EVALUATION OF THE BIOREMEDIATION POTENTIALS OF ABATTOIR WASTES ON OIL CONTAMINATED SOIL

BY

OGBU, CHRIS IKEOKWU (B.Sc., UNIPORT)

(20114770158)

A THESIS SUBMITTED TO THE POST GRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI.

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IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (M.Sc.) IN CHEMICAL QUALITY CONTROL/INDUSTRIAL SAFETY.

MAY, 2016.

CERTIFICATION

This is to certify that the research study "**Evaluation of the Bioremediation Potentials of Abattoir Wastes on Oil Contaminated Soil**" was carried out by **OGBU, CHRIS IKEOKWU** with registration number **(20114770158)** in partial fulfillment of the requirements for the award of the degree of Master of Science (M.Sc.) in Chemical Quality Control/Industrial Safety, in the Department of Chemistry, Federal University of Technology, Owerri, Imo State.

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DEDICATION

This research project is dedicated to Almighty God and also to all the less privileged people in the society.

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ABSTRACT

Agricultural farmlands in an oil producing/prospecting area have been known to give poor yield of farm products. Therefore the remediation of the soil for improved agricultural productivity is necessary. Soil samples from this area were studied and standard methods of analysis were applied in evaluating the soil as 'non oil contaminated", 'oil spilled", and 'Abattoir waste treated" soil samples. The pH was slightly acidic (5.20) before remediation and after remediation a pH of 8.20 was obtained. The Moisture Content was 8.25% initially and was later obtained as 12.91% after remediation. The concentration in mg/Kg of Nitrogen (N), Potassium (K), Phosphorus (P), Total Organic Carbon (TOC), Total Organic Matter (TOM)] and Total Hydrocarbon Content (THC) were 1.51, 2.15, 538, 25.74, 44.50 & 16160 respectively, while after remediation the values were 2.88, 16.71,.1890, 39.04, 67.50, 6071 respectively for treated soils. A ten weeks investigation on the biodegradation of spilled oil revealed 62.43% reduction in Total Hydrocarbon Content (THC) value of the treated soil and 15.34% reduction in THC from the non-treated soil and also showed an enhanced soil restoration for other parameters. Three kinetic models of zero-order, first-order and second-order equations gave good applicability to K and THC, while the first-order and second-order were applicable to P. However, none of the models were appropriate in the description of the kinetics of N, TOC and TOM.

Key words: Environmental degradation, Bioremediation, Bio-stimulating agents, Oil spillage, Abattoir waste, Soil, Kinetics, Niger delta.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Environmental degradation associated with crude oil is a major problem confronting oil-producing countries. The degree of degradation is dependent upon the composition and quantity of priority pollutants and on the configuration of the receiving media. In spite of the public outcry over environmental pollution due to oil exploration, more crude oil wastes are being released into soils and water bodies

In Nigeria, the inhabitants of Niger Delta region where crude oil is produced are the main victims of oil spillage resulting mainly from failures in various media of transportation (pipelines, tankers etc) and oil facilities. This has resulted in explosions leading to deaths and degradation of the ecosystem (Okoli and Akhigbemidu, 2001). The region referred to as the Niger Delta is located at the Delta of the River Niger to the Atlantic Ocean. It covers a land mass of over 70,000 square kilometers, cuts across about 800 oil producing communities, an extensive network of over 900 producing oil wells, 100 flow stations/gas plants as well as over 1,500 kilometers of trunk lines and 45,000 kilometers oil and gas flow lines.

Niger Delta is synonymous with oil pollution recording an average of 221 oil spills per year (Okoko and Ibaba, 1999; Osuji, 2002).The environment being

aquatic; oil sometimes floats on water, where it is dispersed to shorelines by wind and wave action, thus affecting the soil environment. Most of the oil producing communities are however important agricultural lands. Any contact with oil may result in damage to the soil property, plant and animal communities. In soils, petroleum hydrocarbon creates condition which lead to the unavailability of essential plant nutrients such as nitrogen, potassium, and calcium, and the availability of some toxic elements such as arsenic, and lead to death of plants (Akamigbo and Jidere, 2002; Gill, *et. al;* 2003). Over 3% concentration of oil has been reported to be increasingly deterious to soil biota and crop growth (Osuji, 2001).

Crude oil is an extremely complex mixture of aliphatic and aromatic hydrocarbons including volatile components of gasoline, petrol, kerosene, lubricating oil and solid asphaltene residues. The involvement of micro organisms in the cleanup of oil polluted environment has been established as an economic, efficient, versatile and environmentally friendly treatment method (Margesin and Schinner, 2001; Yakubu, 2007). One promising method that has been researched into is the application of chemical fertilizers to augment for the mineral elements, particularly nitrogen and phosphorous (Margesin and Schinner, 1999; Ayotamuno, *et. al;* 2006). The effectiveness of this treatment has however been conflicting (Cunningham and Philip, 2002; Okoko, *et. al;*

2005).This might be due to the heterogeneity of soil amendments and the natural soil constituents (Knaebel, *et. al;* 1994).

Nonetheless in developing countries, fertilizers are not sufficient for agriculture, let alone for cleaning oil spills. It therefore necessitates the search for cheaper, readily available and environmentally friendly options of enhancing petroleum hydrocarbon degraded soil. One such option is the use of abattoir wastes as bio-stimulating agents. Even though some works have been done using animal dung which is a solid waste or part of a solid waste, this research work utilizes the combination of solid waste and Abattoir effluent on crude oil polluted soil of Niger Delta Agricultural farmland.

1.2 Problem Statement

Oil spill is prevalent in our society more especially in the Niger Delta region and its effective removal from contaminated soil is essential because of its environmental problems. It may result in harmful consequences; endanger natural environment and human health and most importantly resulting in poor yield of various farm products. It is explained by their ability to form toxic compounds in soils, superficial and ground water.

It is mainly on the basis of numerous health hazards that this research was conducted to provide a means of removing the contaminants for a healthy living of our people and other living organisms within our environment.

1.3 Aim/Objectives

Aim :

To determine the remediation potential of abattoir waste for soils contaminated with oil as in Niger Delta region.

Objectives :

- To analyse soil for pH, Moisture Content, Nitrogen (N), Potassium (K), Phosphorus (P), Total Organic Carbon (TOC), Total Organic Matter (TOM)] and Total Hydrocarbon Content (THC) parameters before pollution and remediation as well as after.
- To determine the remediation potential of abattoir waste.
- To determine the kinetic model for the remediated soils.

1.4 Scope of Research

This research primarily deals with the bioremediation of crude oil contaminated agricultural land using abattoir waste. Eight soil parameters (pH, moisture content, N, P, K, TOC, TOM and THC) were evaluated before contamination, after contamination and after treatment with the remediating agent. The difference in the obtained values of these parameters was used to evaluate the state of the experimental soil at each stage. A total of 576 sampling

units were used, comprising of 8 parameters in triplicate (that is in 3 different random spots) from 24 sampling points.

1.5 Significance of the Study

This study is necessary as it tends to provide a cheaper, readily available and environmentally friendly option of treatment of contaminated soil, being a major problem in the Niger Delta region.

CHAPTER TWO

LITERATURE REVIEW

2.1: Chemistry of Petroleum

Crude oil or Petroleum is a naturally occurring flammable liquid consisting of a complex mixture of hydrocarbons of various molecular weights and other liquid organic compounds that are found in geological formations beneath the earth's surface. A fossil fuel, it is formed when large quantities of dead organisms, usually zooplankton and algae, are buried underneath sedimentary rock and undergo intense heat and pressure.

Petroleum is recovered mostly through oil drilling. This comes after the studies of structural geology (at the reservoir scale), sedimentary basin analysis, and reservoir characterization (mainly in terms of porosity and permeable structures). It is refined and separated, most easily by boiling point, into a large number of consumer products, from petrol (or gasoline) and kerosene to asphalt and chemical reagents used to make plastics and pharmaceuticals. Petroleum is used in manufacturing a wide variety of materials, and it is estimated that the world consumes about 88 million barrels each day [The Hindu (India), 2001].

The use of fossil fuels such as petroleum can have a negative impact on Earth's biosphere, releasing pollutants and greenhouse gases into the air and damaging ecosystems through events such as oil spills. Concern over the

depletions of the earth's finite reserves of oil, and the effect this would have on a society dependent on it, is a field known as peak oil.

The hydrocarbons in crude oil are mostly alkanes, cycloalkanes and various aromatic hydrocarbons while the other organic compounds contain nitrogen, oxygen and sulphur, and trace amounts of metals such as iron, nickel, copper and vanadium. The exact molecular composition varies widely from formation to formation but the proportions of chemical elements vary over fairly narrow limits as follows:

Composition by weight [Speight J.G. (2007)]

Element	**Percent range**
Carbon	83 to 87%
Hydrogen	10 to 14%
Nitrogen	0.1 to 2%
Oxygen	0.05 to 1.5%
Sulfur	0.05 to 6.0%
Metals	< 0.1%

Four different types of hydrocarbon molecules appear in crude oil. The relative percentage of each varies from oil to oil, determining the properties of each oil.

Composition by weight [Hyne (2001)]

Hydrocarbon	Average	Range
Paraffins	30%	15 to 60%
Naphthenes	49%	30 to 60%
Aromatics	15%	3 to 30%
Asphaltics	6%	Remainder

Crude oil varies greatly in appearance depending on its composition. It is usually black or dark brown (although it may be yellowish, reddish, or even greenish). In the reservoir it is usually found in association with natural gas, which being lighter forms a gas cap over the petroleum, and saline water which, being heavier than most forms of crude oil, generally sinks beneath it. Crude oil may also be found in semi-solid form mixed with sand and water, as in the Athabasca oil sands in Canada, where it is usually referred to as crude bitumen. In Canada, bitumen is considered a sticky, black, tar-like form of crude oil which is so thick and heavy that it must be heated or diluted before it will flow. Venezuela also has large amounts of oil in the Orinoco oil sands, although the hydrocarbons trapped in them are more fluid than in Canada and are usually called extra heavy oil. These oil sands resources are called unconventional oil to distinguish them from oil which can be extracted using traditional oil well methods. Between them, Canada and Venezuela contain an estimated 3.6 trillion

barrels (570×10^9 m^3) of bitumen and extra-heavy oil, about twice the volume of the world's reserves of conventional oil. (“Oil Sands in Canada and Venezuela”, 2008)

Empirical equations for thermal properties of Petroleum include:

Heat of combustion

At a constant volume the heat of combustion of a petroleum product can be approximated as follows:

$$Q_v = 12,400 - 2,100d^2$$ ……(1) [Speight et. al; (2007)]

Where Q_v is measured in cal/gram and d is the specific gravity at 60 °F (16 °C).

Thermal conductivity

The thermal conductivity of petroleum based liquids can be modeled as follows:

$$K = \frac{0.813}{d}[1 - 0.0203(t - 32)]$$0.547……..(2) [Speight et. al;(2007)]

where K is measured in BTU · $hr^{-1}ft^{-2}$, t is measured in °F and d is the specific gravity at 60 °F (16 °C).

Specific heat

The specific heat of a petroleum oils can be modeled as follows:

$$c = \frac{1}{d}[0.388 + 0.00045t]$$…….(3) [Speight et. al; (2007)]

where c is measured in BTU/lbm°F, t is the temperature in Fahrenheit and *d* is the specific gravity at 60 °F (16 °C).

In units of kcal/(kg·°C), the formula is:

$$c = \frac{1}{d}[0.4024 + 0.00081t]$$ ……..(4) [Speight et. al; (2007)]

where the temperature *t* is in Celsius and *d* is the specific gravity at 15 °C.

Latent heat of vaporization

The latent heat of vaporization can be modeled under atmospheric conditions as follows:

$$L = \frac{1}{d}[110.9 - 0.09t]$$ …… (5) [Speight et. al; (2007)]

where L is measured in BTU/lbm, t is measured in °F and d is the specific gravity at 60 °F (16 °C).

In units of kcal/kg, the formula is:

$$L = \frac{1}{d}[194.4 - 0.162t]$$ ……..(6) [Speight et. al; (2007)]

where the temperature *t* is in Celsius and *d* is the specific gravity at 15 °C.

An oil spill is the release of a liquid petroleum hydrocarbon into the environment, especially marine areas, due to human activity, and is a form of pollution. The term is usually applied to marine oil spills, where oil is released into the ocean or coastal waters, but spills may also occur on land. Oil spills may be due to releases of crude oil from tankers, offshore platforms, drilling rigs and

wells, as well as spills of refined petroleum products (such as gasoline, diesel) and their by-products, heavier fuels used by large ships such as bunker fuel, or the spill of any oily refuse or waste oil.

Spilt oil penetrates into the structure of the plumage of birds and the fur of mammals, reducing its insulating ability, and making them more vulnerable to temperature fluctuations and much less buoyant in the water. Cleanup and recovery from an oil spill is difficult and depends upon many factors, including the type of oil spilled, the temperature of the water (affecting evaporation and biodegradation), and the types of shorelines and beaches involved. Spills may take weeks, months or even years to clean up.

2.2: Soil and Its Pollution

Soil is the loose (unconsolidated) mineral or organic matter surface of the earth curst that is capable of supporting plant growth. Soil may be organic or mineral. Organic soils in contrast to mineral soils develop from an accumulation of plant residue that is preserved by the low oxygen environment of shallow and stagnant water. Soil may be few centimeters to several meters thick, most soil are predominantly composed of minerals usually formed by weathering of rocks. These homogenous minerals include quartz, feldspar, micas, hornblendes, calcite and gypsum. Chemically, the soil contains a multitude of organic substances not found in the underlying strata. The soil is composed of five major components-mineral matters; water, air, organic matter and a living population.

The quantity of these constituents is not the same in all soil but varies with the locality.

The soil is plaque with many waste nitrate and phosphorus from overuse or poor management of pesticides, human and animal wastes, municipal and industrial refuse of all types, crude oil pollution due to blow-out, pipeline vandalization, transportation, accident strain. A great percentage of crude oil spills occurs in sensitive environment in onshore and offshore area of Nigeria. The majority of soil-spill incidents occurs in the purely mangrove swamp zones of the offshore areas of the Niger Delta, which constitute the most productive biological area.

2.2.1: Soil factors for growth

At present sixteen (16) chemical elements are known to be essential for the growth of crop plants. These elements are hydrogen, carbon and oxygen from air and water, phosphorus, potassium, sulphur, calcium, iron, magnesium, boron, copper, manganese, zinc, molybdenum, chlorine, from soil and nitrogen from both air and soil.

Soil is the source of thirteen of the sixteen essential plant nutrients. The soil concentrations of these thirteen nutrients and the conditions making them available to plants are of fundamental important to plant growth. Plant nutrient found in the soil are chemical constituent of the soil. The magnitude of the plant growth reflects a composite of many favourable and unfavourable factors.

Favourable growth factors include adequate aeration, water, nutrients, adequate soil depth and proper soil temperature. Unfavourable growth factor are many, among which are toxic levels of certain elements, diseases, harmful insects, adverse temperatures, inadequate or excess sunlight as well as crude oil spillage.

2.2.2: Macro nutrients in the soil

Plants nutrients may be divided into Macro and Micro nutrients. Macro nutrients are elements, which occur in substantial level in plant materials or in fluids in the plant. Micronutrients are element that are essential only in very low levels and generally are required for the function of essential enzymes. The elements generally recognized as essential macronutrients for pants are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium and sulphur, of these carbon, hydrogen and oxygen are obtained by fixing bacteria, and nitrogen may be obtained by some plants directly from the atmosphere. The other essential macronutrients can be obtained from the soil, of these nitrogen, phosphorus and potassium are the most likely to be lacking and commonly added to soils as fertilizers.

Nitrogen mineralization

The soil nutrient, which plants requires in greatest quantity is nitrogen, the element that serves as a keystone of the proteineous matter of living tissue. Yet, despite its critical role in plant nutrient, nitrogen is assimilated almost entirely in inorganic state as ammonium or nitrate. On the other hand, the bulk of

nitrogenous materials found in soil or added in the form of crop residues of the bound elements and the mobilization of the vast reservoir of organically combined nitrogen is essential to the recycling of the nutrients and therefore to soil fertility.

Nitrogen mineralization is the conversion of organic nitrogen to the more mobile, inorganic state. As a consequence of mineralization, ammonium and nitrate accumulate and organic nitrogen disappears. Almost all of the nitrogen found in surface soil horizons is in organic combination.

Phosphorus mineralization

Phosphorus is found in soil, plants and in micro organisms in a number of organic and inorganic compounds. It is second only to nitrogen as a mineral nutrient required by plant and micro organisms, its major physiological role being in certain essential steps in the accumulation and release of energy during cellular metabolism. Agronomically, this element may be incorporated as plant or animal residues. Thus, phosphorus occupies a critical position both in crop production and in biology of soil.

Micro organisms bring about a number of transformations of the element. These include:

a. Altering the solubility of inorganic compounds of phosphorus.

b. Mineralizing organic compounds with the release of orthophosphate.

c. Converting the inorganic available anion into cell protoplasm, an immobilization process analogies to that occurring with nitrogen and

d. Bringing about an oxidation or reduction of inorganic phosphorus compounds.

The existence in soil of a large reservoir of organic phosphorus that cannot be utilized by plants emphasizes the role of micro organisms in converting the organic phosphorus to inorganic forms. Mineralization is generally more rapid in virgin soils than in their cultivated counterparts. Not only is the total amount mobilized greater in virgin areas, but the percentage of total organic phosphorus that is mineralized is higher in virgin than in cultivated land.

The relative percentage of phosphorus made available to plants through the soil is relatively low; it is an essential component of the soil. Phosphorus like nitrogen must be present in simple inorganic form before it can be taken up by the plants. However, in the case of phosphorus, the utilization species in form of phosphate ions, on the pH range that is present in most soils, are the predominant orthophosphate species; this phosphate is most available in soils at pH values near neutrality.

Potassium mineralization

A major cation that plants must obtain from the soil is potassium. Because the quantity in soil is often inadequate, the element is one of the macro nutrients

supplied in chemical fertilizers. In crop residues, the element is not strongly bound in organic combination as experienced in Nitrogen and Phosphorous. However, the element exists mainly in monovalent biological systems and there are thus, few inorganic oxidation and reduction reactions that typify the microbiological transformations of nitrogen, sulphur and iron. The micro flora does have an influence on the level of available potassium, however, the cation is solubilized through the addition of organic or inorganic acids that react with potassium containing materials.

2.3: Bioremediation

Bioremediation is defined as use of biological processes to degrade, break down, transform, and/or essentially remove contaminants or impairments of quality from soil and water. Bioremediation is a natural process which relies on bacteria, fungi, and plants to alter contaminants as these organisms carry out their normal life functions. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases.

Many substances known to have toxic properties have been introduced into the environment through human activity. These substances range in degree of toxicity and danger to human health. Many of these substances directly or indirectly come in contact with the soil. Conventional methods to remove, reduce, or mitigate toxic substances introduced into soil or ground water via

anthropogenic activities and processes include pump and treat systems, soil vapor extraction, incineration, and containment. Utility of each of these conventional methods of treatment of contaminated soil and/or water suffers from recognizable drawbacks and may involve some level of risk.

The emerging science and technology of bioremediation offers an alternative method to detoxify contaminants. Bioremediation has been demonstrated and is being used as an effective means of mitigating: (State of Mississippi, Department of Environmental Quality, 1998).

- hydrocarbons
- halogenated organic solvents
- halogenated organic compounds
- non-chlorinated pesticides and herbicides
- nitrogen compounds
- metals (lead, mercury, chromium)
- radio nuclides

Bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, biostimulation, and bioaugmentation. Bioremediation which occurs without human intervention other than monitoring is often called natural attenuation. This natural attenuation relies on natural conditions and behavior of soil microorganisms that are indigenous to soil. Biostimulation also utilizes indigenous microbial populations to remediate

contaminated soils. Biostimulation consists of adding nutrients and other substances to soil to catalyze natural attenuation processes. Bioaugmentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms.

During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and, through oxidation-reduction reactions, metabolize the target contaminant into useable energy for microbes. By-products (metabolites) released back into the environment are typically in a less toxic form than the parent contaminants. For example, petroleum hydrocarbons can be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The result is formation of carbon dioxide and water (Nester, *et. at;* 2001). When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic (without oxygen) respiration prevails. Generally, inorganic compounds such as nitrate, sulfate, ferric iron, manganese, or carbon dioxide serve as terminal electron acceptors to facilitate biodegradation (State of Mississippi, Department of Environmental Quality, 1998).

Three primary ingredients for bioremediation are: (1) presence of a contaminant, (2) an electron acceptor and (3) presence of microorganisms that

are capable of degrading the specific contaminant. Generally, a contaminant is more easily and quickly degraded if it is a naturally occurring compound in the environment, or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved (State of Mississippi, Department of Environmental Quality, 1998). Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment. Development of biodegradation technologies of synthetic chemicals such DDT is dependent on outcomes of research that searches for natural or genetically improved strains of microorganisms to degrade such contaminants into less toxic forms.

Microorganisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance. Factors that affect success and rate of microbial biodegradation are nutrient availability, moisture content, pH, and temperature of the soil matrix. Inorganic nutrients including, but not limited to, nitrogen, and phosphorus are necessary for microbial activity and cell growth. It has been shown that “treating petroleum-contaminated soil with nitrogen can increase cell growth rate, decrease the microbial lag phase, help to maintain microbial populations at high activity levels, and increase the rate of hydrocarbon degradation” (Walworth, *et. al;* 2005). However, it has also been shown that excessive amounts of nitrogen in

soil cause microbial inhibition. Walworth, *et. al;* (2005) suggest maintaining nitrogen levels below 1800 mg nitrogen/kg H_2O for optimal biodegradation of petroleum hydrocarbons. Addition of phosphorus has benefits similar to that of nitrogen, but also results in similar limitations when applied in excess (State of Mississippi, Department of Environmental Quality, 1998).

All soil microorganisms require moisture for cell growth and function. Availability of water affects diffusion of water and soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. Anaerobic respiration, which produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation, becomes the predominant process. Soil moisture content "between 45 and 85 percent of the water-holding capacity (field capacity) of the soil or about 12 percent to 30 percent by weight" is optimal for petroleum hydrocarbon degradation (US EPA, 2006, "Landfarming").

Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the acceptable range is pH 6 – 8 (US EPA, 2006, "Landfarming"; State of Mississippi, Department of Environmental Quality, 1998).

Temperature influences rate of biodegradation by controlling rate of enzymatic reactions within microorganisms. Generally, “speed of enzymatic reactions in the cell approximately doubles for each 10^{o}C rise in temperature” (Nester *et. al;* 2001). There is an upper limit to the temperature that microorganisms can withstand. Most bacteria found in soil, including many bacteria that degrade petroleum hydrocarbons, are mesophiles which have an optimum temperature ranging from 25 degree C to 45 degree C (Nester *et. al;* 2001). Thermophilic bacteria (those which survive and thrive at relatively high temperatures) which are normally found in hot springs and compost heaps exist indigenously in cool soil environments and can be activated to degrade hydrocarbons with an increase in temperature to 60 degree C. This finding “suggested an intrinsic potential for natural attenuation in cool soils through thermally enhanced bioremediation techniques” (Perfumo, *et. al;* 2007).

Contaminants can absorb to soil particles, rendering some contaminants unavailable to microorganisms for biodegradation. Thus, in some circumstances, bioavailability of contaminants depends not only on the nature of the contaminant but also on soil type. Hydrophobic contaminants, like petroleum hydrocarbons, have low solubility in water and tend to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants (State of Mississippi, Department of Environmental Quality,

1998). Additional research findings of the existence of thermophilic bacteria in cool soil also suggest that high temperatures enhance the rate of biodegradation by increasing the bioavailability of contaminants. It is suggested that contaminants adsorbed to soil particles are mobilized and their solubility increased by high temperatures (Perfuno, *et. al;* 2007).

2.3.1: Types of Bioremediation

There are two major types of bioremediatoion namely:

In Situ Bioremediation

This is a bioremediation technology that requires the treatment of the soil at the place of contamination and without the removal of the contaminated matrix.

Ex Situ Bioremediation

This is a bioremediation technology that requires the removal of the contaminated matrix by excavation and treated elsewhere and therefore more expensive than the in-situ process.

In situ bioremediation causes minimal disturbance to the environment at the contamination site. In addition, it incurs less cost than conventional soil remediation or removal and replacement treatments because there is no transport of contaminated materials for off-site treatment. However, ***in situ*** bioremediation has some limitations: (1) it is not suitable for all soils, (2)

complete degradation is difficult to achieve, and (3) natural conditions (i.e. temperature) are hard to control for optimal biodegradation. ***ex situ*** bioremediation, in which contaminated soil is excavated and treated elsewhere, is an alternative.

Ex situ bioremediation approaches include use of bioreactors, landfarming, and biopiles. In the use of a bioreactor, contaminated soil is mixed with water and nutrients and the mixture is agitated by a mechanical bioreactor to stimulate action of microorganisms. This method is better-suited to clay soils than other methods and is generally a quick process (US EPA, 2006, “Guide”).

Landfarming involves spreading contaminated soil over a collection system and stimulating microbial activity by allowing good aeration and by monitoring nutrient availability (US EPA, 2006, “Landfarming”).

Biopiles are mounds of contaminated soils that are kept aerated by pumping air into piles of soil through an injection system (US EPA, 2006, “Biopiles”).

In each of these methods, conditions need to be monitored and adjusted regularly for optimal biodegradation. Use of landfarming and biopiles also present the issue of monitoring and containing volatilization of contaminants. Like ***in situ*** methods, ***ex situ*** bioremediation techniques generally cost less than conventional techniques and apply natural methods. However, they can require a large amount of land and, similar to ***in situ*** bioremediation, complete

degradation is difficult to achieve, and evaporation of volatile components is a concern (US EPA, 2006, “Landfarming”; US EPA, 2006, “Biopiles”).

If the challenges of bioremediation, particularly of ***in situ*** techniques, can be overcome, bioremediation has potential to provide a low cost, non-intrusive, natural method to render toxic substances in soil less harmful or harmless over time. Currently, research is being conducted to improve and overcome limitations that hinder bioremediation of petroleum hydrocarbons. On a broader scope, much research has been and continues to be developed enhance understanding of the essence of microbial behavior as microbes interact with various toxic contaminants. Additional research continues to evaluate conditions for successful introduction of exogenic and genetically engineered microbes into a contaminated environment, and how to translate success in the laboratory to success in the field (US DOE, 2006).

2.3.2: Advantages of Bioremediation

The use of intrinsic or engineered bioremediation processes offers several potential advantages that are attractive to site owners, regulatory agencies, and the public. These include:

- Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increases in numbers when the contaminant is present; when the contaminant is

degraded, the bio degradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.

- ❖ Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.
- ❖ Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
- ❖ Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment can arise during that transportation.
- ❖ Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste.
- ❖ It can be done either on site or off-site.
- ❖ Reduces the amount of waste to be land-filled.

2.3.3: Disadvantages of Bioremediation

However, there are potential disadvantages to bioremediation as well, these include:

- ❖ May be difficult to control.
- ❖ Amendments introduced into the environment to enhance bioremediation may cause other contamination problems.
- ❖ May not reduce concentration of contaminants to required levels.
- ❖ Requires more time or simply may take several years to remediate.
- ❖ May require more extensive monitoring.
- ❖ May depend on climatic conditions.
- ❖ Lack of (hydraulic) control.
- ❖ Dynamic process, difficult to predict future effectiveness.

