BIOREMEDIATION OF USED ENGINE OIL CONTAMINATED SOIL BY VERMICOMPOSTING

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PhD/Eng/41309/2004-05

DEPARTMENT OF CHEMICAL ENGINEERING
AHMADU BELLO UNIVERSITY, ZARIA

August, 2012

DECLARATION

I hereby declare that this dissertation titled "Bioremediation of Used Engine Oil Contaminated Soil by Vermicomposting" was performed by me in the Chemical Engineering Department of Ahmadu Bello University, Zaria, under the supervision of Dr. I.A. Mohammed-Dabo, Dr S. Ibrahim and Prof J.B. Ameh. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

_____

Alewo Opuada **AMEH**

CERTIFICATION

This dissertation titled Bioremediation of Used Engine Oil Contaminated Soil by Vermicomposting meets the regulations governing the award of the degree of Doctor of Philosophy (Chemical Engineering) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my wife, Phoebe, my children, David and Eliora and to the bride of Christ in diaspora.

ACKNOWLEDGEMENT

To God be the Glory, great things He hath done.

I wish to express my profound appreciation to the members of the supervisory team, Dr I.A. Mohammed-Dabo, Dr S. Ibrahim and Professor J.B. Ameh for their invaluable contributions in developing and completing this work.

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ABSTRACT

This work was aimed at investigating the effect of earthworm (*Eudrilus eugeniae)* and organic amendments on the bioremediation of used engine oil contaminated soil, a process thought to be limited by mass transfer. The use of organic amendment alone facilitated bioremediation. The results obtained showed that the first order bioremediation kinetics was a good description of the process. The effect of surface area and frequency of mixing were also investigated and surface area was found to be an important factor in bioremediation: having a significant effect on k (*P-value* = 0.00045), the first order bioremediation constant. Thus increasing surface area improved mass transfer. The effect of mixing on k was found to be insignificant (*P-value* > 0.436). For the earthworm inoculated system at a used engine oil concentration of 100g used engine oil per kg of soil, the worms recorded survivals of 100, 91.67 and 66.67% in soil contaminated with used engine oil from motorcycle, truck/lorry and motorcar respectively. Investigations into the effect of earthworm inoculation on soil properties indicated that earthworms had no effect on the carbon and nitrogen contents but had significant effects on the moisture retention and aggregate size distribution of the used engine oil contaminated soil (*P-value* of 0.025 and 0.029 respectively). In addition, the water holding capacity and mean weight diameter of the soil were found to increase and decrease respectively with increasing earthworm concentration. The use of earthworms was also found to have no significant effect on the microbial population as both earthworms treated and untreated samples had comparable numbers of microbial count. The effect of initial used engine oil concentration on rate of vermi-assisted bioremediation gave mixed results. At high used engine oil concentrations, the presence of earthworms accelerated the biodegradation process whereas at low initial used engine oil concentration the rate of bioremediation was favored by the absence of worms. It was also discovered that the increase in earthworm concentration favors higher rates of bioremediation. The Investigation into the uptake / elimination kinetics of some heavy metals from various concentrations of used engine oil in (12.5 – 100 g/kg) showed that Zn, Cu, Ni and Cr were eliminated from the bodies of the worms whereas Ti and Mn where accumulated. Furthermore, the metal uptake and elimination kinetics showed good fit to a one-compartment model. Earthworm assisted bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshop indicated that the presence of the worms accelerated the bioremediation process (petroleum hydrocarbon degradation). The rate of bioremediation was found to be a function of the initial total petroleum hydrocarbon content as well as the percentage earthworm survival. The kinetic model made up of the combined first order reaction kinetics and first order catalysts deactivation kinetics was found to be a better fit to bioremediation (with and without earthworm inoculation) than the first order bioremediation kinetics alone in the bioremediation of simulated used engine oil contaminated soil as well as contaminated soil from mechanic workshops.

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LIST OF ABBREVIATIONS/SYMBOLS

a : flux of metals into the earthworm (mg_{metal} kg^{-1} animal day^{-1}); the initial hydrocarbon content in soil (g kg^{-1})

a_E: exposed surface area (cm^2/g)

BAF: bioaccumulation factors (from bulk concentrations)

C_e: external metal concentration (concentration in the soil, µg g^{-1} dry weight)

C_i: C_i is the body concentration (µg g^{-1}dry weight) with time

C_{SS}: calculated steady-state metal concentration, mg kg^{-1}

EW: sample with ten earthworms

k: first order bioremediation constant

k_1: uptake rate constant (from bulk concentrations k_1 soil, g soil ganimal^{-1} day^{-1};

k_2: elimination rate constant (day^{-1})

MR: Methyl-red

MRMX: *moringa oleifera* treated and mixed sample

MRUMX: *moringa oleifera* treated and unmixed sample

NSS: steady state not attained

PCA: plate count agar

PMX: poultry manure treated and mixed sample

PUMX: poultry manure augmented and unmixed sample

TPH: total petroleum hydrocarbon

t_{SS}: calculated time taken to reach steady state, days

UCS: used engine oil uncontaminated soil sample

UEO: used engine oil

VP: VOGES-PROSKAUER

WHC: water holding capacity

X: age of mechanic workshop

MCUW: undiluted motorcycle mechanic workshop soil treated with worms

MCUNW: undiluted motorcycle mechanic workshop soil without worms

MCDW: diluted motorcycle mechanic workshop soil treated with worms

MCDNW: diluted motorcycle mechanic workshop soil without worms

MMUW: undiluted motorcar mechanic workshop soil treated with worms

MMUNW: undiluted motorcar mechanic workshop soil without worms

MMDW: diluted motorcar mechanic workshop soil treated with worms

MMDNW: diluted motorcar mechanic workshop soil without worms

MC0: motorocycle mechanic workshop soil of day 0

MC35: motorcycle mechanic workshop soil on day 35

CHAPTER 1

INTRODUCTION

Thenumber of roadway, railway, air and water vehicles has always been on the increase. Consequently, the quantity of engineoil and other engine fluids (cooling liquids,brake oils, gearbox oils, etc.) used is expected to increase**(Baladincz*et al.*, 2008)**.The lubricating oils used by vehicle engines have to be replaced at least every 20,000 km**(www.daewoobus.com)**. The used oil is either dumped or re-refined and reused.

The term, used engine oil (UEO), refers to any lubricating oil that has served its service purposes in a vehicle and withdrawn from the designed area of application and considered no longer fit for the initial intended purpose. These used lubricants contain a lot of toxic and carcinogenic substances that can cause detrimental effects to the ecosystem. Basically lubricating oils are produced by some refining processes of crude oil. Additives are usually added to the lubricating oil to boost some of the oils properties like viscosity, thermal and oxidation stability. These additives when releasedinto the environment have toxic effect to humans and animals**(Abdullahi and Kawo, 2006)**.

The toxic and carcinogenic components of UEO may also be formed as a result of the thermal decomposition of lubricating materials upon processing, transportation, exploitation and storage. Oxidation of lubricating oil hydrocarbons at the point of application is accompanied by release of free radicals that transform to peroxides, which condense and polymerizeto produce per acids, nephtenic acids, etc. It is widely agreed that toxicity of lubricating oils have significant effects on germination, growth and yield of crops**(Abdullahi and Kawo, 2006)**.

Since the advent of oil exploration, the Nigeria environment has been heavily contaminated with hydrocarbon pollutants, which enters the environment through several routes. Pollution from spent engine oil is more widespread than crude oil pollution especially in urban centers where there are automobiles and generators using this petroleum product to reduce the friction between moving surfaces of engine**(Odjegbaand Sadiq, 2002)**. The presence of these pollutants in the terrestrial and aquatic environments constitutes public health and socio-economic hazards. In Nigeria, about 20 million gallons of waste engine oil are generated annually from mechanic workshops and dischargedcarelessly into the environment **(Adegoroye, 1997)**, out of which only one liter is enoughto contaminate one million gallons of freshwater. Apart from this, UEO renders the environmentunsightly and constitutes a potential threat tohumans, animals and vegetation. Several components of the oil, e.g. solventsand detergents added during the blending process, aliphatic hydrocarbon and polyaromatic hydrocarbons (PAHs) distilled from crude oil, andmetals from engine wear are either toxic in themselves orcan combine with products of combustion to generate carcinogens and endocrine disrupters.

Environmental problems resulting from oil pollution havebeen a major research issue in Nigeria over the last decadeowing to their effect on agriculture, economy, and societyat large. The economic growththat Nigeria has enjoyed as a result of oil revenue hasits drawbacks through exposure of the population in oilproducingareas to environmental contamination, largelydue to the increase in the movement of oil. Spillage of crude oil on soils hinders plant growthand productivity. The retarded plant growthby oil spillage usually results from insufficient aerationcaused by air displacement from the pore spaces betweenthe soil particles by the crude oil. Insufficientaeration also causes root stress, which consequentlyreduces plant growth, and

alwaysresults in leaf chlorosis. Low levelof chlorophyll in leaves of plants grown in soils treatedwith spent engine oil has been reported (**Adenipekun *et al.*, 2008**).

Biological degradation of petroleum hydrocarbon pollutantsand petrochemicals has been reported worldwide. Several researchers have looked at the remediation of oil contaminated soils.**Deshpande *et al.*(1999)** reported the use of surfactants in remediation of oil contaminated soils,**Marja *et al.*(2002)** reported the phytoremediation of soil contaminated with diesel and**Szewczyk and D1ugon´ski (2009)** reported the degradation of Pentachlorophenol and spent engine oil by *Mucor ramosissimus*, etc. In Nigeria, reports on the degradation of usedengine oil have not been widespread.**Adelowo *et al.* (2006)**reported thebio-degradation of used engine oil by locally isolated speciesof *Pseudomonas fragi* and *Achromobacter aerogenes*, while **Adenipekun (2008)** reported the bioremediation of engine oil contaminated soil using a Nigerian white-rot fungus**.**

When environmental conditions (pH, toxicity, etc.) are correctedand microbes are acclimated, the bioremediationrate depends on chemical kinetics within the microbe,the supply of oxygen and nutrients, and the masstransfer of contaminants to the right environment**(Li et al., 1995; Koren *et al.*, 2003)**.This mass transfer rate of oil is in general smallerthan either the oxygen transfer rate or the biodegradation rate and therefore is the controlling step of bioremediation of oil-contaminated soil due to the strong hydrophobicity and low water solubility of these compounds. The engineer's major task is to optimize the environmentalconditions for bioremediation**(Li et al., 1995)**. Another rate-limiting step for bioremediation of polyaromatics hydrocarbon (PAH) -contaminated soil is the strong sorption between the contaminant and

soil.Thus, any factor that works to minimize the effects of these resistances should hasten the bioremediation process.

In spite of the numerous ways in which the activities of earthworm promotes soil health (for example burrowing, soil ingestion, cast formation etc which theoretically is thought capable of influencing bioremediation) relatively few research have been undertaken to study the effects of earthworm on petroleum hydrocarbons contamination removal from soil **(Hickman and Reid, 2008).Hickman and Reid (2008)** enumeratedsome of the effects of earthworm activities which theoretically can enhance bioremediation. These effects include microorganism promotion and dispersal, improved soil structure, fragmented soil particles and increased surface area, organic matter turnover, increased water holding capacity, enhanced nutrient content and availability (with increased C:N ratio), improved O_2 and gas diffusion, increased porosity, increased pH etc.**Maike and Filser (2007)** studied the biodegradation of crude oil contaminated soil using earthworm and reported that earthworms can trigger bioremediation and may be applied in the bioremediation of crude oil contaminated soil of low to moderate concentrations. **Philip *et al.*(1998)**reported that earthworms are sensitive indicators of soil quality and may be used to evaluate the quality of remediated oily soils.

1.1 NULL HYPOTHESIS

The use of earthworm in the bioremediation of used engine oil contaminated soil will not contribute to the rehabilitation of the soil.

1.2 PROBLEM STATEMENT

Environmental (soil, water and air) contamination is a phenomenological problem worldwide. Bioremediation as a cleanup option,though cost effective and environment friendly, suffers the

disadvantage of being relatively very slow – thought to be mass transfer limiting. Furthermore, in spite of the numerous ways in which the activities of earthworm promotes soil health (which theoretically is thought capable of influencing bioremediation), relatively few research efforts have been undertaken to study the use of earthworm as a biocatalyst in the bioremediation of petroleum hydrocarbon contaminated soil.

1.3 JUSTIFICATION

The justifications for this work include:

Used engine oil contamination is wide spread in Nigeria and constitutes a serious environmental hazard.Bioremediation is considered to be cheap (**Aalok *et al., 2008)*** and therefore inviting as a treatment option. Finally, success in the work will provide a faster means of bioremediation using indigenous organisms.

1.4 AIM AND OBJECTIVES

This work is aimed at studying the use of earthworm in enhancing the bioremediation of engine oil contaminated soil.

The set objectives include:

1. To carry out investigation into the effect of earthworm and organic amendments on biodegradation of used engine oil contaminated soil (simulated contamination and contaminated soil from mechanic workshops).
2. To fit the bioremediation data to established bioremediation kinetic equations.
3. To investigate the effect of vermicomposting on microbial biomass.
4. To investigate the effect of vermicomposting on used engine oil contaminated soil moisture retention, metal uptake/elimination, particle sizeand carbon/nitrogen contents.

1.5 RESEARCH LIMITATION

This research was limited by the unavailability of the appropriate standards for total petroleum hydrocarbon (TPH) determination using gas chromatograph as well as the appropriate crucible for elemental analysis of the used engine oil samples.

1.6 SCOPE

1. Testing of soil samples collected from various mechanic workshops,where used engine oil is usually dumped. Physical and chemical properties will be investigated.
2. Preparation and analysis and of organic materials for use as amendment.
3. Investigations into the effect of earthworms on metal uptake (bioaccumulation in earthworm), moisture retention in soil (water holding capacity), soil carbon and nitrogen contents and aggregate size distribution in the bioremediation of used engine oil contaminated soil.
4. Investigationsinto the use of earthworm in the bioremediation of soil samples in which the microorganisms have been stimulated. Parameters to be investigated include the effect of earthworm concentration and used engine oil concentration.
5. Analysis and interpretation of data and testing of data with models established in the literature.
6. Studies on vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshop.

CHAPTER 2

REVIEW OF LITERATURE

The quality of life on Earth is linked inextricably to the overall quality of the environment. In early times, it was believed that there was an unlimited abundance of land and resources; today, however, the carelessness and negligence in the use of these natural endowments have resulted in the degradation of the environment which in turn affects life negatively. The problems associated with contaminated sites now assume increasing prominence in many countries. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today. The problem is worldwide, and the estimated number of contaminated sites is significant **(Cairney, 1993)**. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use.

This chapter gives an extensive review of work on remediation with emphasis on bioremediation and earthworm assisted bioremediation of petroleum hydrocarbon. It covers the theories and hypothesis of bioremediation.

2.1 BIOREMEDIATION

The conventional techniques used for remediation has been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create

significant risks in the excavation, handling, and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. The cap and contain method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability.

A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents.

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result.Because bioremediation

seems to be a good alternative to conventional clean-up technologies research in this field is rapidly increasing.

Bioremediation has been used at a number of sites worldwidewith varying degrees of success. Techniques are improving as greater knowledge and experience are gained, and there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Unfortunately, the principles, techniques, advantages, and disadvantages of bioremediation are not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators**(Vidali, 2001)**.

2.1.1 Principles of Bioremediation

Environmental biotechnology is not a new field; composting and wastewater treatments are familiar examples of old environmental biotechnologies. However, recent studies in molecular biology and ecology offer opportunities for more efficient biological processes. Notable accomplishments of these studies include the clean-up of polluted water and land areas. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities **(Mueller *et al.*, 1996)**.

By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are

transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation we have a process known as bioaugmentation**(Vidali, 2001)**.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

Like other technologies, bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies.

Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions **(Colberg and Young, 1995)** may permit microbial organisms to degrade

otherwise recalcitrant molecules. Alist of some contaminants potentially suitable for bioremediationis shown in Table 2.1.

Table 2.1: Some contaminants potentially suitable for bioremediation

Class of contaminants	Examples	Aerobic	Anaerobic	Potential sources
Chlorinated solvents	Trichloromethylene		+	Drycleaners
	Perchloroethylene			Chemical manufacture
Polychlorinated biphenyls	4-Chlorobiphenyl		+	Electrical manufacturing
	4.4-Dichlorobiphenyl			Power station, Railway yards
Chlorinated phenol	Pentachlorophenol		+	Timber treatment, landfills
BTEX	Benzene Toluene Ethylbenzene Xylene	+	+	Oil production and storage, Gas work sites, Airports, Paint manufacture, Port facilities, Railway yard, Chemical manufacture
Polyaromatic hydrocarbon (PAHs)	Naphthalene Antracene Flourene Pyrene Benzo(a)pyrene	+		Oil production and storage, Gas work sites, Coke plants, Engine works, Landfills, Tar production and storage, Boiler ash, Dump sites, Power stations
Pesticides	Atrazine Carbaryl Carbofuran Coumphos Diazinon Glycophosphate Parathion Propham 2,4-D	+	+	Agriculture, Timber treatment plants, Pesticides, Pesticides manufacture, Recreational areas, Landfills.

Source: Vidali, 2001

2.1.2 Factors of Bioremediation

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environmental factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

2.1.2.1 Microbial Population for Bioremediation Processes

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are an energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. We can subdivide these microorganisms into the following groups:

Aerobic (in the presence of oxygen). Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

Bacillus is a genus of gram-positive rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species can be obligate aerobes or facultative anaerobes, and test positive for the enzyme catalase **(Tumbull, 1990)**. Ubiquitous in nature, *Bacillus* includes both free

living and pathogenic species.Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods.

Few studies have been reported on the roles of *Bacillus spp.* in hydrocarbon bioremediation; although there are several reports on bioremediation of pollutants by the action of *Bacillus spp*. occurring in extreme environments (**Khan and Rizvi, 2011).Ijah and Antai (2003)** reported *Bacillus spp*. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples (30 and 40% crude oil). It has been postulated that *Bacillus spp*. are more tolerant to high levels of hydrocarbons in soil due to their resistant endospores.

Singh and Lin (2008) reported that *Bacillus pumilus* achieved 86.94% of diesel degradation in 2 weeks. **Kebria *et al.* (2009)** reported that a strain of *bacillus* native to Tehran*Bacillus Cereus*,showed great potential for in situ remediation of diesel-contaminated soil in oil refinery sites. *Bacillus cereus*is an organism occurring naturally in most foods and it can cause two differentforms of food poisoning, diarrhoeal and emetic, both caused by toxins produced by the bacteria.**Khan and Rizvi (2011)** cultured and isolated microorganisms from mechanic workshops and reported that the isolate showing maximum oil degrading ability was gram positive *bacillus spp*.

Pseudomonas is a genus of gammaproteobacteria, belonging to the family Pseudomonadaceae containing 191 validly described species. *Pseudomonas aeruginosa* is a versatile Gram-negative bacterium that grows in soil, coastal marine habitats, and on plant and animal tissues.In their review**Das and Chandran (2011)**touched on the use of*Pseudomonas aeruginosa* for degrading sludge hydrocarbons. **Emtiazi *et al*. (2005)** reported the immobilization of *pseudomonas* isolated from a petroleum-contaminated soil for use in petroleum hydrocarbon degradation.

Anaerobic(in the absence of oxygen): Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform.

Ligninolytic fungi: Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs.

Methylotrophs (Aerobic bacteria that grow utilizing methane for carbon and energy): The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatics trichloroethylene and 1,2-dichloroethane **(Allard and Neilson, 1997)**.

For degradation it is necessary that bacteria and the contaminants be in contact. This is not easilyachieved, as neither the microbes nor contaminants are uniformly spread in the soil. Some bacteriaare mobile and exhibit a chemotactic response, sensing the contaminant and moving toward it. Othermicrobes such as fungi grow in a filamentous form toward the contaminant. It is possible to enhancethe mobilization of the contaminant utilizing some surfactants such as sodium dodecyl sulphate (SDS).

Cometabolism is used to describe the transformation of an organiccompound by a microorganism that is unableto use the substrate as a source of energy. Metabolites or transformation products fromcometabolism by one organism can typicallybe used as an energy source by another.

2.1.2.2 Environmental Factors

Nutrients:Although microorganisms are present in contaminated soil, they cannot necessarily be there in thenumbers required for bioremediation of the site. Their growth and activity must be stimulated.Biostimulation usually involves the addition of nutrients and oxygen to help in the growth of indigenous microorganisms.These nutrients are the basic building blocks of life and allow microbes to create the necessaryenzymes to break down the contaminants. These nutrients include nitrogen, phosphorous, and carbon.

Carbon is the most basic element of living forms and is needed in greater quantities than otherelements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells. Phosphorous and sulfur make up 70% of the remainders. The carbon/nitrogen and carbon/phosphorous ratios required for microbial growthis 10:1and 30:1 respectively.

Environmental requirements:Optimum environmental conditions for the degradation of contaminants are reported in Table 2.2.Microbial growth and activity are readily affected by pH, temperature, and moisture. Although microorganisms have been also isolated in extreme conditions, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions. If the soil has too much acid it ispossible to raise the pH by adding lime. Temperature affects biochemical reactions rates, and the rates of many of them double for each 10 °C rise in temperature. Above a certain temperature, however, the cells die. Plastic covering can be used to enhance solar warming in late spring, summer, and autumn. Available water is essential for all the living organisms and irrigation is needed to achieve the optimal moisture level. The amount of available oxygen will determine whether the system is aerobic or anaerobic. Hydrocarbons are readily degraded under aerobic conditions, whereas chlorurate compounds are

degraded only in anaerobic ones. To increase the oxygen amount in the soil it is possible to till or sparge air. In some cases, hydrogen peroxide or magnesium peroxide can be introduced in the environment. Soil structure controls the effective delivery of air, water, and nutrients. To improve soil structure, materials such as gypsum or organic matter can be applied. Low soil permeability can impede movement of water, nutrients, and oxygen; hence, soils with low permeability may not be appropriate for *insitu* clean-up techniques**(Vidali, 2001)**.

Table 2.2: Environmental conditions affecting degradation.

Parameters	Condition required for microbial activity	Optimum value for an oil degradation
Soil moisture	25-28 % of water holding capacity	30-90%
Soil pH	5.5- 8.8	6.5 – 8.0
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10 – 40%
Nutrient content	N and p for microbial growth	C:N:P=100:10:1
Temperature (^{o}C)	15-45	20-30
Contaminants	Not too toxic	Hydrocarbon 5-10% of dry weight of soil
Heavy metals	Total content 2000ppm	700ppm
Type of soil	Low clay or silt content	

Source: (Vidali, 2001)

2.1.3 Bioremediation Strategies

Different techniques are employed depending on the degree of saturation and aeration of an area. *In situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. *Ex situ* techniques are those that are applied to soil and groundwater which

has been removed from the site via excavation (soil) or pumping (water). *Bioaugmentation* techniques involve the addition of microorganisms with the ability to degrade pollutants.

