amendment with capra aegagrus hircus (Goat) Manure. Biotechnology Research International, Volume 2015, Article ID 657349, 7 pages.

Office of Research and Development, EPA, ATTIC Downloadable Documents, available at http://www.epa.gov/bbsnrmrl/attic/documents/html.

Ogoanah SO et al.,(2020), Enhancement of the soil quality of an oil-polluted ultisol using livestock wastes. Notulae Scientia Biologicae 12(2): 387-398.

Peer WA, Baxter IR, Richards EL, Freeman JL, Murphy AS. Phytore mediation and hyperaccumulator plants. In: Tamas MJ, soils environmental pollution, 2004; 130:465-476.

Phulia V, Jamwal A, Saxena N, Chadha NK, Muralidhar, et al.(2013) Technologies in aquatic bioremediation.65-91.

Piehler MF, Swistak JG, Pinckney JL, et al. Stimulation of Diesel fuel biodegradation by indigenous Nitrogen fixing bacterial consortia. Microb Ecol.1999; 38(1):69-78.

Pingping Cai, Zhuo Ning, Yaci Liu, Ze He, Jiansheng Shi and Manlan Niu,(2020), Diagnosing bioremediation of crude oilcontaminated soil and related geochemical processes at the field scale through microbial community and functional genes, Cai et al. Annals of Microbiology (2020) 70:36 https://doi.org/10.1186/s13213-020-01580-x.

Pivetz BE. Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites. EPA/540/S-01/500; USEPA. 2001. Available from: http://www.clu-in.org/ download/remed/epa_540_s01_500.pdf.

Raghuvanshi, S.P.; Singh, R and Kaushik, C.P., (2004), Kinetics studies of Methylene blue dye bio-adsorption on Bagasse, Applied Ecology and Environmental Research, journal, Vol. 2(2), pp; 35 – 43.

Rhykerd RL, Crews B, Mclnnes KJ, et al. Impact of bulking agents, forced aeration and tillage on remediation of oil-contaminated soil. Bioresource Technol.1999; 67(3): 279-285.

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

Salanitro JP, Dorn PB, Huesemann MH, Moore KO, Rhodes IA, Ricejackson LM, et al. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. Environmental Science and Technology. 1997;31:1769-1776. DOI: 10.1021/es960793i

Shah MP, Patel KA, Nair SS, et al. Microbial degradation of Textile Dye (Remazol Black B) by Bacillus spp. ETL-2012. J Bioremed Biodeg. 2013;4(2):1-5.

Shamik, C and Papita, S., (2010), Pseudo-second-order kinetic model for sorption of malachite green onto sea shells; comparison of linear and non-linear methods, the IIOAB Journals (Research Bio-Physics), Vol. 1(3), pp 3-7.

Sharma S. Bioremediation: Features, strategies and applications. Asian Journal of Pharmacy and Life Science. 2012;2:202-213.

Shilpi Sharma (2012) Bioremediation: features strategies and applications.Asian journal of pharmacy and life science 2:202-213.

Simon, M., Autenrieth, R.L., McDonald, T.J., Bonner, J.S.(1999) Evaluation of bioaugmentation for remediation of petroleum in a wetland. Proceedings of 1999 International Oil Spill Conference. American Petroleum Institute, Washington DC.

Stanley Chukwudozie Onuoha, (2013), Stimulated Biodegradation of Spent Lubricating Motor oil in Soil Amended with Animal droppings. Journal of natural science research, ISSN 2224-3186(paper), ISSN 2225-0921(Online), Vol.3, No.12,2013.

Stohl, A.; Klimont, Z.; Eckhardt, S.; Kupiainen, K.; Chevchenko, V.P.; Kopeikin, V.M.; Novigatsky, A.N.(2013),” Black carbon in the arctic: the underestimated role of gas flaring and residential combustion emissions”, Atmos. Chem. Ohys., 13(17): 8833-8855.