2.4: Abattoir

An abattoir has been defined as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, processing and effective preservation and storage of meat products for human consumption [Bell and Oyedemi (2009)]. Animals slaughtered include cattle, sheep, pigs, goats and other equine animals. The killing of animals for community consumption is inevitable in most nations of the world and dated back to antiquity.

Abattoirs generate large amounts of solid waste and effluents such as rumen contents (animal feaces), blood horns, bones, paunch manure and waste water. Abattoirs often have difficulties in disposing of the solid wastes and wastewater in an environmentally acceptable fashion and in many instances untreated rumen contents, blood and/or other Abattoir effluents and wastewater are released into the environment.

The abattoir wastes have the potential to pollute surface waters, underground waters, abattoir/market environment, and consumables around the abattoir, especially when abattoir wastes are not properly treated and disposed off. Abattoir wastes should be managed to achieve allowable effluent standards, odour control, or to exploit the benefits locking in the wastes before safely and economically disposing the ultimate wastes. In order to develop optimized abattoir wastes management strategies that would ensure reduction in environmental pollution in Nigeria, this research recommends the use of abattoir waste as a remediating agent for the cleaning of oil spills mainly in polluted soils of Niger Delta.

2.5: Previous Studies on Bioremediation of oil contaminated Soils

In recent times, there is a tremendous increase in the number of publications resulting from various studies or investigations or researches on

bioremediation in which it has proved to be a useful tool in removing oil from polluted environment. Some of these works are enumerated below:

Ayotamuno, *et. al;* (2006) investigated the bioremediation of petroleum hydrocarbon polluted agricultural soil at various levels of soil tillage in Portharcourt, Nigeria. In their work a combination of field cells involving a control and five treatment cells were evaluated under field conditions in the bioremediation of a petroleum- hydrocarbon polluted agricultural soil over a six-week period. Previous works have indicated that crude oil contamination of soils depletes oxygen reserves in the soils and slows down its diffusion rate to the deeper layers. Hence, this hypothesis was tested in the study by the treatments employed. The treatment option used was the application of mineral fertilizer, and different rates of oxygen exposure through various levels of soil tillage. In the experiments described, conditions of a major spill were simulated by sprinkling crude oil on the cells using perforated cans. The treatment applications were then resorted to and relevant soil physicochemical characteristics monitored at intervals. The results of the study showed an enormous increase in total heterotrophic bacterial (THB) counts in all the treatment cells. The percentage reduction in total hydrocarbon content (88% to 99%) experienced in the cells that received treatment were significantly different from the control. These results highlight the view that the availability of large amounts of oxygen in the soil profile induces accelerated biodegradation of

petroleum hydrocarbons in a polluted agricultural soil and implies that regular tillage of contaminated soils in the presence of nutrients could achieve the decontamination of such soils.

Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cutting was investigated by chaillan, *et. al;* (2005). The work was done using oil drill cuttings and a soil contaminated with weathered crude oils were treated by enhanced biodegradation under tropical conditions in industrial scaled experiments. Oil contaminants were characterized by gas chromatography and mass spectrometry. This allowed for the identification of a mixture of two crude oils in the contaminated soil. After 12 months of bioremediation process, the removal of hydrocarbon reached by biodegradation an extent of 60% although nutrient amendment with elevated concentration of N-urea had highly detrimental effects on the hydrocarbon degrading fungal populations due to the production of toxic concentration of ammonia gas by nitrification. The saturated hydrocarbons were extensively assimilated, though n-alkanes were not completely removed. Aromatic hydrocarbons were less degraded than saturated whereas resin and asphaltene fractions were, surprisingly, partly assimilated. In laboratory conditions, the residual hydrocarbons in the field-treated materials were 15-20% further degraded when metabolic byproducts resulting from biodegradation were diluted or removed.

Ex situ bioremediation of a soil contaminated by mazut (heavy residual fuel oil) was studied by Vladimir, *et. al;* (2011) in which Mazut polluted soil was exposed to bioremediation in an ex situ field-scale(600m^3) study. Re-inoculation was performed periodically with biomasses of microbial consortia isolated from the mazut-contaminated soil. Biostimulation was conducted by adding nutritional elements (N, P and K). The biopile (depth 0.4m) was comprised of mechanically mixed polluted soil with softwood sawdust and crude river sand. Aeration was improved by systematic mixing. The biopile was protected from direct external influences by a polyethylene cover. Part (10m^3) of the material prepared for bioremediation was set aside un-inoculated, and maintained as an untreated control pile (CP). Biostimulation and re-inoculation with zymogenous microorganisms increased the number of hydrocarbon degraders after 50 d by more than 20 times in the treated soil. During the 5 months, the total petroleum hydrocarbon (TPH) content of the contaminated soil was reduced to 6% of the initial value, from 5.2 to 0.3 g /kg dry matter, while TPH reduced to only 90% of the initial value in the CP. After 150 d there were 96%, 97%and 83% reductions for the aliphatic, aromatic, and nitrogen–sulphur–oxygen and asphaltene fractions respectively. The isoprenoids, pristane and phytane, were more than 55% biodegraded, which indicated that they are not suitable biomarkers for following bioremediation. According to the available

data, this is the first field-scale study of the bioremediation of mazut and mazut sediment-polluted soil, and the efficiency achieved was far above that described in the literature to date for heavy fuel oil.

Sang-Jim, *et. al*; (2005), evaluated the bioremediation effectiveness on crude oil contaminated sand. A treatability study was conducted using sea sand spiked with 3% or 6% (w/w) of Arabian light crude oil to determine the most effective bioremediation strategies for different levels of contamination. The sea sand used in the study was composed of gravel (0.1%), sand (89.0%), and silt and clay (10.9%). The water content of the sea sand was adjusted to 12.6% (w/w) for the study. Different combinations of the following treatments were applied to the sand in biometer flasks: the concentration of oil (3% or 6%), the concentration of a mixture of three oil-degrading microorganisms (Corynebacterium sp. IC-10, Sphingomonas sp. KH3-2 and Yarrowia sp. 180, 1×10^6 or 1×10^8 cellsg^{-1} sand), the concentration of the surfactant Tween 80 (1 or 10 times the critical micelle concentration), and the addition of SRIF in a C:N:P ratio of 100:10:3. Three biometer flasks per combination of experimental conditions were incubated, and the performance of each treatment was examined by monitoring CO_2 evolution, microbial activity, and oil degradation rate. The results suggest that the addition of inorganic nutrients accelerated the rate of CO_2 evolution by a factor of 10. The application of oil-degrading

microorganisms in a concentration greater than that of the indigenous population clearly increased biodegradation efficiency. The application of surfactant slightly enhanced the oil degradation rate of the contaminated sand treated with the higher concentration of oil-degrading microorganisms. The initial CO_2 evolution rate was shown to efficiently evaluate the treatability test by providing significant data within a short period, which is critical for the rapid determination of the appropriate bioremediation approach. The measurements of microbial activity and crude oil degradation also confirmed the validity of the CO_2 evolution rate as an appropriate criterion.

A feasibility study was conducted by Barathis and Vasudevan (2003) to evaluate the efficiency of Pseudomonas fluorescens NS1 bioaugmented to stimulate in situ bioremediation of crude oil-contaminated soil with different amendments in treatment units. Pure culture of P. fluorescens NS1 was isolated from a petroleum-contaminated soil. The rate of degradation of petroleum hydrocarbons by the indigenous soil microflora and in the presence of P. fluorescens NS1 was assessed with the addition of nutrients and bulking agents for a period of about 35 days. The study showed that addition of wheat bran as bulking agent rapidly enhanced bioremediation of crude oil-contaminated soil compared to amendments in other treatment units.

A bioremediation of crude oil contaminated soils study was carried out in engineered laboratory biopile systems by Benyahia, *et. al;* (2005). In soil

bioremediation, biopiles are ‘ex situ’ treatment systems that consist of excavated and aerated contaminated soils amended by addition of biological, mineral or organic material depending upon specific needs. When bacteria are added to the contaminated soil undergoing biological treatment, the latter is referred to as a bio-augmented treatment system. Such soils are arranged above ground in a pile fashion and hence the term biopile. The study has shown virtually identical trends in respiration rates when indigenous and commercial added bacteria were employed. However, the bio-augmented experiments yielded much greater respiration rates and lead to a reduction of 75% of the initial oil in just 118 days, compared to 1 year in similar studies in the literature. The benefits of engineered biopile systems include safe operations, facilitated material balance and process controllability. The benefits of bio-augmentation were clearly demonstrated and it was found that when no nutrients are added to the soil, bacteria tend to metabolize hydrocarbons into carbon dioxide and water rather than assimilate carbon in cell growth. Polyaromatics analysis of treated soil in this investigation pointed out that simpler and more bio-available crude oil components were degraded first. A simple sigmoid model for the carbon dioxide generation was developed from the respiration data.

Bioremediation of crude oil contaminated soil by composting was researched by Lihua, *et. al;* (2009). Composting technology was used in the laboratory to simulate the remediation of the crude oil-contaminated soil

collected from Jilin oil field. Consequently the contaminants removal paths and mechanism were discussed. The results showed that this method was effective in the remediation process. After 40 days, 45% crude oil was removed from the soil given the original oil content at 7.00 times 10^4 mgldrkg^{-1}.The rate constant of biodegradation and half life was 0.0333d^{-1} and 20.82d, respectively. Biodegradation was the main way of contaminant removal. Volatilization loss of crude oil was less than 0.1% of initial crude oil content. Among the three ratio of contaminated soil to organic fertilizer (dry weight) with the ratio of 8:2, 7:3 and 5:5 respectively, the highest removal of complete biodegradation of crude oil was observed in the ratio of 7:3, in which the complete biodegradation reached to 17% of the total removal. The rate and degree of biodegradation were highly related to the number and activity of microbe.

The volume of oil in domestic wastewater is increasing each year due to the urbanization and industrial development all around the world. It is of concern that the increase of oil in the wastewater could cause severe impact to the environment and to human health. Bioremediation of oil from domestic wastewater using mixed culture was being studied by Mohd K, *et.al.*, (2008), to overcome this problem. Microorganisms from local palm oil plant are utilized for this study. The ability of the microorganisms to degrade the oil is observed by investigating effect of concentration of the inoculum (g/ml) and the agitation speed (rpm) on oil removal. The optimum condition for these microorganisms to

degrade oil was aimed for the highest volume of oil degraded. From the result obtained it showed that agitation with the speed of 150 rpm gave the best condition for oil removal while the addition of 4g/110ml of inoculum concentration over wastewater and oil volume gave the optimum oil removal. Higher concentration of inoculum cause high oil removal but at highly concentrated inoculum could cause reverse effect. Therefore high agitation also contributes to higher oil removal.

Jelena, *et. al;* (2009), studied the remediation of soil heavily contaminated with crude oil and its product. The aim of this study was to examine the composition of the microbial consortium during the *ex situ* experiment of bioremediation of soil heavily contaminated with crude oil and its products from the Oil Refinery Pancevo, Serbia. After a 5.5-month experiment with biostimulation and bioventilation, the concentration of the total petroleum hydrocarbons (TPH) had been reduced from 29.80 to 3.29 g/kg(89 %). In soil, the dominant microorganism population comprised Gram-positive bacteria from actinomycete-*Nocardia* group. The microorganisms which decompose hydrocarbons were the dominant microbial population at the end of the process, with a share of more than 80 % (range 107 CFU/g). On the basis of the results, it was concluded that a stable microbial community had been formed after initial fluctuations.

Ebuehi, *et. al;* (2005), investigated the Remediation of Crude contaminated Soil by Enhanced Natural Attenuation. In the investigation, the concentrations of nitrogen, phosphorus, total hydrocarbon utilizing bacteria (THUB), total heterotrophic bacteria (THB) and total petroleum hydrocarbon were determined using the remediation by enhanced natural attenuation (RENA) in a crude oil contaminated farmland in Rivers state, Nigeria. A TPH concentration of 1.1004 x10 4mg/kg of the sandy soil was achieved after spiking and tilling. There was a reduction in the TPH level from 300mg/kg after 8weeks, to 282mg/kg after 10weeks.No significant reduction in the TPH level was observed after the 10th week. The nitrogen and phosphorus levels of the sandy soil were 24.6 and 22.8mg/kg respectively. This suggests that the nitrogen and phosphorus levels could no longer support biodegradation at the residual TPH levels of 282mg/kg and 22.8mg/kg after spiking and tilling respectively, which further reduced to 0.12mg/kg and 1.7mg/kg respectively after 10 weeks. The total hydrocarbon utilizing bacteria (THUB) increased from 3.0 x 10^{4}cfu/g to 8.55x10^{4}cfu/g and finally reduced to 5.38 x10^{4}cfu/g, while the total heterotrophic bacteria (THB) reduced from 1.22 x10^{8} cfu/g to 5.98 x 10^{5}cfu/g. Data of the study indicate that remediation enhanced natural attenuation technique could be employed to remediate a farm settlement contaminated by crude oil

Millioli, *et. al;* (2009), worked on the bioremediation of crude oil bearing soil: Evaluatng the effect of Rhamnolid addition to soil toxicity and to crude oil biodegradation efficiency. This work is aimed at evaluating the potentiality of adding a rhamnolipid biosurfactant in a petroleum-bearing soil. For this purpose, dehydrogenase activity and seed germination (*Lactuca sativa*) tests were performed before the biodegradation assays with different concentrations of rhamnolipid (1 to 15mg for 1g of soil). The addition of 1 and 15 mg g-1 of rhamnolipid was harmful to the soil environment. The biodegradation assays were carried out at room temperature during 45 days in bioreactors containing 450g of a polluted soil with different rhamnolipid concentrations varying from 1 to 15 mg g-1. The nutrients were corrected through the addition of NH_4NO_3 and KH_2PO_4, in a nutritional ratio of C: N: P=100:15:1. The humidity was adjusted to 50% of the liquid retention capacity. Besides these assays, a control test was conducted without adding rhamnolipid. TPH (Total petroleum hydrocarbon) removal and seed germination were evaluated at the end of these experiments. When 4 mg g^{-1} of rhamnolipid was used a TPH removal of about 60% was observed. The biosurfactant addition improved all treatments, except for the assays with addition of 1 and 15 mg g^{-1} in which a decrease of the bioremediations rates was observed in the toxicity tests.

The effect of organic fertilizers (spent mushroom, cow dung and poultry droppings) on the bioremediation of hydrocarbon contaminated soil was

investigated on a 28 days study period by Ibiene, *et. al;* (2011). The hydrocarbon contaminated soil was supplemented with the different organic fertilizers and analyzed throughout the study period. Physic-chemical and microbiological parameters like soil pH, moisture content, phosphate, nitrate, % total organic carbon (%TOC), total petroleum hydrocarbon (TPH) total heterotrophic counts, total hydrocarbon utilizing counts (bacteria and fungi) were studied from baseline to the 28th day. The concentrations of phosphate, nitrate, and percentage TOC of the treatments decreased significantly during the study period whereas the controls slight decrease in the parameters. pH values of the treatments were within slight acidity to alkalinity as the control had acidic pH range during the study period. The percentages of TPH loss in the cow dung option poultry droppings option spend mushroom option and control were 97.83%, 98.21%, 99.91% and 27.52%. The hydrocarbon utilizing bacterial isolates from the study include Bacillus, Pseudomonas, Kebsiella, Proteus, Flavobacterium, Clostridium, Micrococcus, Acinetobacter. The hydrocarbon utilizing fungal isolates from the study were Penicillium, Aspergillus, Sacharomyces, Rhizopus, Fusarium and Mucor. This study showed that cow dung, spent mushroom and poultry droppings are effective nutrient sources for bioremediation.

Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles was noted by Jorgensen *et. al;* (1999). Composting of

contaminated soil in biopiles is an ex situ technology, where organic matter such as bark chips are added to contaminated soil as a bulking agent. Composting of lubricating oil-contaminated soil was performed in field scale (5 x 40 m^3) using bark chips as the bulking agent, and two commercially available mixed microbial inocula as well as the effect of the level of added nutrients (N,P,K) were tested. Composting of diesel oil-contaminated soil was also performed at one level of nutrient addition and with no inoculum. The mineral oil degradation rate was most rapid during the first months, and it followed a typical first order degradation curve. During 5 months, composting of the mineral oil decreased in all piles with lubrication oil from approximately 2400 to 700 mg (kg dry w)y1, which was about 70% of the mineral oil content. Correspondingly, the mineral oil content in the pile with diesel oil-contaminated soil decreased with 71% from 700 to 200 mg (kg dry w)y1. In this type of treatment with addition of a large amount of organic matter, the general microbial activity as measured by soil respiration was enhanced and no particular effect of added inocula was observed.

In situ bioremediation of oily sludge contaminated soil by biostimulation of indigenous microbes through adding manure was conducted at the Shengli oilfield in northern China by Liu *et. al;* (2010). After bioremediation for 360 days, total petroleum hydrocarbon (TPH) content was reduced by 58.2% in the treated plots compared with only 15.6% in the control plot. Moreover,

bioremediation significantly improved the physicochemical properties of the soil in the treated plot. Soil microbial counts and community-level physiological profiling were also examined. Manure addition increased TPH degraders and polycyclic aromatic hydrocarbon (PAH) degraders in the contaminated soil by one to two orders of magnitude. The activity and biodiversity of soil microbial communities also increased markedly in the treated plot compared with that of the control. Finally, biotoxicity was used to evaluate the soils and a sharp increase in the EC50 of the soil after bioremediation was observed, indicating that bioremediation had reduced the toxicity of the soil.

Atagana, (2008) researched on compost bioremediation of hydrocarbon-contaminated soil inoculated with organic manure. Contaminated soil (FAO: Lithosol) containing >380 000mg kg^{-1} total petroleum hydrocarbons (TPH) was bioremediated by composting. The soil was inoculated with sewage sludge and incubated for 19 months. The soil was mixed in a ratio of 1:1 (v/v) with wood chips. The soil-wood chips mixture was then mixed in a ratio of 4:1 with sewage sludge. Compost heaps were set up in triplicates on wood pallets covered with double layers of nylon straw sheets. Control experiments which contained the contaminated soil and wood chips but without sewage sludge were set up in triplicate. Moisture, temperature, pH, ash content, C: N ratio of the compost mixture and TPH of the soil was monitored monthly. The concentrations of selected hydrocarbons in the contaminated soil were measured monthly during

the incubation period. Temperature rose to about 58°C in the sewage sludge compost within two months of incubation, while temperature in the control fluctuated between 15 and 35°C throughout the incubation period. Total petroleum hydrocarbons (TPH) was reduced by 17% in the control experiments and 99% in the sewage sludge compost at the end of the incubation period. The concentrations of most of the selected hydrocarbon components were reduced by up to 100% within the same period. Microbial activities were shown to correlate with the reduction in hydrocarbon contents of the soil.

Liu *et. al;* (2011) investigated on bioremediation petroleum hydrocarbon contaminated soil: Effect of strategies and microbial community shift. Biodegradation of petroleum hydrocarbon oil (14,000 mg kg^{-1}) were investigated in six biopiles batches, differing in the remediation strategy: bioaugmentation (selected consortium and kitchen waste were introduced), biostimulation (added with rhamnolipid, high-level, or low-level nutrient), and bioaugmentation plus biostimulation (added both with rhamnolipid and bacterial consortia). After the 140-day operation, the kitchen waste (KW) and the low-level nutrient (NEL) batches achieved the highest total petroleum hydrocarbon degradation efficiency (>80%). The result of the hydrocarbon analysis revealed that the bioaugmentation approaches were the most effective ones in removing aromatic component (64% and 68%), and KW and NEL were the only two approaches that can remove the polar component with positive efficiency, 11%

and 21%, respectively. The terminal-restriction fragment length polymorphism percentage (T-RFLP) abundance applied with non-metric multidimensional scaling indicated a similarity of the bacterial communities during the early fastest remediation stage. The results of the oligonucleotide array targeting the ribosomal internal transcribed spacer (ITS) region, along with the hydrocarbon fractional analysis, indicated a successive degradation completed by the bacterial–fungi consortia. Before Day 70, the bacterial community was dominant in decomposing the saturated and partially aromatic hydrocarbons. After Day 70, the fungal community found to be dynamic and responsible for degradation of the polar hydrocarbons composing of recalcitrant metabolites.

Akpoveta *et. al;* (2011), studied the microbial degradation and its kinetics on crude oil polluted soil. In the research, a pilot study was conducted on soil simulated with crude oil to examine the effects of the hydrocarbon on soil properties, the potentials of exploring soil indigenous microbes and determining suitable conditions for effective degradation of the contaminant as well as evaluating the kinetics of the process. Soil collected from Agbor area of the Niger Delta in southern Nigeria was artificially spiked with 10% brent crude and studied. Control soil, simulated soil and treated soil were all characterised for pH, electrical conductivity, total organic carbon and matter, total nitrogen and phosphorus, texture and heavy metals (Cd, Pb, Ni, V and Cr) using standard analytical methods to determine the effect of crude oil pollution on these

properties. Total petroleum hydrocarbon (TPH) was determined by measuring the amount of parent contaminant left in the soil at intervals in order to establish the efficiency and kinetics if the bioremediation process. Crude oil utilizing bacteria and fungi were also determined using standard microbiological procedures. Crude oil pollution caused a reduction in pH, conductivity and phosphorus level with significant effect in the growth rate of soil heterotrophic microbes, but however did not show any negative effect on the other properties. Crude oil did not affect the levels of the metals in the soil since the simulated soil showed lower metal concentration than the control soil, except for the remediation process which caused an increase in the concentration of Ni and V due to contributions of these metals from the animal waste used. The rate of microbial degradation was found to be dependent on availability of nutrient source and pH, as high biodegradation rate occasioned by an increase in microbial population was favoured between pH 6.7-9.6. Suitable pH condition and nutrient availability will enhance speedy microbial transformation of contaminant. A remediation efficiency of 81.69% was obtained on the sixth week indicating the efficiency and effectiveness of the process. The biodegradation process followed first order with a rate constant of 0.035day-1. Biodegradation isotherm was found to be minus unity expressing the opposite linear relationship between the concentration of the contaminant in the soil (Cs)

and the concentration degraded by the microbes (Cd) at different time intervals for the remediation period.

Major fertility indices - N, P, K, TOC and TOM contents - were examined against the backdrop of physicochemical conditions of pH, temperature, moisture content and electrical conductivity of soils three months after oil spillage at Owaza in the Niger Delta region of Southern Nigeria by Leo, *et.al;* (2007). Evidence of severe hydrocarbon contamination was provided by high extractable hydrocarbon content of $3.4 \times 10^3 – 6.8 \times 10^3$ mg/kg. High soil acidity (low pH of 4.9 – 5.1), low electrical conductivity as well as high temperature and moisture content, all provided evidence of reduced metabolic activities on the affected site which explains the relatively low TOC/TOM values obtained. These conditions generally imply low soil fertility, which in turn implies low agricultural productivity and reduced source of livelihood in the affected area. Based on the results obtained, contingency/remedial measures should include the application of appropriate and sufficient inorganic NPK fertilizer to restore the carbon to nutrient ratios to the optimum required to stimulate and sustain microbial activity; adjustment of the pH to 6.0 – 6.5 by the addition of calcitic lime; stimulation of indigenous microbial growth by cultivating the soil to distribute the nutrients and lime and appropriate aeration of the treatment zone.

CHAPTER THREE

MATERIALS AND METHODS

3.1: Study Area

The study area is Umuibe (Akwete) situated along Obehie-Azumini road in Ukwa East L.G.A of Abia State, an oil producing area. The town is about 30km from Aba, a major commercial city of Abia state in South Eastern Nigeria as shown in figure 3.

The ambient environment of the study area is characterized by annual rainfall of about 2500mm-2850mm and average temperature of about 27^{o}C - 30^{o}C. The vegetation cover is the tropical rain forest.

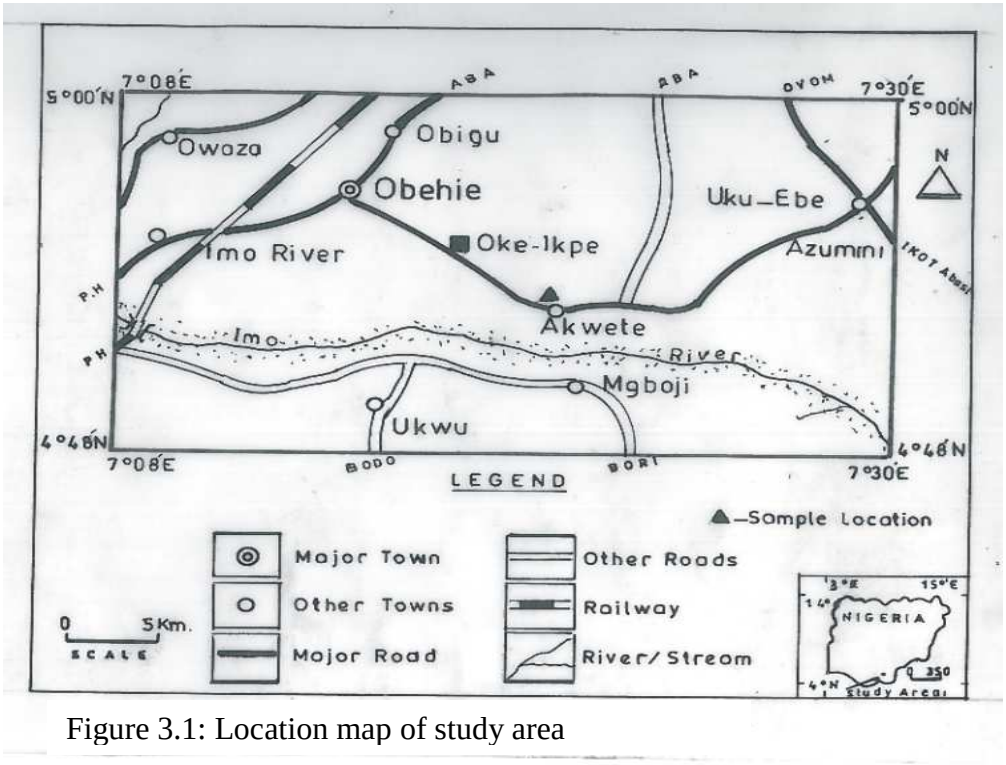

Figure 3.1: Location map of study area

3.2: Materials, Equipment and Apparatus

pH meter, pH and temperature electrodes, Beakers, Volumetric flasks, Glass rods, Drying oven, Weighing balance, Specimen containers, Desiccators, Container handling apparatus, leather hand gloves, tongs or suitable holder for removing and handling hot sample containers, UV Spectrometer, Stopwatch or electric timer, Volumetric flask (100ml, 1L), Pipette, Filter paper, Glass bottles for extraction, Funnel, Spatula, Shaker, Glass Test tubes (10ml), Test tube racks, Water bath suitable for use at 100^{o}C, Cold bath suitable for use at 10-15^{o}C, Sample Vial, Glass bottles for extraction, Funnel, Measuring spoon, Shaker (water bath), Acetic acid, Ammonium hydroxide, Atomic absorption spectrophotometer, Burette, Erlenmeyer flask 250ml, 0.5mm sieve, Weighing balance, Asbestos sheet, Porcelain mortar, Beaker 250ml**,** Fourier Transform Infra Red (FTIR) spectrometer, 100ml volumetric flask, 120ml glass bottles for extraction, Phase separator, Glass funnel, Clean, dry bottles for storing extracts (20ml), 10mm quartz.