2.1.3.1 In situ Bioremediation

These techniques are generally the most desirable options due to lower cost and less disturbance since they provide the treatment without excavation and transport of contaminants. *In situ*treatment is limited by the depth of the soil that can be effectively treated. In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases. The most important land treatments are:

Bioventing: Bioventing is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.

In situ biodegradation: Thisinvolves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment.

Biosparging: Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

Bioaugmentation: Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit:

a. Nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and
b. Most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

2.1.3.2 Ex situ Bioremediation

These techniques involve the excavation or removal of contaminated soil from ground.

Landfarming: Thisis a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attentionas a disposal alternative.

Composting: This technique involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials support the development of a rich microbial population and elevated temperature characteristic of composting.

Biopiles: Biopilesare a hybrid of landfarming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms**(Von Fahnestock*et al.*, 1998)**.

Bioreactors:Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil-bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction

(e.g., vacuum extraction) before being placed in a bioreactor. Table 2.3 shows the advantages and disadvantages of bioremediation methods.

2.1.3.3 Advantages of Bioremediation

Bioremediation enjoys the following advantages:

1. 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 increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.

2. 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.

3. 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.

4. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.

Table 2.3: Summary of bioremediation strategies

Technology	Examples	Benefits	Limitations	Factors to consider
In situ	*In situ* bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	Biodegradative abilities of indigenous microorganisms Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Landfarming Composting Biopiles	Cost efficient Low cost Can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation	See above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhanced mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relative high cost capital Relatively high operating cost	See above Bioaugmentation Toxicity of amendments Toxicity concentrations of contaminants

Source: (Vidali, 2001)

5. Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

2.1.3.4 Disadvantages of Bioremediation

Bioremediation as a cleanup option suffers the following disadvantages:

1. Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptibleto rapid and complete degradation.

2. There are some concerns that the products of biodegradation may be more persistent or toxic thanthe parent compound.

3. Biological processes are often highly specific. Important site factors required for success includethe presence of metabolically capable microbial populations, suitable environmental growth conditions,and appropriate levels of nutrients and contaminants.

4. It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
5. Research is needed to develop and engineer bioremediation technologies that are appropriate forsites with complex mixtures of contaminants that are not evenly dispersed in the environment.

6. Bioremediation often takes longer than other treatment options, such as excavation and removalof soil or incineration.

7. Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation: there is no accepted definition of “clean”, evaluating performance of bioremediation is difficult,and there are no acceptable endpoints for bioremediation treatments.

2.1.4 Phytoremediation

Although the application of microbial biotechnology has been successful with petroleum-based constituents, microbial digestion has met limited success for widespread residual organic and metals pollutants. Vegetation- based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. In natural ecosystems, plants act as filters and metabolize substances generated by nature. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water **(Raskin and Ensley, 2000)**. The term "phytoremediation" is relatively new, coined in 1991. Its potential for encouraging the biodegradation of organic contaminants is promising and requires further research.Phytoremediationtechniques are classified as: phytoextraction, phytotransformation, phytostabilization, phytodegradation, rhizofiltration(based on the contaminant fate).

2.2 EARTHWORM

Earthworms are perhaps the most important soil organisms in terms of their influence on organic matter breakdown, soil structural development, and nutrient cycling, especially in productive ecosystems. Aristotle called them the "intestines of the earth" and the eminent nineteenth century biologist, Charles Darwin, spent many years observing their major influence on the formation of humus and transport of soil **(Darwin, 1881)**.

2.2.1 Earthworm Biology and Classification

Earthworms are terrestrial annelids with bilateral symmetry and corresponding external and internal segmentation. They have a thinly pigmented cuticle bearing setae on all segments except the first two. All earthworms are hermaphroditic. Their gonads are situated in specific segments,

which vary among different taxa. When sexually mature, they develop a swollen area of the epidermis called a clitellum. This region produces a cocoon in which one or more eggs are deposited and then the cocoon is passed over the anterior segments and deposited in or on the soil. The young develop within the cocoon and newly hatched worms resemble adults. The time to hatching and reproductive maturity varies widely among different earthworm species and is influenced by environmental factors. Earthworms are classified within the phylum Annelida and the class Oligochaeta, which consists of as many as 36 families worldwide. About two-thirds of oligochaete families comprise aquatic or semi-aquatic worms, and the remaining families comprise mostly or exclusively terrestrial worms or earthworms. There are over 3500 known earthworm species and it is estimated that the global total may be twice that number. Distinct taxonomic groups have arisen on every continent except Antarctica, and some groups are now distributed throughout the world**(Partrick, 2002)**. Figure 2.1 is a pictorial representation of *Eudrilus Eugenia* an earthworm species commonly found in Northern Nigeria.

2.2.2 Earthworm Ecology

2.2.2.1 Ecological groups

Earthworm species can be grouped according to behavioral, morphological or physiological adaptations that enable them to partition available resources in the soil. The three main life history strategies are termed epi, anecic, and endogeic **(Edward and Bohlen, 1996 and Lee 1985)**.



Figure 2.1: Earthworm (*Eudrilus eugeniae*)

Epigeic worms feed on plant litter, dwell on the soil surface or within the litter layer, tend to be heavily pigmented, and are small- to medium-sized. Anecic worms feed on plant litter and soil, live in nearly vertical permanent burrows, are dorsally pigmented, and large. Endogeic species are soil-feeders, are not heavily pigmented, form extensive horizontal burrow systems, and range in size from small to large. Endogeic worms have been further divided into polyhumic, mesohumic, and oligohumic groups, which are separated, respectively, by the descending importance of organic rich mineral soil in their diet and increasing size. Earthworm species do not always fall clearly into these three main categories and may even exhibit traits of different groups at different life stages or under different environmental conditions.

2.2.2.2 Earthworm communities

Earthworm communities generally consist of from one to six species. The relative abundance and species composition of earthworm communities depend upon soil type, topography, and vegetation, and is also influenced by land use history and earthworm biogeography. Earthworms account for the majority of animal biomass in soil in a wide range of productive ecosystems, from temperate grasslands, pastures and forests to tropical pastures, savannas and rainforests, and many temperate and tropical agroecosystems. They generally do not occur in deserts and arid grasslands or in extreme alpine or boreal habitats. Earthworms are often absent from strongly acidic forest soils with poor litter quality, such as some northern coniferous forests. Many earthworm communities consist of invasive exotic species. In North America, where approximately 100 native earthworm species have been described, at least 45 exotic species have been introduced. Lumbricid earthworms of European origin dominate many North American agroecosystems. A worldwide survey of earthworms in tropical regions reported that a total of 51 exotic and 151 native species commonly occur in tropical agroecosystems **(Fragoso *et al.*, 1999)**.

2.2.3 Effects of Earthworms on Soil Properties

The influence of a given earthworm species on soil properties depends upon that species' life history strategy. For example, the large vertical burrows of anecic earthworms, such as the common nightcrawler *Lumbricus terrestris*, can facilitate preferential flow of water through the soil profile, increasing the transport of water, nutrients, and agricultural chemicals into deeper soil layers **(Subler *et al.*, 1997 and Edward *et al.*, 1992)**. Epigeic species facilitate the breakdown and mineralization of surface litter, whereas anecic species incorporate surface litter deeper into the soil profile. Anecic species also bring soil from deeper soil horizons to the surface, which over a long period of time can change the mineralogy of surface soil. Endogeic species feed on fragmented organic matter and mix it thoroughly through the surface mineral soil.

2.2.3.1 Effects on organic matter breakdown and nutrient cycling

Earthworm activity accelerates the decomposition of plant litter, increases rates of nutrient transformation and plant nutrient uptake, improves soil aggregation and porosity, and enhances water infiltration and solute transport. Earthworms can consume and incorporate large amounts of organic matter into soil (Table 2.4). Such mixing is largely responsible for the formation of mull soils in which surface organic horizons are thoroughly mixed with underlying mineral soil.

Earthworms are a major influence on nutrient cycling in many ecosystems. Although they generally increase the mineralization of soil carbon, earthworms can also decrease mineralization of carbon by contributing to the formation of stable soil aggregates in which carbon is protected from further breakdown.

Table 2.4: Amount of organic matter ingested or incorporated into soil by earthworm populations in different environments

Ecosystem	**Location**	**Type of organic matter**	**Amount consumed or incorporated kgha^{-1}**
Alfalfa field	United States	Alfalfa residue	1,220
Maize field	United States	Maize residue	840
Mixed forest	England	Canopy tree leaves	3,000
Oak forest	Japan	Oak leaves	1,071
Orchard	England	Apple leaves	2,000
Savannah	Ivory coast	Total organic matter	1,300
Tallgrass prairie	United States	Total organic matter	740-8,980

Source: (Patrick, 2002)

Direct fluxes of nutrients through earthworm biomass can be considerable. For example, the turnover of nitrogen (N) through earthworm tissues can be up to 150 kgNha^{-1} yr^{-1}**(Lee, 1985)**. Earthworm casts contain elevated amounts of inorganic N relative to surrounding soil. As a consequence, earthworms can greatly enhance the mineralization of N and can stimulate other N transformations such as denitrification. By increasing bypass flow of infiltrating water, earthworm burrows can increase the amount of N and other nutrients leaching from the soil

profile. Alternatively earthworms can reduce the amount of nutrients lost in surface runoff by increasing rates of water infiltration into the soil **(Subler *et al.*, 1997 and Sharpley *et al.*, 1979)**.

2.2.3.2 Effects on physical properties of soil

The effect of earthworms on soil structure results from the net outcome of their feeding and burrowing activities. Earthworms ingest soil particles and organic matter, mixing these two fractions together and egesting them as surface or subsurface casts. Once egested, soil in casts can be eroded due to impact of rainfall or can form stable soil aggregates through a variety of stabilizing mechanisms. Earthworms generally improve the aeration and porosity of soil through formation of burrows and by increasing the proportion of large aggregates in the soil, and their effects are especially important in poorly structured or reclaimed soil.

By increasing rates of water infiltration, earthworms can reduce the amount of surface runoff. Alternatively, earthworms can increase erosion by removing the protective cover of surface litter, increasing surface sealing and depositing surface casts, which can be carried downslope during heavy rains. Some tropical species actually increase soil bulk density and decrease infiltration by producing compact soil casts. Overall, the effects of earthworms on soil structure are considered to improve soil fertility.

2.2.3.3 Effects on plant growth

The majority of studies examining the influence of earthworms on plant growth have reported that earthworms stimulate plant growth, although some studies have reported no effect or even a negative effect of earthworms on plant growth.

Earthworms have been shown to increase production of shoots and grain in a variety of field trials and greenhouse experiments. Introduction of earthworms into reclaimed polders and pastures resulted in large increases in forage quantity and quality **(Hoogerkamp*et al.*, 1983)**. Beneficial effects of earthworms on plant growth may be due to increased nutrient and water availability, improved soil structure, stimulation of microorganisms or formation of microbial products that enhance plant growth, or possibly through direct production of plant growth promoting substances.

2.2.3.4 Undesirable Effects of Earthworms

Despite the documented and putative beneficial effects of earthworms on nutrient dynamics, soil structure, and fertility, some aspects of earthworm activities are considered undesirable **(Edward and Bohlen, 1996)**. These include removing and burying surface residues that would otherwise protect soil surfaces from erosion; producing fresh casts that increase erosion and surface sealing; depositing castings on the surface of lawns and golf greens or irrigation ditches where they are a nuisance, or in pastures where they interfere with haying operations; dispersing weed seeds in gardens and agricultural fields; transmitting plant or animal pathogens; increasing losses of soil nitrogen through leaching and denitrification; and increasing soil carbon loss through enhanced microbial respiration. It is the net outcome of their positive and negative effects that determines whether earthworms are to be considered pests or benefactors in any given situation, and they are generally considered to be of great benefit to soil properties and fertility.

2.2.4 Earthworms and Soil Management

2.2.4.1 Earthworms and soil reclamation

Introduction of appropriate earthworm species or encouraging natural population through the addition of suitable amendments can increase the rate of soil improvement and genesis of soil structure of reclaimed land. Introduction of European earthworms into pasture in New Zealand and Australia, as well as in Dutch polders, greatly facilitated improvements in soil structure and plant productivity **(Hoogerkamp*et al.*, 1983)**. There has also been some success in introducing earthworms into reclaimed mining sites and in reclaimed peat with beneficial effects on soil structural development, nutrient cycling, and productivity **(Edward and Bohlen, 1996)**.

Earthworms can be used to assess toxicity in terrestrial systems and the survival rate of theworms, or changes in other parameters such as biomass, can be used to calculate an LC_{50} value(lethal concentration to 50% of the population) for test chemicals spiked into soil. This type ofinformation can be useful in predicting the likely toxicological effect of oily drill cuttings on thesoil environment and may be useful for calculating application rates for fluids with differenttoxicological characteristics if the oily cuttings are to be land farmed**(Getliff *et al*., 2002)**.

Studies have also been conducted on the use of earthworms in the remediation of spent drilling fluidcontaminated soil. When drill cuttings and other organic materials are fed to earthworms such as*Eisenia foetida* the oil content of the cuttings is rapidly degraded and the mixture converted into ahigh quality soil-like compost that can be used as a soil improver or fertilizer **(*Getliff et al*., 2002)**. In addition, treatment of crude oil contaminated soil was also indicative of

a significant decrease in the TPH concentration in treatments with earthworms, compared with the samples without worms**(Maike and Filser, 2007)**.

2.2.4.2 Influence of agricultural practices on earthworm populations

Earthworms can be affected favorably or adversely by different agricultural practices. Whereas heavy cultivation is detrimental to earthworm populations, reduced and notillage agricultural practices promote the growth of earthworm populations. Organic amendments, such as animal or green manures, also stimulate the growth of earthworm populations. Inorganic fertilizers may benefit earthworm populations by increasing the production of crop residues, but their effects are not as great as the effects of organic fertilizers. Long-term application of inorganic fertilizers may adversely affect earthworm populations due to soil acidification or other changes in the soil environment. Liming can benefit earthworm populations in some instances.

2.2.4.3 Effects of pesticides and soil pollutants on earthworms

Pesticides have widely varying effects on earthworm populations, with many chemicals having little or no toxicity toward earthworms and others exhibiting acute toxicity **(Edward and Bohlen, 1992)**. In general, the carbamate insecticides and soil fumigants are very or highly toxic to earthworms. Herbicides in general show low toxicity toward worms, although there are some exceptions. Organochlorine and organophosphorous insecticides have varying levels of toxicity. Soil contamination with organic pollutants, heavy metals, and acid precipitation can be detrimental to earthworm populations. Most toxic organic soil pollutants are highly toxic to earthworms. Heavy metals vary in their toxicity toward earthworms. In addition to acute negative effects of some heavy metals on earthworms, sublethal heavy metal concentrations can cause declines in earthworm growth and reproduction. Heavy metals can accumulate in earthworm tissues,which is potentially problematic for movement of heavy metals into higher

trophic levels, because of the wide array of animals that prey on earthworms. Earthworm species vary in their tolerance of acid soil conditions but some reports have noted a decline in earthworm populations in response to large amounts of acid deposition.

2.2.5 *Eudrilus Eugeniae*

The earthworm species, *Eudrilus eugeniae* commonly referred to as the West African night crawler, occurs all over the world but mostly in West African regions (**Shagoti 1985; Segun 1998**). It is largely found in West Africa from Ghana to Nigeria to West Cameroun and Gabon. It grows well at a temperature of more than 25^{o}C but best at 30^{o}C (**Viljoen and Reinecke, 1992**), attaining maximum weight, length and number of segments in about 15 to 20 weeks (**Rodriguez and Lapiere, 1992**). It ranges in size from about 10cm in length to huge specimens of over 12cm and size may depend on habitat (**Segun, 1998**). The total number of segments in *Eudrilus eugeniae* varies from about 80 to over 100 with the location of a thick cylindrical collar – the clitellum between segments 13 – 20 (**Pirrone, 1985**). It has a purple sheen and the posterior segments are evenly tapered to a point (**Blackburn, 1989**).

2.2.6 Vermicomposting

Vermicomposting is biooxidation and stabilization of organic material involving the joint action of earthworms and microorganisms in which negligible organic waste resources are converted into nutrient rich plant growth media i.e. vermicompost. Studies reveal that epigeics seem well fitted for such kind of operations, because of their surface activity, their ability to colonize organic material quickly, and in the process to convert it into useful compost and their ability to minimize malodour formation. However, involvement of earthworms in composting process

decreases the time of stabilization of the waste and produces an organic pool with energy reserves as vermicompost. The composting biology and ecology of earthworms in waste resources are controlled by several abiotic and biotic factors: temperature, moisture, pH, substrate, feed and its palatability, particle size, earthworm density and fecundity, which directly or indirectly influence the composting potential of the composting species. Several earthworm species e.g. *Eisenia fetida, Eisenia andrei, Eudrilus eugeniae, Perionyx excavatus*have been identified as potential candidates for managing organic waste resources. World-wide spread *E. fetida*was and still remains a favoured earthworm species for vermicomposting operations due to their wide range of tolerance for environmental variables. However, in tropical and sub-tropical conditions earthworm: *P. excavatus, P. sansibaricus,* and*E. eugeniae*appeared as best vermicomposting species. The life cycle parameters of above tropical species has been investigated in respect to their environmental requirement like temperature, moisture, fecundity and density pressure, pH, but influence of culture material or substrate quality on their life cycle parameters is less considered. Although, animal dung is recognized as a suitable earthworm culture media, other organic waste materials from agriculture and industry have also proved successful. The chemical structure of a given culture media for large scale vermiculture practices is always important. Apart from having high concentrations of nutrients, plant wastes contain important secondary metabolites e.g. polyphenols, which directly or indirectly influence the composting potential as well as growth patterns of given composting species during vermicomposting practices. The impact of such kind of wastes on life cycle parameters of composting worms must be of a research priority**(Suthar, 2007)**.

2.2.7 Effect of Earthworm Activities on Soil Propertiesand Bioremediation

When soil becomes contaminated, thecontaminants become entrapped and sorbed onto and into soilmineral and organic matter fractions rendering them inaccessible and thus unavailable for microbial interaction. Earthwormactivity has the potential to release these residues and could thus potentially enhance bioremediation performance.

Both single strains and consortia of microorganisms added to contaminated soils usually produce little added value in terms of degradative ability over the soil's indigenous microorganisms, which are often only limited in their contaminant degradation capacities due to the soil's suboptimal status. Promotion of the indigenous microorganisms is thus a primary aim and would occur via optimization and enhancement of both abiotic and biotic conditions; something that earthworms could enhance.

2.2.7.1 Abiotic Effects

In bioremediation, it is often necessary to enhance and maintain moisture, oxygen and nutrient levels, whilst also ensuring they can be homogenously dissipated. Whilst time consuming, labour intensive and expensive methods can be utilized to aid in the optimization of these variables, there might exist a relatively low input, low technological tool available to undertake this work: as earthworms move throughout the soil environment, their resulting burrows (burrowing being species specific) act as input points of, and preferential pathways for, water and particle movement **(Shipitalo and Le Bayon, 2004; Kretzshmar, 2004; Dominguez, 2004)**, and nutrient flow and aeration **(Dominguez 2004)**.

As earthworms ingest and digest soil, a further positive effect upon the soil environment occurs; that of the mechanical breakdown of soil particles **(Edwards and Bohlen, 1996; Bohlen et al., 2004; Kersante et al., 2006)**.It could be hypothesized that this action might assist in the release

of soil associated (aged) organic contaminants **(Barois et al., 1993)**. Suchactivities have also been shown to increase soil organic matter surface area **(Edwards and Bohlen, 1996)**, whichwill have an effect upon the fraction of material exposed for microbial interaction **(Dominguez, 2004)**, potentially resulting in increased contaminant degradation. These aspects are worthy of serious research and consideration with respect to increasing degradation potential and offsetting the residual concentration problem, which often limits final bioremediation success **(Hickman and Reid, 2008)**.

In cast formation, it is known that there are many subsequent positive effects upon the improvement of the soil environment and soil structure. These include increased soil porosity **(Bolan and Baskaran, 1996; Shipitalo and Le Bayon, 2004)**, increased oxygenation, moisture retention **(Edwards and Bohlen,1996; Schaefer and Filser, 2007)**, improved soil fertility via accelerated organic matter decomposition, and improvement of the availability of nutrients **(Edwards and Bohlen, 1996; Lavelle et al., 2004**). Thedeposition of these casts upon the burrow walls, within burrows or on the soil surface can significantly differ in chemical and physical composition to the surrounding soil in terms of altered C:N ratios and higher pH **(Bolan and Baskaran, 1996; Edwards and Bohlen, 1996; Brown and Doube, 2004; Shipitalo and Le Bayon, 2004)**. There could thus be potential for the use of earthworms casts (either directly applied to contaminated soils, or being produced as earthworms reside within contaminated soils) to firstly improve chemical conditions to aid bioremediation and, secondly, improve the overall soil condition. Some of these effects upon the soil environment have been investigated within soil/land rehabilitation **(Lowe and Butt, 2003; Butt et al., 2004)**, specifically the increases in soil fertility and structure, leading to increased soil 'health'. Thus, not only might soils benefit from earthworm input in terms of reduced contaminant concentrations, but also

from improved soil 'health'; both aspects being fully in line with the recently proposed **EU Soil Framework Directive (2006)**.