Swannell RP, Lee K, McDonagh M.(2004) Field evaluations of marine oil spill bioremediation. Microbiological Reviews. 1996;60:342-365.

Swannell, R.P.J., Lee, K., and McDonagh, M. (1996) Field evaluations of marine oil spill bioremediation. Microbiological Reviews, 60(2), 342-365.

Thapa B, Kumar KCA, Ghimire A. A review on bioremediation of petroleum hydrocarbon contaminants in soil. Kathmandu University Journal of Science, Engineering and Technology. 2012;8:164170. DOI: 10.3126/kuset. v8i1.6056.

Thavasi R, Jayalakshmi S, Banat I M (2011) Application of biosurfactant produced from peanut oil cake by Lactobacillus delbrueckiiin biodegradation of crude oil. Bioresource Technology 102:3366-3372.

The library of Congress(2006). ‘’History of the oil and gas industry’’. Business and Economics Research Advisor (5/6).

Townsend, R.T., Bonner, J.S., Autenrieth, R.L.(1999) The effect of bioremediation on microbial populations in an oil-contaminated coastal wetland. Proceedings of 1999 International Oil Spill Conference. American Petroleum Institute, Washington DC.

Tsutsumi, H., Kono, M., Takai, K., Manabe, T. (2000) Bioremediation on the shore after an oil spill from the Nakhodka in the Sea of Japan. III. Field test of a bioremediation agent with microbiological cultures for the treatment of an oil spill. Marine Pollution Bulletin, 40, 320-324.

Tyagi M, da fronseca MM, de Carvalho CC. Bio-augmentation and bio-stimulation strategies to improve the effectiveness of bioremediation processes. Biodegradation.2010; 22(2):231-241.

U.S. Air-force Environics Directorates of the Armstrong laboratory, U.S. Air-force center for environmental excellence, 1995, Manual. Bioventing Principles and Practices, EPA/540/R-95/534a.

U.S. Air-force Environics Directorates of the Armstrong laboratory, U.S. Air-force center for environmental excellence, 1995, Manual. Bioventing Principles and Practices, EPA/540/R-95/534b.

U.S. Environmental protection Agency (USEPA),2003. Aerobic Biodegradation of oily wastes. A field Guidance Book for federal on-science coordinators, Version 1.0, October 2003. Region 6 south central Response and prevention branch.

U.S. Environmental protection Agency (USEPA),2017. How to evaluate alternative cleanup technologies for underground storage tank site: A Guide for corrective action plan reviewers, chapter V: Land farming, Land and Emergency Management 540IR, EPA510-B-17-003.

U.S. Environmental protection Agency (USEPA)1993. Bioremediation using the land treatment concept. U.S. Environmental protection Agency office of research and development, Washington, D.C.

Ugboma, P.P,. 2015, Environmental Degradation in Oil Producing Areas of Niger Delta Region, Nigeria.ISSN:2225-8590(print)

Venosa, A. D., Suidan, M. T., Wrenn, B. A., Strohmeier, K. L., Haines, J. R., Eberhart, B. L, King, D.W., and Holder, E. (1996) Bioremediation of experimental oil spill on the shoreline of Delaware Bay. Environmental Science and Technology, 30, 1764-1775.

Vergetis E. Oil Pollution in Greek Seas and Spill Confrontation MeansMethods. Greece: National Technical University of Athens; 2002.

Vidali M. Bioremediation: An overview. Pure and Applied Chemistry,2001; 73(7): 1163 -1172.

Wang Q, Zhang S, Li Y, Klassen W (2011) Potential Approaches to improving Biodegradation of hydrocarbons for bioremediation of crude oil pollution. Environ Protection J. 2:47-55.

Yang S Z, Jin H J, Wei Z, He RZ, Ji YJ, et al.(2009) Bioremediatiom of oil spills in cold environments: A review. Pedosphere 19:371-381.

Yuh-Shan, H.O., (2006), Review of second-order models for adsorption systems, Journal of Hazardous materials, vol. B 136, pp 681-689.