3.3: Sampling and sample preparation

An uncultivated farmland with no record of crude oil contamination of about 250cm x 750cm dimension was used for this research and divided into 75 cells or grid plots. The composite soil sample from each cell was analysed and the baseline data recorded for reference. Each cell of about 50cm x 50cm

dimension was spilled with 1.25 litres of crude oil obtained from an oil location at Owaza in Ukwa West L.G.A of Abia State. The crude oil was obtained for the purpose of this research work only and the research was carried out during the dry season (November, 2012 - February, 2013) when there was little or no rainfall.

A total of 24 cells of the farmland were contaminated at regular intervals with the 1.25 litres of crude oil, while the remaining 51 cells were untouched. Few days after, the contaminated cells were tilled on the surface to a depth of 2cm with knives, shovels, cutlasses etc and sprinkled with water. The surface tillage and the sprinkling of water help to soften the soil and ensure proper mixing of soil with the remediating agent.

1 week after oil spillage, 12 of the contaminated cells chosen at random were treated with 0.5kg of abattoir waste. The abattoir waste consists of the dungs, pieces of bones, blood, urine, waste water etc. Other oil spilled cells were left without adding the abattoir waste to serve as control experiment.

Table 3.1: Grid System of Sampling

250cm

(1) P+R		(2) P		(3) P+R
(4) P		(5) P+R		(6) P
(7) P+R		(8) P		(9) P+R
(10) P		(11) P+R		(12) P
(13) P+R		(14) P		(15) P+R
(16) P		(17) P+R		(18) P
(19) P+R		(20) P		(21) P+R
(22) P		(23) P+R		(24) P

750cm

[ ] = Cell without oil spill

[P] = Crude oil spilled cell without the abattoir waste (control)

[P+R] = Crude oil spilled cell with the abattoir waste

Soil samples were collected periodically for analysis. This was done using a hand dug soil auger. The cells were augured to 20cm depth in random triplicate (that is three different random spots). The samples collected were bulked together (composite soil samples) and put in amber (coloured) bottle for analysis involving hydrocarbon (THC), and other bottles for other parameters (pH, moisture content, N, P, K, TOC, TOM) to guarantee quality results. The samples were taken to the laboratory for analysis. The analysis of remediated soil samples took a period of 10 weeks and samples were checked for various parameters at intervals of 2 weeks.

3.4: Soil Analysis

The physicochemical characteristics of various soil samples including pH, moisture content, nitrogen, phosphorus, potassium, total organic carbon (TOC), total organic matter (TOM), and hydrocarbon content (THC) were determined in triplicate (i.e. three replicate) using standard analytical methods described below. It should be noted that the mean of the triplicate analysis were used as the true values.

3.4.1: Determination of pH

This involved the use of American Standard Test Method (ASTM D 4972 for soil) with the steps outlined as follows:

Testing Procedure

Calibration

1. The pH meter was calibrated against three buffer solutions
2. Buffers pH 4.0, pH 7.0 and pH 10.0

Sample Analysis

1. 20.0g of the fresh soil sample was weighed into 50.0ml beaker and 20.0ml of distilled water added to it.
2. It was thoroughly stirred at first with a glass rod and allowed to stand for 30 minutes.
3. The electrodes of the meter were inserted into the partly settled suspension.
4. The pH and temperature values were recorded after 180 seconds.
5. The electrodes were thoroughly rinsed with distilled water and blotted dry with soft tissue paper between readings.

3.4.2: Determination of Soil Moisture Content

Test Method

This involved the use of American Standard Test Method (ASTM D 2216-92 for moisture content of soils) with the steps outlined as follows:

Testing Procedure

1. The weight of the clean and dry specimen container (petri-dish) was determined and recorded.
2. 20g of the moist sample was placed in the container.
3. The mass of the container and the moist soil sample was determined using weighing balance.
4. The container with moist material was placed in the drying oven at 110^0C ± 5^0C and dried to a constant weight (at least 12 hours).
5. The container was brought out and cooled in a desiccator.
6. The mass of the container and oven dried sample was determined and recorded.

Calculation

$$W = \frac{Mi - Md}{Mi - Mtin}$$

Where

W = moisture content (%)

Mi = mass of the container and wet specimen, g

Md = mass of the container and oven dry specimen, g

Mtin = mass of container, g

3.4.3: Determination of Nitrate Nitrogen in Soil/Sediment by Spectrophotometry

Test Method

This involved the use of EPA's Sampling and Analysis Methods, edited by Lawrence H. Keith, 2nd edition, 1996 with the steps outlined as follows:

Reagents/Chemical

- Extracting solution (distilled water)
- Sodium chloride solution (30%): 30g NaCl was dissolved in distilled water and dilute to 100ml.
- Sulphuric acid solution: 4 parts H_2SO_4 was carefully added to 1 part water (e.g. 100ml H_2SO_4 to 25ml water). Cooled and kept tightly stoppered to prevent absorption of atmospheric moisture.
- Bruicine-Sulphanilic acid reagent: 1g bruicine sulphate $(C_{23}H_{26}N_2O_4)_2H_2SO_4.7H_2O$ and 0.1g sulfanilic acid $(NH_2C_6H_4SO_3H.H_2O)$ were dissolved in 70ml hot distilled water. 3ml concentrated HCl was added, cooled, mixed, and diluted to 100ml with distilled water and stored in a dark bottle at 5^oC.
- Potassium nitrate stock solution: 0.185g of anhydrous potassium was dissolved in water and diluted to 250ml (1ml = 0.10mg of NO_3-N)

- Nitrate working standard solution: 10ml of the stock was diluted to 100ml with distilled water. 1ml = 10mg Nitrate.
- Acetic acid (1+3): 1 volume glacial acetic acid (CH_3COOH) was diluted with 3 volumes of distilled water.
- Sodium hydroxide (1N): 40g of NaOH was dissolved in distilled water. Cooled and diluted to 1L.

Testing Procedure

Sample Preparation

a. The sample was mixed using a glass rod or stainless steel spatula.
b. The composite sample was dried in the sun or in the oven at 105 ± 2^oC for two hours.
c. The dried material was disaggregated by gently crushing any lumps in a mortar to pass through 2mm stainless steel sieve.

Sample Extraction

a. 1.00g of the soil sample was weighed into a shaking bottle.
b. 25ml of distilled water (extracting solution) was added to it.
c. The solution was shaken for 10 minutes on a rotary shaker and then filtered.

Sample Analysis

1. 5.00ml of the extract was pipetted into a 10ml test tube.

2 1.0ml of the 30% NaCl was added to the test tube.

3 The contents were mixed by swirling and the rack was placed in a cold bath (10-15^{o}C).

4 5ml of the 4:1 H_2SO_4 was pipetted into the test tube and mixed by swirling.

5 The tube was allowed to cool before continuing.

6 0.25ml brucine-sulphanilic acid was added to the tube and mixed by swirling.

7 The rack of the tube from the hot water bath was later removed and immersed in the cold water bath and allowed to reach thermal equilibrium (20-25^{o}C).

8 Absorbance of the Nitrate was measured at 410nm using 1cm cell with UV spectrometer and concentration of the Nitrate was determined from the Nitrate calibration curve.

Data Processing and Reporting

Calculation

$$NO_3^- N(mg/kg) = \frac{\text{mg } NO_3^- \text{ from the cal. graph x vol. of extractant (ml)}}{\text{Vol. of extract (ml) x sample weight (g)}} \times 1000$$

3.4.4: Determination of Phosphate in Soil/Sediment by Spectrophotometry

Reagents/ Chemicals

- Ammonium molybdate reagent: 25g $(NH_4)_6$ $MO_7O_{24}4H_20$ was dissolved in 200ml of distilled water in a beaker and warmed slightly to dissolve).
- 280ml Conc. H_2S04 was added carefully (with mixing and cooling) to 40ml of distilld water.
- The molybdate solution was filtered into the acid mixture, mixed thoroughly and made up to 1L when cooled.
- Stannous chloride reagent (0.5g $SnCl_2$, $2H_2O$ was dissolved in 250ml of 2% v/v HCl).
- Extractant (2.5% acetic acid, i.e. 25ml of glacial acetic acid was diluted to 1L).

Testing Procedure

Sample Extraction

25.00ml of the extracting solution (2.5% acetic acid) was added to 1.00g of soil sample and shook for 30 minutes and then filtered through a filter paper.

Sample analysis

1. 10.00ml of the extract was transferred into 50ml volumetric flask.

2. The extract was diluted with distilled water until the flask was about two third full.

3. 2.00ml ammonium molybdate reagent was added and mixed.

4. 2.00ml stannous chloride reagent was also added, mixed and diluted to 50ml mark and then allowed to stand for 30 minutes.

5. Absorbance of phosphate was measured at 700nm using 10mm cell in the UV/visible spectrometer and the concentration of the phosphate was determined from the phosphate calibration curve.

PO_4- p (mg/kg) = $\frac{\text{C (mg) x solution volume (1) x1000}}{\text{Aliquot x sample weight (g)}}$

Where, C=mg PO_4^{-3} obtained from the calibration graph using the UV/visible spectrometer and vision software version 3

1000 = conversion factor to kg

Aliquot = $\frac{\text{Volume (ml) of extract used for analysis}}{\text{Volume (ml) of extractant used for the extraction.}}$

3.4.5: Determination of Potassium by Ammonium Acetate Extraction

Test Method

This involved the use of 1N Ammonium acetate (NH_4OAc**)** at pH 7.0 to extract basic cations (calcium, Ca; magnesium, Mg; potassium, K and sodium, Na) from the soil.

Reagents

Extracting Solution-Ammonium acetate--NH_4OAc @ pH 7.0)

58 mL of acetic acid ($HC_2H_30_2$), 95.5%, 1.05 sp. gr. was poured into 500 mL of deionized water. 70 mL of ammonium hydroxide (NH_40H), 0.9 sp. gr. was added and mixed. pH was adjusted to 7.0 ± 0.05 with acetic acid or ammonium hydroxide. The mixture was diluted to one liter with deionized water.

Test Procedure

Sample Extraction

2 g of <10 mesh air dry soil was weighed into an extraction flask. 20 mL of extracting solution was added and shook for 5 minutes on a shaker, filtered, and the filtrate collected and diluted to 250 mL mark.

Sample Analysis

Concentration of potassium was measured from the filtrate using Atomic Absorption Spectrophotometer (AAS).

Data processing

Calculation

$$\text{Potassium (mg/kg)} = \frac{\text{Conc.(mg/l)} \times 250 \times 1000}{\text{Weight of sample (g)}}$$

Where

250 = the vol. (in ml) of the volumetric flask in which the filtrate was made up to 1 L.

3.4.6: Determination of Total Organic Carbon/Matter

Test Method

This involved the use of British Standard Test Method (BSTM 1377 for soil) with the steps outlined as follows:

Reagents

- Potassium dichromate ($K_2Cr_2O_7$), 1N: 49.04g of reagent-grade $K_2Cr_2O_7$ (dried at 105^oC) was dissolved in distilled water and diluted to 1L in a volumetric flask.
- Concentrated Sulphuric acid (H_2SO_4)
- Ferrous sulphate heptahydrate ($FeSO_4$.7H2O) solution, 0.5N: 140g of reagent grade $FeSO_4.7H_2O$ was dissolved in distilled water, 15ml of conc. Sulphuric acid added, and the solution cooled and diluted to 1L in a volumetric flask. OR,
- Ferrous ammonium sulphate hexahydrate $(NH_4)_2$ $SO_4.FeSO_4.6H_2O$) solution (0.5N): 196.07g of reagent grade $(NH_4)_2SO_4.FeSO_4.6H_2O$) was

dissolved in distilled water, 50ml of conc. Sulphuric acid added, and the solution cooled and diluted to 1L in volumetric flask.

- Indicator: Phenanthroline-Ferrous complex (0.025M): 14.85g of o-phenanthroline monohydrate and 6.95g of ferrous sulphate heptahydrate or, 9.80g of ferrous ammonium sulphate hexahydrate was dissolved in distilled water and dilute to 1L.

Testing Procedure

Sample Preparation

a. The sample was mixed using a glass rod or stainless steel spatula.
b. The composite sample was dried at $105^{o}C$ for two hours.
c. The dried sample was cooled and disaggregated by gently crushing any lumps or clumps in a mortar.
d. The crushed sample was passed through 0.5mm sieve.

Sample Analysis

a. 0.2g of the sieved sample was weighed into a 250ml wide mouth Erlenmeyer flask or beaker.
b. 10ml of 1N $K_2Cr_2O_7$ solution was added and the flask swirled gently to disperse the sample in the solution.
c. 20ml of concentrated H_2SO_4 was added rapidly to the solution directing the stream into the suspension.

d. The flask was swirled gently until sample and reagents were mixed, then more vigorously for about 1 min.
e. The flask was allowed to stand on a sheet of asbestos in a fume hood for about 30mins.
f. 5 to 6 drops of the indicator was added and titrated with 0.5N $FeSO_4$ to maroon colour.

Data Processing

Calculation:

$$\text{Organic Carbon (g/kg)} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq } FeSO_4) \times 0.003 \times 1000 \times 1.3}{\text{Weight of water free sample(g)}}$$

[Walkey, A. (1947)]

Total Organic Matter (g/kg) = Total organic carbon (g/kg) x 1.729

Where,

Meq $K_2Cr_2O_7$ = 1N x 10ml

Meq $FeSO_4$ = 0.5N x volume of titrant in ml

0.003 = Milliequivalent weight of carbon

1.30 = Correction factor

1000 = Conversion factor to kg

3.4.7: Determination of Total Hydrocarbon Content in Soil/Sediment by Infra-Red Spectrophotometry

Test Method This involved the use of American Standard Test Method (ASTM D3921 for soil) with the steps outlined as follows:

Reagent

- Isooctane: Spectroscopic grade
- Tetrachloroethylene
- Silica gel
- Sodium sulphate anhydrous

Testing Procedure

Sample Handling and Preparation

a. The soil sample was mixed using a glass rod or spatula, to minimize stratification effects due to differential rates of setting and also to obtain representative sample.
b. The composite sample was dried in the oven at $105^{o}C \pm 2^{o}C$ for two hours.
c. The dried material was disaggregated by gently crushing any lumps in a mortar.

Sample Extraction

a. 5.0g of sample was weighed into a 100ml glass for extraction.

b. 20ml of Tetrachloroethylene was added into the bottle (glass) and shook for three hours.

c. The solid was allowed to settle and the extract transferred into a clean bottle through a glass funnel lined with pre-treated phase separator or glass wool which does not allow aqueous phase (water) to pass through.

Sample Analysis

The hydrocarbon in the extract was determined using FTIR spectrometer.

Data Processing and Reporting

The actual THC concentration (mg/kg) is deduced as follows

$$= \frac{\text{instrument reading (mg/l) x volume of extract (ml)}}{\text{Weight of sample (g)}}$$

3.5: Kinetic Studies of the Bioremediation

Kinetics is very essential in the understanding of the rate of bioremediation of contaminated soils, more especially the rate of biodegradation of hydrocarbons (THC) in crude oil contaminated soils, being a major pollutant in farmlands of Niger-Delta areas. Zero-order, first-order and second-order kinetic models were applied to the experimental result (bioremediation data) to produce a rate mechanism of the bioremediation process. Zero-order reactions are those reactions in which the concentrations of the reactants remain constant during the course of the reaction and, therefore, the rate is independent of the concentration

of such reacting molecules. In the first-order reaction, with a single reactant, the rate of reaction is directly proportional to the concentration of reactant. For a second-order reaction, the rate depends on two variable concentration terms which may or may not be same. These models were assessed based on the closeness of their regression coefficients to unity (one). The regression coefficient (R^2) can therefore be used to indicate if a model is applicable or not to the bioremediation process.

3.5.1: Zero-Order Kinetic Model

Zero-order kinetics was applied on the experimental result to determine the rate of bioremediation process. The linear form of the zero-order equation is given as:

$$C_o - C_t = K_o t$$

Where C_o (mg/kg) is the initial concentration of parameter, C_t (mg/kg) is the concentration of the parameter at a given time t, K_o (day^{-1}) is the zero order rate constant of the bioremediation given by the slope of the graph. The applicability of this equation was tested by a linear plot of $C_o - C_t$ against t. This was applied to six parameters (N, P, K, TOC, TOM, & THC) to determine their rate constants and regression coefficients. (See table 4.2 and figures 4.9 to 4.20 below for both treated samples and control experiments.)

3.5.2: First-order kinetic model

First-order kinetics was also applied to the experimental data to evaluate the rate of bioremediation process. The linear form of the first-order equation is given as:

$$\ln (C_t/C_o) = -K_1 t$$

Where C_t (mg/kg) is the concentration of the parameter at a given time t, C_o (mg/kg) is the initial concentration of parameter, K_1 (day^{-1}) is the first-order rate constant of the bioremediation given by the slope of the graph. . The applicability of this equation was tested by a linear plot of $\ln (C_t/C_o)$ against t. This was applied to six parameters (N, P, K, TOC, TOM, & THC) to determine their rate constants and regression coefficients. (See table 4.3 and figures 4.21 to 4.32 below for both treated samples and control experiments.)

3.5.3: Second-order kinetic model

Second-order kinetics was as well applied to the experimental data to determine the rate of bioremediation process. The linear form of the second-order equation is given as:

$$1/C_t - 1/C_o = K_2 t$$

Where C_t (mg/kg) is the concentration of the parameter at a given time t, C_o (mg/kg) is the initial concentration of parameter, K_2 (day^{-1}) is the second-order

rate constant of the bioremediation given by the slope of the graph. . The applicability of this equation was tested by a linear plot of $1/C_t - 1/C_o$ against t. This was also applied to the six parameters (N, P, K, TOC, TOM, & THC) to determine their rate constants and regression coefficients. (See table 4.4 and figures 4.33 to 4.44 below for both treated samples and control experiments.)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.I: Results

The hypothesis that crude oil contamination of agricultural soils limits the availability of some soil parameters especially the basic soil nutrients (N,P,K) has been substantiated by the findings of this research work. This can be observed from the result of the study presented in table 4.1, and also the effect of treatment of the polluted soils with Abattoir was as in tables 4.2 to 4.6. Bioremediation kinetics result was as well presented in tables 4.7 to 4.10 as shown below.

values of the Studied Parameters

TABLE 4.1: Soil physicochemical characteristics before crude oil contamination and one week after contamination

No. of Trials	pH (H_2O) at 23.0 °C		Moisture Content		Nitrogen		Phosphorus		Potassium		TOC		TOM		THC	
	B/C	A/C_1	B/C	A/C_1	B/C	A/C_1	B/C	A/C_1	B/C	A/C_1	B/C	A/C_1	B/C	A/C_1	B/C	A/C_1
1	5.30	5.20	8.45	8.29	3.28	1.51	2.69	2.15	626	540	8.70	25.74	15.18	44.50	10.00	16198
2	5.31	5.22	8.49	8.25	3.22	1.53	2.61	2.24	622	538	8.50	26.20	14.70	45.78	10.20	16160
3	5.29	5.18	8.41	8.21	3.25	1.49	2.53	2.06	618	536	8.30	25.28	14.22	43.22	9.80	16122
Mean	5.30	5.20	8.45	8.25	3.25	1.51	2.61	2.15	622	538	8.50	25.74	14.70	44.50	10.00	16160
SD	±0.01	±0.02	±0.04	±0.04	±0.03	±0.02	±0.08	±0.09	±4.00	±2.00	±0.20	±0.46	±0.48	±1.28	±0.20	±38.00

B/C = Before Contamination, A/C_1= One Week after Contamination, SD= Standard Deviation. All units are in mg/Kg except moisture content (%) and pH

TABLE 4.2: Soil physicochemical characteristics two weeks after remediation and control experiment

No. of Trials	pH (H_2O) at 23.0 °C		Moisture Content		Nitrogen		Phosphorus		Potassium		TOC		TOM		THC	
	A/R_2	EC	A/R_2	EC	A/R_2	EC	A/R_2	EC	A/R_2	EC	A/R_2	EC	A/R_2	EC	A/R_2	EC
1	6.21	5.20	11.90	8.29	1.83	1.52	6.44	2.15	805	562	32.69	25.74	57.50	45.70	13185	15512
2	6.22	5.16	11.85	8.26	1.81	1.48	7.12	2.08	807	566	33.25	26.20	56.50	45.90	13192	14754
3	6.23	5.25	11.95	8.23	1.85	1.44	6.78	2.22	809	558	32.97	25.28	57.00	45.50	13199	15550
Mean	6.22	5.20	11.90	8.26	1.83	1.48	6.78	2.15	807	562	32.97	25.74	57.00	45.70	13192	15512
SD	±0.01	±0.05	± 0.05	± 0.03	± 0.02	±0.04	±0.34	± 0.07	± 2.00	± 4.00	± 0.28	± 0.46	± 0.50	±0.20	± 7.00	± 449.00

A/R_2= Two Weeks after Remediation, EC= Experimental Control, SD= Standard Deviation. All units are in mg/Kg except moisture content (%) and pH

TABLE 4.3: Soil physicochemical characteristics four weeks after remediation and control experiment

No. of Trials	pH (H_2O) at 23.0 °C		Moisture Content		Nitrogen		Phosphorus		Potassium		TOC		TOM		THC	
	A/R_4	EC	A/R_4		A/R_4	EC	A/R_4	EC	A/R_4	EC	A/R_4	EC	A/R_4	EC	A/R_4	EC
1	6.75	5.22	12.40	8.29	2.62	1.50	9.16	2.46	1132	594	36.06	27.36	63.49	47.60	10623	15018
2	6.85	5.20	12.35	8.20	2.57	1.52	9.14	2.38	1134	588	36.03	27.30	62.30	47.30	10618	115019
3	6.95	5.24	12.30	8.38	2.67	1.54	9.12	2.30	1130	591	36.00	27.42	61.11	47.00	10628	15020
Mean	6.85	5.22	12.35	8.29	2.62	1.52	9.14	2.38	1132	591	36.03	27.36	62.30	47.30	10623	15019
SD	±0.10	±0.02	±0.05	±0.09	±0.05	±0.02	±0.02	±0.08	±2.00	±3.00	±0.03	±0.06	±1.19	±0.30	±5.00	±57735.00

A/R_4= Four Weeks after Remediation, EC= Experimental Control, SD= Standard Deviation. All units are in mg/Kg except moisture content (%) and pH

TABLE 4.4: Soil physicochemical characteristics six weeks after remediation and control experiment

No. of Trials	pH (H_2O) at 23.0 °C		Moisture Content		Nitrogen		Phosphorus		Potassium		TOC		TOM		THC	
	A/R_6	EC	A/R_6	EC	A/R_6	EC	A/R_6	EC	A/R_6	EC	A/R_6	EC	A/R_6	EC	A/R_6	EC
1	7.20	5.25	12.00	8.27	2.83	1.52	13.15	4.10	1377	618	34.17	27.69	59.60	47.80	8139	14623
2	7.30	5.28	11.92	8.21	2.78	1.54	13.12	3.80	1401	620	34.23	27.61	59.20	49.90	8130	14621
3	7.40	5.22	12.08	8.24	2.88	1.56	13.18	3.50	1389	622	34.29	27.65	58.80	45.70	8148	14619
Mean	7.30	5.25	12.00	8.24	2.83	1.54	13.15	3.80	1389	620	34.23	27.65	59.20	47.80	8139	14621
SD	±0.10	±0.03	±0.08	±0.03	±0.05	±0.02	±0.03	±0.30	±12.00	±2.00	±0.06	±0.04	±0.40	±2.10	±9.00	±2.00

A/R_6= Six Weeks after Remediation, EC= Experimental Control, SD= Standard Deviation All units are in mg/Kg except moisture content (%) and pH

TABLE 4.5: Soil physicochemical characteristics eight weeks after remediation and control experiment

No. of Trials	pH (H_2O) at 23.0 °C		Moisture Content		Nitrogen		Phosphorus		Potassium		TOC		TOM		THC	
	A/R_8	EC	A/R_8	EC	A/R_8	EC	A/R_8	EC	A/R_8	EC	A/R_8	EC	A/R_8	EC	A/R_8	EC
1	9.79	5.30	13.00	8.26	2.74	1.56	17.27	4.92	1523	666	38.80	28.22	63.65	48.80	7025	13991
2	9.67	5.26	13.45	8.19	2.77	1.53	17.33	4.90	1521	660	36.84	28.00	63.75	49.00	7015	14015
3	9.55	5.22	12.55	8.33	2.80	1.50	17.30	4.88	1519	663	34.88	28.44	63.70	48.60	7035	14003
Mean	9.67	5.26	13.00	8.26	2.77	1.53	17.3	4.90	1521	663	36.84	28.22	63.70	48.80	7025	14003
SD	±0.12	±0.04	±0.45	±0.07	±0.03	±0.03	±0.03	±0.02	±2.00	±3.00	±1.96	±0.22	±0.05	±0.20	±10.00	±12.00

A/R_8= Eight Weeks after Remediation, EC= Experimental Control, SD= Standard Deviation. All units are in mg/Kg except moisture content (%) and pH

TABLE 4.6: Soil physicochemical characteristics ten weeks after remediation and control experiment

No. of Trials	pH (H_2O) at 23.0 °C		Moisture Content		Nitrogen		Phosphorus		Potassium		TOC		TOM		THC	
	A/R_{10}	EC	A/R_{10}	EC	A/R_{10}	EC	A/R_{10}	EC	A/R_{10}	EC	A/R_{10}	EC	A/R_{10}	EC	A/R_{10}	EC
1	8.20	5.36	12.91	8.18	2.96	1.61	16.71	4.95	1890	705	39.23	28.44	67.50	48.80	6067	13677
2	8.18	5.28	12.95	8.24	2.80	1.58	16.75	4.90	1895	715	38.85	28.22	67.10	47.75	6071	13685
3	8.22	5.20	12.87	8.30	2.88	1.55	16.67	4.85	1885	695	39.04	28.00	67.90	49.85	6075	13681
Mean	8.20	5.28	12.91	8.24	2.88	1.58	16.71	4.90	1890	705	39.04	28.22	67.50	48.80	6071	13681
SD	±0.02	±0.08	±0.04	±0.06	±0.08	±0.03	±0.04	±0.05	±5.00	±10.00	±0.19	±0.22	±0.40	±1.05	±4.00	±4.00

A/R_{10}= Ten Weeks after Remediation, EC= Experimental Control, SD= Standard Deviation. All units are in mg/Kg except moisture content (%) and pH

TABLE 4.7: Zero-order kinetic parameters

Parameter	Nitrogen	Phosphorous	Potassium	TOC	TOM	THC
K_0	-0.016	-0.200	-18.25	-0.092	-0.16	127.4
R^2	0.672	0.922	0.982	0.758	0.759	0.952
			CONTROL			
K_0	-0.001	-0.057	-2.557	-0.031	-0.055	33.41
R^2	0.848	0.917	0.990	0.898	0.900	0.993

TABLE 4.8: First-order kinetic parameters

Parameter	Nitrogen	Phosphorous	Potassium	TOC	TOM	THC
K_1	-0.006	-0.017	-0.014	-0.002	-0.002	0.014
R^2	0.645	0.915	0.960	0.757	0.757	0.984
			CONTROL			
K_1	-0.006	-0.016	-0.004	-0.001	-0.001	0.002
R^2	0.561	0.910	0.994	0.893	0.895	0.993

TABLE 4.9: Second-order kinetics parameters

Parameter	Nitrogen	Phosphorous	Potassium	TOC	TOM	THC
K_2	-0.003	-0.001	-1.0×10^{-5}	-7.0×10^{-5}	-4.0×10^{-5}	2.0×10^{-6}
R^2	0.619	0.887	0.907	0.746	0.759	0.995
			CONTROL			
K_2	-0.000	-0.005	-6.0×10^{-6}	-4.0×10^{-5}	-2.0×10^{-5}	2.0×10^{-7}
R^2	0.848	0.896	0.998	0.889	0.893	0.992

TABLE 4.10: Comparison of Coefficient of regression (R^2) for the kinetic studies

Parameter	Nitrogen	Phosphorous	Potassium	TOC	TOM	THC
Zero-order	0.672	0.922	0.982	0.758	0.759	0.952
First-order	0.645	0.915	0.960	0.757	0.757	0.984
Second-order	0.619	0.887	0.907	0.746	0.759	0.995
			CONTROL			
Zero-order	0.848	0.917	0.990	0.898	0.900	0.993
Fssirst-order	0.561	0.910	0.994	0.893	0.895	0.993
Second-order	0.848	0.896	0.998	0.889	0.893	0.992

4.2: Discussion

4.2.1: pH

Crude oil pollution on the soil caused a reduction in pH from 5.30 to 5.20. The observed reduction in pH was similar to the findings of Osuji and Nwoye (Osuji and Nwoye , 2007). A reduction in pH implies increased acidity which is a problem for agricultural soils because many metal cations are soluble and available in the soil solution at very low pH including Cd, Cu, Hg, Ni, Pb and Zn (McBride, 1994). The resulting increased acidity could be due to the fact that hydrocarbons contain many free cations causing them to have properties of weak acid (Akpoveta, *et. al;* 2011).