2.2.7.2 BioticEffects

Itis known that earthworms interact with soil microorganisms (fungi, bacteria and actinomycetes) on three broad spatial scales (burrow linings, casts and earthworm gut or intestine) **(Brown and Doube, 2004)**. For example, mucus and cast deposition on burrow walls, and other organic carbon sources transferred through the burrow systems promote the increase and distribution of microorganisms in earthworm burrows **(Farenhorst *et al.*, 2001)**, whilst mucus, urine and glucose can result in increases in microbial biomass **(Scheu,1987)**. This is of relevance because an increase in microbial biomass is linked to increased microbial catabolic activity **(Meharg, 1996)**. Higher microbial numbers, diversity and activity are also known to be related to passage of microorganisms through the earthworm gut, as well as the promotion therein, and the 'awakening' of dormant gut flora **(Brown *et al.*, 2000; Brown and Doube, 2004)**. Importantly, the increased gut associated microflora are then excreted throughout the media within earthworm casts and via microbial adheration to earthworm skin **(Edwards and Bohlen, 1996)**, whilst the transit and dispersal mechanisms associated with the water flow (**Kretzshmar, 2004)** also help to further dissipate microorganisms. Such actions are clearly relevant to any bioremediation methodology desiring the spatial incorporation of microorganisms capable of organic contaminant degradation. Earthworm casts are therefore generally much richer in microbial numbers in comparison to the initially ingested material. Forexample, **Scheu (1987)** reported an increase of 90% in respiration rate infresh casts, whilst, **Teotia *et al.* (1950)** reported bacterial counts of32 million/g compared to 6–9 million/g for the surrounding soil. This isdue not only to the aforementioned intestinal promotion of microorganismsbut also to the inherently high soil

organic matter levels,resulting in further microbial activity and proliferation (**Brown andDoube, 2004)**. Dynamics and succession of microorganisms withinearthwormcasts is complex, and relies heavily upon many factors suchas type of food ingested, gut passage time and ingested and inherent gutmicroorganisms. Further discussion upon these factors, numbers ofmicroorganisms in earthwormcasts, and the effect of earthworms upon microorganisms on the meso and macroscale, are further discussed by **Edwards and Bohlen (1996)**, and **Brown and Doube (2004)**. It has also been noted that water soluble **(Edwards and Bohlen, 1996)** low molecular weight **(Barrois and Lavelle, 1986; Lavelle *et al*., 1993)** organic compounds such as enzymatic fluid and mucus based solutions are added to earthworm gut contents during digestion, **(Brown and Doube, 2004)**, which also subsequently stimulate microbial activity both in the gut and in the egested casts **(Edwards and Bohlen, 1996)**. It is known that pre-exposure, or pre-induction of microorganisms to contaminants, or groups of contaminants, can result in subsequent increased degradation rates **(Carmichael and Pfaender, 1997; Reid *et al*., 2002)**. Therefore, these intestinal organic compounds might help 'prime' the microorganisms in the gut to break down more complex organic compounds in the ingested soil. Earthworm casts have had a strong presence within this particular approach, and relevantly, the application of earthworms within waste management involves the digestion of a wide range of organic wastes **(Edwards and Bohlen,1996)** into vermicast/compost. Vermidigestion is an area rapidly increasing in interest and research due to the use of the nutrient and microbially rich casts as plant growth media, plant pest and disease suppressant and itsusewithin horticulture **(Edwards and Arancon, 2004)**. Such amaterialmaywell have a placewithin remediation, andwill be discussed subsequently. Pertinent reviews of earthworm feeding ecology **(Curry and Schmidt, 2007)** and vermidigestion, and the

use of earthworm casts have been undertaken elsewhere **(Edwards and Bohlen, 1996; Dominguez, 2004; Edwards and Arancon, 2004)**.

Intheory, it is the earthworm's combined mechanical activity upon the soil and subsequent promotion of microorganisms that would be of most benefit to remediation efforts, as opposed to uptake/accumulation of organic contaminants. It has been shown that earthworm body burdens of organic contaminants can typically be low **(Kelsey and Alexander, 1997; White *et al*., 1999; Gevao *et al*., 2001)** and is unlikely to prove to be a worthwhile tool in bioremediation. Table 2.5summarizes the discussed positive earthworm actions upon the soil environment.

Table 2.5: The effect of earthworm activities on the soil environment

Burrowing/ Movement	Burrow creation	Soil ingestion, Digestion and excretion	Earthworm casts
Improved soil structure	Improved O_2 and gas diffusion	Breakdown of particles	Fragmented soil particles and increased surface area
Increased porosity		Release of organic residue	Increased water holding capacity
Release of nutrient	Improved moisture drainage and dispersal	Microorganism dispersal and transport	Higher SOM
Organic matter turnover	Improved nutrient movement	Organic matter turnover	Enhanced nutrient content and availability, with increased C:N ratio
Improved fertility	Microorganism promotion and dispersal	Addition of high energy water soluble organic compounds	Increased pH
Microorganism promotion and dispersal		Microorganism promotion	Microorganism promotion

(*Source:* Hickman and Reid, 2008).

In addition to the effects of earthworms upon microbial biotic effects, a number of studies have highlighted direct earthworm biotic effects in the form of feeding behaviors upon contaminant fate. For example, whilst **Ma *et al.* (1995)** noted that leaf litter food made only minimal difference in polycyclic aromatic hydrocarbons (PAH) loss extents, they did note that bioaccumulation of these PAHs was greatly enhanced via food limitation, thus it is a possibility that earthworms increase their oral intake of soil particles when driven by hunger stress. When investigating total petroleum hydrocarbons (TPH) losses in with-earthworm systems that either had or had not received food, **Schaefer *et al.* (2005)** noted that residual TPH in the with-food systems was greater, however, these were not significant values. These results suggest that if

earthworms were applied to suboptimal contaminated soils lacking in organic matter, a greater degree of biological interaction might ensue resulting in greater extents of loss. In further support of this, **Haimi *et al.* (1992)** stated that the lower the organic matter content, the higher the bioaccumulation, which obviously implies increased soil association. This result was confirmed by **Peters *et al.* (2007)** who noted significantly greater uptake of p,p-DDE from soil than from compost by earthworms (*E. fetida* and *L. rubellus*).

2.2.7.3 Earthworm and bioremediation of petroleum and crude oil hydrocarbons

Whilst a relatively large amount of research has been undertaken investigating the effects of earthworms upon agrochemicals, less has been undertaken upon the effects on petroleum hydrocarbons (petroleum and crude oil hydrocarbons) **(Hickman and Reid, 2008)**.

In contrast to the agrochemical studies, many of the subsequent studies are descriptive investigations as opposed to mechanistic ones. However, to highlight the increased interest of earthworm inclusion in bioremediation for hydrocarbons, a recent paper elucidated upon the co-application of compost and earthworms for bioremediation **(Ceccanti *et al.*, 2006)**: the work investigated biochemical processes, including microbial enzyme activity and carbon dioxide evolution, which took place during bioremediation treatment of oil refinery sludge with (1) a mixture of microorganisms, enzymes and nutrients (2) compost only, and (3) compost with earthworms (E. fetida; 10 per kg soil) for 3 months. Their aim was to use these treatments and variables to stimulate soil microbial biomass and therefore degradation ability to explain both microbial activity and the intensity of microbial metabolism. They concluded that the reduction in TPH of the oil refinery sludge was greatest in the treatment with earthworms present (reduction of 50.3%), followed by compost only (reduction of 39.5%), highlighting the

usefulness of the co-application of compost and earthworms. Importantly, they stated that the result could be due to earthworm action and/or soil microbial stimulation via compost addition and earthworm casts. An earlier study **(Schaefer, 2001)** had already concluded that increased microbial catabolic activity due to *E. fetida* presence was responsible for the loss of 91% (1074 mg kg^{-1} to 96 mg kg^{-1}) of crude oil contamination when present for 56 days. Thiswas in comparison to the without-earthworm treatments, which showed no significant difference in concentrations over the same period. Interestingly, it was in the soils with the highest initial concentration that degradationwas fastest.Such results are not always the rule, for example, **Callaham *et al.* (2002)** observed no difference in TPH concentrations associated with *E. fetida* or wheat treatments when applied to petroleum contaminated soils, whilst **Stom *et al.* (2003)**, who investigated the effect of earthworm (*E. fetida*) application (n=5) to crude oil contaminated soils (25 g kg^{-1}) for 40 days with the co-application of microorganisms also observed minimal losses (~15 g kg^{-1} loss for the microbial inoculation and earthworm co-application; ~9 g kg^{-1} loss for the microbial inoculum alone, and only ~3 g kg^{-1} loss for earthworm only treatments). However, in the treatments with earthworms, it was noted that soil structure and porosity were improved, thus facilitating oil losses via the introduced microorganisms. However, little methodological data was provided, thus, full understanding of the underlying mechanisms cannot be discussed. **Schaefer *et al.* (2005)** investigated the effects of differing earthworm species (10 worms/1 kg treatment) (*L. terrestris, Allolobophora chlorotica* and *E. fetida*) on the microbial community within a crude oil contaminated soil (10,000 mg kg−1 TPH) for 28 days. This is one of the few studies that have been undertaken with a view to specifically utilising earthworms for bioremediation of organic contaminants. It was noted that both respiration and concentration of microbial biomass was significantly enhanced, with observed changes not only in microbial

numbers and activity but of taxonomic groups, whilst TPH concentrations were significantly reduced (TPH reduction of 30–42% in samples with *L. terrestris*, 31–37% in samples with *E. fetida* and of 17–18% in samples with *A. chlorotica* with an average control TPH concentration decrease of a non-significant 9–17% between test start and test end). Whilst positive earthworm usefulness within bioremediation was noted, there were differences in results between earthworm species due to ecological behavioural differences, thus reinforcing the importance of choosing species suited to specific environmental conditions. **Schaefer and Filser (2007)** further reported upon their earlierwork **(Schaefer *et al.*, 2005)**, which utilized the three differing earthworm species to effect TPH losses in crude oil contaminated soils. Interestingly, they noted that there was more degradation products in the with-earthworm soils, indicating that degradation of the longer chain aliphatics (C29–C36) had occurred, thus resulting in an increase of shorter chain aliphatics (C19–C29). Such an observation was reported by **Prince *et al.* (2007)**, who stated that within a complex mixed gasoline mixture; larger n-alkanes were more readily degraded than the smaller n-alkanes. Further to this, **Schaefer and Filser (2007)** introduced organic additives (coffee grains, horticultural waste (compost) and brewery mash) (as 10% mass) in combination with earthworms (*L. terrestris*) to assess microbial crude oil (5000 mg kg−1) degradation over 28 days. Whilst increased microbial respiration was observed in all treatments, only significant TPH degradationwas noted in the earthworm only treatments (decrease of 29%) and the mixed brewery mash treatment with earthworms (19%), whilst the brewery mash without earthworms resulted in a decrease of 35%. Other additives had no significant effects upon TPH degradation. It was noted by the authors that the earthworms did not interact or feed on the additives, thus minimizing the co-application potential. They concluded that the microorganisms preferred the more easily degradable organic additives as opposed to the more complex hydrocarbons.

Considering that compost proves to be useful in bioremediation procedures and its use as a remediation amendment in combination with earthworms has been demonstrated by **Ceccanti *et al.* (2006)**, it was surprising that the other additives did not determine any significant TPH decreases. **Tomoko *et al.* (2005)** also investigated the addition of the earthworm (*E. fetida*) and varying organic wastes to an oil contaminated soil and found that earthworms alone significantlydecreased oil contents in comparison to the control. Earthworms used to remediate hydrocarbon contaminated drilling fluids and cuttings has been investigated **(Getliff *et al.*, 2002)**. However, as opposed to direct earthworm inclusion to hydrocarbon impacted soils to effect remediation, the process described by **Getliff *et al.* (2002)** involves adding the drilling wastes to other greenwastes, altering the C:N ratio and the moisture content and adding the mixture to worm beds as feed. Worm beds were mounds 88 m×3 m covered with polypropylene-backed felt mat, with moisture and aeration capabilities. The drilling materials/greenwasteswere applied to the mounds once a week at an average depth of 15–30 mm. The worms (*E. fetida*) integrate with the applied material and consume it within 5–7 days. Interestingly, the digested material (wormcast,or vermicast/compost) can be harvested and packaged for fertilizer or as a soil improver. Over an experimental period of 45 days and at application of 30% w/w (drill cuttings/organic material), 50% w/w or 70% w/w, significant TPH reductions were observed. At 30% application, TPH decreased from 1900 mg/kg to less than 60 mg/kg in 45 days, whilst at 50% application TPH decreased from 2100 mg/kg to below detection limit and at 70% application, TPH decreased from 20,000 mg/kg to 1500 mg/kg. However at a tested application of 100% drill cuttings, the earthworms did not associate with the material and thus degradation was minimal. The difficulty in the removal of recalcitrant hydrocarbons and the lack of biodegradability by inherent microorganisms of high molecular weight compounds is known

(Atlas, 1995; Alexander, 2000). This aspect of bioremediation is often a final limiting factor. **Martin-Gil *et al.* (2007)** has recently investigated the use of earthworms (*E. fetida*) and vermicomposting in the treatment of the high molecular weight hydrocarbons asphaltens, from the Prestige oil spill. Green waste (cow bed and potato peelings) (total mass 80%) was added to heavy fuel oil (20%), which then underwent a composting process. Subsequent to the composting process, earthworms were added at a density of 330 g/m^2in a treatment vessel $0.40\times0.50\times0.12$ m^3 for 6 months. Definite values of asphaltens reduction were not given, but they did state that their results revealed that the microorganisms living in either the earthworm intestines or vermicompost substrate can possibly mineralize and thus eliminate asphaltens.

2.2.8 Earthworms and Heavy Metal Uptake in Contaminated Soil

Within the field of ecological risk assessment, it is widely recognized that earthworms are an important component of the soilbiota. For this reason, much work has focused on metal accumulationand toxicity to earthworms.Earthworms have been reported as being able to accumulate heavy metals, from soil, in their bodies, complex the metals with other compounds thereby making them less toxic**(Jain and Singh, 2004)**.It is also supposed that the activities of earthworms increase the mobility and bioavailability of heavy metals in soils **(Wen *et al.*, 2004)**.Earthworms constitute more than 90% of the invertebrate biomass in soils **(Ireland, 1983)** and are suggested to be an appropriate tool to predict bioaccumulation in the terrestrial food chain **(Rhett *et al.*, 1988; Hinton and Veiga, 2002)**. Several studies have shown that earthworms accumulate trace metals such as Cu, Pb and Zn **(Ma, 1982; Ireland, 1983; Morgan and Morgan, 1988; Bengtsson and Transvik 1989)**.

Heavymetal accumulation in earthworm tissue has been reported to be dependent not only on the concentration of the substrate they consume, but also on ecological and species-specific physiological properties of different earthworms, such as efficiency of detoxification mechanisms, gut morphology, quantity of metal-binding ligands, consumption rates of the food material and feeding behavior **(Ma, 1982; Morgan and Morgan, 1992, 1999)**.

Furthermore, previous studies on metal uptake by earthworms show that the magnitude of accumulation and steady-state tissue metal concentrations are a function of either total or ‘‘available’’ metal concentration in the soil. The available metal concentrations are controlled by soil parameters such as pH, organic matter content, clay content, cation exchange capacity and the concentration of Fe- and Al-oxyhydroxides**(Nahmani *et al.*, 2009)**.The uptake of lead by earthworm showed a linear increase with time **(Davies*et al.*, 2003)**.

However, surprisingly little is known about the kinetics of uptakeand excretion of metals by earthworms**(Nahmani *et al.*, 2009)**. The majority of availabledata focus on Cd, Cu, Pb and Zn **(Spurgeon and Hopkin, 1999; Conder *et al.*, 2002; Vijver *et al.*, 2005)** though data exist for otherelements such as Mn, Cs, I **(Sheppard *et al.*, 1997**), As, Cr, Ni**(Peijnenburg *et al.*, 1999)** and Co **(Crossley *et al.*, 1995)** as well.

Various researchers have studied and derived models that predict metal uptake and excretion kinetics by earthworms**(Nahmani *et al.*, 2007)**. Notable amongst these are;

1. **Spurgeon and Hopkin (1999)**:

$$C_{iworm}(t) = C_i(0) + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \quad (2.1)$$

$$C_{iworm}(t) = C_i(0) + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) - \frac{a}{k_2}\left(1 - e^{-k_2 (t_c - t)}\right) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots . (2.2)$$

Where: t_c= time at which animals were transferred to clean soil (days)

Equation (2.1) holds for $t < t_c$: (accumulation phase)

Equation (2.2) holds for $t > t_c$: (elimination phase)

2. **Van Straalen *et al.*, 2005**

A one-compartment model was used to analyze the development of earthwormbody concentrations with time, and to calculate uptake and elimination rate constants. Equation (2.3) was used for essential elements and Equation (2.4) for non-essential.For non-essentialmetals this initial metal load may be excreted when the earthworm istransferred to the test soil.

$$C_{iworm}(t) = C_i(0) + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots . \ldots \ldots . \ldots \ldots (2.3)$$

$$C_{iworm}(t) = C_i(0)e^{-k_2 t} + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots .. \ldots \ldots (2.4)$$

Where: $C_{iworm(t)}$ = metal concentration in the earthworm (µg g^{-1} dry weight) at time t (days), a = flux of metals into the earthworm (mg_{metal} kg^{-1} animal day^{-1}),k_2 = elimination rate constant (day^{-1}). $C_i(0)$ is the initial body concentration (µg g^{-1}dry weight), which is measured in the animals before exposure.

2.3 SOIL

Soils are made up of four basic components: minerals, air, water, and organic matter. In most soils, minerals represent around 45% of the total volume, water and air about 25% each, and organic matter from 2% to 5%. The mineral portion consists of three distinct particle sizes classified as sand, silt, or clay. Sand is the largest particle that can be considered soil. Sand is largely the mineral quartz, though other minerals are also present. Quartz contains no plant nutrients, and sand cannot hold nutrients, they leach out easily with rainfall. Silt particles are much smaller than sand, but like sand, silt is mostly quartz. The smallest of all the soil particles is clay. Clays are quite different from sand or silt, and most types of clay contain appreciable amounts of plant nutrients. Clay has a large surface area resulting from the plate-like shape of the individual particles. Sandy soils are less productive than silts, while soils containing clay are the most productive and use fertilizers most effectively. *Soil texture* refers to the relative proportions of sand, silt, and clay. A loam soil contains these three types of soil particles in roughly equal proportions. A sandy loam is a mixture containing a larger amount of sand and a smaller amount of clay, while a clay loam contains a larger amount of clay and a smaller amount of sand. Another soil characteristic -soil structure - is distinct from soil texture. *Structure* refers to the clumping together or, aggregation, of sand, silt, and clay particles into larger secondary clusters.

Goodstructure is apparent when the soil crumbles easily in the hand. This is an indication that the sand, silt, and clay particles are aggregated into granules or crumbs. Both texture and structure determine pore space for air and water circulation, erosion resistance, looseness, ease of tillage, and root penetration. While texture is related to the minerals in the soil and does not change with agricultural activities, structure can be improved or destroyed readily by choice and timing of farm practices.

Largequantities ofpollutants have continuously been introduced intoecosystems as a consequence of urbanization andindustrial processes. Metals are persistent pollutants thatcan be biomagnified in the food chains, becomingincreasingly dangerous to human beings and wildlife.Therefore, assessing the concentrations of pollutants indifferent components of the ecosystem has become animportant task in preventing risk to natural life and publichealth. Heavy metals enter into the environment mainlyvia three routes namely: (i) deposition of atmosphericparticulate, (ii) disposal of metal enriched sewagesludges and sewage effluents and (iii) by-products frommetal mining process. Soil is one of the repositories foranthropogenic wastes. Biochemical processes canmobilize them to pollute water supplies and impact foodchains. Heavy metals such as Cu, Cr, Cd, Ni, and Pb arepotential soil and water pollutants. Globally, the problemof environmental pollution due to heavy metals hasbegun to cause concern in most large cities since thismay lead to geoaccumulation, bioaccumulation andbiomagnifications in ecosystems. The Nigerian soil quality standard by the environmental regulatory body is presented in Table 2.6.

Table 2.6: Soil Quality Standards

Parameters	**Guideline value**	
	(mg/kg dry weight)	**% composition**
Arsenic	20	0.00200
Barium	400	0.04000
Cadmium	3	0.00030
Chromium (Cr^{+6})	100	0.01000
Cobalt	50	0.00500
Copper	100	0.01000
Lead	164	0.01640
Mercury	4	0.00040
Molybdenum	40	0.00400
Nickel	70	0.00700
Tin	50	0.00500

Zinc	421	0.04210
Benzene	0.1	0.00001
Toluene	0.1	0.00001
Xylene	0.1	0.00001
Styrene	0.1	0.00001
Hexane	0.5	0.00005
Heptanes	0.5	0.00005
Flourine	100	0.00100
Cyanide	5	0.00050
Phenol	10	0.00100

Source: National Environmental Regulation (2009).

2.3.1 The Importance of Soil Organisms

An acre of living topsoil contains approximately 900 pounds of earthworms, 2,400 pounds of fungi, 1,500 pounds of bacteria, 133 pounds of protozoa, 890 pounds of arthropods and algae, and even small mammals in some cases**(Preston, 2004)**. Therefore, the soil can be viewed as a living community rather than an inert body. Soil organic matter also contains dead organisms, plant-matter, and other organic materials in various phases of decomposition. Humus, the dark-colored organic material in the final stages of decomposition, is relatively stable. Both organic matter and humus serve as reservoirs of plant nutrients; they also help to build soil structure and provide other benefits. The type of healthy living soil required to support humans now and far into the future will be balanced in nutrients high in humus, with a broad diversity of soil organisms. It will produce healthy plants with minimal weed, disease, and insect pressure. Nativeprairies and forests soils are tilled by soil organisms. The soils are covered with a layer of plant litter and/or growing plants throughout the year. Beneath the surface litter, a rich complexity of soil organisms decompose plant residue and dead roots, then release their stored nutrients slowly over time. In fact, topsoil is the most biologically diverse part of the earth. Soil-dwelling organisms release bound-up minerals, converting them into plant-available forms that

are then taken up by the plants growing on the site. The organisms recycle nutrients again and again with the death and decay of each new generation of plants. There are many different types of creatures that live on or in the topsoil. Each has a role to play. While a great variety of organisms contribute to soil fertility, earthworms, arthropods, and the various microorganisms merit particular attention.

Type specimens of bacterialstrains used in bioremediation exist in various repositoriesor are commerciallyavailable and are usually covered by patent rights. Eachof these strains may yield dramatic results in vitro forspecific target compounds. However, the overall successof such strains in treating a wide range of contaminants*in situ* remains limited. The reintroduction of indigenousmicroorganisms isolated from a contaminated site afterculturing seems to be a highly effective bioremediationmethod, especially when microbial growth is supplementedby oxygen and fertilizers. Thus, bioremediationis normally achieved by stimulating theindigenous microbiota (naturally occurring microorganisms).Stimulation is achieved by the addition ofgrowth substrates, nutrients, terminal electron acceptor,electron donors, or some combination therein, resultingin an increase in contaminant biodegradation and biotransformation**(Yehuda, 2002)**.

2.4 PETROLEUM HYDROCARBON AND BIOREMEDIATION

Bioremediation, a process that utilizes the capability of microorganismto degrade toxic waste, is emerging as a promising technology for thetreatment of soil and groundwater contamination. The technology is very effectivein dealing with petroleum hydrocarbon contamination. Some microorganisms can decompose or transform the chemical substancespresent in petroleum and petroleum derivatives. A single microorganism can degrade only certain types of petroleumcompounds, but a mixed population – microbial community enables a higher levelof

degradation. Moreover, some substances can be decomposed only by co-metabolism**(Jelena *et al.*, 2009)**.

Given favorable environmental conditions, all naturalorganic compounds degrade. Petroleumis, in one sense, a natural product, resulting from theanaerobic conversion of biomass under high temperatureand pressure. Most of its components are subject tobiodegradation, but at relatively slow rates. Petroleumhydrocarbons can be divided into four classes: saturates,aromatics, asphaltenes (phenols, fatty acids, ketones,esters, porphyrins), and resins (pyridines, quinolines,carbazoles, sulfoxides, amides).Hydrocarbons differ in their susceptibility to microbial attack and generally degrade in the following order of decreasing susceptibility: n-alkanes >branched alkanes >low-molecularweightaromatics >cyclic alkanes **(Perry, 1984)**.Most common pathway of alkane degradation isoxidation at the terminal methyl group; alkane →alcohol → fatty acid → ketone → CO_2 and H_2O.

Microorganisms are capable of aerobicallymetabolizing single-ring aromatic hydrocarbonsubiquitous in the subsurface. PAHs with two or three rings such asnaphthalene, anthracene, and phenanthrene aredegraded at reasonable rates when O_2 is present. PAHs with four rings such as chrysene, pyrene,and pentacyclic compounds are highly persistent and are considered recalcitrant. The ease with which microbes degrade organic compounds is presented in Table 2.7.The biodegradation of hydrocarbons is generally an aerobic process.As high as 20% of all soil microbes (bacteria, fungiand yeast) are capable of degrading aliphatichydrocarbons.

Table 2.7: Ease of biodegradability of some organic compounds.

Hydrocarbons	**Ease of degradation**

Simple hydrocarbons, C1 – C15	Very easy
Alcohols, phenols, amines	Very easy
Acids, esters, amides	Very easy
Hydrocarbons, C12 – C20	Moderately easy
Ethers, monochlorinated hydrocarbons	Moderately easy
Hydrocarbons, >C20	Moderately difficult
Multichlorinated hydrocarbons	Moderately difficult
PAHs, PCBs, pesticides	Very difficult

Petroleum-based products are the major source ofenergy for industry and daily life. Petroleum is also theraw material for many chemical products, such asplastics, paints, and cosmetics. The transport of petroleumacross the world is frequent and the amounts ofpetroleum stocks in developed countries are enormous.Consequently, the potential for oil spills is significant.

The volume of spills usually exceeds the inherent remediationcapacity for any given environment, resulting ina significant ecological impact. The rate of biodegradation depends on both physicochemical and biological variables.The ability of hydrophobic organic compounds to be solubilized and transported into the immediate vicinity of bacterial cells capable of metabolizing them is potentially the rate-limiting step in bioremediation.

2.5 LUBRICATION AND ENGINE OILS

Lubrication is simply the use of a material to improve the smoothness of movement by reducing friction. The immediate result is reduced wear and reduced heat generation.

Lubricating oils are used for the majority of applications. They may be classified according to their viscosities and any special properties imparted to them by additives. Oils whose base stocks are derived primarily from crude oil refining are called mineral or petroleum oils. Petroleum oils may be further classified as being paraffinic or naphthenic based on the types of hydrocarbons comprising the base stock. Oils that have been manufactured by chemical synthesis such as polymerization are called synthetic oils. Additivesmay be blended into the base stock to impart special properties to the finished product**(Baladincz*et al.*, 2008)**. Some common lubricant additives are given in Table 2.8.

Pure mineral oil has good lubricating properties, but it is the additives in the oil that give it its characteristics and enhance its lubricating properties to enable it to meet modern requirements.Different oils and different materials and loading conditions can create vastly different coefficients of friction that can affect performance and longevity of an engine and other mechanical components. A few basic key functions of a motor oil is to reduce friction under all extremes of operating condition, prevent corrosion of internal engine components and provide for cooling via transfer of heat.

Table 2.8: Common lubricant additives

Additives	Compound Used	Function
Extreme Pressure (EP)	Sulphur-Phosphorus compounds	Increase load carrying ability
Antioxidant	Hindered Phenols, Amines	Prolonged oil life by slowing oxidation
Antifoam	Silicones	Control foaming in forcefeed systems
Anticorrosion	Zinc dithiophosphate (ZDDP)	Foam anticorrosion film
Detergent or Dispersant	Calcium sulphonates	Prevent deposition of carbon
Alkalinity	Calcium carbonate, Magnesium Sulphonates	Neutralize acidic engine byproducts.
Antiwear	Zinc dithiophophates (ZDDP)	Reduce wear.
Pour Point Depressant	Methacrylate polymers	Lower pour point
VI Improver	Organic Polymers	Improve IV

Source: Naval Ship's Technical Manual, 1999.

Oxidation is the most important form of chemical breakdown of a motor oil and itsadditives. The chemicals in a motor oil are continuously reacting with oxygen inside anengine. The effects of oxidation due to this reaction as well as the by-products ofcombustion produce very acidic compounds inside an engine. These acidic compoundslead to corrosion of internal engine components, changes in oil viscosity, formation of varnish,sludge and other insoluble oxidation products that can cause performance and durabilitydegradation of engines over a period of time. The products of oxidation are lessstable than the original base hydrocarbon molecular structure and as they continue to beattacked by these acidic compounds can produce varnish and sludge.

The environmental problem caused by the high quantity of used lubricating oil is currently attempted to be resolved simultaneously from multiple sides. On one hand, it is an important criteria to ensure the biodegradability of modern engine oils and other industrial oils.Onthe other hand, increase of the oil replacement period also constitutes one of the main objectives of research and development activity. Apart from these, several modern processeswere also developed for the effective recycling of used engine oils and other industrial oils**(Baladincz*et al.*, 2008)**.

2.5.1 Properties of used engine oil

Used engine oil is of variable composition depending on the composition ofthe original oil and the degree of degradation. Used engine oils contain contaminating materials due to wearing and combustion, and their chemical composition also differs from that of new oils due to oxidation and other chemical reactions. Most important contaminants materials in used engine oils are as detailed below.

2.5.1.1 Combustion products

Water: In the case of a perfect process, engine fuel combusts into carbon monoxide and water. In normal operating conditions the steam vents through the exhaust system, though after cold-start a part of the water may get also into engine oil and aid in sludge formation at the bottom of oil sump.

Carbon black: In diesel engines, carbon black also forms as a product of combustion, a part of which gets into engine oil.

Engine fuel: a part of the non-combusted engine fuel accumulates also in engine oil.

2.5.1.2 Contaminations originating from wearing

Metals: majority of metal particles coming from the cylinder wall and from the piston get into the engine oil. Such particles may include: iron (400-800 mg/kg), chromium (30-50 mg/kg), copper (2-5mg/kg), magnesium (150-400 mg/kg), nickel (2-7 mg/kg), calcium (600-2000mg/kg), aluminium (300-800 mg/kg), arsenic (0-5 mg/kg) etc. Other parts of the metals originated from additives that are originally in engine oil (for example zincdialkyl-dithio-phosphates)**(Baladincz*et al.*, 2008)**.

Dust: Dust and other solid contaminating materials (mainly silicates) get into engine oil through air filter and promote sludge formation.

2.5.1.3 Oxidation products

Lubricantsalso contain oxidation products forms as a result due to the heat load exercised in normal operating conditions within engine space. Theyform complexes with metals and are basically organic acids.

2.5.1.4 Halogenised hydrocarbons

Totalhalogenic content of used engine oils is between 500-1000 mg/kg in general. These may include substances such as PCB's (polychlorinated Biphenyls).

Other contaminats may include polycyclicaromatic hydrocarbons (PCAH's) and glycols.

Due to the contaminating materials present in relatively high amount, certain analytical and application properties of used engine oils change as well. Its extent is also affected by the composition of engine fuel (for example: the vegetable oil fatty acid methyl ester content of

diesel gas oils). Properties such as viscosity (of used engine oils), significantly decreases with the increase of engine fuel concentration.

2.5.2 Used Oil and the Environment

Most of the concern about used oil relates to poor management practices some of which are:

a. Throwing oil in the garbage: This may result in groundwater contamination if it goes to landfills with inadequate liners.
b. Storm sewer disposal: This may create oil sheens that have negative effects on biota and may cause clogging of lines and pumps.
c. Road oiling: This may kill plants and grasses in the immediate vicinity of use and contaminate groundwater through the release of petroleum hydrocarbons.
d. Burning as fuel in small commercial boilers: This is generally safe except in the case of high contaminant levels or inadequate pollution control devices.

2.6 CELL GROWTH KINETICS IN A BATCH REACTOR

When living cells are added to a solution of essential nutrients at a suitable temperature and pH the cells grow. For unicellular organisms, increase in biomass (mass of living matter) is accompanied by increase in the number of cells present.

Associated with cell growth are two other processes: uptake of some material from the cell's environment and release of metabolic end products into the surroundings.

In a typical batch reactor the number of living cells varies with time, as shown in Figure 2.2. After a lag phase, where no increase in cell number is evident, a period of rapid growth ensues, during which the cell number increases exponentially with time. Since cell multiplication cannot

continue indefinitely in a closed vessel, a stationary phase follows the period of exponential growth during which the population achieves its maximum size. Eventually a decline in cell numbers occurs during the death phase.Each phase is of importance in design: to minimize the length of the lag phase, maximize the rate and length of the exponential phase, to slow down the onset of transition to stationary phase.

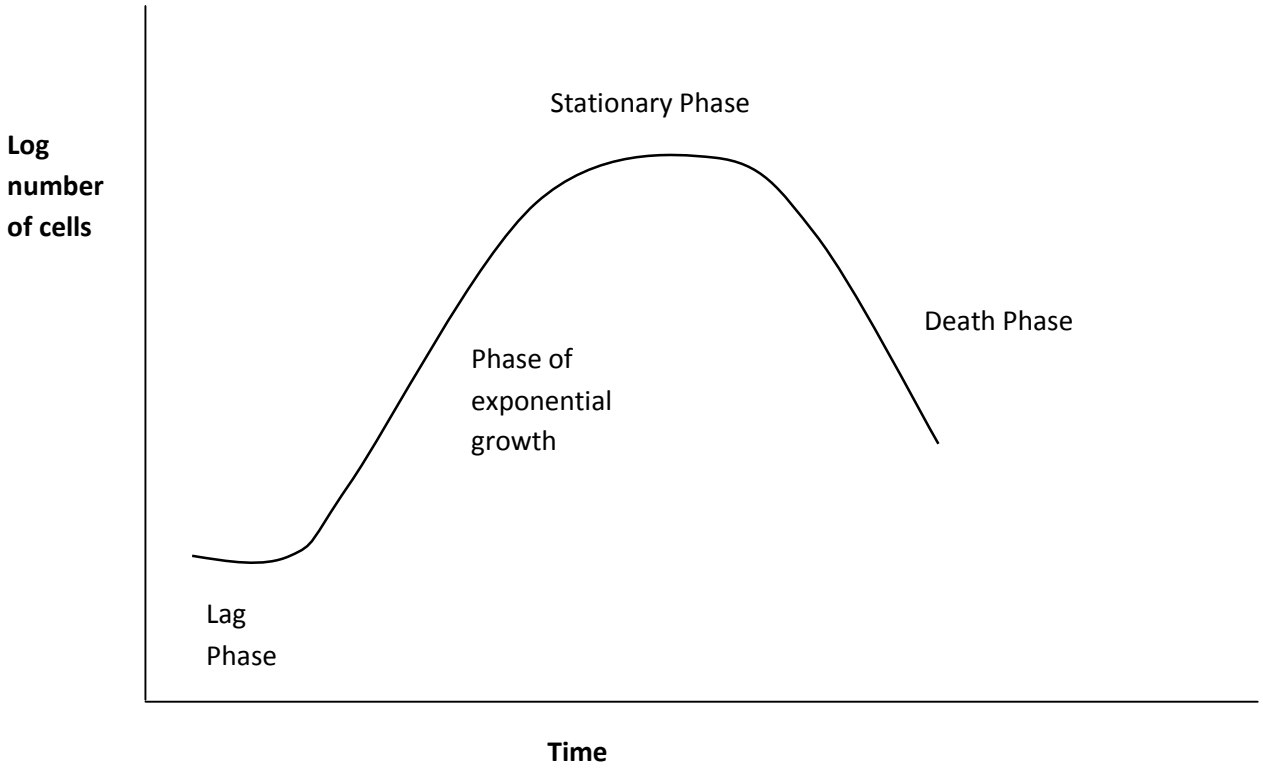


Figure 2.2: Typical growth curve for batch cell cultivation

The length of the lag phase depends on changes in nutrient composition. The shock of a switch to a new environment has effects on the living cells: the cells have to adapt to the new environment necessarily resulting in a time interval of insignificant cell division rate. Multiple lag phases may

sometimes be observed when the medium contains multiple carbon sources. This phenomenon is known as diauxic growth and is caused by a shift in metabolic patterns in the midst of growth.

At the end of the lag phase, the population of microorganisms is well adjusted to the environment. The cells can then multiply rapidly. The equation

$$\frac{dx}{dt} = \mu x \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.6)$$

with $x = x_o$ at $t = t_{lag}$

describes the increase in cell numbers during this period and

$$x = x_o e^{\mu(t-t_{lag})} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.6)$$

$t > t_{lag}$

Thus only μ is required to characterize the population during exponential batch growth. Useful growth kinetic data may be obtained from batch experiments confined to the experimental growth phase.

During the stationary phase, some cells are dividing while others die. Often the dead cells lyse (break open) and the carbohydrates, amino acids and other components freed from the lysed cells are then used as nutrients by the remaining living members of the population. Eventually due to nutrient depletion and toxic product buildup, the population cannot sustain itself and the death phase sets in.

Usually death of the population is assumed to follow an exponential decay:

$$x = x_s e^{-k_d t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.7)$$

Where t denotes the time elapsed after the onset of the death phase.

Various kinetic models have been proposed to account for the various transient growth situations of cell population. They are generally grouped as structured and unstructured growth kinetic models. A structured model attempts to explicitly describe intracellularprocesses in both a structural and physiological sense, and thus offersthe most realistic representation of a cell. Structured models are usuallysingle-cell models that look in some detail at individual processes and reactions.However, whereas cell behavior to varying growth conditions is notthoroughly understood, a structured model is not applicable to most of celllines, for example to CHO cells. Therefore, the majority of the kinetic modelsin literature are categorized as unstructured: they rely on the global relationshipsbetween cell growth and environmental properties of the culture,and neglect intracellular processes.

2.7 KINETICS OF BIOREMEDIATION OF USED ENGINE OIL CONTAMINATED SOIL

Biological processes may be classified as fermentations, elementary physiological processes and the action of living entities. Fermentations are reactions wherein a raw organic feed is converted into a product by the action of microbes or by the action of enzymes. The key distinction between the two types of fermentation is that in enzyme fermentation the catalytic agent, the enzyme, does not reproduce itself, but acts as an ordinary chemical, while in microbial fermentation the catalytic agent, the cell or microbe, reproduces itself. Within the cell, it is the enzyme which catalyses the reaction, just as in enzyme fermentation (**Levenspiel, 1999**). Bioremediation may thus be classified as microbial fermentation.

Bioremediation of used engine oil contaminated soil by microorganisms has been shown to be a function of several parameters some of which include the pH, nature of soil, moisture content, nature of bacteria consortium, temperature, quantity and nature of organic amendment, presence of heavy metals etc.

The first order bioremediation kinetics has been shown to describe the change in TPH with time in the process of bioremediation**(Yeung *et al.*, 1997; Adesodun and Mbagwu, 2008)**:

$$y = ae^{-kt} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(2.8)}$$

In Equation (2.8), y is the residual hydrocarbon content in soil(g kg^{-1}),

a is the initial hydrocarbon content in soil(g kg^{-1}),

k is the biodegradation rate constant(day^{-1}),and

t is time (day).

Half life was computed as

$$Halflife = \frac{\ln(2)}{k} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(2.9)}$$

The factors involved in bioremediation among other include: the nature and type of organisms, moisture content, temperature, degree of agitation, degree of aeration, nature, type and concentration of organic amendment. Since more than one phase is usually present, the movement of material from phase to phase must be considered in the rate equation. Thus the rate expression in general will incorporate mass transfer terms in addition to the usual chemical kinetics term **(Levenspiel, 1999)**. This implies that the mass transfer terms and the usual

chemical kinetics terms are incorporated in *k*. Examples of frequencies of mixing that have been used in bioremediation studies to improve aeration include: twice a month **(Bagherzadeh-Namazi *et al*,. 2008)**, twice a week **(Margesin *et al.*, 2000)**, every alternative day **(Vasudevan and Rajaram, 2001)** and every day **(Maike and Filser, 2007)**.

2.8 ENVIRONMENTAL BIOTECHNOLOGY AND THE CHEMICAL ENGINEER

Over the past few decades large quantities of industrial pollutants have been discharged into the environment. Thosestructurally related to natural compounds, are readily degraded or removed by microorganisms found in soil and water. However, there is also an increasing number of novel industrial compounds rarely found in nature. These xenobiotic compounds are usually removed slowly and tend to accumulate in the environment. They can accumulate and cause severe environmental problems due to their high degree of toxicity.

The development of alternative, economical and reliable biological treatments becomes increasingly important because of the problems associated with pollutant treatment by conventional methods, such as incineration or landfills. Although natural microorganisms collectively exhibit remarkable evolutionary capabilities to adapt to a wide range of chemicals, natural evolution occurs at a relatively slow rate, particularly when the acquisition of multiple catalytic activities is necessary. In these cases, the acceleration of these events via genetic engineering/processing engineering is helpful since the desirable traits can be carefully designed and controlled. The drive toward this goal represents the essence of environmental biotechnology**(Chen *et al*., 2005)**.

Environmental biotechnology deals with the utilization of microorganisms to improve environmental quality. Although the field of environmental biotechnology has been around for decades, starting with the use of activated sludge and anaerobic digestion in the early 20th century by civil engineers, the introduction of new technologies from modern microbiology and molecular biology has enabled engineers and scientists to tackle the more contemporary environmental problems such as detoxification of hazardous chemicals. Chemical engineers are uniquely poised to contribute in this emerging area since many of the potential solutions require a combined perspective from modern biology and process engineering, two areas where chemical engineers excel**(Chen *et al.*, 2005)**.

For example, the realization of environmental biotechnology into practical solutions requires the implementation of process design, which is the foundation of the chemical engineering discipline. The same is also true at the cellular level, where the functions of cells are determined primarily by networks of specific catalytic reactions. The nature and activities of these networks are dictated by the genetic information, thereby defining the ways in which engineers can influence cellular functions and metabolic capabilities toward the design of improved biocatalysts for environmental remediation**(Bailey, 1991)**. Although, superficially these strategies seem distant from traditional chemical engineering, a deeper inspection reveals that the design of biological catalysts, based on defined techniques from biochemistry and biology is indeed parallel to our understanding of chemical kinetics, transport, separation, and control. In addition, advances in genomics and proteomics are providing opportunities to predict, in a quantitative manner, the potential manipulations necessary. Even though chemical engineers are well prepared to contribute new research directions in environmental biotechnology, only a few are working in this area today. Fortunately, the number of chemical engineers showing interest is

growing every year and with the recent research emphasis on biotechnology, it is easy to envision that many others will join this exciting research area in the near future **(Chen *et al.*, 2005)**.

2.9 SAFETY CONSIDERATIONS

The safety measures required for this research work include:

1 Handling of used engine oil contaminated soil: Used engine oils are thought to contain carcinogenic substances as such would require being prevented from coming into contact with the skin. Laboratory coat, hand gloves, and safety goggles must be worn. To prevent inhalation of soil particles, nose mask should be worn.

2 Laboratory coat, hand gloves, and safety goggles must be worn. There should be provision for a large quantity of water for washing in case of an accident.

3 Handling of poultry manure: the area around the experimental rig should be well ventilated to take care of the foul smell from the manure.

4 Care should be exercised in handling toluene and dichloromethane. Appropriate nose mask is recommended.

CHAPTER 3

MATERIALS AND METHODS

This chapter gives the description of equipment and instruments used in the course of this investigation. Details of the experimental proceduresemployed are also presented.

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3.1 EQUIPMENT

The major equipment used in the course of the study were:

1. pH meter (Kent EIL 7055)
2. Oven (Tamson, Heraeus T500)
3. Gas Chromatograph (GC) (GCMS-QP2010 Plus Shimadzu, Japan)
4. Spectrophotometer (B. Bran Scientific and Instrument Company, 722 – 2000 20D spectrometer)
5. X-ray fluorescence (MiniPal PW4025, Philips Analytical)
6. Weighing balance (Berkel, max wt 15kg)
7. Analytical balance (Adam PW 184)
8. Vacuum pump (Edwards, ES 100)
9. Shaker (*Tamson,* Holland)
10. Sieve shake GRAVQUICK (A/S) EFL2 MK3
11. Colony counter (Stuart Scientific Colony Counter, Model No: R000102672)
12. Microscope (Olympus, Model 280317, max mag x 1000)
13. Thermometer (0 – 100^{o}C)

3.2 APPARATUS

1. Hand trowel
2. Evaporating dishes
3. Sieves
4. Glass funnel
5. Muslin cloth
6. Plastic containers with tight fitting lids (2liters).
7. Spatula
8. Measuring cylinders (5, 10, 20 and 250mls)
9. Syringes (2, 5 and 10mls)
10. Sample bottles
11. Filter papers (Whatman No 42)
12. Plastic containers of various cross-sectional areas (70 – 250cm^2)
13. Stop watch (Hanhatl mechanical analog stopwatch)
14. Glass rod
15. Sieves
16. Bent glass rod
17. Flat bottom flask (50, 250 and 500mls)
18. Bijou bottle
19. Pipettes
20. Glass rod

3.3 MATERIALS

The major materials used were:

1. Earthworms
2. Cattle dung (from National Animal Production and Research Institute (NAPRI), Ahmadu Bello University, Zaria)
3. Poultry manure (from National Animal Production and Research Institute (NAPRI), Ahmadu Bello University, Zaria)
4. Toluene (Analytical grade, BDH Chemicals Ltd England)
5. Dichloromethane (Analytical grade, BDH Chemicals Ltd England)
6. Nutrient Agar (Oxoid England procured from HADDIS, Samaru)
7. MacConkey Agar (Oxoid England procured from HADDIS, Samaru)
8. Calcium carbonate (Analytical grade, BDH Chemicals Ltd England)
9. Chromic acid (Analytical grade, BDH Chemicals Ltd England)
10. Deionized and distilled water
11. Soil (petroleum hydrocarbon contaminated and uncontaminated soil samples)
12. Normal saline
13. Plate count agar (Oxoid England procured from HADDIS, Samaru)
14. Citrated human plasma (from Microbiology Department A.B.U. Zaria)
15. Hydrogen peroxide (BDH, from HADDIS, Samaru)
16. Simmon's citrate agar (Oxoid England procured from HADDIS, Samaru)
17. Peptone water (Oxoid England procured from HADDIS, Samaru)
18. Kovac's reagent (Oxoid England procured from HADDIS, Samaru)

19. Methyl-red VOGES-PROSKAUER Broth(Oxoid England procured from HADDIS, Samaru)
20. Alpha naphthol (BDH Chemicals Ltd England, from HADDIS, Samaru)
21. Ethanol (Analytical grade, BDH Chemicals Ltd England)
22. Potassium hydroxide(Analytical grade, BDH Chemicals Ltd England)
23. Filter paper

3.4 COLLECTION OF SOIL SAMPLES, MANURE, *MORINGA* LEAVES, CASSAVA PEELS AND EARTHWORMS

Soil samples from mechanic workshops were collected and analyzed to confirm that they were trulycontaminated with petroleum hydrocarbon. This section presents details of the investigation.