A significant increase in pH (from 5.20 to 9.67), 8 weeks after remediation, before dropping in the 10th week was observed (see fig. 4.1 below). The observed increase in pH was due to the bioremediation process which removed the contaminant and introduced some salts and ions from the animal waste since they contain them. The rise in pH from acidity (5.20) to alkalinity (9.67) during bioremediation process was due to the animal waste used which contains elements like calcium, potassium, magnesium and phosphorus that are alkaline in nature (Akpoveta, *et. al;* 2011). In the control experiment there was no noticeable increase after remediation (5.20-5.28).

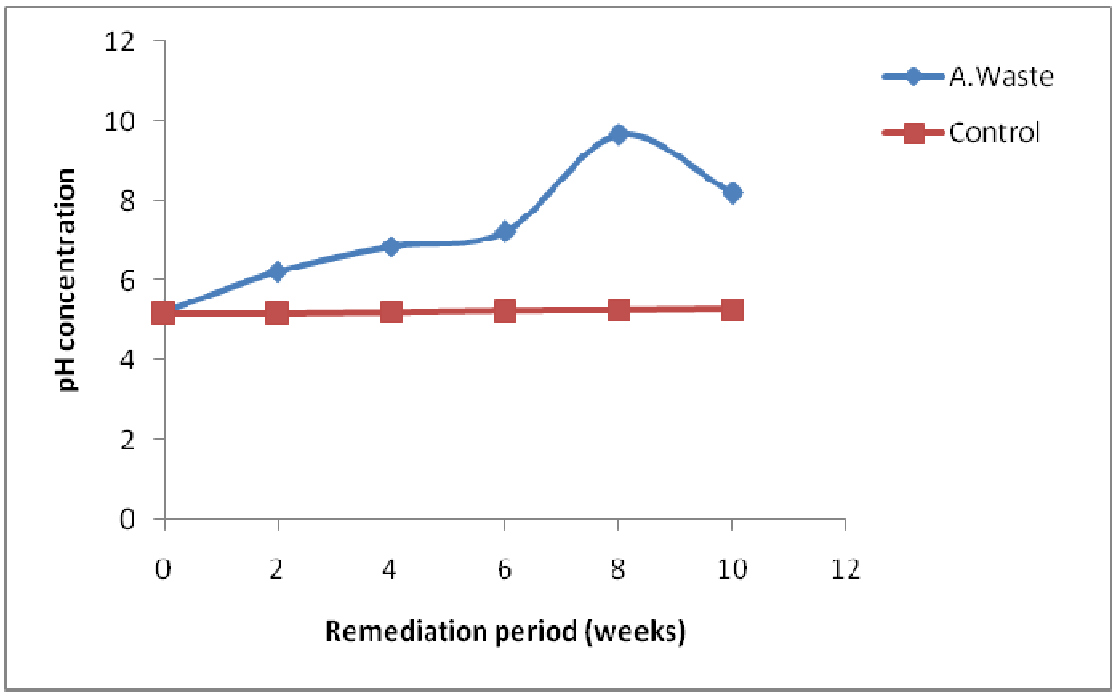

FIGURE 4.1: Soil pH profile as a function of remediation period

4.2.2: Moisture Content

Soil moisture dropped from 8.45% to 8.25% after crude oil contamination. This is expected because in heavily polluted soils water droplets adhere to the hydrophobic layer formed, and this prevents wetting of the inner parts of the soil aggregate. A similar observation was made by Odokuma and Dickson (Odokuma and Dickson, 2003).

As the cells were remediated through the introduction of animal waste (abattoir waste), the moisture content increased and later dropped slightly. It however rose again after 6 weeks (see fig 4.2 below). The rise in moisture content during remediation process from 8.25% to 12.91% in 10 weeks period may be probably as a result of the water from the effluent and also the solid waste. It could also be attributed to the improvement in the humidity of the environment as a result of intermittent rainfall within the period under investigation. In the control, there was no significant change within the period under investigation (8.25% - 8.24%).

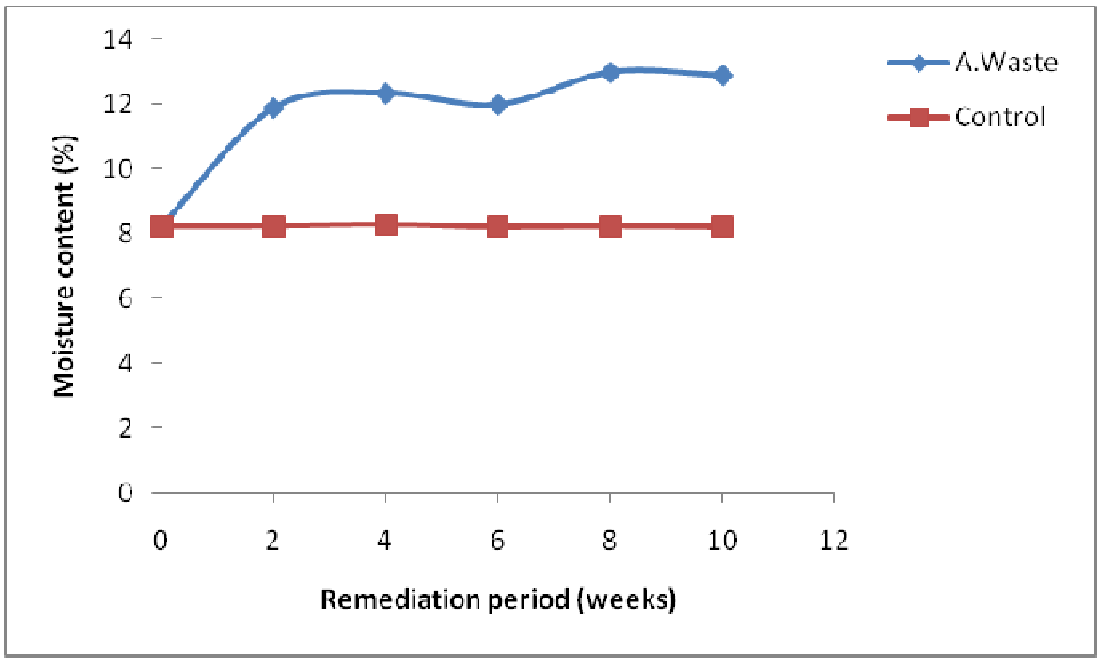

FIGURE 4.2: Moisture content profile as a function of remediation period

4.2.3: Nitrogen

After crude oil contamination the soil nitrogen reduced from 3.25mg/kg to 1.51mg/kg. The reduction in the concentration of nitrogen in the contaminated site suggests that the process of nitrification might have reduced following the incidence of oil contamination or spillage. According to Odu, *et. al;* (1985), oil-degrading or hydrocarbon utilizing microbes such as Azobacter Spp normally become more abundant while nitrifying bacteria such as nitrosamines Spp become reduced in number. This probably explains the relatively lower values of nitrogen (N) obtained for the contaminated soils.

By the addition of the remediating agent (abattoir waste), the nitrogen in the contaminated soil increased from 1.51mg/kg to 2.88mg/kg within 10 weeks period of the investigation (see fig 4.3). The quick nitrogen mineralization may due to the nutrients in the animals waste (abattoir waste) that was converted to soil nitrogen by some microbes in the soil. In the control experiment there was no significant change within the period under investigation (1.51mg/kg to 1.58mg/kg).

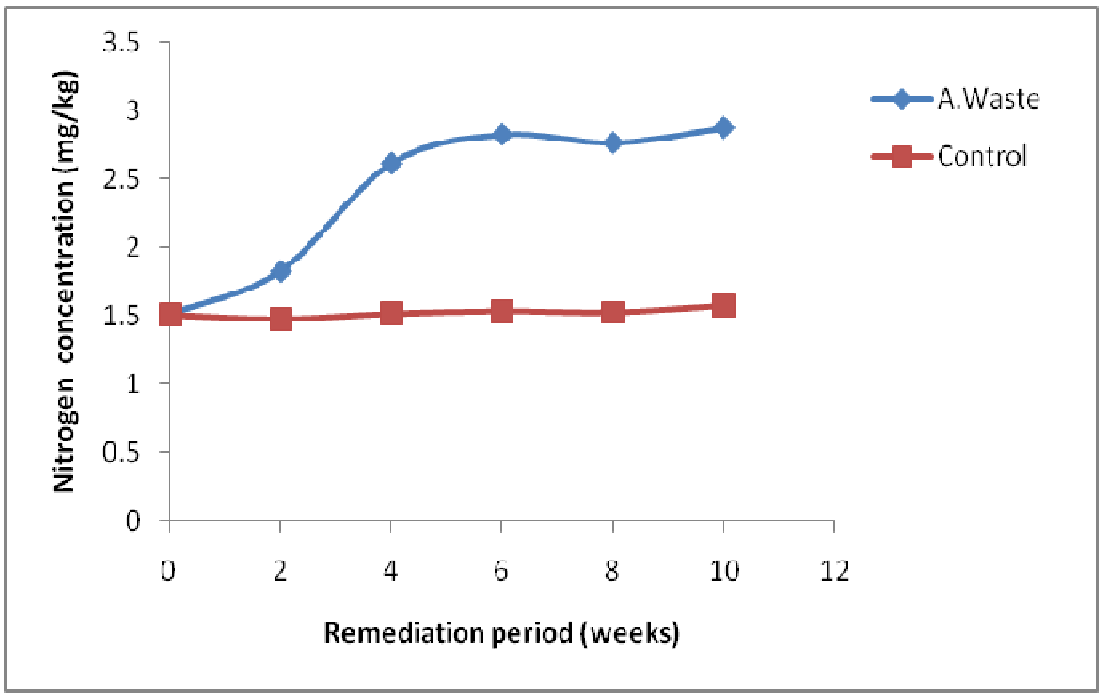

FIGURE 4.3: Nitrogen profile as a function of remediation period

4.2.4: Phosphorous and Potassium

A reduction in both parameters was observed when the soil was contaminated with the crude oil. The reduction in phosphorous level from 2.61mg/kg to 2.15mg/kg could be due to possible oxidation of free phosphorous in the soil to phosphate because hydrocarbons can act as election acceptors or oxidizing agent due to the presence of oxygen in them thereby producing a reducing environment. Also potassium reduced from 622mg/kg to 538 mg/kg on the additional of the crude oil to the soil.

Significant increase in phosphorous (2.15mg/kg to 16.71mg/kg) and potassium (538mg/kg to1890mg/kg) was observed after the bio-remediation

process (see fig 4.4 and 4.5). This is because the animal waste used contains among others piece of bones, ground into powdered form, with high content of cations like calcium, magnesium, phosphorus, potassium and nitrogenous nutrients. In the control experiment for both, there was a noticeable increase mainly in phosphorous (2.15mg/kg to 4.90mg/kg) and in potassium (538mg/kg to 705mg/kg) within the period under investigation. The remediation is natural and probably as a result of microbial activities in the soil.

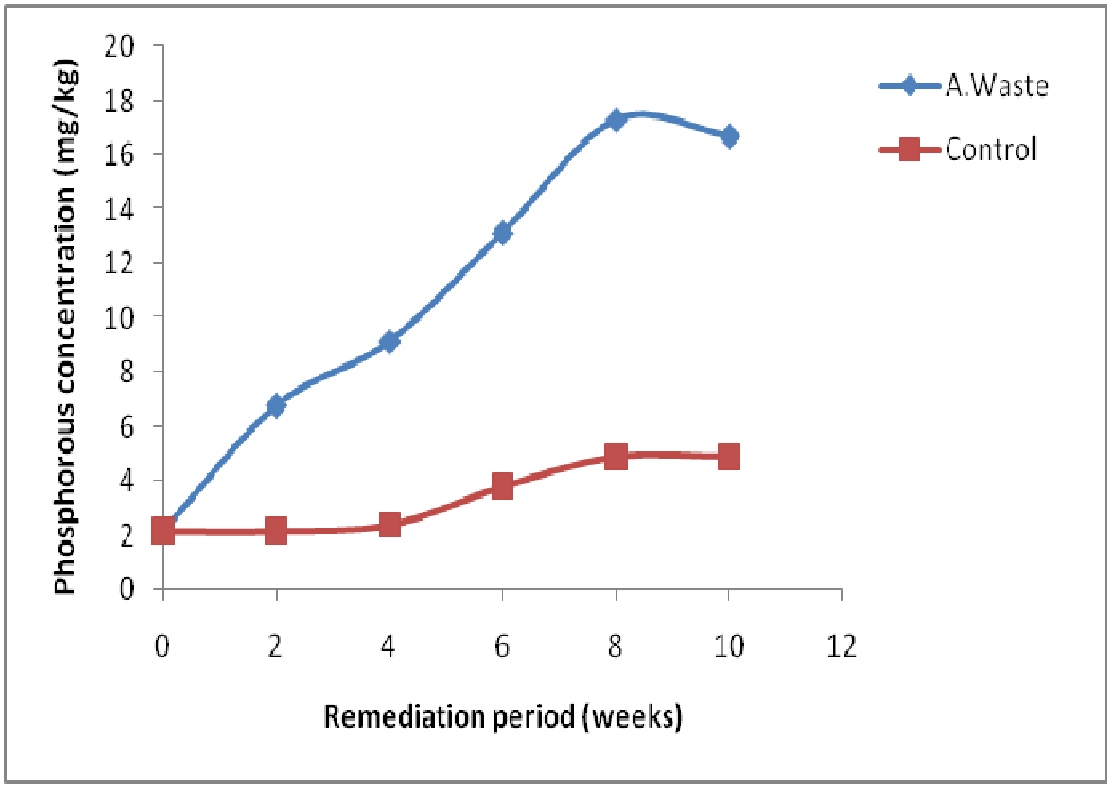

FIGURE 4.4: Phosphorous profile as a function of remediation period

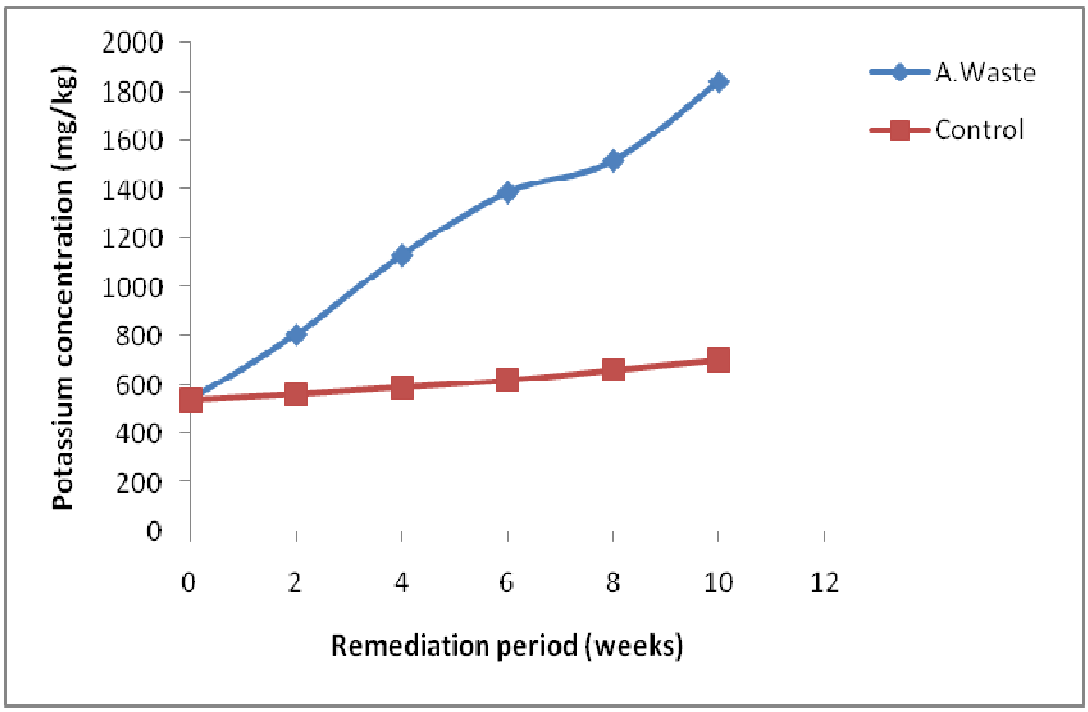

FIGURE 4.5: Potassium profile as a function of remediation period

4.2.5: TOC and TOM

Soil properties such as TOC (8.50mg/kg to 25.74mg/kg) and TOM (14.7mg/kg to 44.5mg/kg) increased on additional of the crude oil and subsequently increased after the bioremediation process as seen in table 4.1 above. The observed increased on introduction of crude oil could be due to the fact that crude oil is highly carbonaceous. This is in line with the statement of Ak poveta, *et. al;* (2011). The carbons or hydrocarbons in the crude oil are being attacked by the microbes in the soil (e.g. hydrocarbon degrading bacterial) which convert them to organic matter.

When the abattoir waste was added to the soil as a remediating agent, more microbes were added which oxidized or broke further the crude hydrocarbons releasing more organic matter to the soil. Hence, more increase in organic matter (TOM) from 44.5 mg/k to 67.5 mg/kg and also in organic carbon (TOC) from 25.74mg/kg to 39.04mg/kg during remediation as both are related (see fig 4.6 and 4.7). Besides, the abattoir waste is very rich in certain nutrients which when converted to soil nutrients increases the organic matter composition of the soil. In the control experiments, there was a slight increase in both TOC and TOM as a result of natural bioremediation of the soil by some microbes present is the soil.

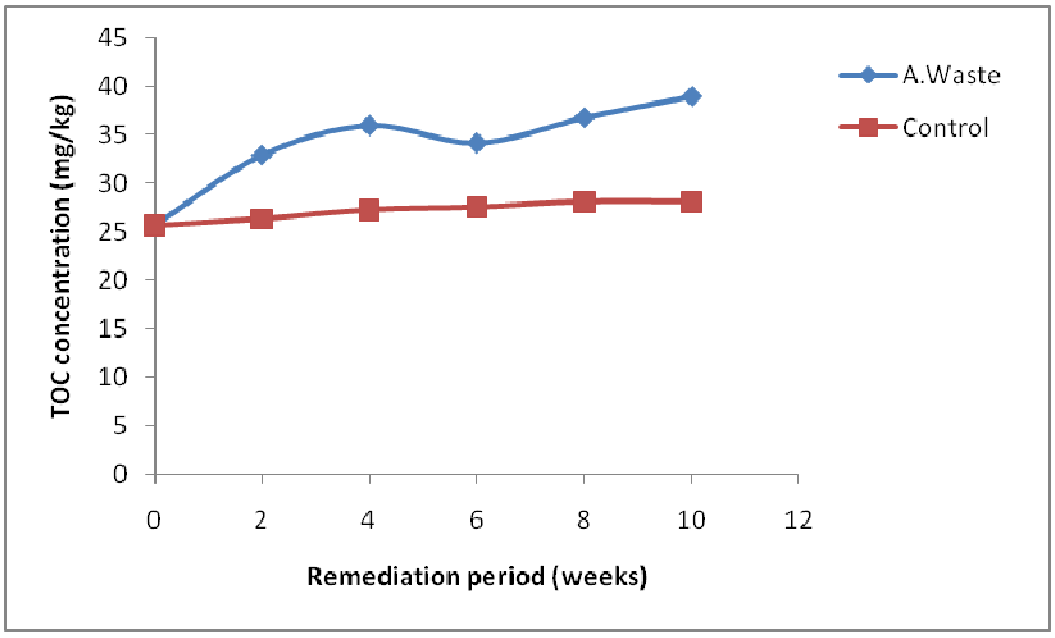

FIGURE 4.6: Total organic carbon profile as a function of remediation period

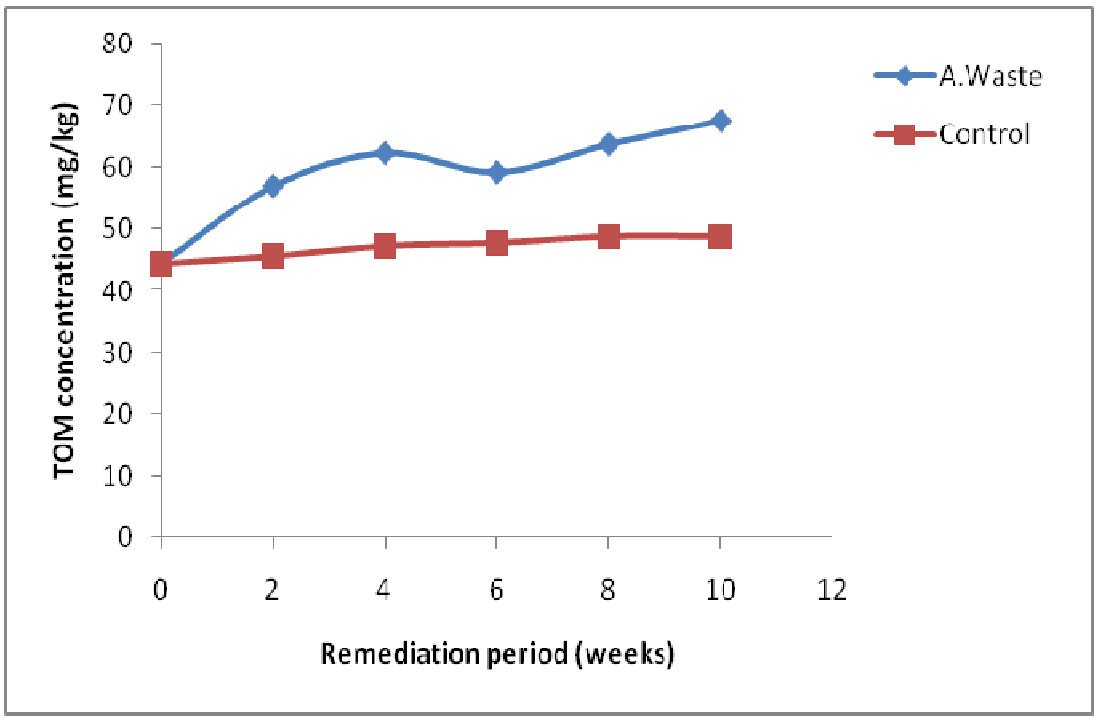

FIGURE 4.7: Total organic matter profile as a function of remediation period

4.2.6: Total Hydrocarbon Content (THC)

THC increased greatly from < 10mg/kg to 16160mg/kg after the soil was contaminated with crude oil. This is due to the fact that crude oil contains high proportion of hydrocarbons compared to other constituents.

On the application of the abattoir waste to the contaminated soil, there was a gradual reduction in THC values within the period under investigation. A ten weeks investigation on the biodegradion of crude oil in the contaminated soil reveals that THC reduced from 16160mg/kg to 6071mg/kg.

This is equivalent to 62.43% reduction. Also there was a noticeable reduction of THC in the control experiment from 16160mg/kg to 13681mg/kg,

equivalent to 15.34% reduction. The marked reduction in the percentage of THC in the treated soil was quite different from the one observed in the control under the same period. This is in line with the findings of Akpoveta, *et. al;* (2011). It contains the view that biodegradation is impeded by nutrient deficiency.

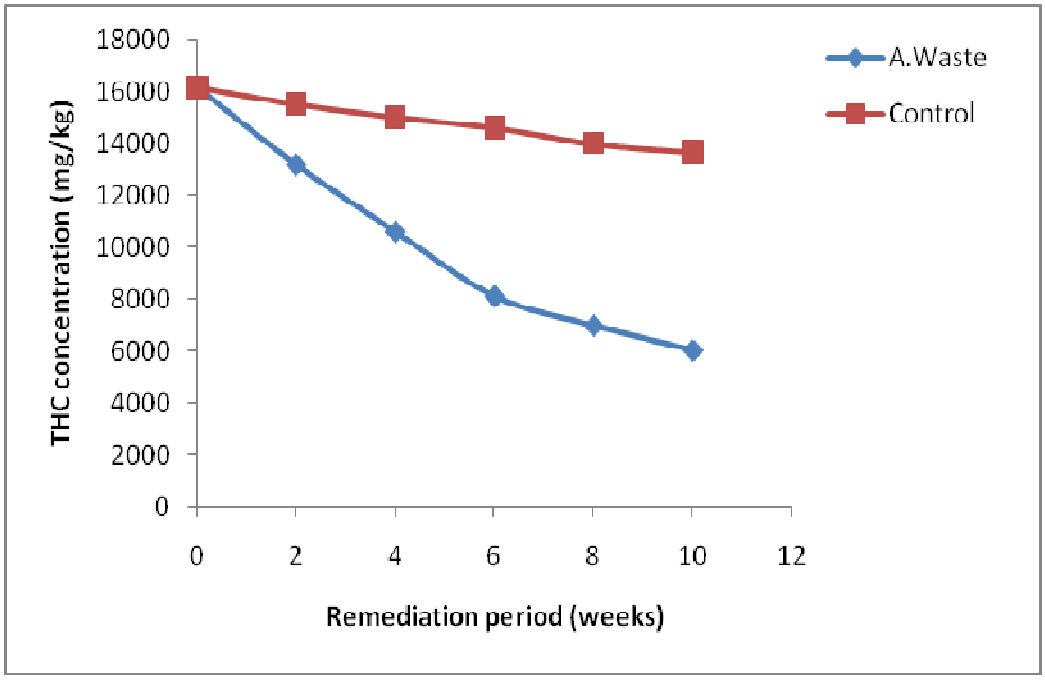

FIGURE 4.8: Total hydrocarbon content (THC) profile as a function of remediation period.