3.4.1 Enumeration of Mechanic Workshops

A tour was made round Zaria town, Nigeria (Latitude: 11° 4' 0 N, Longitude: 7° 42' 0 E) and mechanic workshops were enumerated namely: motorcar (MM), motorcycle (MC) and trucks/lorries (HD). The workshop managers were interviewed for vital information like the age of the workshop, number of times the site was sand filled, if any, and if UEO was usually dumped on the soil.The area coveredis shown on the Google Earth image presented in Figure 3.1.

3.4.1.1 Collection of Soil Samples

Based on the spread and the number of the various types of workshops along the enumeration route, soil samples were collected from MC, MM and HD so as to give a fair representation of what obtains in the soil in mechanic workshops in Zaria. In sample collection, soil was collected

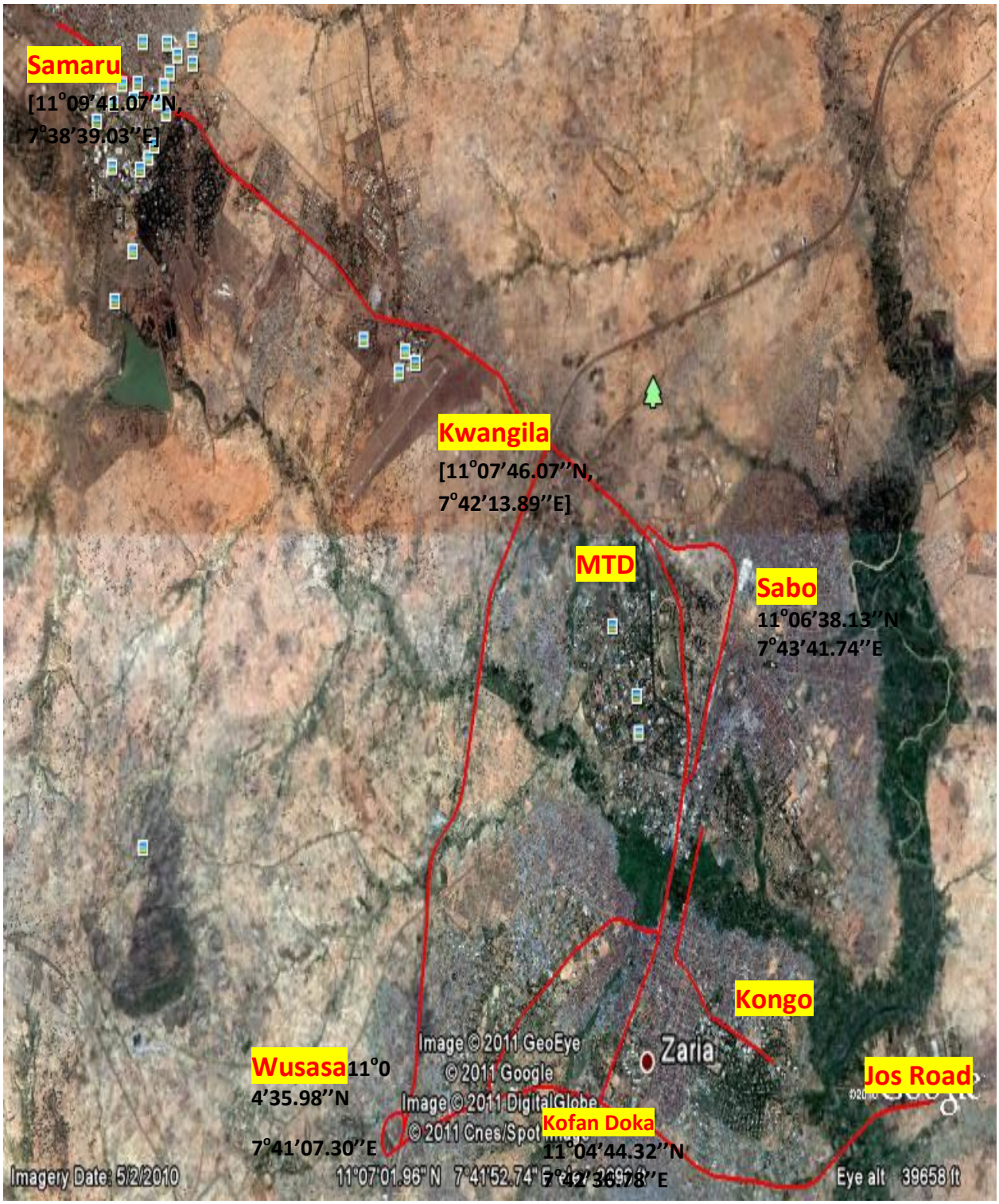


Figure 3.1: Google Earth map of Zaria (Latitude: 11° 4' 0 N, Longitude: 7° 42' 0 E) showing the area covered during the enumeration of mechanic workshops

from the centre of the workshop using a hand trowel to dig to a depth of six (6) centimeters (that is the top soil, where the concentration of the contaminants is expected to be high). Samples were collected into cleansterilized plastic containers.

Used engine oil uncontaminated soil (UEO free soil) was collected from the farmland of Ahmadu Bello University, Zaria in clean plastic containers.

From six (6) motorcar mechanic workshops, twelve (12) liters of usedengine oil were collected (two (2) liters per mechanic workshop) and kept in cleaned plastic containers (4 liter cans). This was also done for used engine oil from motorcycle and truck/lorries (diesel engine vehicles)mechanic workshops.

Earthworm was collected following the recommendation by **Uba *et al.*(2009)**that earthworm may be easily obtained in Zaria by digging into soil.Adult earthworms(with clitellum) ranging in weight from 0.3 to 0.5 gram each, were collected by digging into the earth at Samaru Extension, Zaria, Nigeria. Samples of the worms were submitted to the Department of Biological Sciences, Landmark University, Kwara State, Nigeriafor identification. The remainder was used for study within 72 hours after collection.

Poultry manure and cattle dung were collected from the National Animal Production and Research Institute (NAPRI), Ahmadu Bello University (ABU), Zaria. Leaves of *Moringa oleifera* where procured from Samaru village, Zaria and identified in the Department of Biological Sciences, ABU, Zaria. Fresh cassava tubers were procured from Samaru market and peeled. The cassava peels was soaked in water in a closed plastic container for 1 week to allow for elimination of cyanide that might be present. The fermented peels were then air dried, crushed and sieved (≤5mm).

3.4.2 Analysis of Soil and Organic Amendments

The following analyses were performed on all the soil samples obtained from the mechanic workshop.

3.4.2.1 Elemental Composition

The determination of the elementalanalysis of all samples was performed using an ED-XRF machine the MiniPal PW4025 (Philips Analytical). The Procedure for Analysis of samples using EDXRF is presented in **Appendix B**.

3.4.2.2 Soil Texture

Soil textural classification of contaminated and uncontaminated soil samples was done using the hydrometer method **(Iwuafor *et al.* 1990)**described in **Appendix A**

3.4.2.3 Microbial Count

Microbial count was determined using the plate count method **(Talaro and Talaro, 2002)**. Serially diluted samples (0.1 millilters) were plated on nutrient agar mediumsupplemented with 50µg/ml nystatin to suppress the growth of fungi. Triplicate plates were incubated at 30°C for 24 hours before the colonies were counted.

3.4.2.4 Total Petroleum Hydrocarbon (TPH)

The TPH of the soil samples was determined using the toluene cold extraction method **(Adesodun and Mbagwu, 2008)**. 5gm of soil from mechanic workshop was placed in a 200 milliliters beaker into which was added 50 milliliters of toluene. The mixture was manually stirred continuously for 30 minutes, left to stand in a fume cupboard for 2 hours and afterwards

filtered using Whatman No 42 filter paper. The residue (soil) was allowed to dry in an oven at 50^{o}C. The TPH was computed as:

$$TPH\ (g/kg) = \frac{(initial\ weight\ of\ soil - weight\ of\ soil\ after\ solvent\ extraction)\ in\ g}{Initial\ weigh\ of\ soil\ in\ kg} \ldots\ldots\ldots 3.1$$

3.4.2.5 Carbon,Nitrogen and Phosphorus Content

The carbon, nitrogen and phosphorus contents of the soil samples were analysed using Walkley-Black **(Iwuafor *et al.* 1990)**, Kjedahl **(Iwuafor *et al.* 1990)**and Bray No 1 **(Iwuafor *et al.* 1990)** methods respectively. Details of the procedure are shown in **Appendix A**. The materials used as organic amendment (manures,cassava peels and *moringa oleifera* leaves)were also analyzed for carbon, nitrogen and phosphorus contents following the same procedure as those used for the soil samples.

3.4.2.6 Soil pH Determination

Ten grams of sieved (≤ five millimeters) and air-dried soil was weighed into a 50milliliters capacity beaker and 25milliliters of distilled water added. The content was stirred manually for 30 minutes with a glass rod and left to stand for an 1 hour. The electrode of the pH meter (Kent EIL 7055) was then inserted and the pH read.

3.4.2.7 Determination of Water Holding Capacity (WHC) and Moisture Content

35g of soil was placed in percolation tube (to give 15 cm of soil) and compacted by gentle tapping. Water was added until the water level stood at about 5 cm above the soil level. The tube was covered and left to stand for 2 days. After this period, the top half-inch of soil was discarded and wet soil was weighed into a pre-weighed evaporating dish. The sample was placed in oven at

110^{o}C for 24 hours. It was then removed and weighed to obtain bone dry weight of the sample. Percent water holding capacity was calculated as **(Srivastava and Thakur, 2006):**

$$\boldsymbol{waterholdingcapacity(WHC)} = \frac{\boldsymbol{wetweight(g) - bonedryweight(g)}}{\boldsymbol{bonedryweight(g)}} \ldots\ldots\ldots\ldots\ldots\ldots \mathbf{3.2}$$

For soil moisture content determination, a watch glass was weighed (W1). About10g of soil was placed on the watch glass and the combined weight of glass and soil recorded (W2). The sample (watch glass and soil) was placed in an oven at 105^{o}C and left for 48 hours. After the 48 hours drying time the weight of the sample (watch glass and dry soil) was recorded (W3). The moisture content of the soil was computed as:

$$\boldsymbol{moistute\ content(MOC)} = \frac{\boldsymbol{W2 - W3}}{\boldsymbol{W3 - W1}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{3.3}$$

3.4.2.8 Elemental Analysis of Simulated Used Engine Oil Contaminated Soil

Five different concentrations (12.5, 25, 50, 75, 100g/kg) of used engine oil contaminated soil were prepared and thoroughly mixed. Elemental analysis was then conducted on each sample using EDXRF.

3.5 BIOREMEDIATION USING ORGANIC AMENDMENTS

3.5.1 Soil Contaminated in the Laboratory

The uncontaminated soil samples were contaminated with 10% (wt/wt) used engine oil. As was done with the soil sample from mechanic workshop, weighted soil samples in different sized

containers were biostimulated using 10% (wt/wt) cow dung. Control samples were also prepared. Allsamples were maintained at 60% water holding capacity and at room temperature.

To make soil up to 60% water holding capacity Wo grams of water (calculated using Equation 3.3) was added to Ws grams of soil.

$$\boldsymbol{Wo = (0.6 * (WHC) - MOC) * Ws} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{3.3}$$

Where, WHC = water holding capacity

MOC = moisture content

At intervals of time (twice a week), all samples were tilled and makeup water added. The TPH and pH of the samples were determined every two weeks, using the toluene cold extraction method as described by **(Adesodun and Mbagwu, 2008)** and pH meter (Kent EIL 7055) respectively.For the TPH determination,soil sample (5g) was weighed into a 50 milliliterscapacity flask and 10 milliliters of toluene (AnaLar grade) was added. After shaking for 30 minutes on a shaker **(***Tamson,* Holland**)** and allowing to stand for an hour in an air tight sample bottle, the supernatant was extracted using a syringe and its absorbance measured at four hundred and twenty nanometers (420nm) using spectrophotometer (B. Bran Scienticific and Instrument Company). The TPH of the soil with time was estimated using a calibration curve prepared by recording the absorbance of supernatants obtained by mixing five (5) grams of UEO contaminated soil obtained from soil with known concentration of UEO contaminat (1 to 20grams UEO/kg of soil) with 10ml of toluene.

3.5.2 Soil from Mechanic Workshop

Weighted soil samples in containers of different cross-sectional-areas were mixed with 10% (wt/wt) manure and the moisture content maintained at 60% water holding capacity. The manure considered for use was cow dung. A control sample without manure (but with all other parameters – quantity of soil, moisture content and geometry of container - kept constant) was also prepared. The moisture content was maintained at 60% water holding capacity and TPH followed with time as in **Section 3.5.1**.

3.5.3 Effect of Surface Area

Five hundred grams of the uncontaminated soil was weighted into six (6) containers of different cross-sections to give surface area to weight of soil ratio of 0.143, 0.147, 0.243, 0.278, 0.349 and 0.428cm^2/g. The soil was made up to 60% water holding capacity and thoroughly mixed. Fifty grams of used engine oil was added to each container and mixed. The organic material used as amendment was *Moringa oleifera* leaves. Twenty five grams each of dry pulverized *Moringa oleifera* leaves and poultry manure was then added and again thoroughly mixed. All samples were maintained at room temperature (≈ 29^oC) and moisture content of 60% water holding capacity by weighing twice a week and adding make up deionized water when necessary. The TPH of each sample was followed with time using gravimetric method. This involved collecting soil and drying at room temperature for 72 hours. Ten milliliters of toluene was then mixed with five grams(5g) of the dried soil sample, covered and left to stand for 24 hours. The supernatant was extracted using a syringe and 10 milliliters of fresh toluene was added. After 24 hours the supernatant was again removed and the soil sample placed in an oven at 60^oC to remove any remaining toluene. The weight of soil (M_1) was then determined. The TPH was calculated as:

$$TPH(g/kg) = \frac{5 - M_1}{5} * 1000 \quad \ldots \ldots \quad 3.3$$

The composition of the used engine oil was determined by mixing two grams of dry contaminated soil (at time, t = 0) with 5milliliters of dichloromethane and analyzing the supernatant after settling using GC-MS (GCMS-QP2010 Plus Shimadzu, Japan).The initial column oven temperature was kept at 60°C for five minutes and then raised by 20°C per minute to 140°C and then at 5°C per minute to 200°C. It was held at 200°C for 5 minutes and then raised by 4°C per minute to 280°C. The total run time was 49 minutes.

GC-MS analysis was conducted after 21 weeks of bioremediationfor the sample with optimum rate.

3.5.4 Effect of Mixing

Five hundred grams of soil was placed in a plastic container. The ratio of the exposed surface area to the weight of soil was 0.428cm^2 per gram of soil. The soil was made up to 60% water holding capacity and 50 grams of used engine oil added. The mixture was thoroughly mixed with 50 grams of poultry manure. Two samples labeled PUMX and PMX were prepared, both in duplicates. All samples were maintained at 60% water holding capacity for twenty two (22) weeks. After the initial mixing of the content of the container (on day 0) PUMX samples remained unmixed throughout the period of the study (22 weeks). For PMX samples, the mixture was manually mixed every three days (twice a week) for 5min/day. The initial TPH of all samples was determined and this was repeated at intervals of time during the period of the study

so as to follow the progress of biodegradation with time. The procedure was repeated using pulverized *Moringa oleifera* leaves as the organic amendment. Themixed and unmixed samples were called MRMX and MRUMX respectively. A control sample (NA) was prepared with similar quantities of used engine oil, soil and water but with no organic amendment for both set of experiment (i.e. with poultry manure and *M. oleifera*). All samples were covered with muslin cloth and incubated in the open laboratory condition (≈29^{o}C).

TheTPH of all samples were determined as described in **Section 3.5.3**. Thecompositions of the used engine oil contaminant wasalso determined using GCMS. Samples analyzed using GCMS were NA (at t = 0 weeks and 22 weeks) and PUMX and MRMX (at t = 22 weeks).

3.6 EFFECT OF EARTHWORM INTRODUCTION INTO USED ENGINE OIL CONTAMINATED SOIL

This section presents the procedure used in investigating the effect of the use of earthworms for bioremediation. Adult earthworms (with clitellum) were used for all investigations involving worms. The average weight of the worm was found to 0.4g. Investigations were first carried out using simulated used engine oil contaminated soil to generate information on the most important parameters to consider in earthworm (*E. eugeniae*) assisted bioremediation of petroleum hydrocarbon contaminated soil. Secondly, the effect of earthworm inoculation on the bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshops was investigated.

3.6.1 Earthworms Survival Assessment

Used engine oil is thought to contain some toxic organic compounds and heavy metals **(Obidike, 1985)**. As a result, an initial investigation was conducted to investigatethe used-engine-oil concentrations tolerable to earthworm. The test substrates were prepared according to the OECD guidelines for testing of chemicals **(OECD, 1984)**. The soil sample collected was sieved (≤5mm) to remove coarse stones and to ensure homogeneity. 0.5kilograms of soil was weighed into plastic test containers. The soil was made up to 60% water holding capacity using deionised water. The soil samples were contaminated with various concentrations of the motorcycle UEO (5, 10, 20, 30, 40, 50 and 100 per kg of soil). The mixture was thoroughly mixed manually. Used engine oil was collected from six (6) different motorcycle mechanic workshops, so six replicas were prepared. 5grams of poultry manure was then added to the soil and thoroughly mixed. After mixing, 1gram of the manurewas littered on the surface. Worms were kept on moist filter paper for three hours before being placed in test containers so they can void their gut contents. They were then rinsed,quickly killed and dried for elemental analysis. Tenearthworms (*E. eugeniae*) each were introduced into each test container and the weight of each test container recorded. Adult earthwormswith clitellum,were considered having an average weight of 0.47grams. At intervals of time (after every 2 days), the samples were examined. The number of living earthworms in each sample was recorded and the dead worms removed. The setup used for investigations involving earthworm inoculation is shown in Figure 3.2.

The moisture content of 60% water holding capacity was maintained throughout the test period by weighing, to determine moisture loss, and adding make up deionised water. The test containers were covered with muslin cloth firmly held in place to prevent the worms escaping.

Testing was done in continuous light (to ensure that worms remain in the test medium throughout the duration of test).The samples were monitored for a total of 14 days. This procedure was repeated using cow dung in place of poultry manure. The above procedure was repeated for used engine oil collected from motorcar and diesel engine vehicles. Control samples, without engine oil was also prepared for each run.

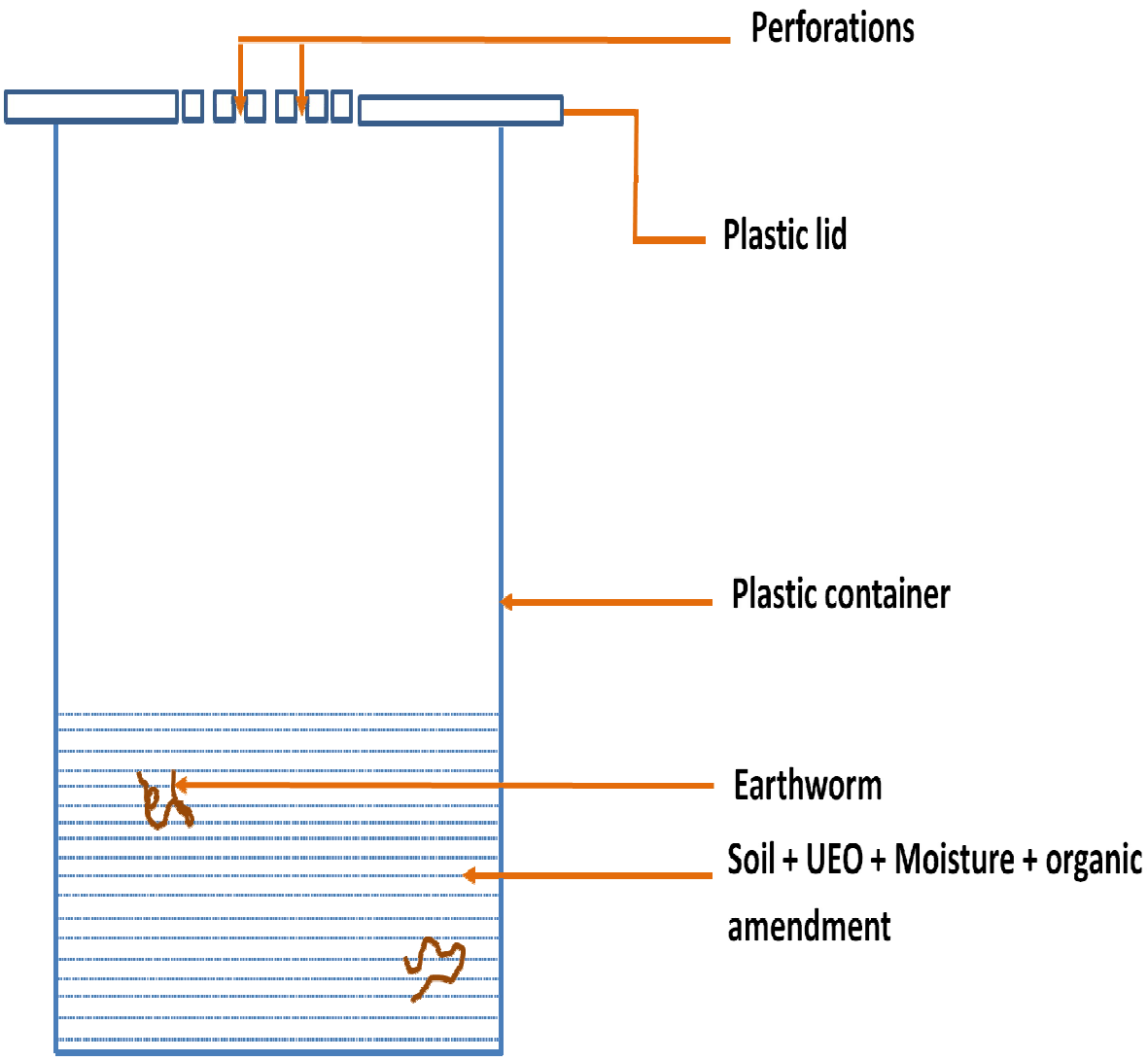


Figure 3.2: Setup used for earthworm inoculation studies

3.6.2 Effect of Earthworm on the Carbon and Nitrogen Content, Moisture Retention and Aggregate Size Distribution of Used Engine Oil Contaminated Soil

Five samples were prepared each containing 40grams of used engine oil in 400grams of soil made up to 60% water holding capacity. The samples were kept in plastic containers covered with muslin cloth. 10grams of cow dung was added to each and the mixture thoroughly homogenized. The soil samples were labeled 0W, 4W, 8W, 12W, and 16W according to the number of worms the contained (i.e. 0, 4, 8, 12 and 16 worms respectively). All samples were prepared in duplicates. Samples were monitored for 6 weeks. The moisture content was maintained at 60% water holding capacity by weighing every day to determine the weight loss and adding makeup deionized water.

3.6.2.1 Carbon Nitrogen Content

Starting from the day the samples were prepared, 10g of the contaminated soil was collected from each sample and the carbon and nitrogen contents determined using the Walkley-Black and the Macro-Kjedahl methods respectively. This was repeated every seven (7) days for six weeks.

3.6.2.2 Water Holding Capacity and Aggregate Size Distribution

At the end of six weeks, the water holding capacity and the aggregate size distribution of each sample was determined. The water holding capacity was determined as described in **Section 3.4.2.7.**In the determination of aggregate size distribution, 300grams of dried bulk soil was weighted and transferred into the nest of sieves consisting sieves of diameters (D_i) 2.00, 0.66, 0.50, 0.25 and 0.05millimeters. The nest of sieves was placed on a mechanical sieve shaker (GRAVQUICK (A/S) EFL2 MK3) and shaken for 60 seconds after which the weight of soil

retained in each was determined. Aggregate size distribution was calculated as proportion of soil retained in each sieve**(Hajabbasi and Hemmat, 2000)**using:

$$\boldsymbol{MWD} = \sum(\boldsymbol{P_i D_i}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{3.4}$$

Where,

$$\boldsymbol{P_i} = \frac{\boldsymbol{weight\ of\ soil\ retained\ on\ sieve(g)}}{\boldsymbol{total\ weight\ of\ soil\ taken(g)}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{3.5}$$

3.6.3 Effect of UEO Concentration on Bioremediation

1 kilogram of soil contaminated with 5g UEO was mixed with 5grams of poultry manure. 1gram of the manure was littered on the surface **(Schaefer and Juliane, 2007)**. The moisture content of the soil was made up to 60% water holding capacity using deionized water. The soil, manure, UEO and water were thoroughly mixed manually in a plastic test container. This was repeated using other concentrations of UEO contaminated soil (10, 15 and 20g UEO/kg soil). 10 earthworms were introduced into each sample. The test containers were covered with muslin cloth to prevent worms escaping. The total petroleum hydrocarbon (TPH) of the soil (g/kg) was determined every two weeks for a total of 6 weeks using the toluene cold extraction method as described by **Adesodun and Mbagwu (2008).**

3.6.4 Effect of Worm Concentration on Bioremediation and Microbial Count

1 kilogram of soil was spiked with 6grams of poultry manure, contaminated with 20grams of UEO and 5 earthworms were introduced. Other samples were also produced but with varying

numbers of earthworms introduced (0, 10, 15 and 20). At intervals of two (2) weeks, microcosms were prepared from each sample by mixing 5g of soil collected from four different areas of the spiked soil (so as to obtain a composite sample) for isolation and enumeration of bacteria and determination of TPH. TPH was determined using the toluene cold extraction method. The microbial count of the samples at the start of the experiment and after every two week was determined using the plate count method **(Talaro and Talaro, 2002)**. The culture media used was nutrient agar. Serially diluted samples (0.1 ml) were plated on nutrient agar medium (Oxoid) supplemented with 50µg/ml nystatin to suppress the growth of fungi. Triplicate plates were incubated at 30°C for 24 hours before the colonies were counted. Samples were incubated for 42 days.

GCMS analysis was conducted at t = 0 (for sample without worms) and at t = 42 days (for sample without worm and for sample with 20 worms).

3.6.5 Effect of Mixing/Tilling Versus Earthworm Assisted Bioremediation

This investigation was aimed at comparing the effect of the activities of earthworm with manual tilling/mixing in the process of bioremediation. Four samples were prepared in which 5grams of UEO was added to 500grams of soil and thoroughly mixed in plastic test containers. 5grams of poultry manure was added and again thoroughly mixed manually. Water was added to 60% water holding capacity and the content of the container was again thoroughly mixed. 15 earthworms were added to one test container and 10 earthworms to another.One of the remaining two was labeled MX (mixed) as the contents of the container were regularly mixed while the other was labeled UMX (unmixed) as it was unstirred throughout the test period, save for the initial mixing when the content of the test container was thoroughly mixed. The test containers where covered with muslin cloth to allow air in-flow and preventing earthworms from escaping.

At intervals of two (2) weeks, microcosms were prepared from each sample by mixing 5 grams of soil collected from four different areas for isolation and enumeration of bacteria and determination of TPH. The process lasted 14 weeks and all samples where produced in duplicates.GCMS analysis was conducted at time t = 0 weeks and at t = 14 weeks (for mixed sample and sample with 15 worms).

3.6.6 The Effect of Manure Concentration on Bioremediation

Five initial concentration of manure were considered for this investigation namely 0, 1, 2, 3 and4% manure (%wt/wt manure per soil). Each test sample contained 500grams of soil and 7.5grams of UEO. They contents of the containers where thoroughly mixed and made up to 60% water holding capacity using de-ionized water. Poultry manure was added and thoroughly mixed. 10 earthworms where then introduced into each test container apart from the one having 4% manure. At intervals of two weeks a 5grams microcosm was prepared and analyzed for TPH using the toluene old extraction method. All samples where produced in duplicates.

3.6.7 Heavy Metals Uptake and Elimination in Earthworm in Used Engine Oil Contaminated Soil

The procedure followed was adopted from the **OECD 317 (2010)**. Five different concentrations of used engine oil contaminated soil were prepared using 40, 30, 20, 10 and 5grams of used engine oil in 400grams of uncontaminated soil. The mixtures were kept in clean plastic containers. The soil was made up to 60% water holding capacity and spiked with 10grams of cow dung. Twenty (20) earthworms were added to each sample and on days 0, 5, 10, 15 and 20 five earthworms were removed and prepared for XRF analysis. Earthworms were introduced

without prior conditioning in the laboratory. In preparing the worms for analysis, on the set days, the worms were removed from the soil and placed on wet filter papers so as to void their guts for a period of 24 hours. Theywere then quickly killed by freezing at 0^{o}C. The dead worms were then sun dried for four (4) hours before being transferred to an oven at 60^{o}C for 12 hours. Elemental analysis of the carcasses of the worms was then conducted using XRF. The soil samples were maintained at 60% water holding capacity throughout the test period by weighing everyday and adding make up deionized water when necessary.

Elemental analysis of the various used engine oil contaminated soil before earthworm introduction (at time zero) was determined by drying 5grams of each soil sample in open air (26 – 31^{o}C) for 72 hours and analyzing using EDXRF. The soil samples were similarly analyzed, at the end of the investigation (day 20).All samples were prepared in duplicates.

At the end of the investigation, the % drop in TPH for all samples was determined using the toluene cold extraction method.

The Procedure for Analysis of samples using EDXRF is presented in **APPENDIX B**.

3.6.8 Effect of Earthworm on the Bioremediation of Mechanic Workshop Soil

This investigation was conducted using petroleum hydrocarbon contaminated soil from a motorcycle and motorcar mechanic workshop.Large particles were removed from the soil samples. The TPH and water holding capacities of the soil samples were determined using Equation 3.1 and 3.2 respectively.GCMS analysis was also conducted on the dichloromethane extract obtained using the freshly collected mechanic workshop soil samples. 500g of soil from each source was weighted and placed in clean plastic containers. Deionised water was then added to make up 60% water holding capacity and thoroughly mixed manually. 50gm of

fermented cassava peels (prepared as reported in **Section 3.4.1**) was added and again thoroughly mixed. A second sample was similarly prepared. The TPH of the mixture was determined using the toluene cold extraction method. 10 earthwormswas added to one of the samples. Both samples were maintained at 60% water holding capacity by recording the drop in weight every other day and adding makeup deionized water. All samples were prepared in duplicates.A control sample was also prepared containing neither worms nor the fermented cassava peels but it was also maintained at 60% water holding capacity. The TPH of all samples was followed using the toluene cold extraction method. Microbial count and identification were also determined at set intervals of time.

The TPH of the sample from each type of mechanic workshop soil considered was reduced by diluting with uncontaminated soil from a farmland (reported in **Section 3.4.1**). The TPH and water holding capacities of these mixtures were determined. Two samples were also prepared (as was done for the undiluted), one with organic amendment alone and the other with organic amendment and worms. A control sample was also prepared containing neither cassava peels nor worms. All samples were prepared in duplicates and maintained at 60% water holding capacity. The TPH of all samples was followed using the toluene cold extraction method. Microbial count and identification were also determined at set intervals of time. At the end of the investigation (after 35 days) GCMS analysis was conducted on the earthworm inoculated diluted motorcycle mechanic workshop soil sample.

Elemental analysis using EDXRF analysis of soil samples at the beginning and end of the experiments (after 35 days of inoculation) was also conducted on the samples in which the worms were still alive).

3.6.9 Total Bacterial Count and Characterization of Bacterial Flora

Samples considered for this investigation included PMX, MRMX (of **Section 3.5.4**)and at 22 weeks of bioremediation and the samples with 30 earthworms/kg of soil amended with poultry manure (of **Section 3.6.5**)at 14 weeks of bioremediation, also samples from Section 3.6.8.

Twenty five (25) grams of soil sample was mixed with 225mls of normal saline (NaCl + H_2O) (1:10). Ten fold dilution was then prepared, that is 1ml of the diluted sample (stock) was transferred into 9ml of sterile normal saline (10^1) using a sterile pipette, this was repeated to obtain 10^2, 10^3, 10^4 and 10^5 dilutions respectively. Using a sterile pipette, 0.1milliliters of 10^5dilution was aseptically transferred into sterile plate count agar (PCA) plates in duplicates.

With the aid of a sterile bend glass rod, the inoculum was spread on the surface of the culture medium (PCA). The inoculated plates were incubated at 37^{o}C for 24 hours. The numbers of bacterial colonies were counted by using a colony counter and recorded as colony forming units per gram (cfu/g).

Total colony forming unit per gram was computed using Equation 3.6:

$$\boldsymbol{Colony\ forming\ per\ gram} = \frac{\boldsymbol{Number\ of\ colonies\ counted} * \boldsymbol{Dilution\ factor}}{\boldsymbol{volume\ of\ inoculum}} \ldots\ldots\ldots\ldots \mathbf{3.6}$$

The isolates were Gram stained and observed under the microscope to determine the gram reaction, morphology and the presence of spores. The organisms were identified and characterized. Gram-reaction, motility, shape and color of colony, catalase and oxidase activities, were checked.

Colonies to be identified were picked from the nutrient agar plates and maintained on slants of the same medium. These were subsequently used for various biochemical tests which include motility test, coagulase, catalase, citrate utilization, methyl red voges proskaner and indole tests.

Motility test:The motility medium was inoculated by making a fine stab with a straight wire containing the isolates to a depth of 1-2 centimeters short of the bottom of the tube. The inoculated medium was then incubated at $37^{o}C$ for 24 – 48 hours. However, for motile organisms, the line of inoculation was not defined and the rest of the medium was somewhat cloudy. For non motile organisms, growth was restricted to the line of inoculation, which remained sharply defined, the rest of the medium remaining clear.

Coagulase test: Two drops of physiological saline about 2centimeterswere placed on a clean glass slide that was divided into two with grease pencil. A colony was carefully emulsified in each drop of saline after which a drop of citrated human plasma was added on one side and mixed. The side was held up and tilted back and forth for 1 minute.Clumpingof cells of bacterial suspension with plasma was regarded as a positive coagulase test.

Catalase test:**A** drop of 3% hydrogen peroxide was placed on a glass slide, a bit of growth from the slant was removed and placed on the drop of hydrogen peroxide. Bumbling and frothing indicated a positive catalase test.

Citrate utilization:A freshly prepared slant of Simmon's citrate agar was inoculated and put in a bijou bottle and incubated for 48 – 72 hours. The development of a deep blue color was recorded as a positive reaction.

Indole test:Each test organism was grown in 5ml peptone water for 24 hours after which Kovac's reagent was added (about 4 drops) and shaken. The development of a red color in the reagent layer above the broth within one minute was recorded as positive reaction.

Methyl-red VOGES-PROSKAUER (MR – VP) test:MR – VP broth was inoculated with the isolated colony and incubated at 37^{o}C for least 24 – 48 hours, after which 1ml of the broth was transferred into a bijou bottle, 2 – 3 drops of the methyl red was added to this quantity. A red color on addition of the indicator signifies a positive methyl red test. A yellow coloration signifies a negative result. 15 drops of 5% alphanaphthol in ethanol and 5 drops of 40% potassium hydroxide (KOH) were added to the rest of the broth in the original tube.The cap of the tube was loosened and placed in a sloppy position. The development of a red color starting from the liquid-air interphase, within one hour indicates VP positive test.Nocolor change signifies a negative VP test.

CHAPTER 4

RESULTS AND DISCUSSIONS

In this chapter, the results of the various investigations carried out in Chapter Threeare presented and discussed.

4.2 SOIL ANALYSIS

4.1.1 Analysis of Soil from Mechanic workshops

In this section the investigation into pollution in mechanic workshops is presented and discussed.

4.1.1.1 Enumeration of Mechanic Workshop for Assessment of Petroleum Hydrocarbon Contamination.

The data obtained from the enumeration of auto mechanic workshop is presented in Table 4.1.As shown in the table, a total of 129 mechanic workshops sites (where used engine oil isusually withdrawn and dumped into the soil) where identified. The shaded boxes indicate the type of mechanic workshop selected for soil analysis and the location.

Based on the distribution in Table 4.1, soil samples were collected from nine (9) motorcar mechanic workshops, nine (9) motorcycle mechanic workshops and seven (7) truck/lorry mechanic workshops. 3 motorcycle mechanic workshops were selected between Samaru and Kwagila, three (3) in Sabo and three (3) from Wusasa to Jos road. In the case of motorcar workshops, 3 workshops were selected between Samaru and Kwagila, 3 in Sabo and three (3) from Wusasa to Jos road andfor the trucks/lorries mechanic workshops one (1) was selected along Jos road and 6 between Wusasa and Kwagilla.

Table 4.2 presents the information obtained from the managers of the workshops on interview: The location of the various mechanic workshops is given as well as the age, possible sources of soil petroleum hydrocarbon contamination and number of times the workshop had been refilled since inception.

Table 4.1: Identification and enumeration of mechanic workshops in and around Zaria.

Location		DISTANCE (km)	MM	MC	HD
FROM	**TO**				
NUGA Gate	**Kwagilla**	7.59	19	5	1
Kwagilla	**Wusasa Bridge**	7.59	6	0	7
Wusasa	**Kofa-Doka**	3.58	10	7	0
Kofa-Doka	**MTD**	5.25	1	1	0
MTD	**Kwagilla**	1.47	8	2	0
MTD-thru Sabo	**PZ**	3.36	4	22	0
Kofa-Doka	**Jos road**	4.76	8	6	2
Shagalinku Junction	**Kongo Junction**	2.95	5	5	0
PZ	**KONGO**	2.80	6	4	0
TOTAL			67	52	10

MM is Motor mechanic workshop, MC is Motorcycle mechanic workshop, HD is Truck/lorry mechanic workshop.

Table 4.2: Description of mechanic workshops from which soil samples were obtained.

Designation	Location	Age of workshop	Petroleum hydrocarbon dumped on soil	Refilling	Comments
MC1	Bassawa road	11 years	Petrol, grease, engine oil	No refill	
MC2	Dogon Iche garage, Samaru	10yrs	"	No refill	
MC3	Bala's garage, Bassawa Rd, Samaru	28yrs	Petrol, grease, engine oil	No refill	Works generators – gasoline and diesel
MC4	Amo's garage, NTC roundabout, Sabo.	10yrs	Petrol, grease, engine oil	No refill	
MC5	Umar Farouk Garage, Aminu Rd, Tudun Wada	6yrs	Petrol, grease, engine oil	1 refill at inception	
MC6	Emma's workshop, Crescent str. Sabo	6 yrs	Petrol, grease, engine oil	No refill	
MC7	Bakin Makabarta, Jos Rd	6 yrs	Petrol, grease, engine oil	No refill	
MC8	Ban Zau Zau, Jos Rd	15yrs		Refills: marshy area	
MC9	Mikailu workshop, Jos Rd	2 yrs		No refill	
MM1	Olu motor mechanic garage, Danraka str. Samaru	27yrs	ATF, gear oil, grease, engine oil, petrol	No refill	
MM2	Oga Jimoh garage along Bassawa Rd, Palladan	38yrs	ATF, gear oil, grease, engine oil, petrol	No refill	
MM3	Kan Dutse workshop, Hanwa	25yrs	"	No refill	
MM4	Adaka Mugani Garage, Jos Rd.	22yrs	ATF, gear oil, grease, engine oil, petrol	No refill	
MM5	Mustpha garage, Jos Rd	5yrs	"	No refill	
MM6	Master Engineering workshop, Jos Rd	18 yrs	"	No refill	

Table 5.2 continued

MM7	Amita mechanic workshop, Wusasa Rd	4 yrs	"	No refill	
MM8	Ganuwa garage, Kofa Dokar, Wusasa Rd	20yrs	"	No refill	UEO is poured on the ground when there are no buyers
MM9	Alhaji Aminu Abdulazeez workshop, Dan Magaji	20 yrs	"	No refill	
HD1	Anchau filling station, Gwugi, Jos Rd.	13yrs	Engine oil, gear oil, diesel, hydraulic, grease	No refill	
HD2	Madarkachi Engineering Workshop , Jos Rd (tractor workshop)	18yrs	Gear, diesel, engine oil	No refill	
HD3	Two brother mechanic Engineering Workshop, Dan Magaji	7 yrs	"	No refill	
HD4	Tipper garage, Dan Magaji	3yrs	"	No refill	UEO is used in the tipping system
HD5	Nwansika mechanical construction company, Zaria-Kaduna Express way	35 yrs	Penetrating oil, gear oil, engine oil, hydraulic	No refill	
HD6	Dan Mallam garage, Zaria-Kaduna express way	30yrs	"	No refill	
HD7	Garage Alhaji Saleh Turu, Dan Magaji	15 yrs	"	No refill	
			"		

MC 1 - 9: 1st to 9th motorcycle mechanic workshop, MM 1 -9: 1st to 9th motorcar mechanic workshop, HD 1 – 7: 1st to 7th truck/lorry mechanic workshop

4.1.1.2 Elemental Composition of Soil Samples

The elemental analysis of the soil samples collected from the various mechanic workshops are presented in Table 4.3, 4.4 and 4.5. In Table 4.6, the mechanic workshop soil samples were grouped by age, X, of the mechanic workshop in years. The average composition was computed for the various age categories.The concentration of heavy metals in the soil samples are generally higher than typical safe limit levels for leafy or root vegetables as well as for gardens and children contact **(http://www.*al-labs-eastern.com*, 2011)**. The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be naturally degraded like organic pollutants and have the tendency to persist in the ecosystem or accumulated in different parts of the food chain **(Igwe *et al*., 2005 and Smejkalova *et al*., 2003)**. Thus when UEO contaminated soil is considered for remediation, the heavy metal content becomes an issue. The oxides of certain elements such as Re, Eu, V,and K did not show any appreciable change. Using Two-Way ANOVA, at $\alpha = 0.05$, the *P*-values for effect of age and type of mechanic workshop on the increase in heavy metal content (from Ti through to Nb in Table 4.3) were found not to be significant (0.12 and 0.23 respectively).

The heavy metal contents of the soils from mechanic workshops generally were found to be above the optimum heavy metal content (see Table 2.2) recommended for bioremediation. It is thus important to take the heavy metal content of these soils into consideration when bioremediation is envisaged.

Table 2.6 presents the Nigeria's soil quality regulatory limits for chemicals, pharmaceuticals, soaps and detergents sectors (National Environmental Regulation, 2009).

Table 4.3: Elemental analysis of UEO contaminated soil from motorcar mechanic workshops.