4.2.7: Kinetic Analysis

The values of the parameters obtained for the bioremediation kinetics are shown in tables 4.7 to 4.10 while Figs 4.9 to 4.44 showed the kinetic model plots obtained for N, P, K TOC, TOM and THC. Looking at the table 4.10 for the comparison of regression coefficients (R^2) for the six parameters (N, P, K, TOC, TOM & THC), it was observed that all the three kinetic models (zero-order,

first-order and second-order kinetics models) gave good fit to the bioremediation data of Potassium (K) and Total hydrocarbon content (THC) by both the abattoir waste and control. This is evident by the values of their linear regression coefficient (R^2) greater than 0.9 (i.e. very close to unity). However, in the case of phosphorus only the zero-order and first-order kinetic models were appropriate in the description of the bioremediation process, while none of the three models was able to fit to the bioremediation data of N, TOC, & TOM, evident by the values of their regression coefficients (R^2) much less than one.

This implies the applicability of the three models (zero-order, first-order and second-order kinetic models) in the description of the kinetics of K & THC, while the zero-order and first-order kinetic models were applicable in the description of the bioremediation process of P. It also implies the un-applicability of the three models for Nitrogen, TOC & TOM.

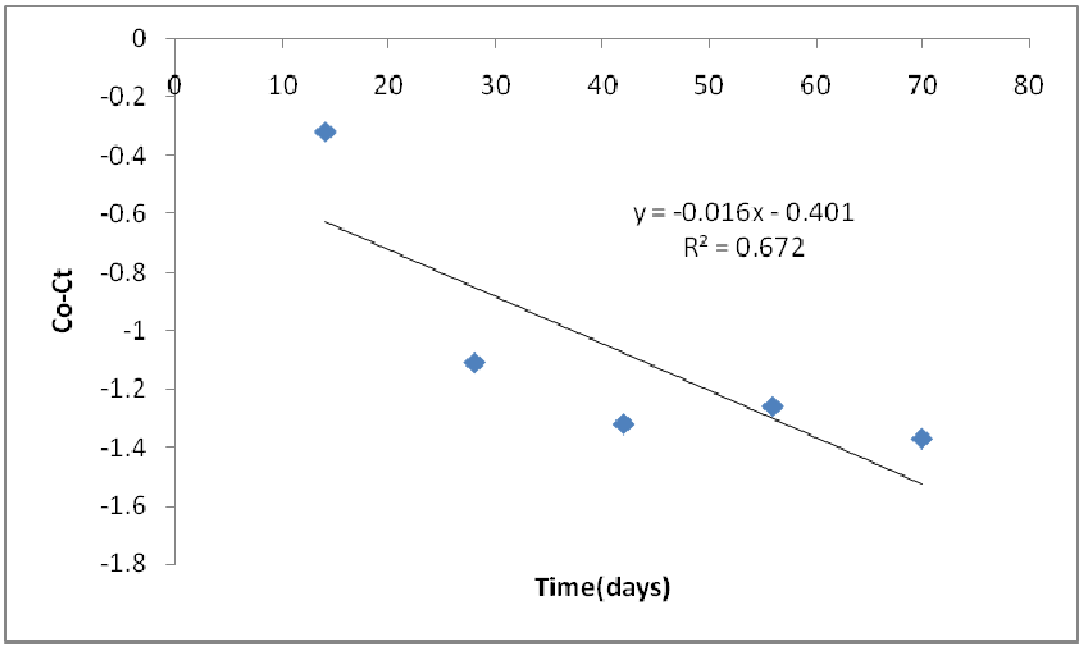

FIGURE 4.9: Zero-order kinetics for Nitrogen using abattoir waste

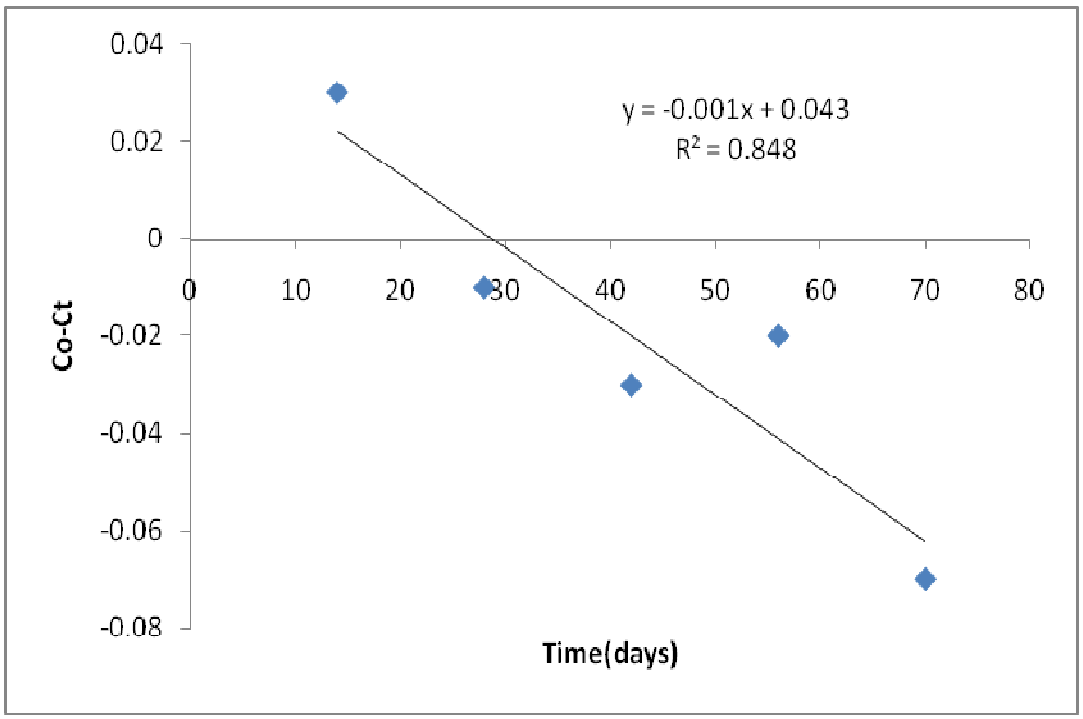

FIGURE 4.10: Zero-order kinetics for Nitrogen at controlled condition

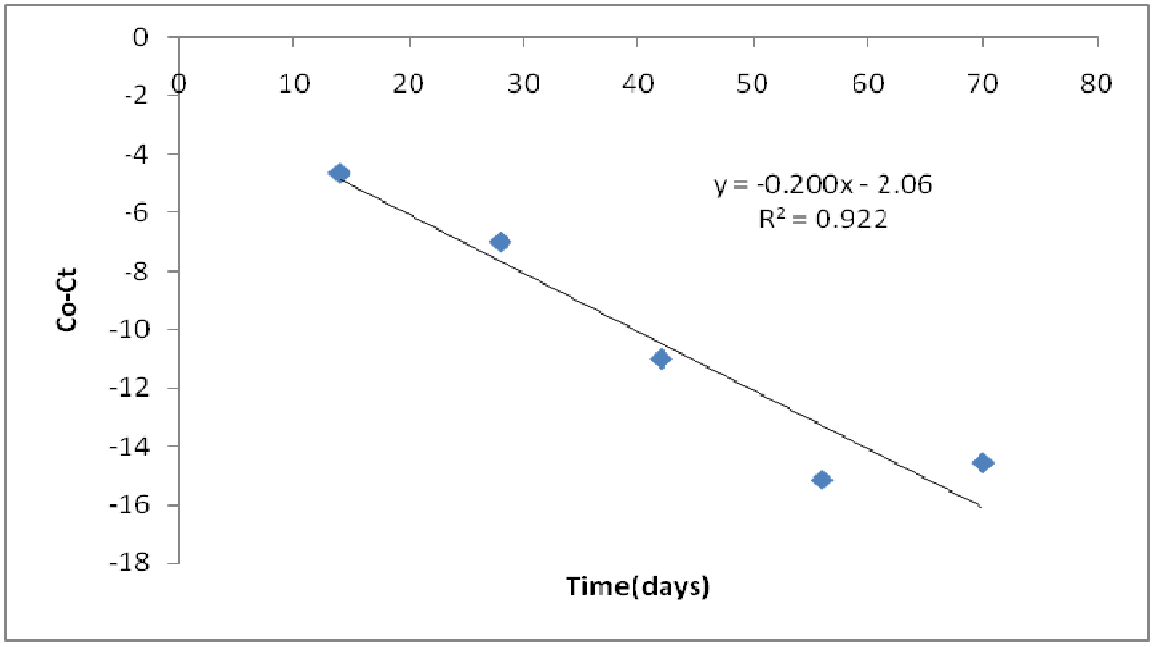

FIGURE 4.11: Zero-order kinetics for Phosphorous using abattoir waste

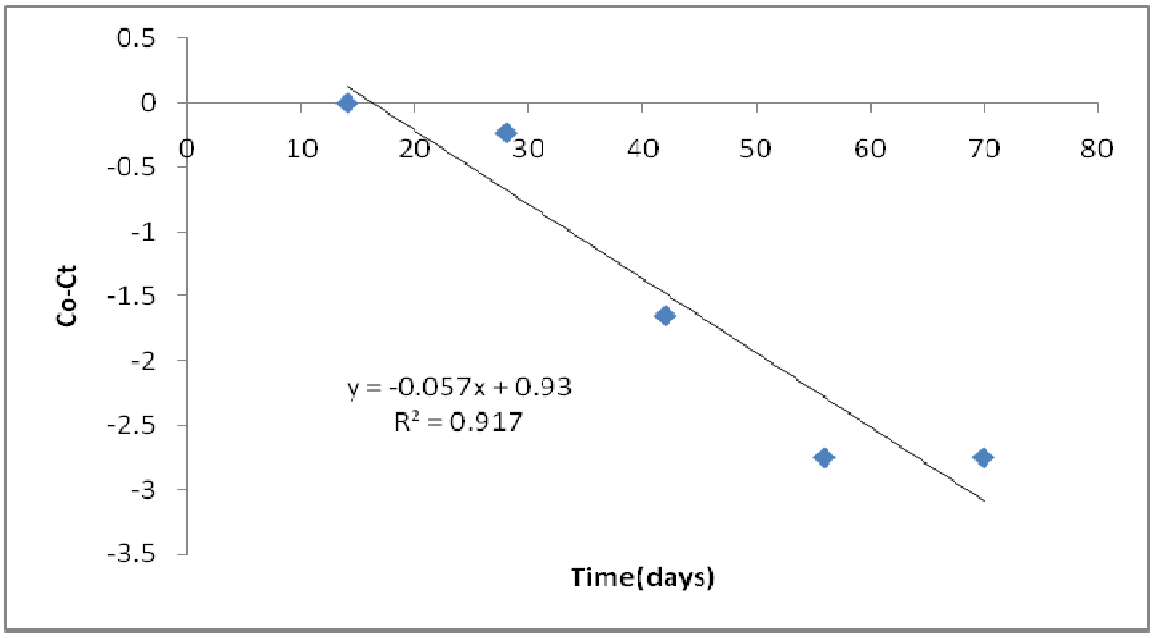

FIGURE 4.12: Zero-order kinetics for Phosphorous at controlled condition

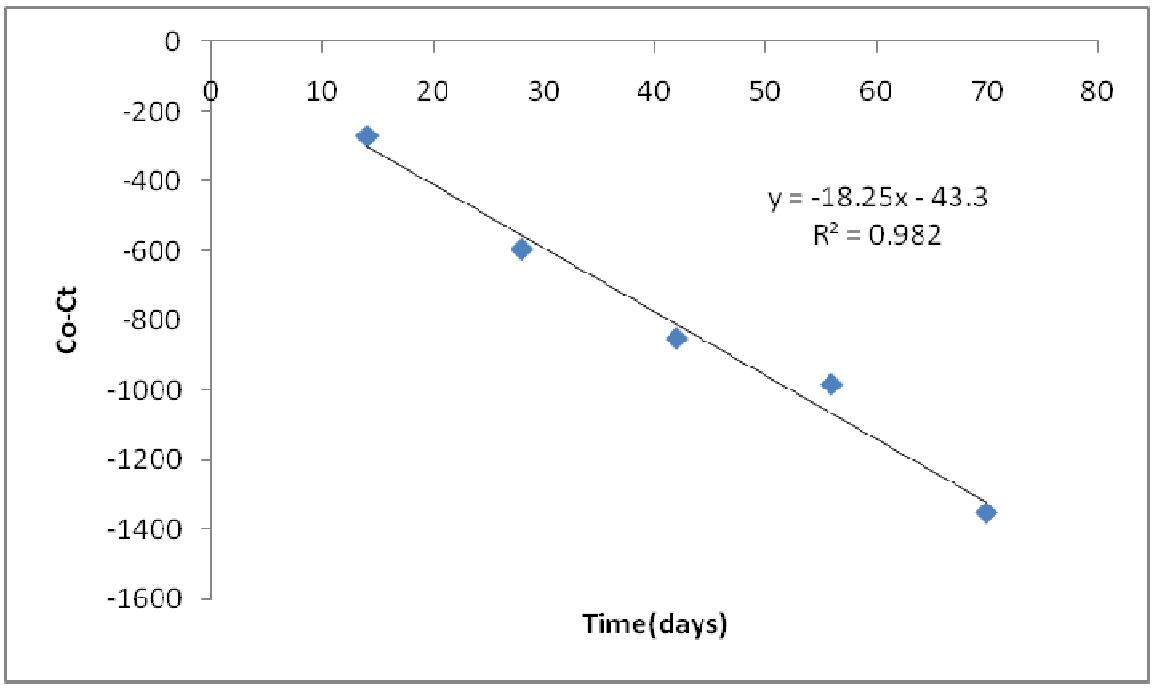

FIGURE 4.13: Zero-order kinetics for Potassium using abattoir waste

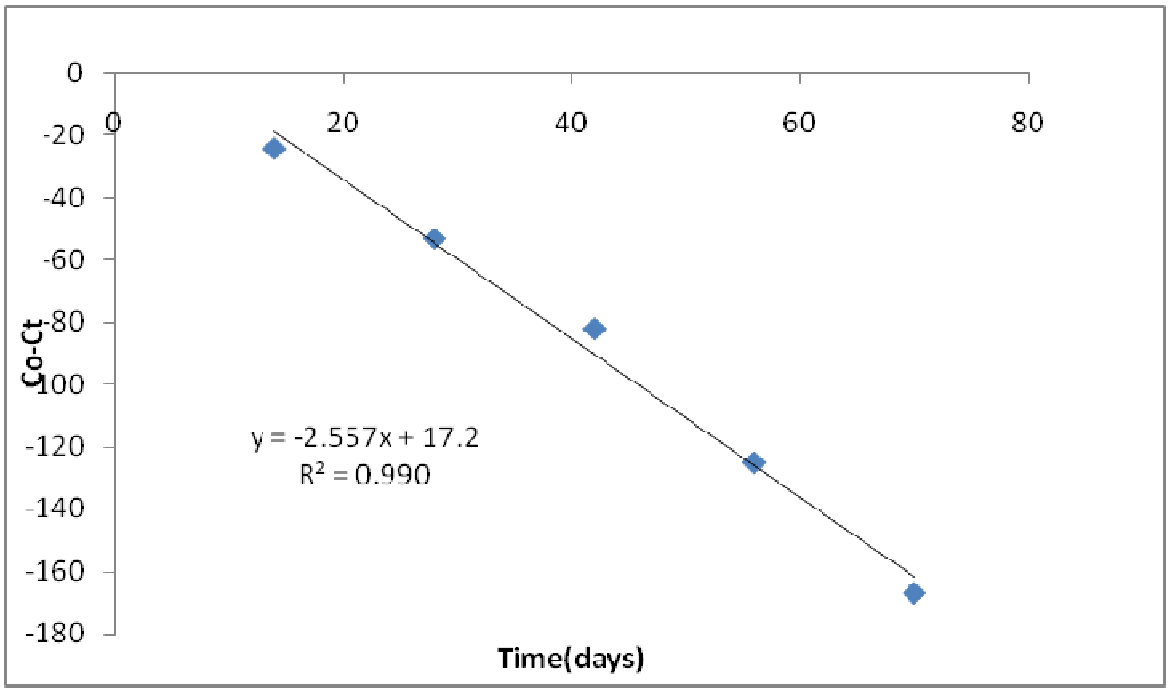

FIGURE 4.14: Zero-order kinetics for Potassium at controlled condition

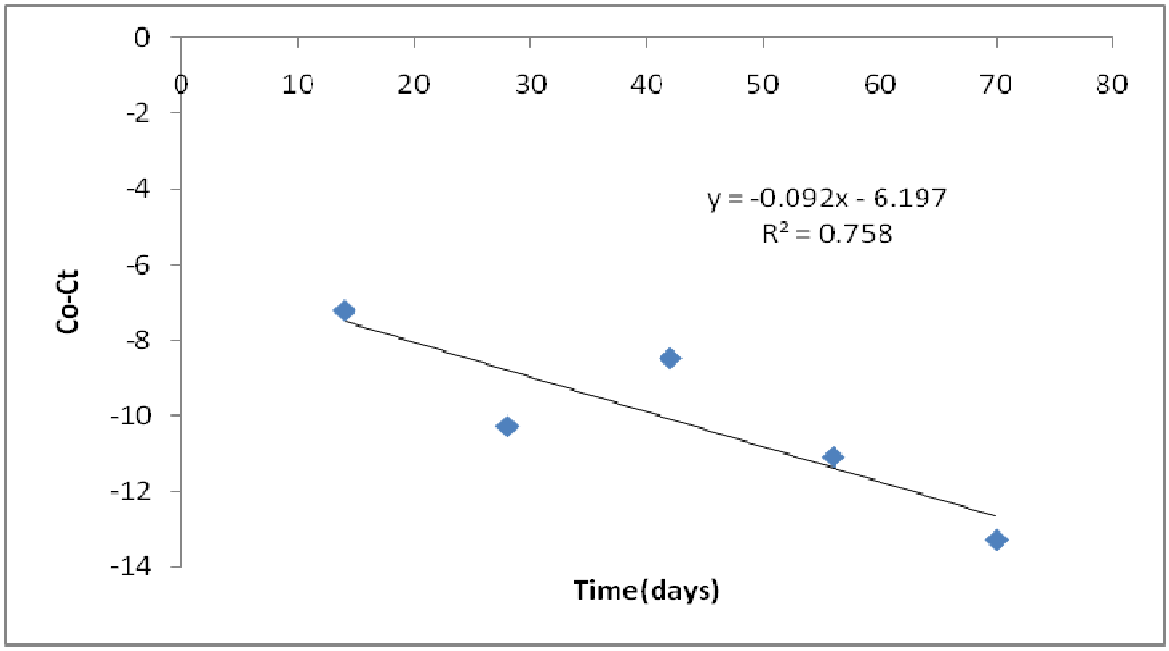

FIGURE 4.15: Zero-order kinetics for TOC using abattoir waste

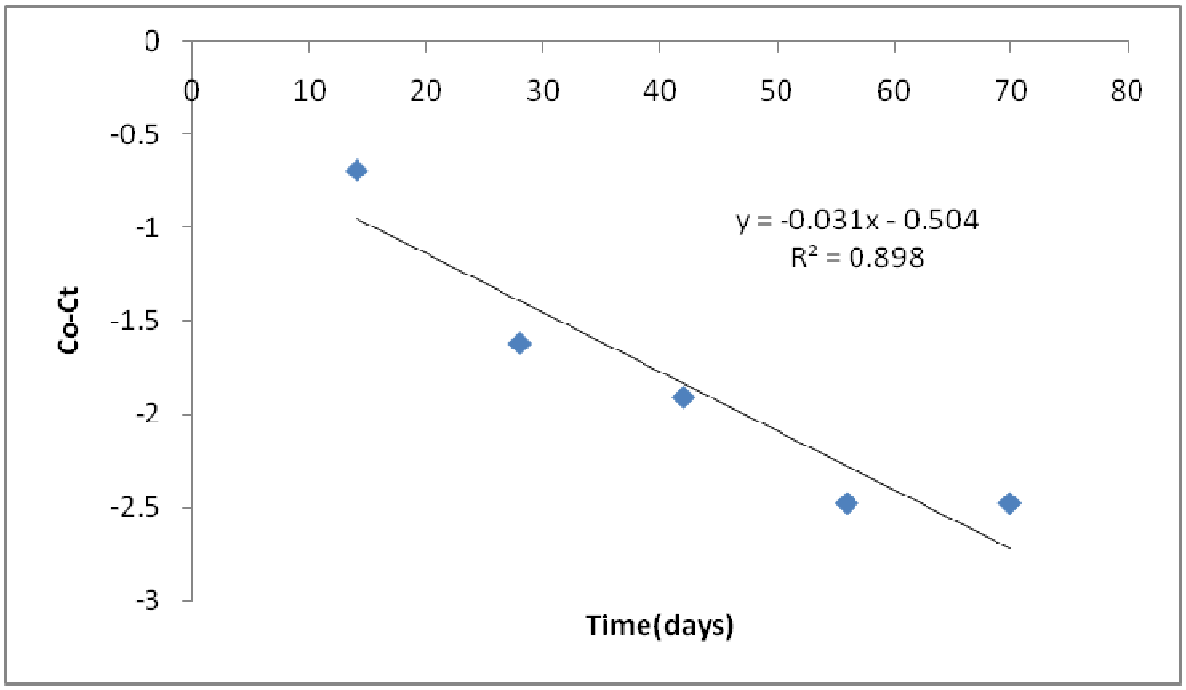

FIGURE 4.16: Zero-order kinetics for TOC at controlled condition

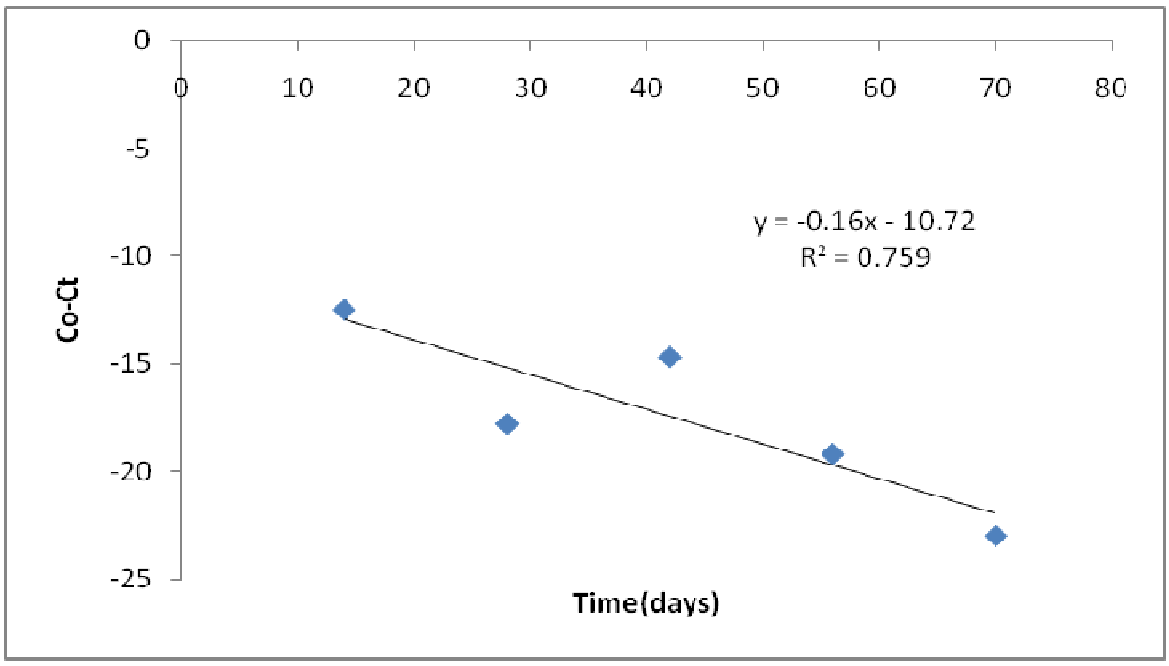

FIGURE 4.17: Zero-order kinetics for TOM using abattoir waste

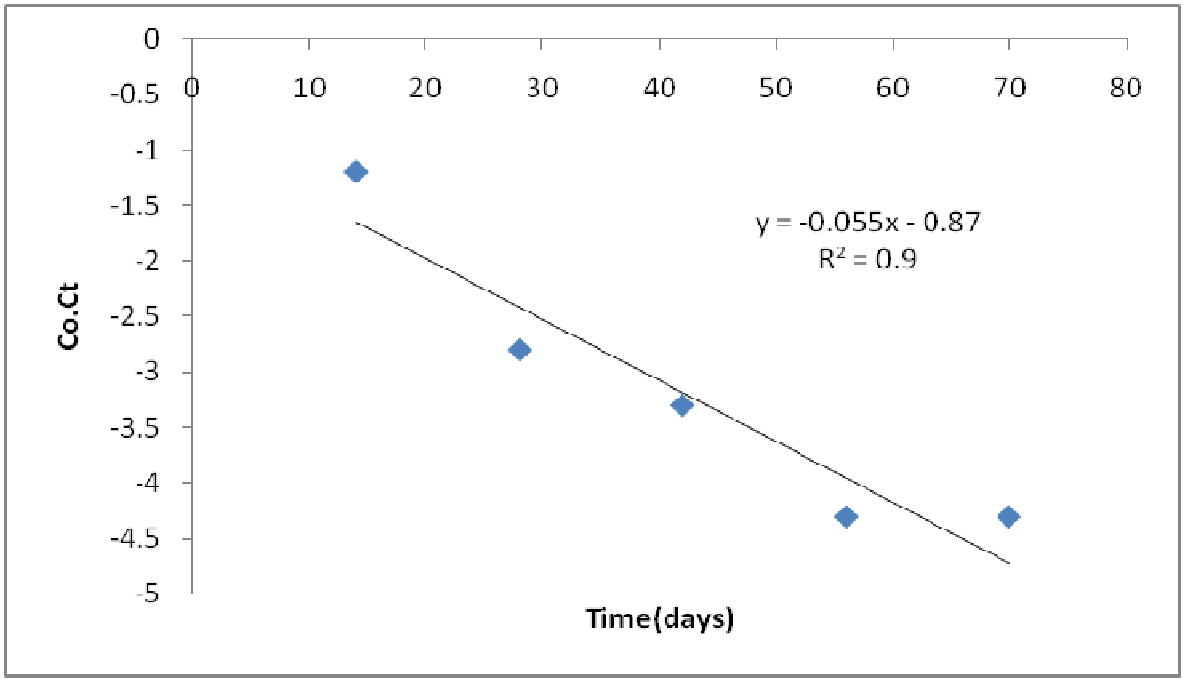

FIGURE 4.18: Zero-order kinetics for TOM at controlled condition

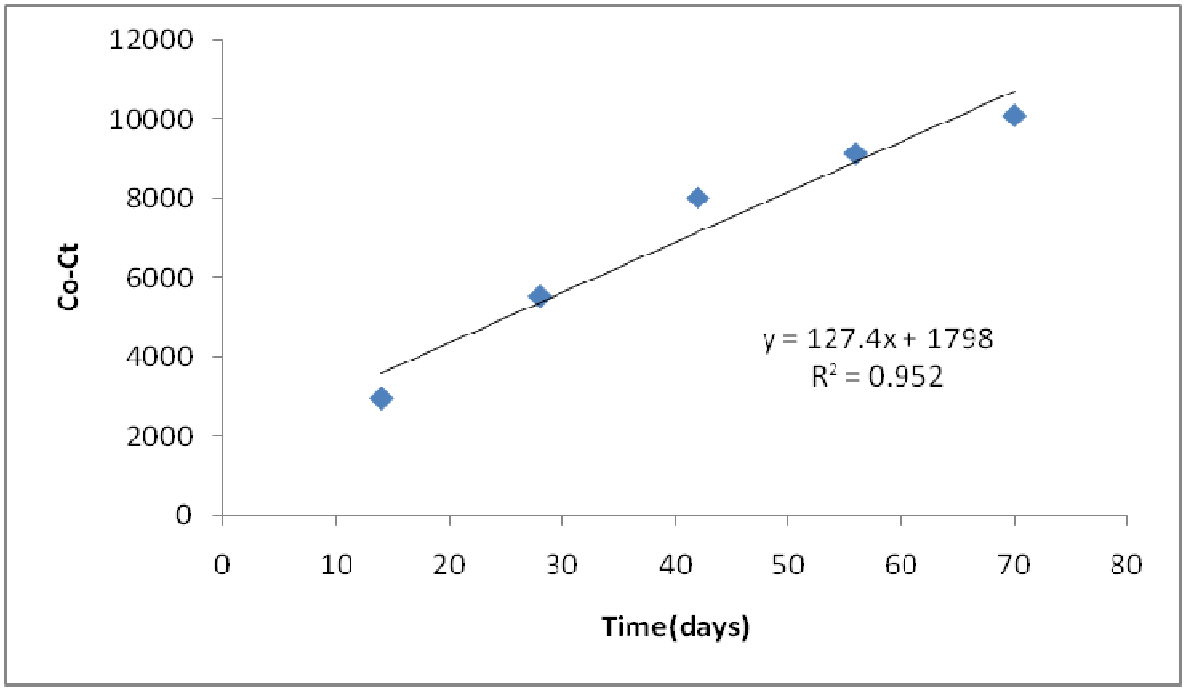

FIGURE 4.19: Zero-order kinetics for THC using abattoir waste

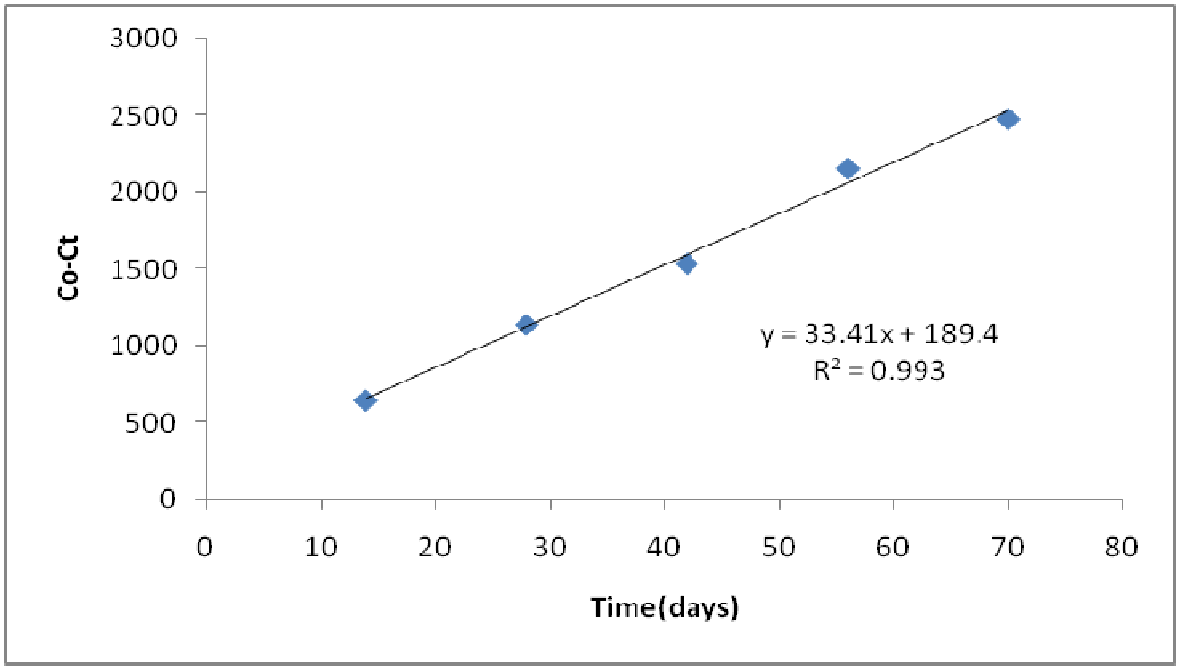

FIGURE 4.20: Zero-order kinetics for THC at controlled condition

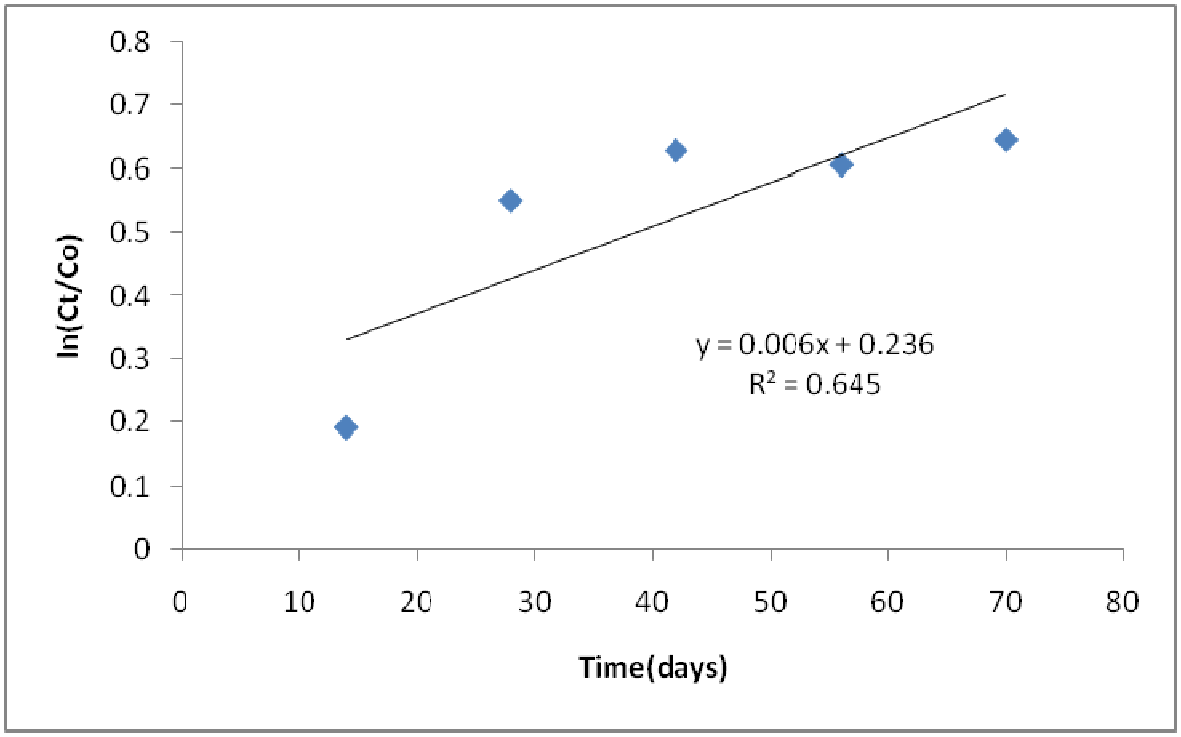

FIGURE 4.21: First-order kinetics for Nitrogen using abattoir waste

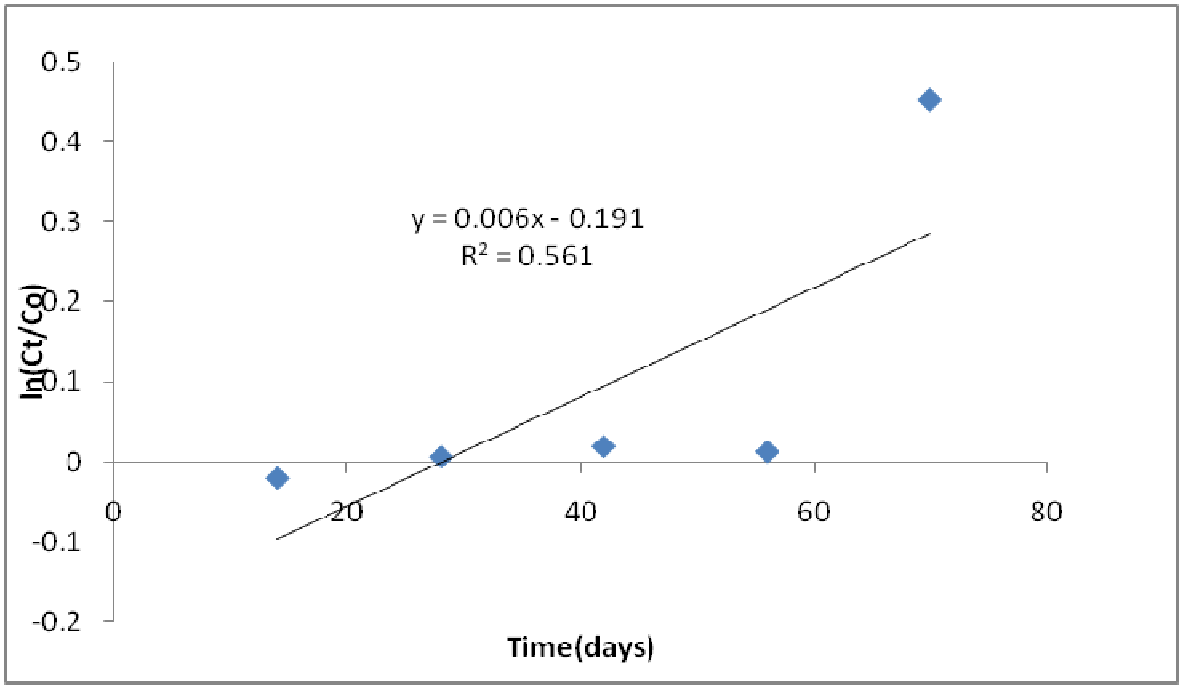

FIGURE 4.22: First-order kinetics for Nitrogen at controlled condition

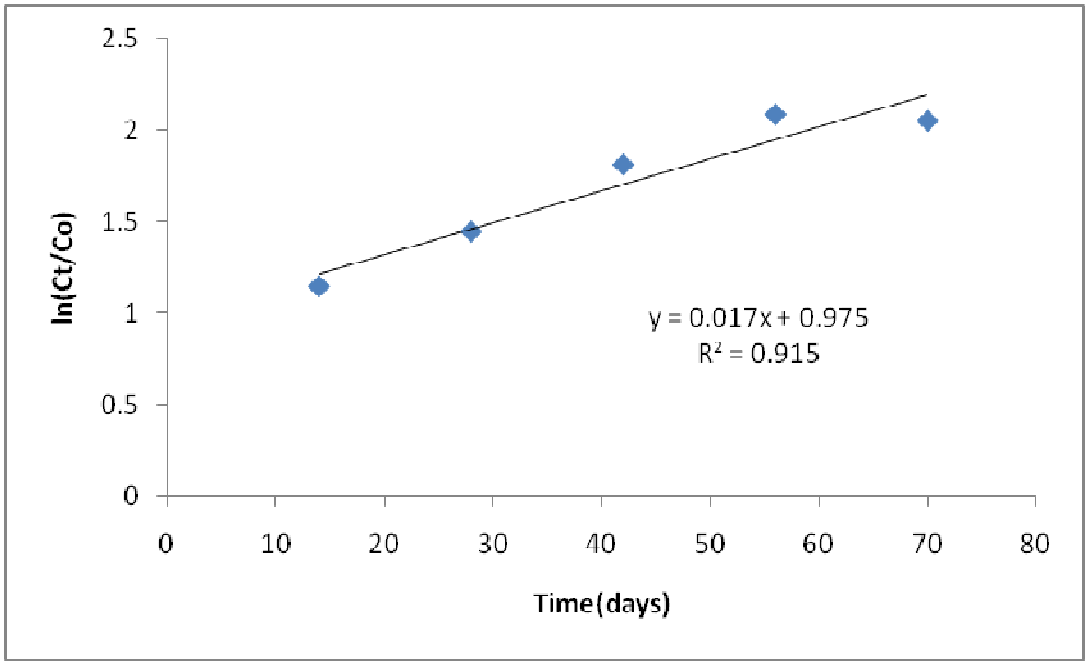

FIGURE 4.23: First-order kinetics for Phosphorous using abattoir waste

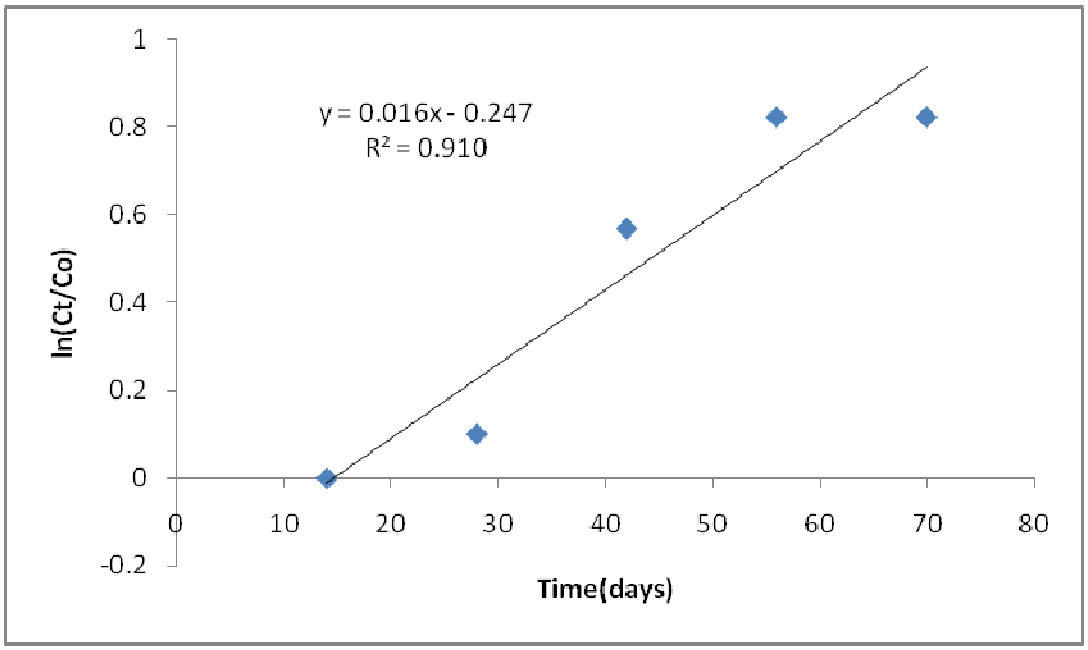

FIGURE 4.24: First-order kinetics for phosphorous at controlled condition

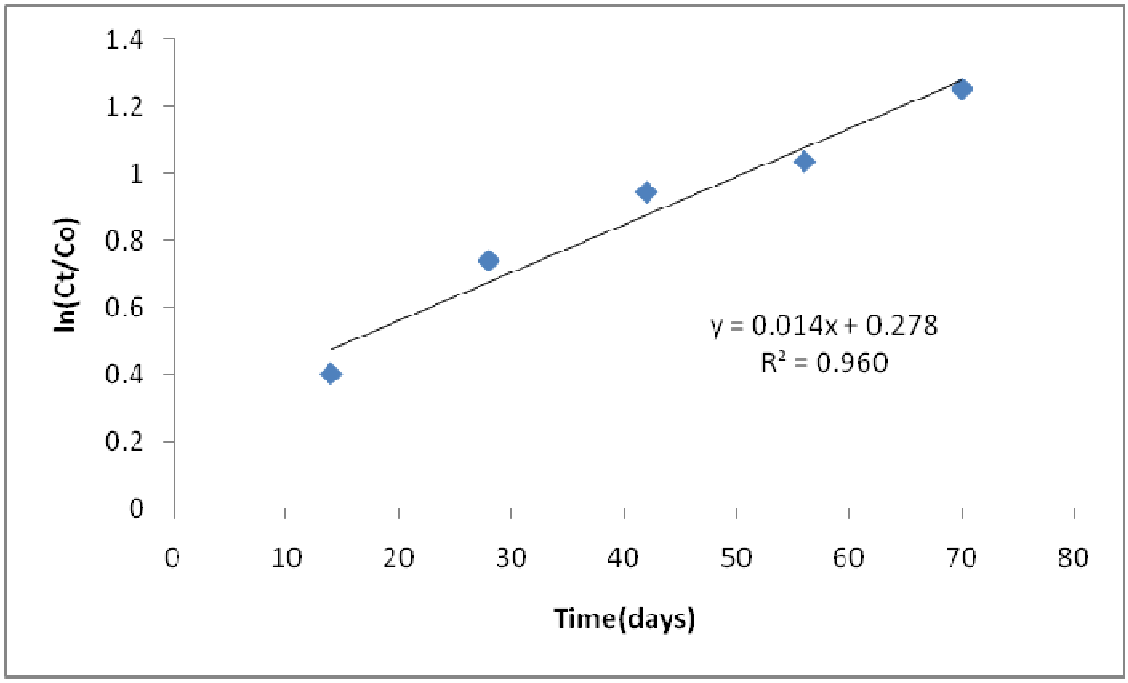

FIGURE 4.25: First-order kinetics for Potassium using abattoir waste

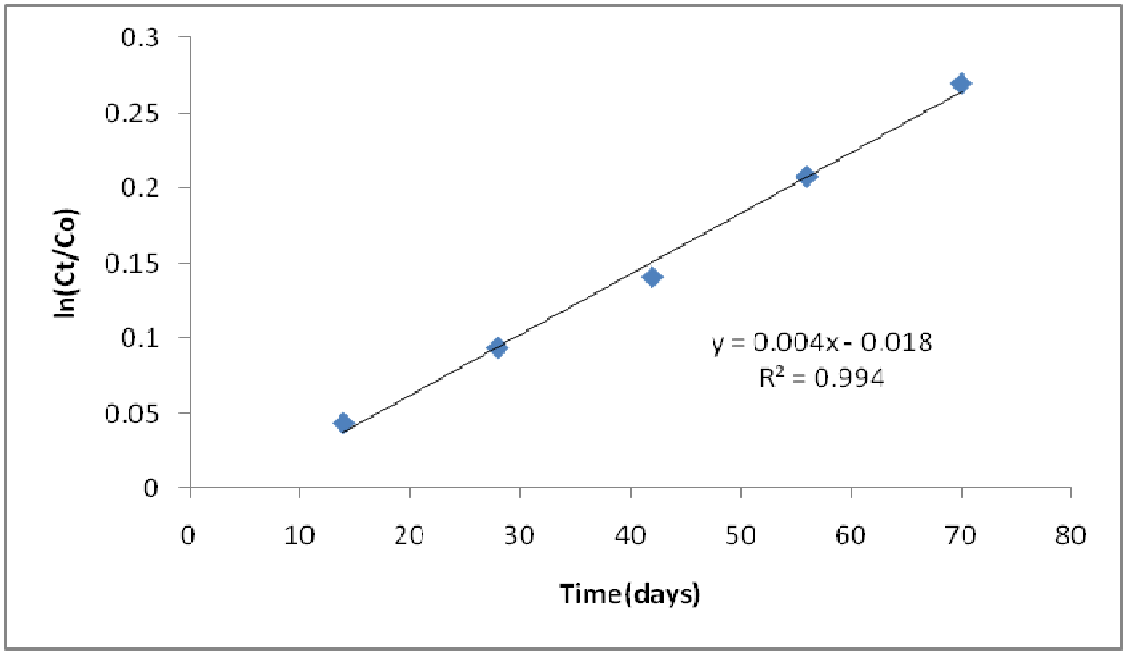

FIGURE 4.26: First-order kinetics for Potassium at controlled condition

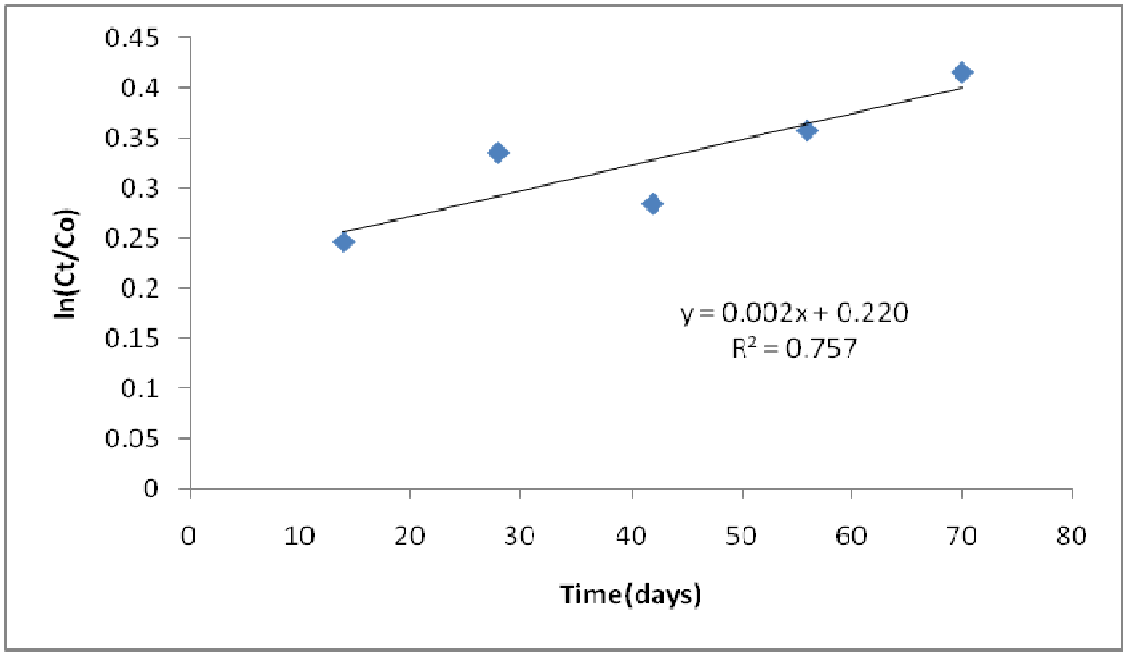

FIGURE 4.27: First-order kinetics for TOC using abattoir waste

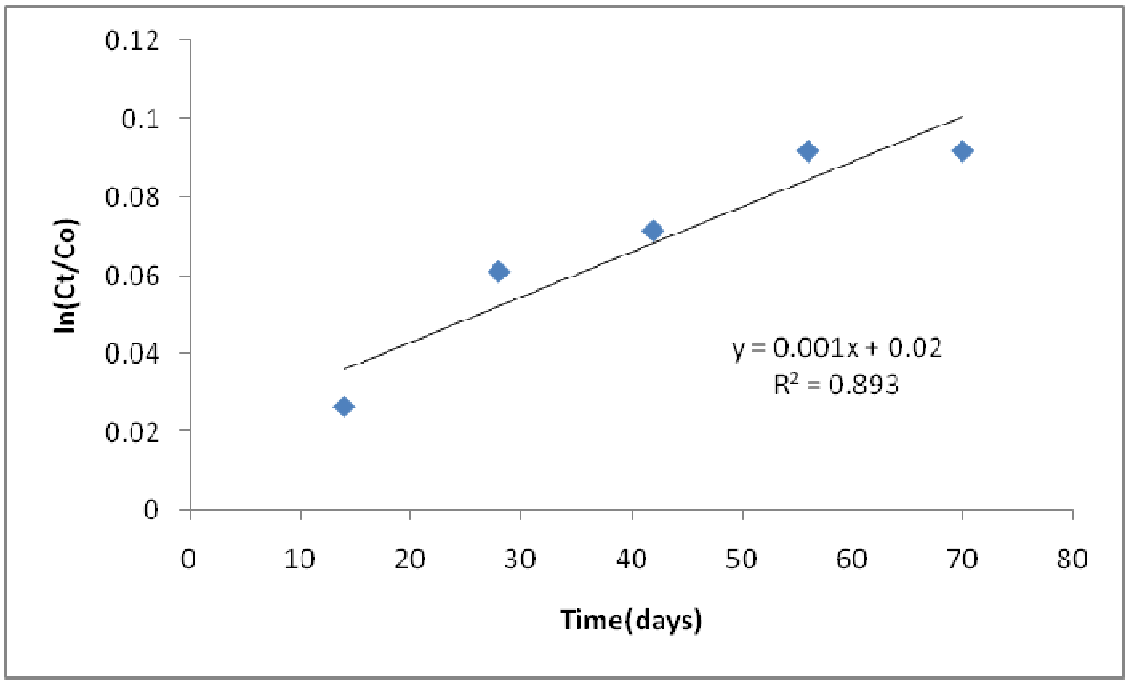

FIGURE 4.28: First-order kinetics for TOC at controlled condition

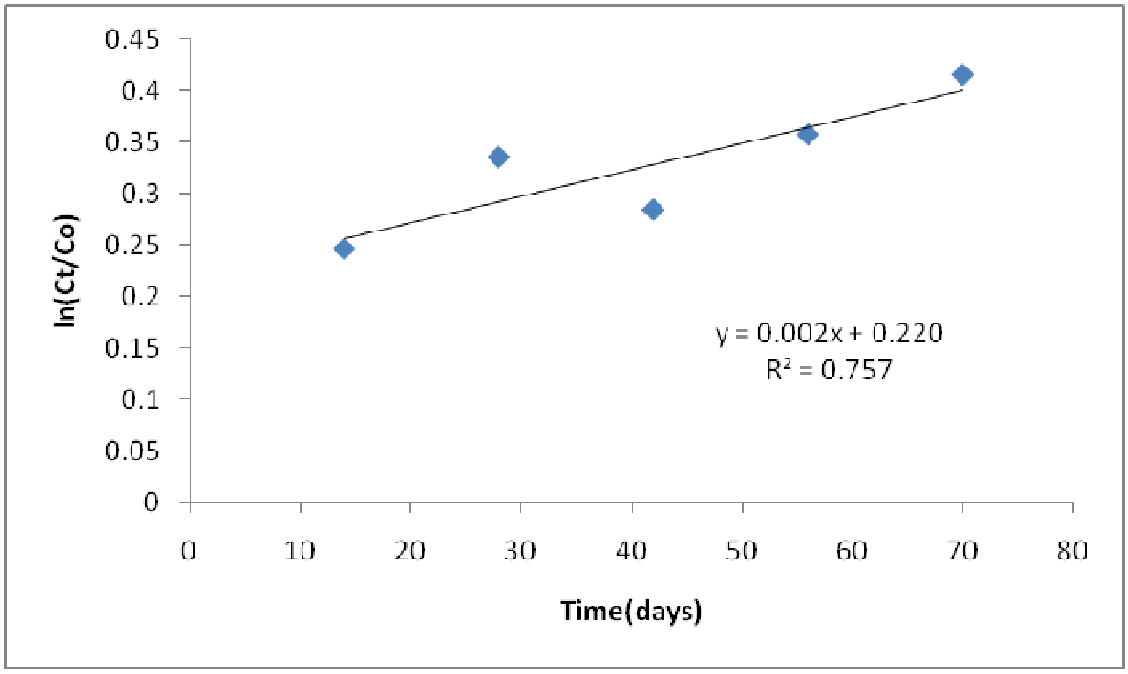

FIGURE 4.29: First-order kinetics for TOM using abattoir waste

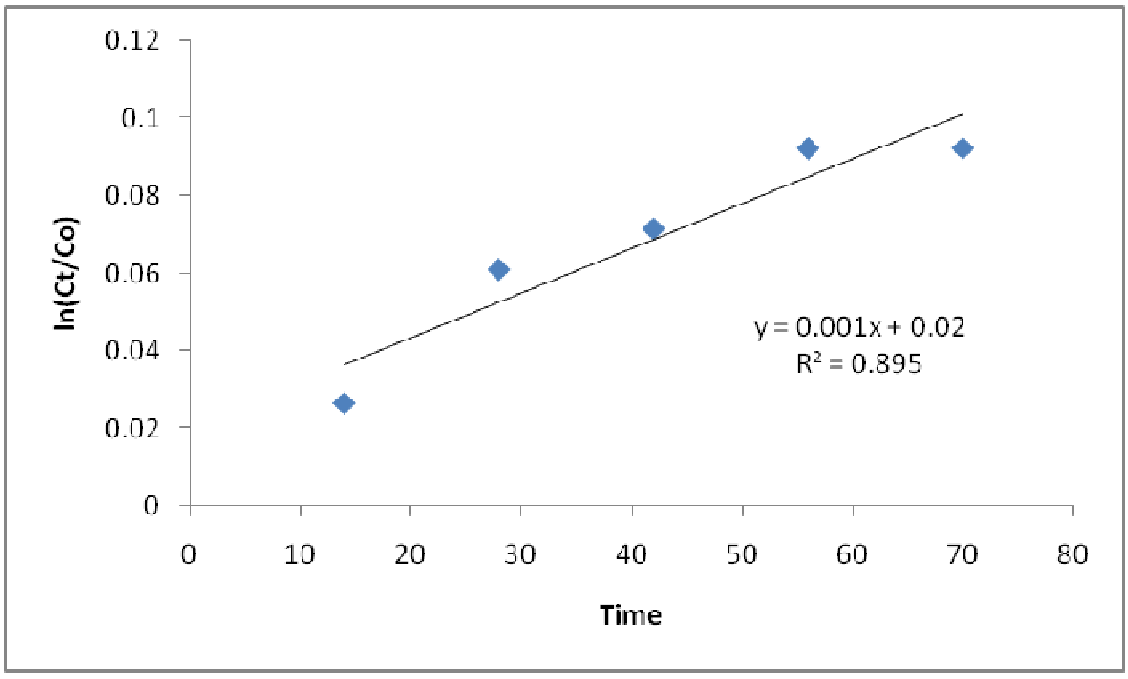

FIGURE 4.30: First-order kinetics for TOM at controlled condition

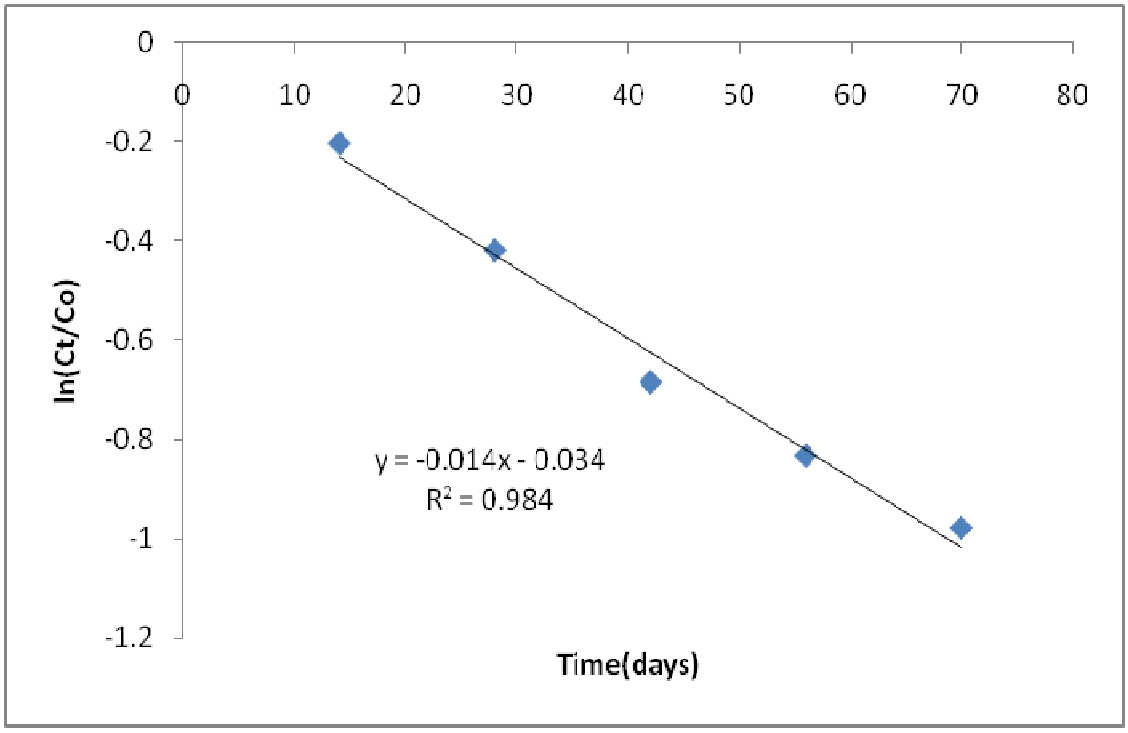

FIGURE 4.31: First-order kinetics for THC using abattoir waste

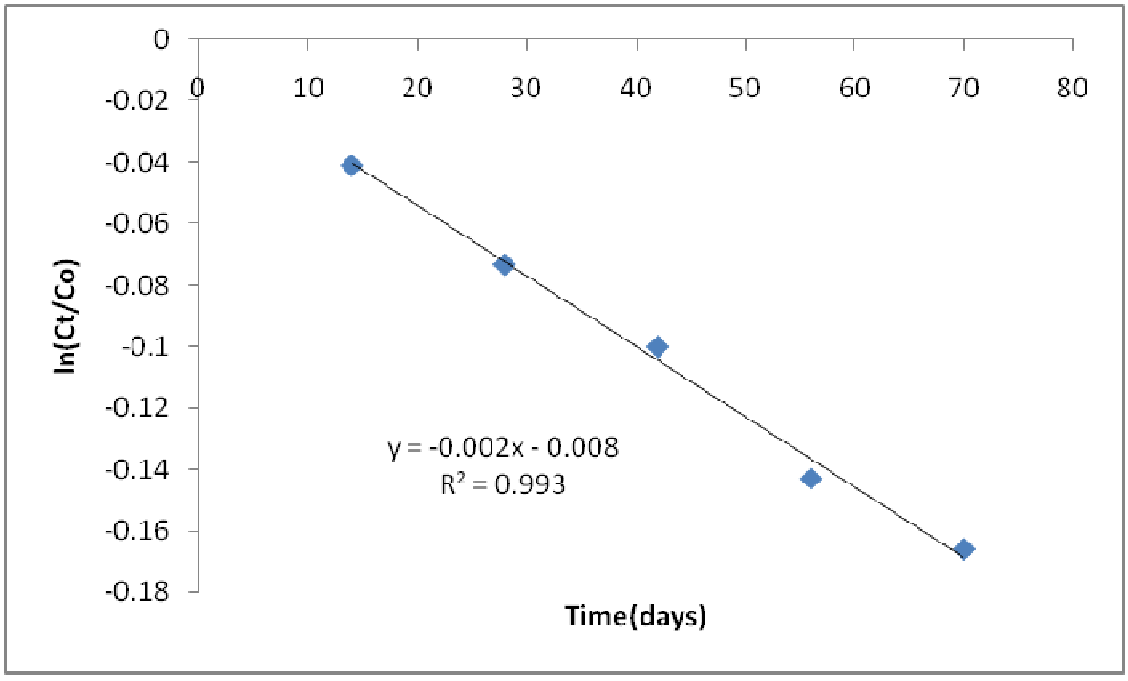

FIGURE 4.32: First-order kinetics for THC at controlled condition

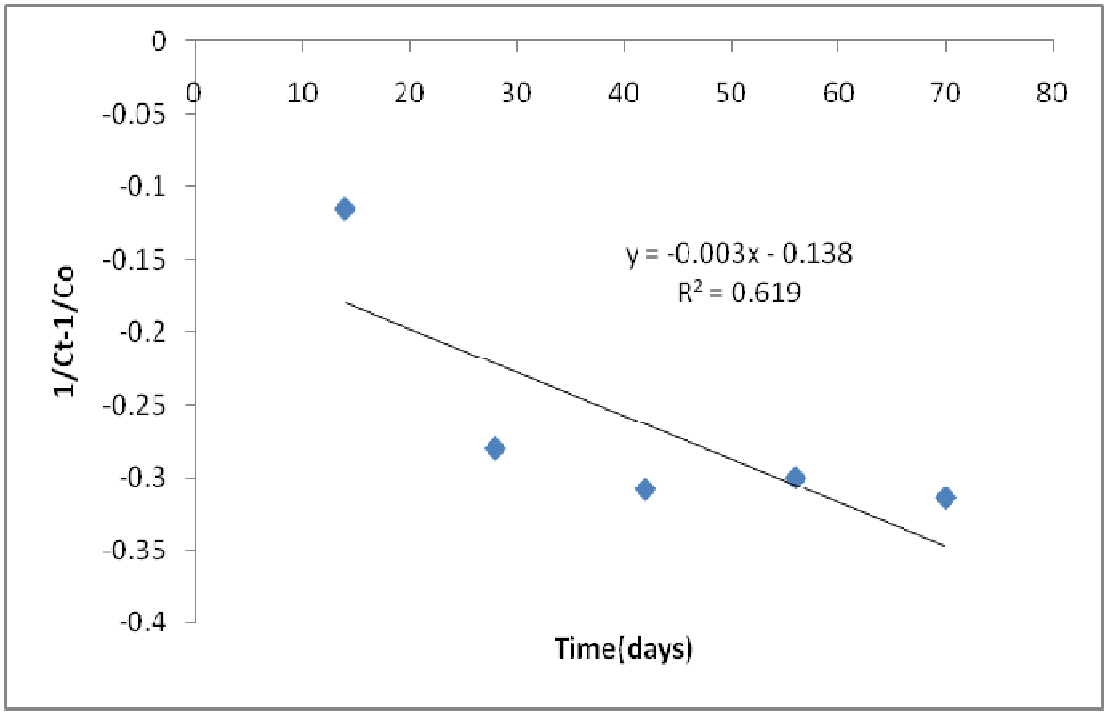

FIGURE 4.33: Second-order kinetics for Nitrogen using abattoir waste

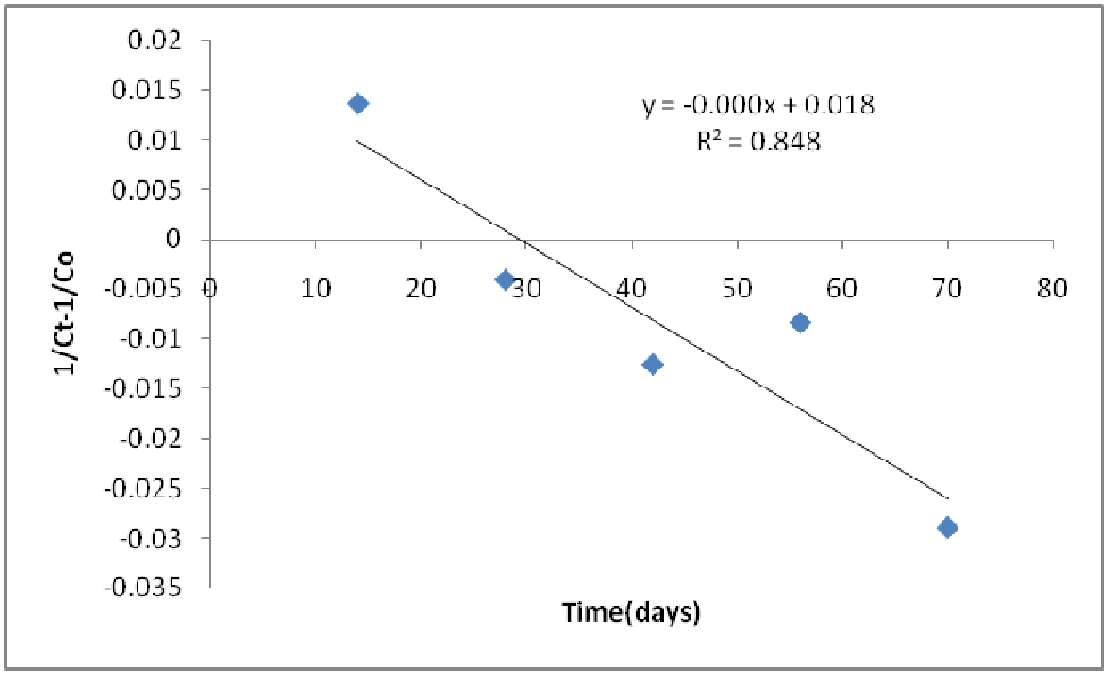

FIGURE 4.34: Second-order kinetics for Nitrogen at controlled condition

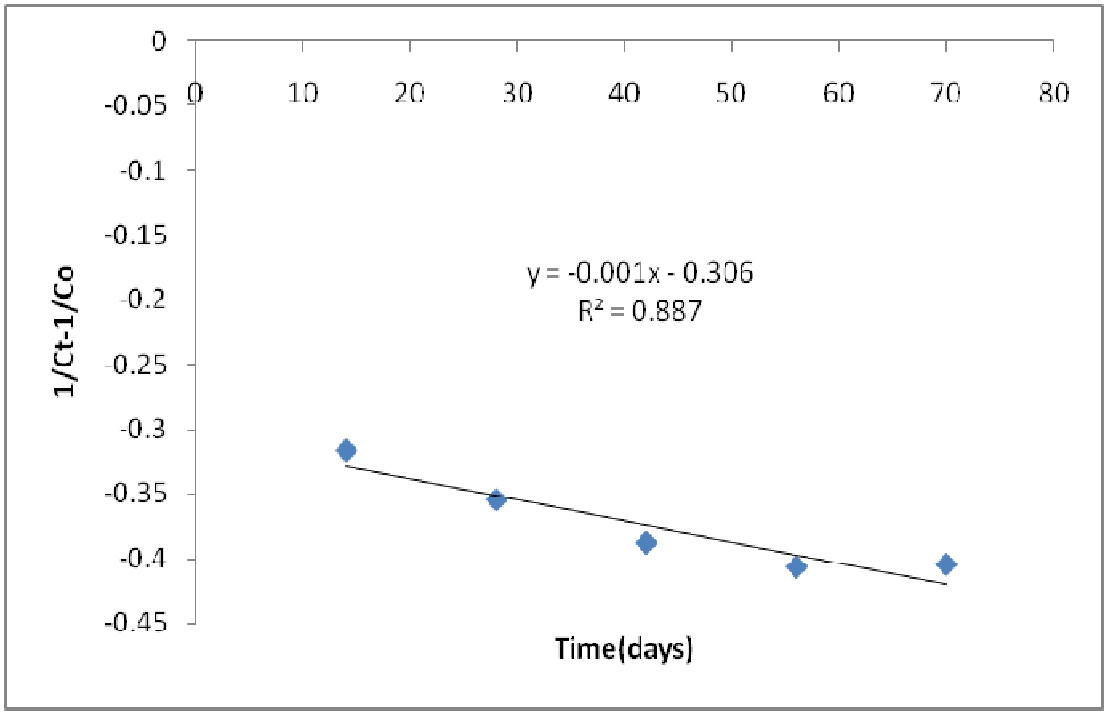

FIGURE 4.35: Second-order kinetics for phosphorous using abattoir waste

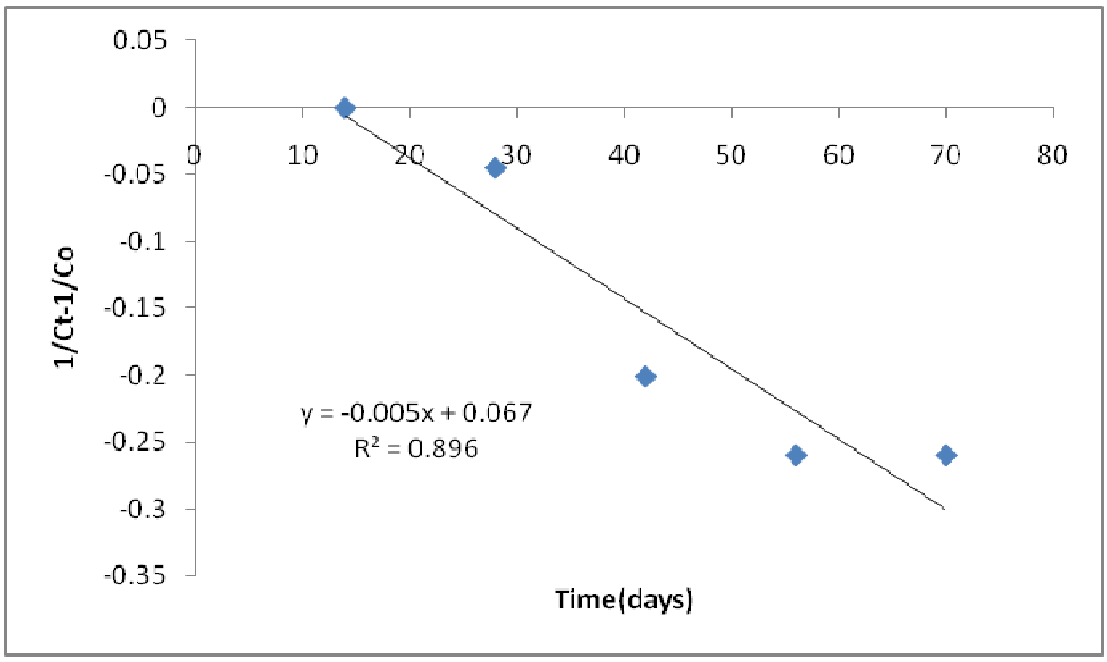

FIGURE 4.36: Second-order kinetics for Phosphorous at controlled condition

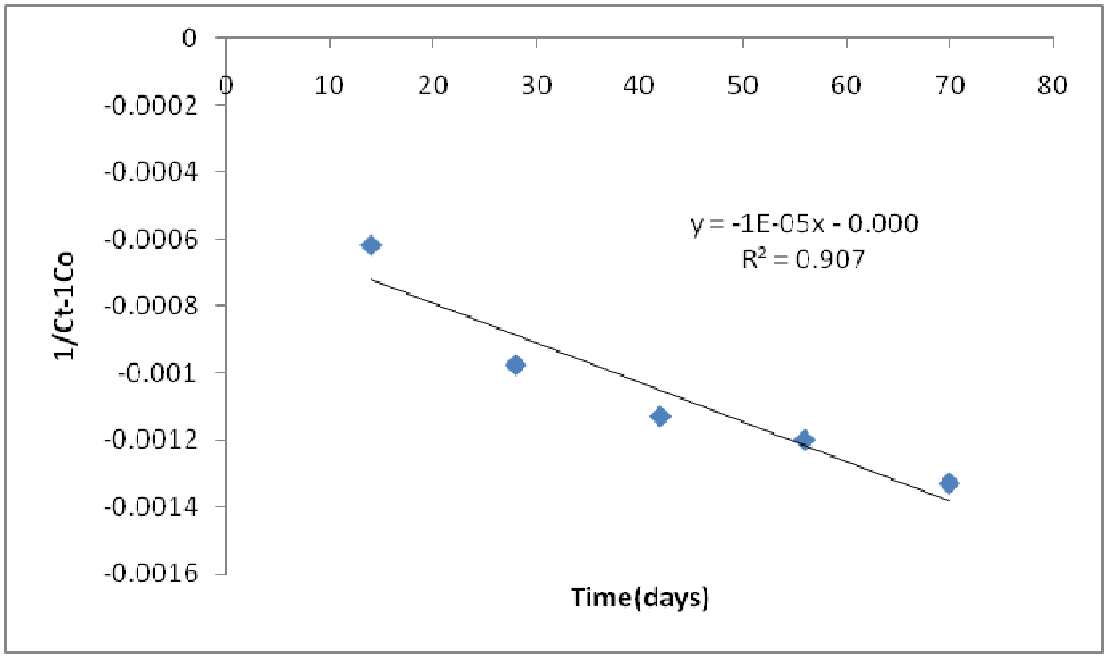

FIGURE 4.37: Second-order kinetics for Potassium using abattoir waste

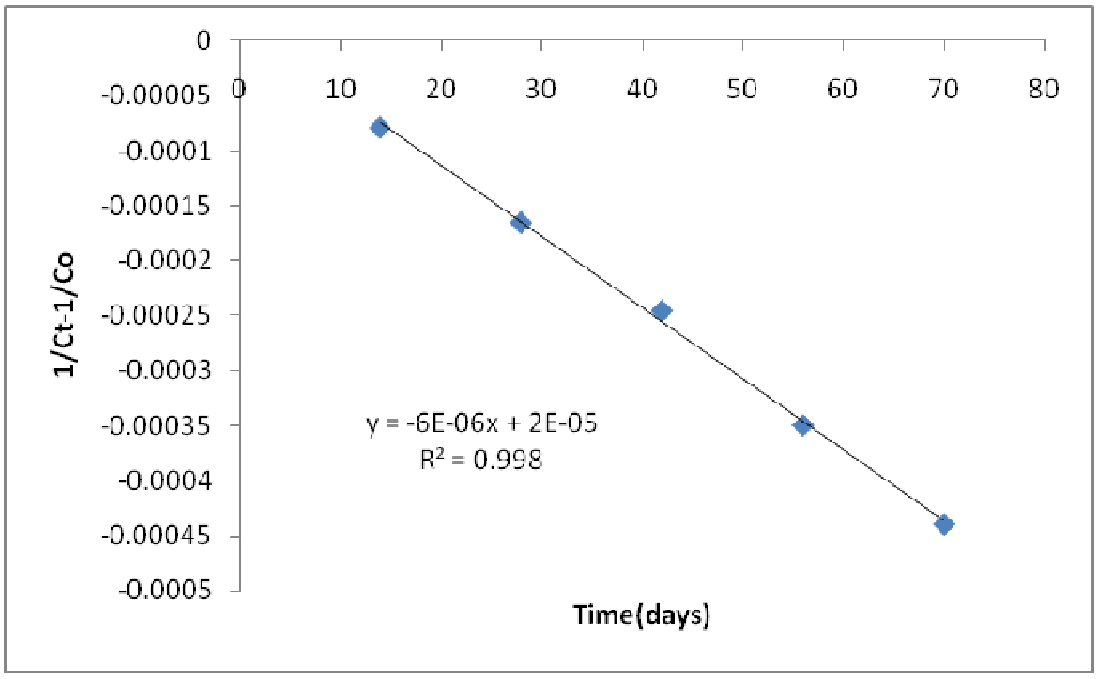

FIGURE 4.38: Second-order kinetics for Potassium at controlled condition

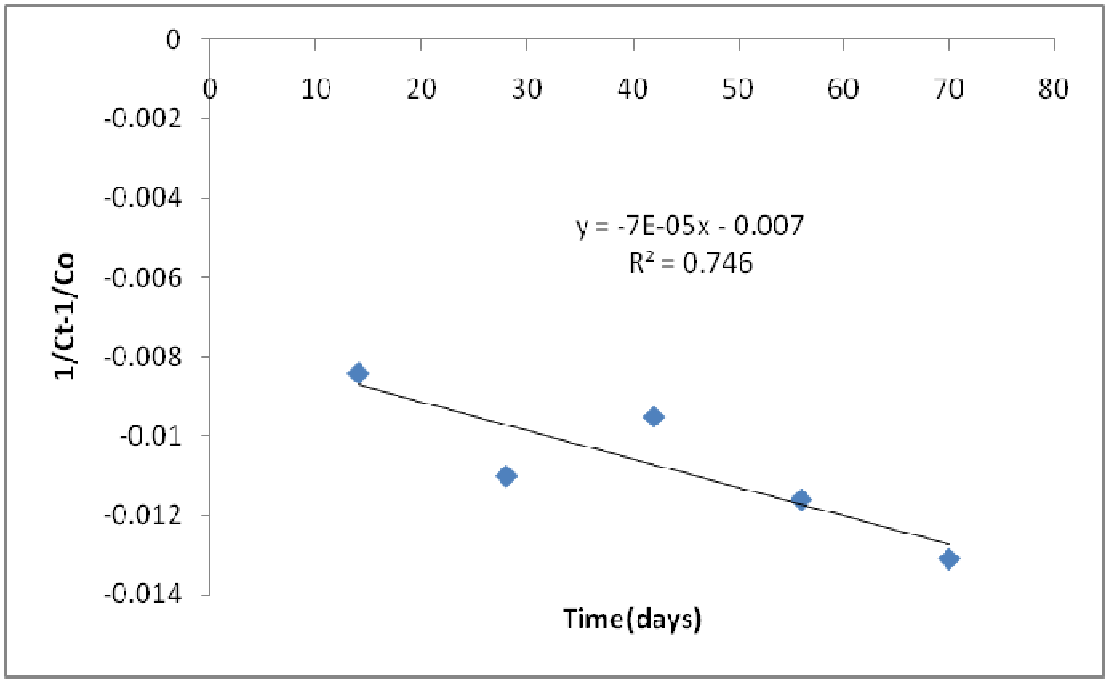

FIGURE 4.39: Second-order kinetics for TOC using abattoir waste

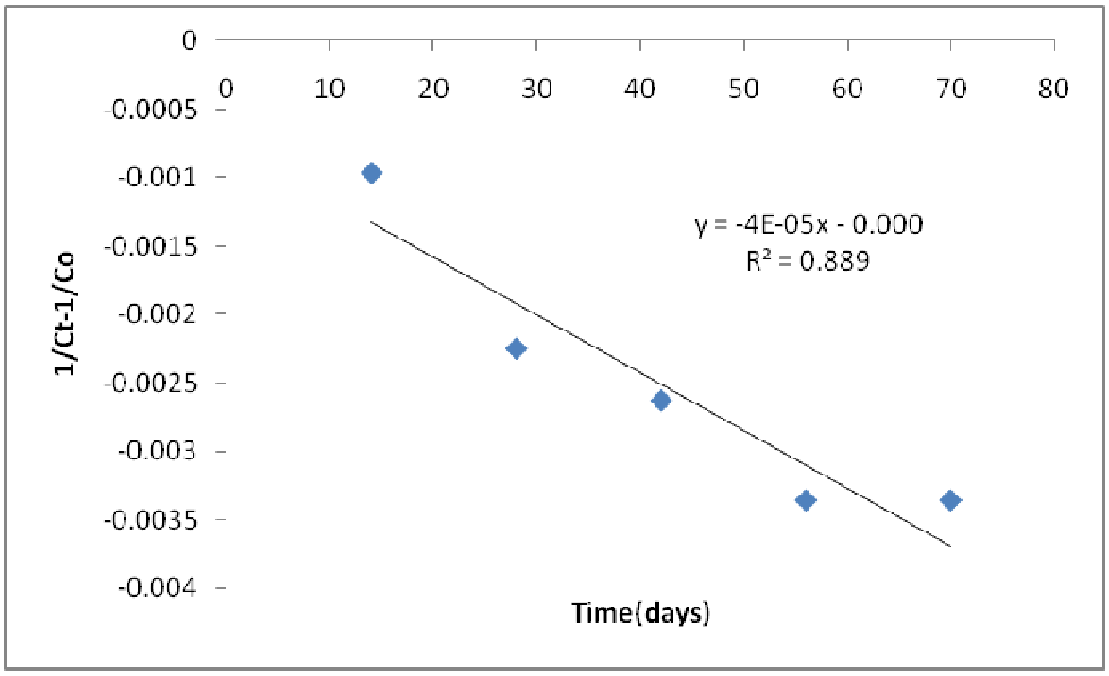

FIGURE 4.40: Second-order kinetics for TOC at controlled condition

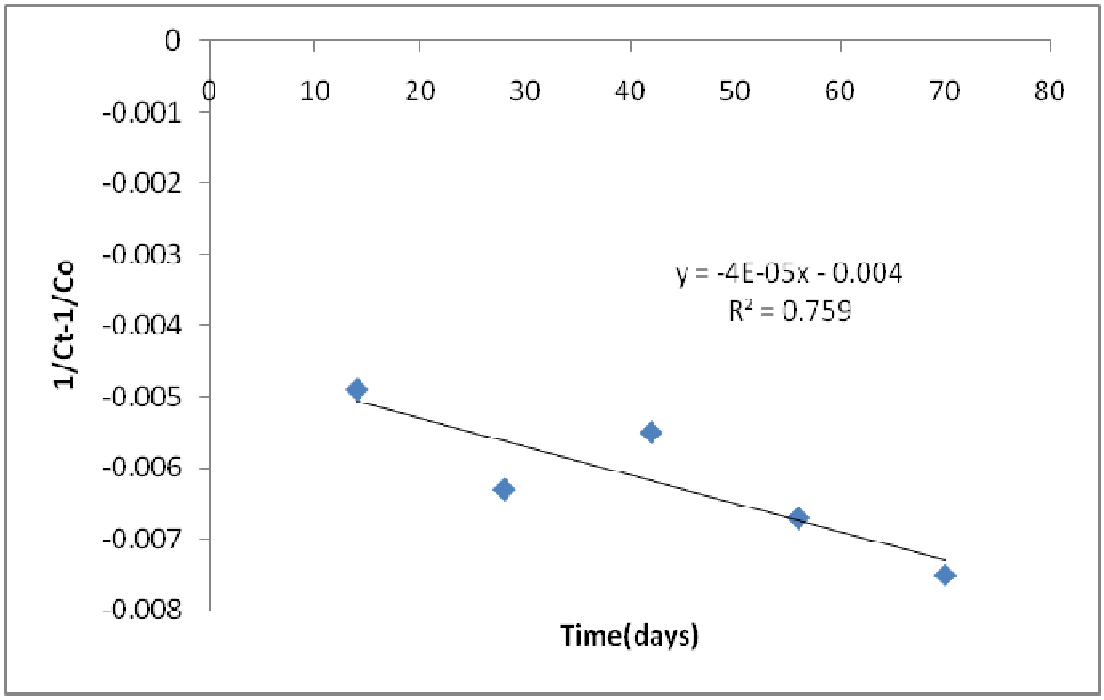

FIGURE 4.41: Second-order kinetics for TOM using abattoir waste

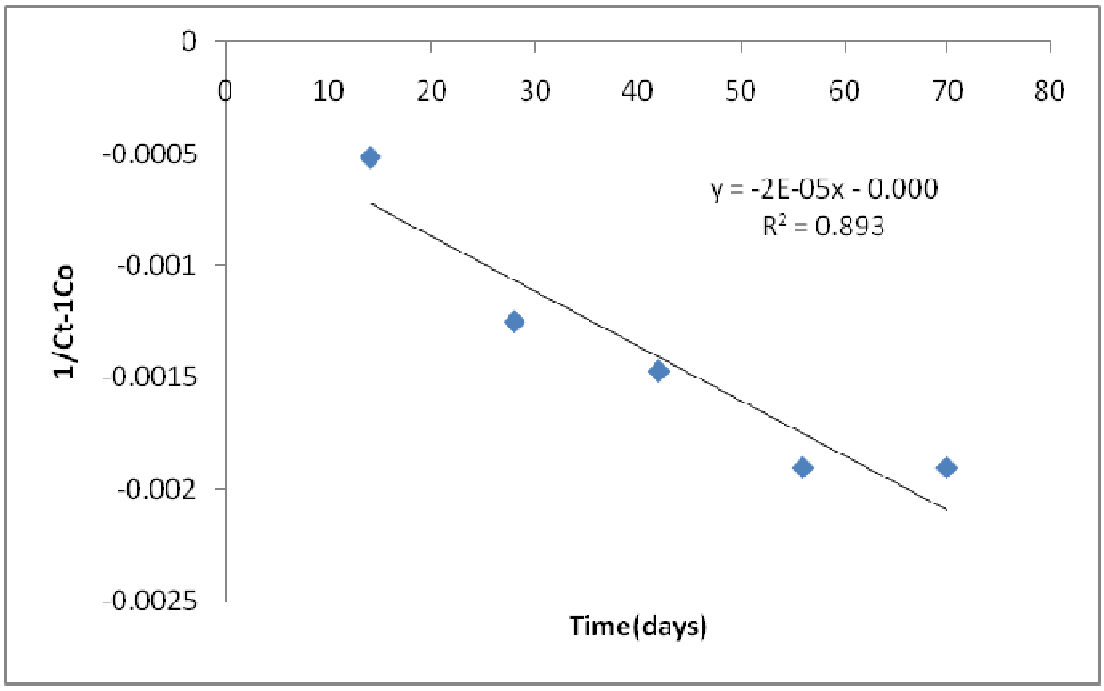

FIGURE 4.42: Second-order kinetics for TOM at controlled condition

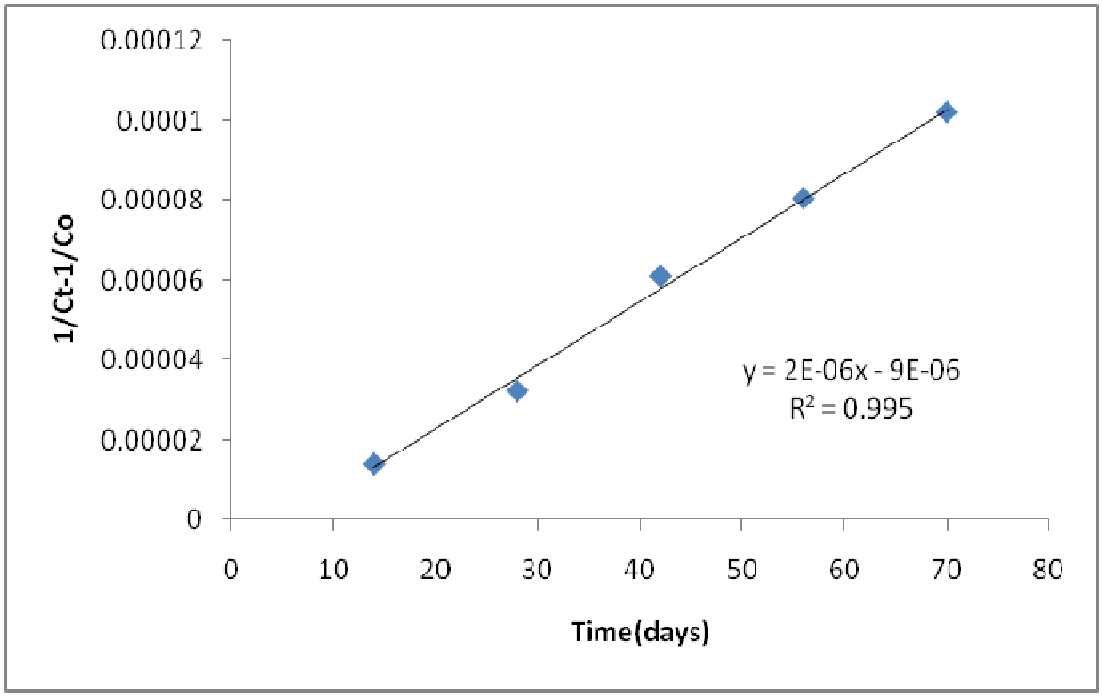

FIGURE 4.43: Second-order kinetics for THC using abattoir waste

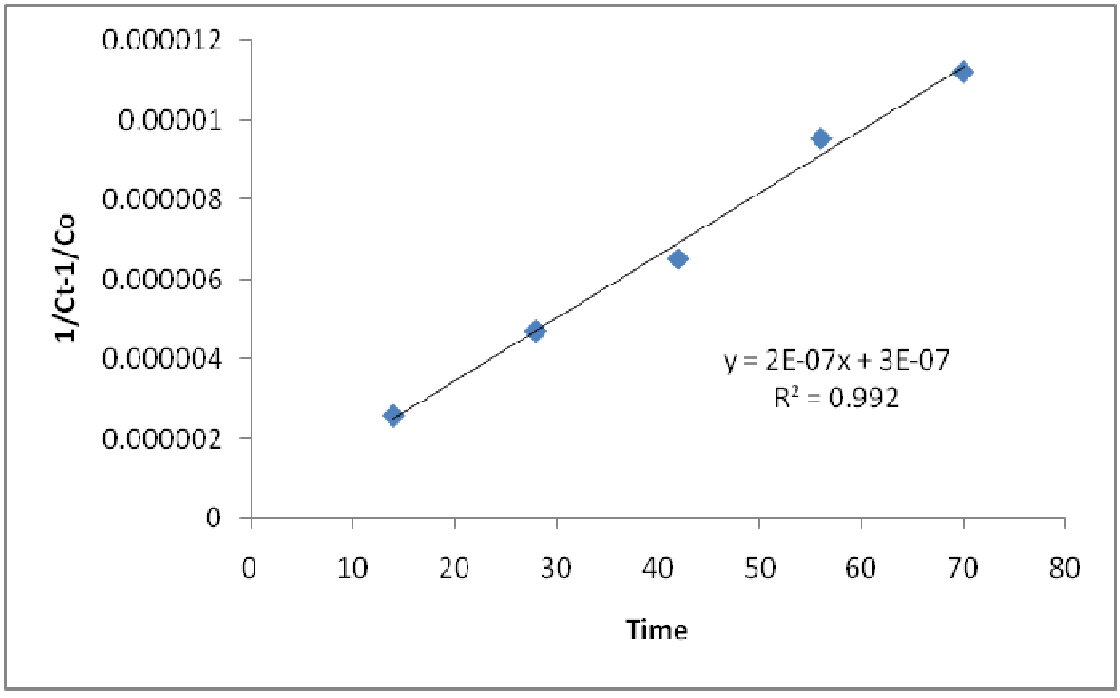

FIGURE 4.44: Second-order kinetics for THC at controlled condition

CHAPTER FIVE

CONCLUSION, RECOMMENDATION AND CONTRIBUTION TO KNOWLEDGE

5.1: Conclusion

It can be concluded from the study that crude oil contamination causes a reduction in pH, moisture content, nitrogen (N), phosphorous (P) and potassium (K), and an increase in total organic carbon (TOC), total organic matter (TOM) and total hydrocarbon content (THC) levels of the soil. The study revealed that the biodegradation of total hydrocarbon content as well as bioremediation of other parameters investigated upon is probably due to the availability of microbes and animal nutrients, which in this case was provided by abattoir waste (animal waste). The microbes are capable of breaking the hydrocarbon bonds of the crude oil while the animal nutrients are converted to soil nutrients mainly in form of N, P, K and TOM. Thus, the use of abattoir waste to stimulate petroleum hydrocarbon degradation in the soil could be one of the environmentally friendly ways of remediating natural ecosystem contaminated with crude oil. It could also be seen from the study that mere reliance on microbial transformation of the contaminant without providing suitable conditions for optimum and speedy degradation or bioremediation , could take a very long time for significant remediation to be achieved. The minimal THC depletion observed in control justified this view.

5.2: Recommendation

This research work therefore recommends the use of waste materials (abattoir wastes) on contaminated agricultural soils, since this could achieve the

decontamination of such soils and thus improving the growth of plants for the overall well-being of man that depend on them for food and other uses.

5.3: Contribution to Knowledge

Contaminated soil restoration (remediation), soil nutrient fortification and efficient waste management technique has been established through this study. The used abattoir waste is very cheap, readily available and environmentally friendly and therefore generally accepted.

REFERENCES

Akamigbo, F. O. R. and Jidere, C. M. (2002). "Carbon Nitrogen Dynamics in Organic Waste Amended Crude Oil Polluted Wetland Soil". Agro-Science. 3(1) pp 20-26.

Akpoveta, O. V., Egharevba F., Medjor O. W., Osaro K. I. and Daniel, E. (2011). " Microbial Degradation and its Kinetics on Crude Oil Polluted Soil". Research journal of Chemical Sciences. Vol. 1(6) pp 8-14.

Atagana, H. I. (2008). "Compost Bioremediation of Hydrocarbon-Contaminated Soil Inoculated with Organic Manure". Afri. Journal of Biotechnology. 7(10) pp 1516-1525.

Ayotamuno, M. J., Kogbara, R. B., Ogaji, S. O. T. and Probert, S. D. (2006). "Bioremediation of a Crude Oil Polluted Agricultural Soil at Port-Harcourt, Nigeria. Appl. Energy 83 pp 1249-1257.

Ayotamuno, M .J., Kogbara, R. B. and Agunwamba, J. C. (2006). "Bioremediation of a Petroleum Hydrocarbon Polluted Agricultural Soil at Various Levels of Soil Tillage in Port-Harcourt, Nigeria. Nig. J. tech. 25(1) pp 44-51.

Barathis, S. and Vasudevan, N. (2003). "Bioremediation of Crude Oil Contaminated Soil by Bioaugmentation of Pseudomonas Fluorescence NSI". J. Environ. Sci. Health. A. Tox. Hazard. Subst. environ. Eng. 38(9) pp 1857-1866.

Bello, Y. O. and Oyedemi, D. T. A. (2009). African J. Biotechnol. 31 pp 11-16.

Benyohia, F., Abdulkarim, M., Zekari, A., Chaalal, O. and Hasanain, H. (2005) "Bioremediation of Crude Oil Contaminated Soils". Pro. Laf. And Env. Prot. 83 pp 364-370.

Beskooki, V. P., Cvijovic, G. G., Milic, J., Ilic, M., Miletic, S., Solevic, T., and Vivic, M. M. (2011). "Ex-situ Bioremediation of a Soil contaminated by Mazut (Heavy Residual Fuel Oil) – A Field Experiment". Chemosphere 83 pp 34-40.

Chaillan, F., Chaineau, C. H., Point, V., Saliot, A. and Oudot, J. (2006) "Factors Inhibiting Bioremediation of Soil contaminated with weathered oils and Drill cuttings". Environmental Pollution 144 pp 255-265.