	MM1	MM2	MM3	MM4	MM5	MM6	MM7	MM8	MM9
					% Composition				
Al_2O_3	11.00	12.00	10.00	14.00	11.00	0.00	15.00	12.00	0.00
As_2O_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BaO	0.25	0.34	0.27	0.10	0.17	0.17	0.10	0.10	0.24
CaO	8.11	4.08	6.93	2.64	5.69	4.17	3.91	8.75	4.93
Cr_2O_3	0.075	0.10	0.07	0.06	0.06	0.06	0.09	0.07	0.07
CuO	0.22	0.22	0.17	0.13	0.15	0.17	0.17	0.13	0.19
Eu_2O_3	0.00	0.00	0.07	0.10	0.00	0.00	0.00	0.00	0.09
Fe_2O_3	14.82	19.42	12.50	14.05	11.80	11.20	21.58	17.60	13.2
K_2O	3.74	3.95	5.20	3.49	4.95	5.77	2.29	3.37	5.06
MnO	0.11	0.19	0.12	0.09	0.16	0.10	0.16	0.11	0.15
Nb_2O_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NiO	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.02	0.00
OsO_4	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
PbO	0.4	0.94	0.41	0.14	0.12	0.22	0.35	0.23	0.29
Rb_2O	0.07	0.09	0.08	0.04	0.07	0.08	0.05	0.06	0.09
Re_2O_7	0.00	0.00	0.05	0.01	0.03	0.02	0.01	0.04	0.00
RuO_2	0.96	1.20	0.95	0.61	0.87	0.90	0.68	0.90	0.92
SiO_2	56.50	52.80	59.10	61.60	60.70	72.90	51.30	53.40	70.2
SO_3	1.00	1.60	0.96	0.88	1.50	1.40	1.10	0.83	1.30
SrO	0.09	0.07	0.07	0.03	0.07	0.06	0.04	0.05	0.06
ThO_2	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.12	0.15
TiO_2	1.75	1.84	1.75	1.46	1.81	1.74	1.80	1.54	1.90
V_2O_5	0.055	0.06	0.04	0.05	0.05	0.05	0.08	0.06	0.06
Y_2O_3	0.06	0.06	0.05	0.04	0.06	0.06	0.05	0.05	0.06
Yb_2O_3	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00
ZnO	0.35	0.68	0.24	0.16	0.21	0.25	0.20	0.26	0.38
ZrO_2	0.56	0.54	0.59	0.47	0.64	0.61	0.63	0.43	0.71

MM 1 -9: 1st to 9th motorcar mechanic workshop

Table 4.4: Elemental analysis of UEO contaminated soil from motorcycle mechanic workshops.

	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8	MC9
	% Composition								
Al_2O_3	12.00	0.00	11.00	13.00	12.00	9.70	12.00	15.00	12.00
As_2O_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BaO	0.10	0.24	0.10	0.10	0.10	0.20	0.20	0.21	0.23
CaO	6.29	6.93	3.61	2.79	3.86	8.35	4.18	6.65	5.15
Cr_2O_3	0.08	0.12	0.08	0.08	0.07	0.10	0.11	0.06	0.08
CuO	0.11	0.17	0.18	0.16	0.14	0.18	0.21	0.10	0.13
Eu_2O_3	0.00	0.00	0.1	0.00	0.08	0.00	0.00	0.00	0.06
Fe_2O_3	11.50	17.20	17.82	18.21	12.24	21.90	21.89	13.30	23.27
K_2O	5.50	4.77	3.51	4.19	3.22	3.93	4.15	5.14	5.50
MnO	0.15	0.19	0.15	0.13	0.16	0.19	0.21	0.14	0.17
Nb_2O_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NiO	0.06	0.09	0.13	0.04	0.03	0.05	0.05	0.04	0.05
OsO_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PbO	0.09	0.19	0.20	0.33	0.12	2.11	0.16	0.00	0.14
Rb_2O	0.10	0.15	0.08	0.14	0.04	0.12	0.13	0.12	0.17
Re_2O_7	0.00	0.09	0.02	0.00	0.00	0.00	0.00	0.02	0.00
RuO_2	1.74	2.77	1.30	1.76	0.75	2.30	2.11	1.48	1.90
SiO_2	56.50	61.90	57.4	54.70	61.50	46.4	50.40	53.60	47.50
SO_3	2.30	1.60	1.50	0.10	3.50	1.00	1.00	1.60	0.60
SrO	0.11	0.15	0.06	0.09	0.04	0.15	0.11	0.14	0.14
ThO_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TiO_2	1.69	1.64	1.29	1.99	1.53	1.60	1.69	1.50	1.55
V_2O_5	0.05	0.05	0.06	0.08	0.05	0.06	0.07	0.04	0.05
Y_2O_3	0.10	0.15	0.08	0.12	0.05	0.14	0.12	0.10	0.12
Yb_2O_3	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00
ZnO	0.33	0.41	0.23	0.40	0.29	0.50	0.47	0.30	0.43
ZrO_2	0.96	1.18	0.74	1.03	0.45	1.03	1.02	0.81	0.75

MC 1 - 9: 1st to 9th motorcycle mechanic workshop

Table 4.5: Elemental analysis of UEO contaminated soil from truck/lorry mechanic workshops.

	HD1	HD2	HD3	HD4	HD5	HD6	HD7
	% Composition						
Al_2O_3	0.00	0.00	0.00	10.00	13.00	0.00	12.00
As_2O_3	0.00	0.00	0.01	0.00	0.00	0.00	0.00
BaO	0.25	0.42	0.20	0.23	0.17	0.43	0.30
CaO	3.45	6.99	2.42	3.69	4.90	5.56	4.21
Cr_2O_3	0.13	0.09	0.08	0.10	0.09	0.11	0.13
CuO	0.17	0.29	0.32	0.19	0.21	0.625	0.20
Eu_2O_3	0.00	0.10	0.00	0.10	0.00	0.00	0.10
Fe_2O_3	17.80	20.30	10.50	22.67	18.83	21.1	22.42
K_2O	6.90	7.28	5.71	3.49	2.67	5.96	3.74
MnO	0.11	0.21	0.09	0.19	0.11	0.2	0.12
Nb_2O_5	0.00	0.00	0.00	0.00	0.00	0.084	0.00
NiO	0.05	0.04	0.05	0.04	0.03	0.077	0.02
OsO_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PbO	0.19	0.32	0.20	0.42	0.17	0.62	0.28
Rb_2O	0.23	0.00	0.19	0.14	0.08	0.21	0.11
Re_2O_7	0.03	0.04	0.03	0.03	0.03	0.07	0.00
RuO_2	3.13	2.99	2.05	2.20	1.56	3.14	1.50
SiO_2	62.60	55.80	74.20	51.90	55.00	55.4	50.50
SO_3	0.60	1.20	0.80	0.60	0.89	1.9	1.80
SrO	0.12	0.17	0.10	0.10	0.06	0.15	0.09
ThO_2	0.20	0.00	0.00	0.00	0.00	0.00	0.00
TiO_2	2.27	2.13	1.83	1.85	1.38	2.13	1.56
V_2O_5	0.09	0.07	0.06	0.05	0.06	0.075	0.06
Y_2O_3	0.18	0.21	0.12	0.13	0.09	0.17	0.09
Yb_2O_3	0.00	0.00	0.00	0.00	0.01	0.00	0.03
ZnO	0.21	0.41	0.18	0.34	0.25	0.49	0.37
ZrO_2	1.29	0.95	0.90	1.15	0.80	1.49	0.75

HD 1 – 7: 1st to 7th truck/lorry mechanic workshop

Table 4.6: Elemental Analysis of used engine oil contaminated soil from mechanic workshops

Elements	MC			MM			HD		
	% Composition								
	X≤10	10<X≤20	20<X	X≤10	10<X≤20	X<20	X≤10	10<X≤20	20<X
Al	5.175	7.143	5.820	6.878	2.116	6.217	2.646	1.587	3.439
S	0.520	0.780	0.600	0.520	0.472	0.444	0.280	0.360	0.560
Ba	0.161	0.143	0.090	0.125	0.152	0.215	0.197	0.215	0.269
Ca	3.724	4.625	2.580	3.431	4.253	3.889	2.187	2.616	3.738
Cr	0.062	0.048	0.055	0.055	0.048	0.055	0.062	0.062	0.068
Cu	0.136	0.080	0.080	0.128	0.128	0.152	0.208	0.136	0.336
Eu	0.009	0.000	0.043	0.000	0.013	0.017	0.022	0.022	0.000
Fe	13.361	8.665	12.453	11.663	9.783	10.622	11.593	10.573	13.955
K	3.560	4.415	2.913	3.004	3.925	3.402	3.818	3.718	3.585
Mn	0.139	0.116	0.116	0.124	0.093	0.101	0.108	0.085	0.124
Nb	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
Ni	0.039	0.039	0.102	0.024	0.016	0.016	0.031	0.0236	0.039
Os	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pb	0.473	0.046	0.186	0.223	0.232	0.436	0.288	0.186	0.371
Rb	0.112	0.101	0.074	0.055	0.064	0.064	0.156	0.0828	0.129
Re	0.015	0.008	0.015	0.015	0.015	0.015	0.023	0.015	0.038
Ru	1.666	1.390	1.122	0.673	0.786	0.803	1.839	1.649	2.029
Si	25.097	25.714	26.811	26.158	30.595	26.858	29.451	19.726	25.784
Sr	0.093	0.110	0.051	0.042	0.051	0.051	0.085	0.085	0.093
Th	0.000	0.000	0.000	0.272	0.0791	0.000	0.000	0.044	0.000
Ti	1.001	0.959	0.773	1.085	1.037	1.019	1.103	0.892	1.0546
V	0.033	0.0224	0.0336	0.034	0.034	0.028	0.028	0.034	0.039
Y	0.094	0.079	0.063	0.047	0.047	0.047	0.102	0.094	0.102
Yb	0.009	0.000	0.000	0.000	0.000	0.009	0.000	0.009	0.009
Zn	0.337	0.249	0.185	0.169	0.241	0.289	0.209	0.201	0.297
Zr	0.674	0.659	0.548	0.474	0.429	0.340	0.755	0.555	0.851

(**X:** the age of the workshop (in years)

Comparison of Tables 4.6 and 2.6 indicated that apart from As, Cd, Co, Hg and Mo which fell below detection level, all other metals in the mechanic workshop soil were found to be in excess of the Nigerian recommended limits. The Al_2O_3 content of some of the soil samples fell below

detection (MM6, MC2, HD1 etc). This is unexpected as Al is a normally a component of soil mineral. This observed absence of Al may be as a result of alteration of the composition of the top soil by physical or chemical process resulting from activities in the mechanic workshop (for example land filling, battery-acid leaching, etc).

4.1.1.3 Other Physicochemical Properties of Soil from Mechanic Workshop

Table 4.7 presents other physicochemical properties of contaminated soil from mechanic workshop.

Soil textural class:Soil textural analysis indicated that 11.1% of the soil samples for MC were found to be sandy, 55.6% loamy sand and 33.3% were sandy loams. 85.7% of the soil samples for MM were found to be sandy while the remaining 14.3% was found to be loamy sand while 88.9% of the soil samples for HD were found to be sandy and the remaining 11.1% was found to be loamy sand.These suggest that there is a relationship between the mechanic workshop type and the texture of the soil. A greater percentage of the soil samples collected from MM and HD (85.7 and 88.9% respectively) were sandy. The sandy nature of the oil contaminated soils is in agreement with the findings of **Al-Sanad *et al.* (1995)** and **Ijimdiya (2010)** that oil contamination leads to a decrease in fine fractions. The most probable reason for this could be the fact that the oil affected the physic-chemical characteristics of the soil particularly the fine fractions. The observed soil textural class may however be the nature of the soil in and around the workshops even before contamination.There was no direct relationship between the textural class of soil sample and the age of the workshop.

Table 4.7: Physicochemical properties and microbial count of soil from mechanic workshops.

Designation	% C	% N	%P	C/N ratio (g/g)	TPH (g/kg)	Soil Textural class	Microbial count, cfu/kg ($*10^5$)
MC1	6.80	0.16	0.30	43.04	165.80	Loamy Sand	125.00
MC2	6.00	0.28	0.37	21.43	90.91	Loamy Sand	140.00
MC3	6.00	0.18	0.26	34.29	193.09	Loamy Sand	150.00
MC4	3.49	0.16	0.88	22.09	111.67	Sandy Loam	120.00
MC5	6.58	0.14	0.46	47.00	183.36	Sand	145.00
MC6	11.85	0.14	0.28	84.64	111.12	Sandy Loam	105.00
MC7	8.48	0.23	0.23	37.19	93.92	Loamy Sand	150.00
MC8	8.08	0.12	0.37	65.69	145.62	Sandy Loam	130.00
MC9	9.04	0.16	0.21	57.21	77.87	Loamy Sand	135.00
MM1	5.44	0.18	0.35	31.09	96.38	Loamy Sand	143.00
MM2	10.81	0.14	0.42	77.21	137.74	Sand	142.00
MM3	4.45	0.16	0.33	28.17	101.60	Sand	145.00
MM4	5.00	0.12	0.25	40.65	70.31	Sand	144.00
MM5	6.00	0.14	0.30	42.86	103.07	Sand	143.00
MM6	4.65	0.16	0.28	29.43	63.80	Sand	135.00
MM7	5.79	0.12	0.23	47.07	89.78	Sand	130.00
MM8	5.40	0.11	0.18	51.43	74.41	Sand	120.00
MM9	6.38	0.19	0.40	33.06	133.41	Sand	120.00
HD1	8.52	0.18	0.18	48.69	84.19	Sand	135.00
HD2	9.04	0.12	0.18	73.50	99.56	Sand	110.00
HD3	6.38	0.11	0.33	60.76	92.30	Loamy Sand	80.00
HD4	5.20	0.11	0.23	49.52	130.34	Sand	140.00
HD5	4.69	0.12	0.19	38.13	84.50	Sand	143.00
HD6	11.53	0.16	0.19	72.98	120.62	Sand	120.00
HD7	10.29	0.12	0.29	83.66	127.99	Sand	140.00

MC 1 - 9: 1st to 9th motorcycle mechanic workshop, MM 1 -9: 1st to 9th motorcar mechanic workshop, HD 1 – 7: 1st to 7th truck/lorry mechanic workshop

Earthworms generally prefer loamy soils **(Delate *et al.*, 2003)** therefore, in the bioremediation of UEO contaminated soil from mechanic workshops using earthworms, the contaminated soil may have to be amended with appropriate quantities of loam or clay soil so as to make the soil better suited for earthworm survival.

All samples irrespective of mechanic workshop type recorded similar microbial counts falling within the range 80 x 10^5 cfu/g – 150 x 10^5 cfu/g.

4.1.1.4 Microbial count

The mean values of the microbial count from the various types of mechanic workshops are presented in Table 4.8. According to **Granatstein and Bezdicek (2003)** there needs to be at least a 10-fold difference in microbial number for changes in microbial count to be considered meaningful because of variability in measuring organisms. Table 4.8 therefore shows that MM, MC and HD have similar number of bacteria and would perform the same if all else was equal.

Table 4.8: Microbial count of UEO contaminated soil from mechanic workshops.

	MC	MM	HD
Microbial count, cfu/g * 10^5	133.33± 15.00	135.78± 10.17	124.00 ± 22.88

MM: motor mechanic workshop, MC: motorcycle mechanic workshop, HD: truck/lorry mechanic workshop

Healthy soils will have a Microbe count of 500-800 million per gram of soil, whereas soils that are near sterile conditions will have a count of 4 million microbes per gram**(http://www.microlifefertilizer.com/resource.htm)**. The mean values of the microbial counts of Table 4.8 indicate that the soils in mechanic workshops are a far cry from being healthy, having bacteria counts 30 times less than the values considered healthy.

4.1.1.5 Total Petroleum Hydrocarbon

The mean values for TPH for the various types of mechanic workshops are presented in Table 4.9. The TPH of all soil samples from mechanic workshops were found to be high: the value

exceeds the safe limit of 500 mg/kg set by the Nigeria Ministry of the Environment and therefore, needs intervention for public safety and environmental health**(Abdulsalam *et al*., 2011).** It can be seen that MC has the highest TPH. This may be as a result of (1) the soil type: loamy soils have a higher holding capacity than sandy soil as such oil will not drain through easily: textural classification of the soil from the mechanic workshops showed that the soil samples from MM and HD workshops were predominantly sandy whereas the MC were mostly loamy sand or sandy loam, (2) that the UEO from motorcycles finds less use compared to those from motorcars and trucks/lorries and are thus wholly dumped oil the soil.

Table 4.9: Total petroleum hydrocarbon of UEO contaminated soil from mechanic workshops.

	MC	MM	HD	UCS
TPH, g/kg	130.37 ± 42.71	96.72 ± 26.03	105.64 ± 20.23	0.00±0.00

MM: motor mechanic workshop, MC: motorcycle mechanic workshop, HD: truck/lorry mechanic workshop UCS: uncontaminated soil

4.1.1.6 Carbon/Nitrogen Ratio

The mean values for carbon/nitrogen ratio for soil samples collected from mechanic workshop (MC, MM and HD) and an uncontaminated soil sample (UCS) is presented in Table 4.10.

The resultsshow that all contaminated soil samples had a C/N at least three times greater than that of the uncontaminated soil sample suggesting that the activities at the mechanic workshop must have increased the carbon content of the soil. The table also shows that the soil samples are grossly deficient in Nitrogen hence the large C/N ratio. It has been reported that a high C/N ratio

stimulates increased microbial activity and that growth in microbial population is limited by lack of nitrogen **(Sullivan, 2004)**. Therefore, C/N of the soil samples is not unconnected with the effect of the oil contamination on microbial count herein reported: HD which has the highest C/N ratio of 61.03 and also the least microbial count 124.00 (Table 4.8).

Table 4.10: The carbon/nitrogen ratio of soil samples from mechanic workshop

	MC	MM	HD	UCS
C:N ratio	45.84 ± 20.64	42.330 ± 15.43	61.03 ± 16.43	12.00 ± 0.00

MM: motor mechanic workshop, MC: motorcycle mechanic workshop, HD: truck/lorry mechanic workshop

4.1.2 Analysis of Uncontaminated Soil Sample

In this section,the result of the analysis of soil sample from a farmland (largely expected to be free of petroleum hydrocarbon contamination) is presented and discussed.

4.1.2.1 Elemental Analysis

The elemental analysis of the petroleum hydrocarbon-uncontaminated soil considered is presented in Table 4.11. Comparison of Tables 4.6 and 4.11 shows that irrespective of type of mechanic workshop (MM, MC or HD) there is a general increase in the % concentration of heavy metal in the soil samples when compared to the values for uncontaminated soil sample (UCS). Comparison with Table 2.6 showed that most of the regulated metals were within safe limits. It could be noted that Pb, Hg and As fell below detection, while Cr and Cu contents of the soil were higher than the set limits in Table 2.6. It is important to note that EDXRF analysis

gives the net elemental composition as distinguished from AAS which gives a better indication of the fraction of the metal bioavailable.

Table 4.11: Elemental analysis of petroleum hydrocarbon-free Soil

Elements	% composition
Al	6.349
Ba	0.000
Ca	0.293
Cr	0.021
Cu	0.064
Eu	0.022
Fe	3.291
K	3.469
Mn	0.046
Nb	0.000
Ni	0.008
Os	0.015
Pb	0.000
Rb	0.046
Re	0.023
Ru	0.423
S	0.084
Si	35.500
Sr	0.025
Th	0.000
Ti	0.929
V	0.028
Y	0.031
Yb	0.000
Zn	0.008
Zr	0.281

4.1.2.2 Physicochemical properties of uncontaminated soil sample

Other physicochemical properties of the uncontaminated soil sample used are presented in Table 4.12. The soil textural class was determined to be silt-loam. This texture favors earthworm

(*Eudrilus eugeniae*) habitation **(Oboh *et al.*, 2007).**The corresponding water holding capacity of the soil was found to be 0.41gwater/g dry soil. The pH indicated the soil was very slightly acidic.

Table 4.12: Physicochemical properties of uncontaminated soil sample.

Properties	Soil
% Nitrogen	0.11
% Carbon	1.26
% Phosphorus	1.68
pH	6.7
Textural class	Silt Loam
WHC (g/g bone dry soil)	0.41

4.1.3 Analysis of SimulatedUsed Engine Oil Contaminated Soil

In this section, the result of the analysis of simulated used engine contaminated soil is presented and discussed.

4.1.3.1 Elemental Analysis

Table 4.13 shows that the concentration of used engine oil did not significantly affect the elemental composition of the mixture as contamination level was increased from 25 to 100 gUEO/kg soil.

Table 4.13: Elemental Analysis of simulated used engine oil contaminated soil.

	Used Engine Concentration (g UEO/kg soil)				
	12.5	**25**	**50**	**75**	**100**
Al_2O_3	14.4	11.6	11.6	11	13.1
SiO_2	70.6	67.4	68.2	65.9	65.7
P_2O_5	0.67	0.43			0.41
SO_3	0.2	0.26	0.2	0.2	0.71
K_2O	4.06	5.08	5.19	5.4	4.83
CaO	0.558	0.599	0.561	0.6	0.754
TiO_2	1.43	1.87	1.78	2	1.97
V_2O_5	0.07	0.11	0.095	0.091	0.093
Cr_2O_3	0.044	0.08	0.069	0.11	0.06
MnO	0.077	0.077	0.071	0.073	0.096
Fe_2O_3	7.444	10.93	10.32	12.1	11.27
NiO	0.013	0.042	0.039	0.064	0.029
CuO	0.014	0.053	0.051	0.079	0.036
ZnO	0.004	0.037	0.042	0.054	0.042
Ga_2O_3	0.006	0.04		0.04	0.039
MoO_3	0		0.2	0.6	
Rh_2O_7	0.11	0.8	0.79	1.1	0.46
BaO		0.37	0.43	0.52	0.29
Re_2O_7		0.1	0.1	0.1	0.16
PtO_2		0.04		0.02	
IrO_2	0.13		0.24		
Au	0.11				

(the blank spaces signify 'not detected')

Comparison of Tables 4.13 and 4.6 shows that the metal content of soils from mechanic workshops were higher than the simulated contaminated soil. This may suggests that the elevated metals contamination contents of soil obtained from mechanic workshop may be as a result of continuous dumping of used engine oil over a period of time or may be as a result of other activities taking place in the mechanic workshop.

4.2 CHARACTERIZATION OF ORGANIC AMENDMENT AGENTS AND IDENTIFICATION OF EARTHWORM SPECIES USED

4.2.1 Analysis of Organic Amendment Agents

The moringa leaves were identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria, as *moringa oleifera* of the family *moringaleae*.The carbon, nitrogen and phosphorus contents of the various materials poultry manure, cow dung, *moringa oleifera* leaves and cassava peels are presented in Table 4.14. The properties of the manures (poultry manure and cow dung) were found to be consistent with values obtained from literature **(Marsh and Campling, 1970 and Amanullah, 2007)**. Nitrogen content of *moringa oleifera* (3.59%) leaves fell within the range 3.064 – 4.608% reported by **Jongrungruangchok *et al.*, (2010)**.The composition of the cassava peels is similar to reported literature values **(Yoonan and Kongkiattikajorn, 2004)**.

Table 4.14: Carbon, Nitrogen and phosphorus content of materials used as organic amendment.

Properties	Cow dung	Poultry manure	*M. oliefera* leaves	Fermented Cassava peels
% Nitrogen	1.14	2.98	3.59	0.74
% Carbon	56.65	45.58	37.67	48.84
% Phosphorus	1.87	1.90	1.85	0.07

4.2.2 Identification of Earthworms Used

The worms collected for this work were identified in the Department of Biological Sciences, Landmark University, Kwara State, Nigeria as *Eudrilus eugeniae*.