Cuningham, C. J. and Philip, J. C. (2002). “Comparison of Biostimulation in ex-situ Treatment of Diesel Contaminated Soil”. Land Contam. Reclam. 8 pp 261-269.

Ebuechi, O. A. T., Abibo, I. B., Shekwolo, P. D., Sigismund, K. I., Adoki, A., Okoro I.C. (2005) “Bioremediation of Crude Oil Contaminated Soil by Enhanced Natural Attenuation Technique”. J. Appl. Sci. Environ. Mgt. 9(1) pp 103-106.

Essien, O.E. and John, I. A. (2010). “Impact of Crude-Oil Spillage Pollution and Chemical Remediation on Agricultural Soil Properties and Crop Growth”. J. Appl. Sci. Environ. Manage. 14(4) pp 147-154.

Gill, L. S., Nyawuame, H. G. K., Eihkhamelor, A. O. (2003). “Effect of Crude Oil on the Growth and Anatomical Features of Chromolena Odorota. L.K. & R.

Hyne, Norman J. (2001). Nontechnical Guide to Petroleum Geology, Exploration, Drilling, and Production. PennWell Corporation. ISBN 0-87814-823-X

Ibiene, A. A., Orji, F. A., Ezidi, C. O and Ngwobia C. L. (2011). “Bioremediation of Hydrocarbon Contaminated Soil in the Niger Delta using Spent Mushroom Compost and other Organic Wastes”. Nig. Journal of Agric., Food and Environment. 7(3) pp 1-7.

Jelena, S. M., Vladimir, P. B., Mila, V. I., Samira, A. M .A., Gordana, D. G. and Mivoslav, M. V. (2009) “Bioremediation of Soil Heavily Contaminated with Crude Oil and its Product: Composition of the Microbial Consortium”. J. Serb. Chem. Soc. 74(4) pp 455-460.

Jorgensen, K. S., Puustinen, J. and Suortti, A. M. (1999). “Bioremediation of Petroleum Hydrocarbon Contaminated Soil by Composting in Biopiles”. Environmental Pollution. 107(2000) pp 245-254.

Knaebel, D. B., Federie, T. W., Mc Avoy, D. C. and Vestal J. R. (1994). “Effects of Mineral and Organic Soil Constituents on Microbial Mineralization of Organic Compounds in Natural Soil. Appl. Environ. Microbial. 60 pp 4500-4508.

Leo, C. O. and Iruka N. (2007). “An Appraisal of the impact of Petroleum Hydrocarbons on Soil Fertility: The Owaza Experience”. Afri. J. of Agric Res. 2(7) pp 318-324.

li-hua, M., Jiangtao, H. and Ye, L. (2009) “Bioremediation of Crude Oil Contaminated Soil by Composing. Env. Sci. Info. Appl. Tech. 2 pp 27-31.

Liu, P.C., Chang T.C., Whang L., Kao, C., Pan, P., Cheng, S. (2011). “Bioremediation of Petroleum Hydrocarbon Contaminated Soil: Effect of Strategies and Microbial Community Shift”.

Liu, W., Luo, Y., Teng, Y. and Li, Z. (2010). “Bioremediation of Oil Sludge-Contaminated Soil by Stimulating Indigenous Microbes”. Environ. Geochem Health. 32 pp 23-29.

Margesin, R. and Schinner, F. (1999). “A Feasibility Study for the in-situ Remediation of a Former Tank Farm”. World J. Microbial. Biotechnol 15 pp 615-622.

Margesin, R. and Schinner, F. (2001). “Bioremediation (Natural Attenuation and Biostimulation) of Diesel Oil Contaminated Soil in an Alpine Glacier Sking Area”. Appl. Environ. Microbial. 67 pp 3127-3133.

McBride, M.B. (1994). “Environmental Chemistry of Soils”. New York, Oxford University Press, 406.

Millioli, V. S., Servulo, E. L. C., Sobral, L. G. S. and Decarvalho D. D. (2009). “Bioremediation of Crude Oil-Bearing Soil: Evaluating the Effects of Rhamnolipid Addition to Soil Toxicity and Crude Oil Biodegradation”. Global Nest Journal: 11(2) pp 181-188.

Mohd, K., Nizam, B. and Mohd Z. (2008). Bioremediation of Oil from Domestic Waste Water using Mixed Culture: Effects of Inoculum Concentration and Agitation speed”. Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Paharg.

Nester, E., Eugene, W., Densie, G. A., Evans Roberts Jr, C., Nancy, N. P. and Murtha, T. (3rd Ed.) “A Human Peerspective”. New York: McGraw-Hill.

Odu, C. T. I., Nwoboshi, L. C. and Esuruoso, O. F. (1985). “Environmental Studies (Soils and Vegetation) of the Nigerian Agip Oil Company Operation Areas”. In: Proceedings of an International Seminar on the Petroleum Industry and the Nigerian Environment, NNPC, Lagos, Nigeria. pp. 274-283.

Odukuma, L. O. and Dickson, A. A. (2003). “Bioremediation of Crude Oil Polluted Tropical Rainforest Soil”. Global Jour. Of Environ. Sci. Vol. 2, pp. 29-40.

"Oil Sands in Canada and Venezuela". Infomine Inc.. 2008. http://oilsands.infomine.com/countries/. Retrieved October 2, 2008.

Okoko, J. C. E., Amadi, N. and Odu, C. T. I. (2005). "Effects of Soil Treatments Containing Poultry Manure on Crude Oil Degradation in a Sandy Loam". Appl. Ecol. Environ. Res. 3 pp 47-53.

Okoko, K. A. B. and Ibaba, I. S. (1999). "Oil Spillages and Community Distribution: The S.P.D.C. and Niger Delta Experience". Journal of Oil and Politics. 2 pp 65-69.

Okoli, J. U. and Akligbmidu, C. O. (2001). "Failure Prevention Strategies in Pipeline Operations in the Niger Delta Area of Nigeria". NJEM Vol. 2. No 3. pp. 44.

Osuji, L. C. (2002)."Some Environmental Hazards of Oil Pollution in the Niger Delta, Disciplinary Studies 3(1) pp 11-17.

Osuji, L.C. (2001). "Total Hydrocarbon Content (THC) of Soil Fifteen Months After Eneka and Isiokpo Oil Spills in the Niger Delta, Nigeria". Journal of Applied Science and Environmental Management. 5(2) pp 35-38.

Perfuno, A., Ibrahim, M.B., Roger, M. and Luigi, V. (2007) "Thermally Enhanced Approaches for Bioremediation of Hydrocarbon-Contaminated Soils". Chemosphere 66 pp 179-184.

Sam-Jim, K., Choi, D. K., Sim, D. S. and Oh, Y. S. (2005) "Evaluation of Bioremediation Effectiveness on Crude Oil Contaminated Sand. Chemosphere 59 pp 845-852.

Speight, J. G. and Ancheyta, J. (2007). Hydroprocessing of Heavy Oils and Residua. CRC Press. ISBN 0-8493-7419-7.

Speight, James G. (1999). The Chemistry and Technology of Petroleum. Marcel Dekker. ISBN 0-8247-0217-4.

State of Mississipi (1988). "Fundamental Principles of Bioremediation". Department of Environmental Quality.

The Hindu (India) [March 1,2011] "Libyan Tremors Threaten to Rattle the Oil World". Lttp://www/hindu.com/2011/03/01/stories/2011030155921100. htm.

United States Department of Energy, Office of Science (USDOE) (2006). "Bioremediation Research Needs".

United States Environmental Agency (USEPA) (2006). “A Citizen’s Guide to Bioremediation”.

United States Environmental Agency (USEPA) (2006). “Biopiles”.

United States Environmental Agency (USEPA) (2006). “Land Farming”.

Walkey, A. (1947). A critical examination of a rapid method for determination of Organic Carbon in soils –Effect of variations in digestion conditions of inorganic soil constituents. Soil Sci. 63: 251- 257.

Walworth, J., Pond, A., Snape, I., Rayner, J., Ferguson, S., and Harvey, P. (2005). “Fine Tunning Soil Nitrogen to Maximize Petroleum Bioremediation”. ARCSACC pp 251-257.

Yakubu, M. B. (2007). “Biodegradation of Lagoma Crude Oil using Pig Dung”. Afr. J. Biotechnol. 6 pp 2821-2822.

APPENDICES

APPENDIX 1: Data for the plot of kinetics parameters (zero, first & second orders) for Nitrogen (Co = 1.51)

T (days)	Ct	Co – Ct	ln (Ct/Co)	1/Ct – 1/Co
14	1.83	-0.32	0.192	-0.115
28	2.62	-1.11	0.551	-0.280
42	2.83	-1.32	0.628	-0.308
56	2.77	-1.26	0.606	-0.300
70	2.88	-1.37	0.645	-0.314
Control				
14	1.48	0.03	-0.020	0.0136
28	1.52	-0.01	0.0066	-0.0041
42	1.54	-0.03	0.0196	-0.0126
56	1.53	-0.02	0.0131	-0.0084
70	1.58	-0.07	0.0453	-0.0290

APPENDIX 2: Data for the plot of kinetics parameters (zero, first & second orders) for Phosphorous (Co = 2.15)

T (days)	Ct	Co – Ct	ln (Ct/Co)	1/Ct – 1/Co
14	6.78	-4.63	1.148	-0.317
28	9.14	-6.99	1.447	-0.355
42	13.15	-11.00	1.811	-0.388
56	17.30	-15.15	2.085	-0.407
70	16.71	-14.56	2.050	-0.405
Control				
14	2.15	0	0	0.000116
28	2.38	-0.23	0.101	-0.0448
42	3.80	-1.65	0.569	-0.201
56	4.90	-2.75	0.823	-0.260
70	4.90	-2.75	0.823	-0.260

APPENDIX 3: Data for the plot of kinetics parameters (zero, first & second orders) for Potassium (Co = 538)

T (days)	Ct	Co – Ct	ln (Ct/Co)	1/Ct – 1/Co
14	807	-269	0.405	-0.000618
28	1132	-594	0.743	-0.000976
42	1389	-851	0.948	-0.00113
56	1521	-983	1.039	-0.00120
70	1890	-1352	1.256	-0.00133
Control				
14	562	-24	0.0436	-0.0000786
28	591	-53	0.0939	-0.000165
42	620	-82	0.141	-0.000245
56	663	-125	0.208	-0.000349
70	705	-167	0.270	-0.000439

APPENDIX 4: Data for the plot of kinetics parameters (zero, first & second orders) for TOC (Co = 25.74)

T (days)	Ct	Co - Ct	ln (Ct/Co)	1/Ct – 1/Co
14	32.97	-7.23	0.247	-0.0084
28	36.03	-10.29	0.336	-0.0110
42	34.23	-8.49	0.285	-0.0095
56	36.84	-11.1	0.358	-0.0116
70	39.04	-13.3	0.416	-0.0131
Control				
14	26.43	-0.69	0.0264	-0.000964
28	27.36	-1.62	0.0610	-0.00225
42	27.65	-1.91	0.0715	-0.00263
56	28.22	-2.48	0.0919	-0.00336
70	28.22	-2.48	0.0919	-0.00336

APPENDIX 5: Data for the plot of kinetics parameters (zero, first & second orders) for TOM (Co = 44.5)

T (days)	Ct	Co - Ct	ln (Ct/Co)	1/Ct – 1/Co
14	57.0	-12.5	0.247	-0.0049
28	62.3	-17.8	0.336	-0.0063
42	59.2	-14.7	0.285	-o.0055
56	63.7	-19.2	0.358	-0.0067
70	67.5	-23.0	0.416	-0.0075
Control				
14	45.7	-1.2	0.0266	-0.000518
28	47.3	-2.8	0.0610	-0.00125
42	47.8	-3.3	0.0715	-0.00147
56	48.8	-4.3	0.0922	-0.00190
70	48.8	-4.3	0.0922	-0.00190

APPENDIX 6: Data for the plot of kinetics parameters (zero, first & second orders) for THC (Co = 16160)

T (days)	Ct	Co - Ct	ln (Ct/Co)	1/Ct – 1/Co
14	13192	2968	-0.203	0.0000139
28	10623	5537	-0.419	0.0000322
42	8139	8021	-0.685	O.0000609
56	7025	9135	-0.833	0.0000804
70	6071	10089	-O.979	0.000102
Control				
14	15512	648	-0.0409	0.00000258
28	15019	1141	-0.0732	0.00000470
42	14621	1539	-0.100	0.00000651
56	14003	2157	-0.143	0.00000953
70	13681	2479	-0.166	0.0000112