4.3 EFFECT OF ORGANIC AMENDMENT ON THE PROPERTIES OF USED ENGINE OIL CONTAMINATED SOIL

4.3.1 Bioremediation Using Manure

In this section, investigation into the effect of organic amendment on the degradation of petroleum hydrocarbon in mechanic workshop contaminated soil and soil from laboratory simulated contamination is presented and discussed.

4.3.1.1 Biostimulation Using Cattle Dung

The Total Petroleum Hydrocarbon (TPH) versus time data is presented in the linearized first order kinetic form inFigures 4.1and 4.2. The first order kinetic model is given as:

$$\boldsymbol{Y = ae^{-kt}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (\mathbf{4.1})$$

On linearization this gives:

$$\boldsymbol{ln(Y) = ln(a) - kt} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (\mathbf{4.2})$$

or,

$$\boldsymbol{ln\left(\frac{a}{Y}\right) = kt} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (\mathbf{4.3})$$

Where:

t = bioremediation time (weeks)

Y = the TPH (g/kg) at various t

a = the initial TPH (g/kg)

The first order kinetics as stated in **Section 2.7** has been reported to give good fits to bioremediation kinetics. The R^2 values for the liberalized first order model generally > 0.9 indicated a good fit. The *k* values, as shown in Tables 4.15 and 4.16were found to increase with increase in surface area of soil directly open to air/weight of soil ratio (a_E) as the only parameter that was varied for the pair of samples shown in both table was the surface a_E.Increase in surface area translates to increased aeration of the soil or generally, reduction in the mass transfer resistance, which is the limiting step in bioremediation. Comparison of Tables 4.15 and 4.16showsthat the rate of bioremediation are seemingly higher for the soil samples obtained from mechanic workshop than for those contaminated in the laboratory. This was probably as a result of better acclimatization of the microbes as they had been in contact with used engine oil for a longer period (the mechanic workshop had been in existence for 10 years) than the soil samples contaminated in the laboratory.This acclimatization would result in the reduction or possible elimination of the lag phase (see Figure 2.2). However, since the a_E considered for the mechanic workshop soil were not the same with those used for the soil contaminated in the laboratory, a definite comparison cannot be made.

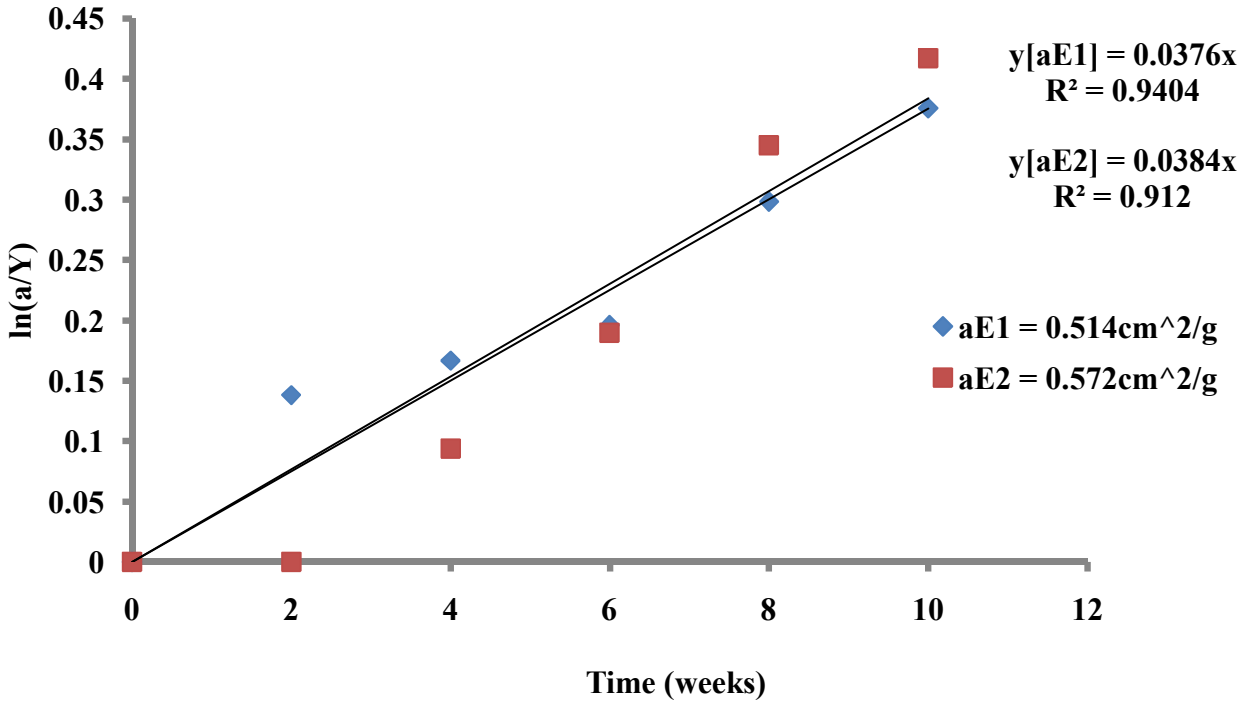


Figure 4.1: Bioremediation of UEO contaminated soil (from mechanic workshop) by biostimulating with cattle dung as a function of surface area

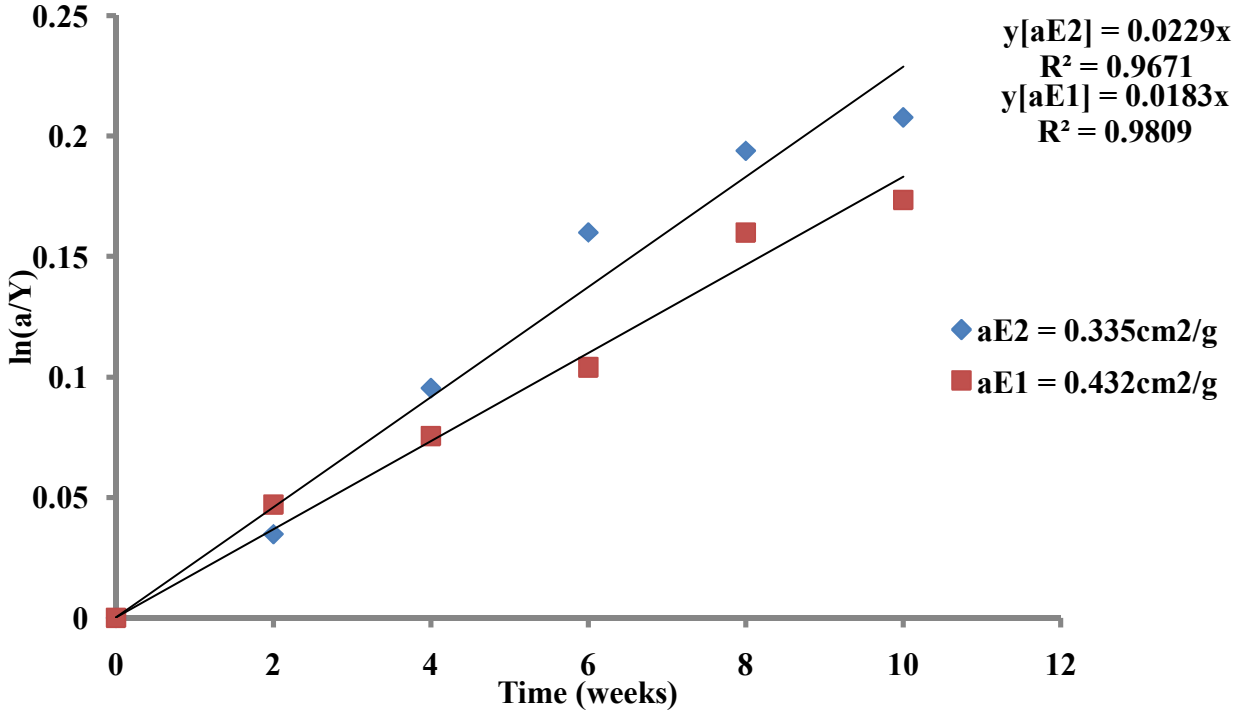


Figure 4.2: Bioremediation of UEO contaminated soil (laboratory simulated contaminated soil) by biostimulating with cattle dung as a function of surface area

Table 4.15: Effect of biostimulation (using cow dung) on bioremediation of UEO contaminated soil (contaminated soil obtained from mechanic workshop).

Biostimulant	*k*, week^{-1}	a_E, cm^2/g	R^2
Cow dung	0.0384	0.572	0.912
Cow dung	0.0376	0.514	0.9404

Table 4.16: Effect of biostimulation (using cow dung) on bioremediation of UEO contaminated soil (soil contaminated in the laboratory).

Biostimulant	*k*, week^{-1}	a_E, cm^2/g	R^2
Cow dung	0.0229	0.423	0.9671
Cow dung	0.0183	0.335	0.9809

4.3.1.2 Effect of Surface Area on bioremediation kinetics

Figure 4.3shows the results of the investigation into the effect of surface area on the rate of bioremediation. They are presented as a linearized form of the first order kinetics.The R^2 values for all samples indicate a good fit, being generally high (>0.959). The *k* values for the various surface areas (a_E) are presented in Table 4.17. The table shows that *k* increases with increasing a_E with a corresponding general increase in the drop in TPH value after 21 weeks also shown in Table 4.17. As a_E increased from 0.143 to 0.243cm^2/g, *k* increased from 0.023 to 0.033 per week. However with further increase in a_E, *k* remained fairly constant.This is suggestive of an optimum

a_E for bioremediation systems.The increase in *k* with increasing a_E may probably be due to greater availability of O_2 required (improved aeration) for aerobic degradation and release of biodegradationproducts, for example volatiles and CO_2 which may be undesirable for bioremediation. Since the major rate determining step in the bioremediation process is mass transfer (solubility of petroleum hydrocarbon in water), it follows that aeration is an important factor in bioremediation. This probably is as a result of enhanced biosurfactant production with improved aeration, in agreement with **Saharan *et al.*(2011)** who reported that aeration is an important parameter to be optimized in the production of biosurfactants. Microorganisms are known to produce biosurfactants which are substance that can reduce surface tension and facilitates the solubilization of oil in water thereby resulting in improved mass transfer.

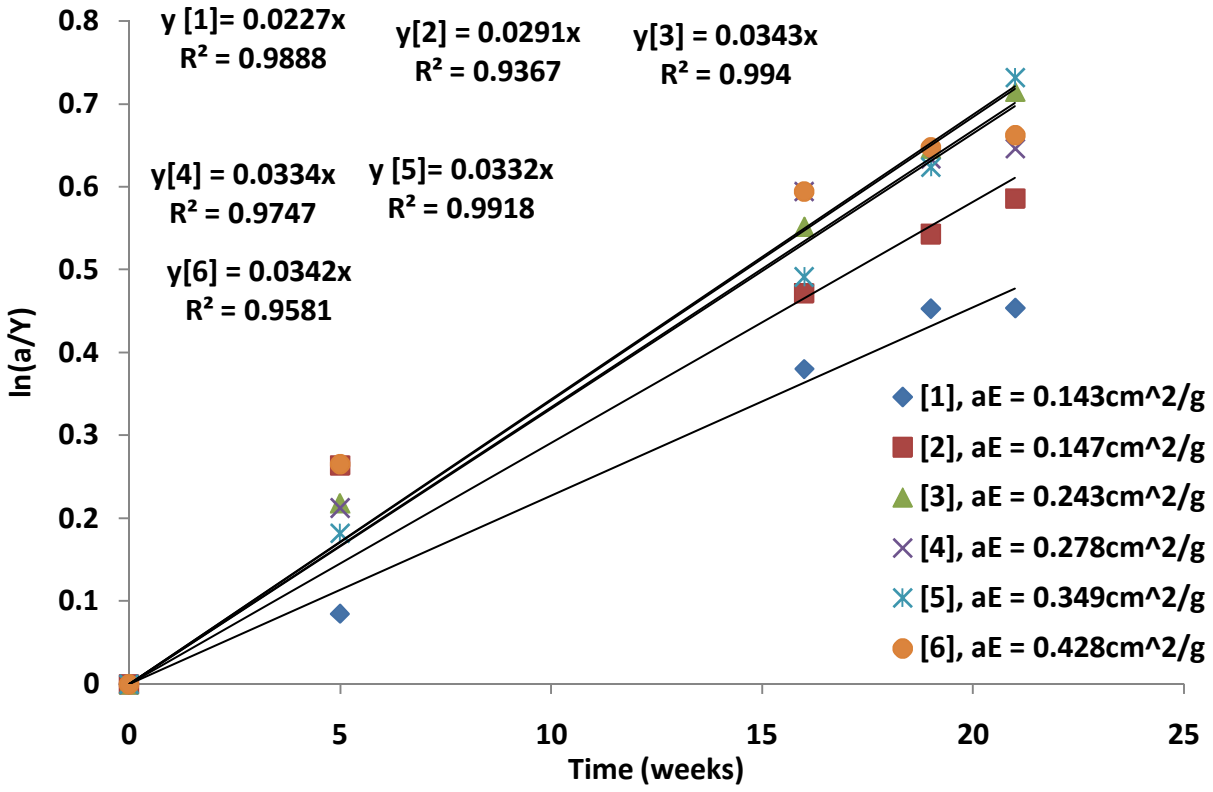


Figure 4.3: Effect of surface area on bioremediation kinetics of used oil contaminated soil

For the effect of surface area, the chromatogram (Figures 4.4 and 4.5) and the MS analysis presented are for the sample with the largest surface area 0.428cm^2/g at time 0 and 21 weeks (at the start and finish of the investigation). The TPH result of Table 4.17 for Sample 6 indicated a 48.44% drop and the GC-MS result of Figures 4.4 - 4.5 and Table 4.18 showed that by 21 weeks of bioremediation, a lot of peaks (corresponding to hydrocarbons) had disappeared from the sample and that all the hydrocarbons constituting C30 and above had been degraded whereas, the C9 – C19 and C19 – C29 fraction increased. Similar observation was reported by **Prince *et al.* (2007)**, who stated that within a complex mixed gasoline mixture; larger n-alkanes were more readily degraded than the smaller n-alkanes.

Table 4.17: Effect of surface area on the bioremediation kinetics.

Sample	a_E, cm^2/g	k, $week^{-1}$	% drop in TPH after 21 weeks
1	0.143	0.023	36.44
2	0.147	0.029	44.33
3	0.243	0.034	51.06
4	0.278	0.033	47.56
5	0.349	0.033	51.87
6	0.428	0.034	48.44

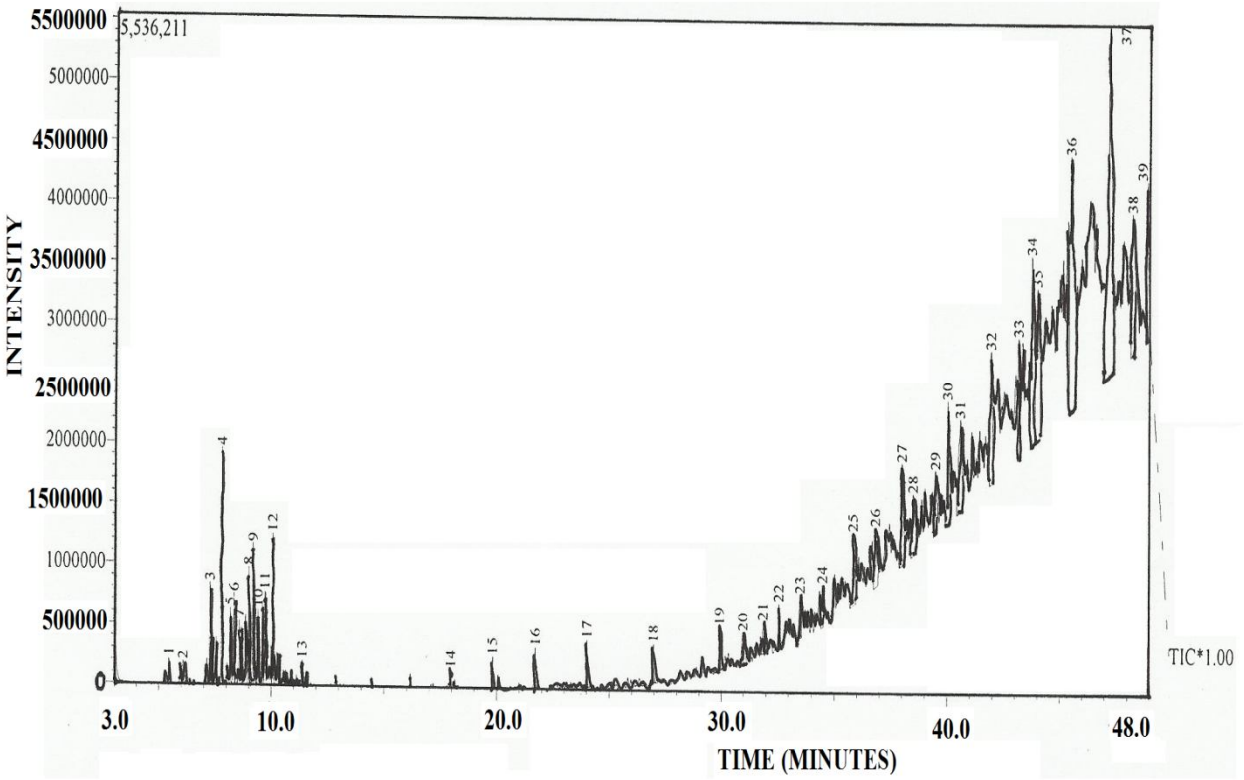


Figure 4.4: GC of extract from used engine oil contaminated soil at t = week 0

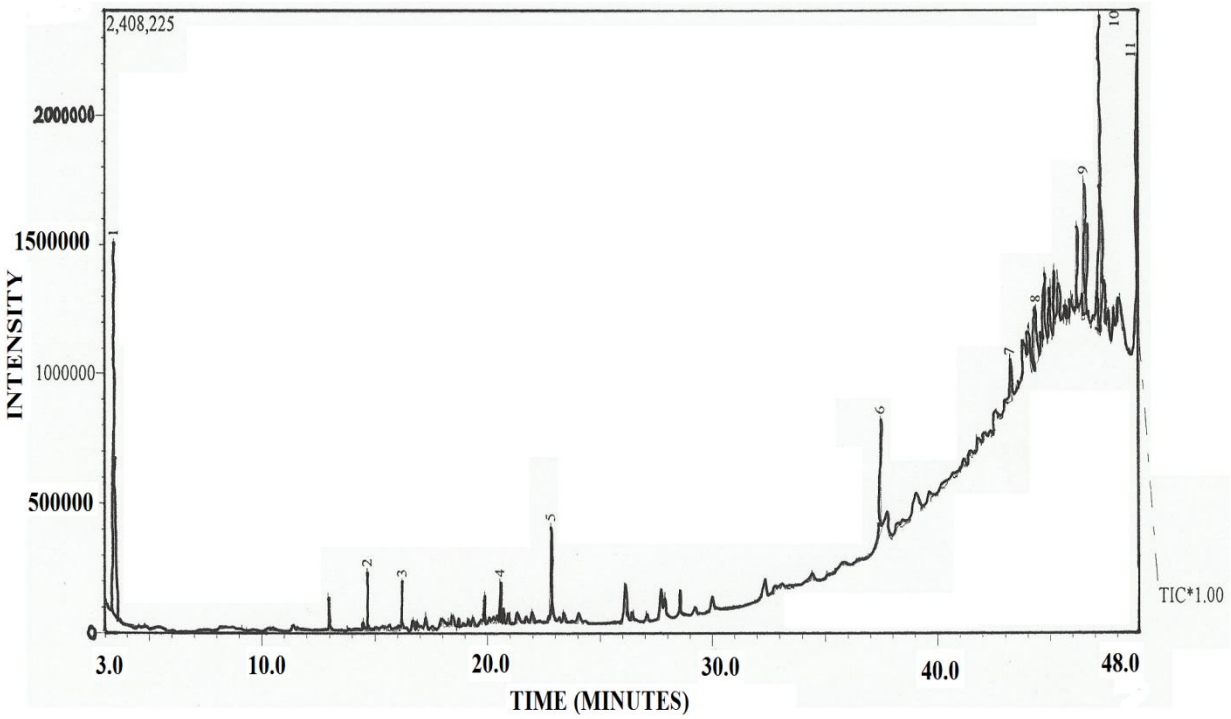


Figure 4.5: GC of extract from sample 6 (area = 0.428cm^2/g) at week 21

Table 4.18: Composition of used engine oil contaminated soil for sample with surface area of 0.428g/cm^2.

	Composition of UEO	
	0 weeks	21 weeks
Components		**Sample6**
C9 - C19	15.8	60.37
C20 - C29	26.42	39.62
C30 and Above	56.78	0

4.3.1.3 Effect of Mixing (agitation) on the rate of bioremediation

Figure 4.6 presents the result of the investigation into the effect of manual tilling on the bioremediation kinetics. The graph is also plotted in the form of the linearized first order kinetics. The values of *k* and % TPH drop after 22 weeks, as a function of degree of mixing, are shown in Table 4.19. The R^2 values for the samples amended with poultry manure (>0.977) were higher than those amended with *Moringa oleifera* leaves (<0.880). This may be attributed to the initial slow rate of the samples amended with*Moringa oleifera*leaves (the initial slow rate being probably as a result of the antimicrobial properties of *Moringa* leaves).

It is worthy of note that in spite of the antimicrobial properties of *Moringa oleifera* leaves **(Dahot, 1998; Chen, 2009)**, it was able to effect a higher degree of TPH removal than the control sample. Comparison of the *k* values and the drop in TPH values after 22 weeks indicated that mixing every three days (for 5min/day) had no significant effect on TPH removal. For poultry manure as well as *Moringa oleifera* leaves amended processes, both the mixed and unmixed samples had very similar *k* and TPH values. These results are in agreement with the findings of **Maike and Filser (2007)**. Though generally agreed that biosurfactant production

increases with stirring and aeration, instances have been reported where agitation has no or a negative influence on biosurfactant production. For example *N. erythropolis* and *A. calcoaceticus* produce less biosurfactant due to the increase ofshear stress (**Saharan *et al.*, 2011**).

The frequency of mixing used in this investigation was every three days (twice a week) for 5min/day. Examples of frequencies of mixing that have been used in bioremediation studies to improve aeration include: twice a month **(Bagherzadeh-Namazi *et al.*, 2008)**, twice a week **(Margesin *et al.*, 2000)**, every alternative day **(Vasudevan and Rajaram, 2001)** and every day **(Maike and Filser, 2007)**.

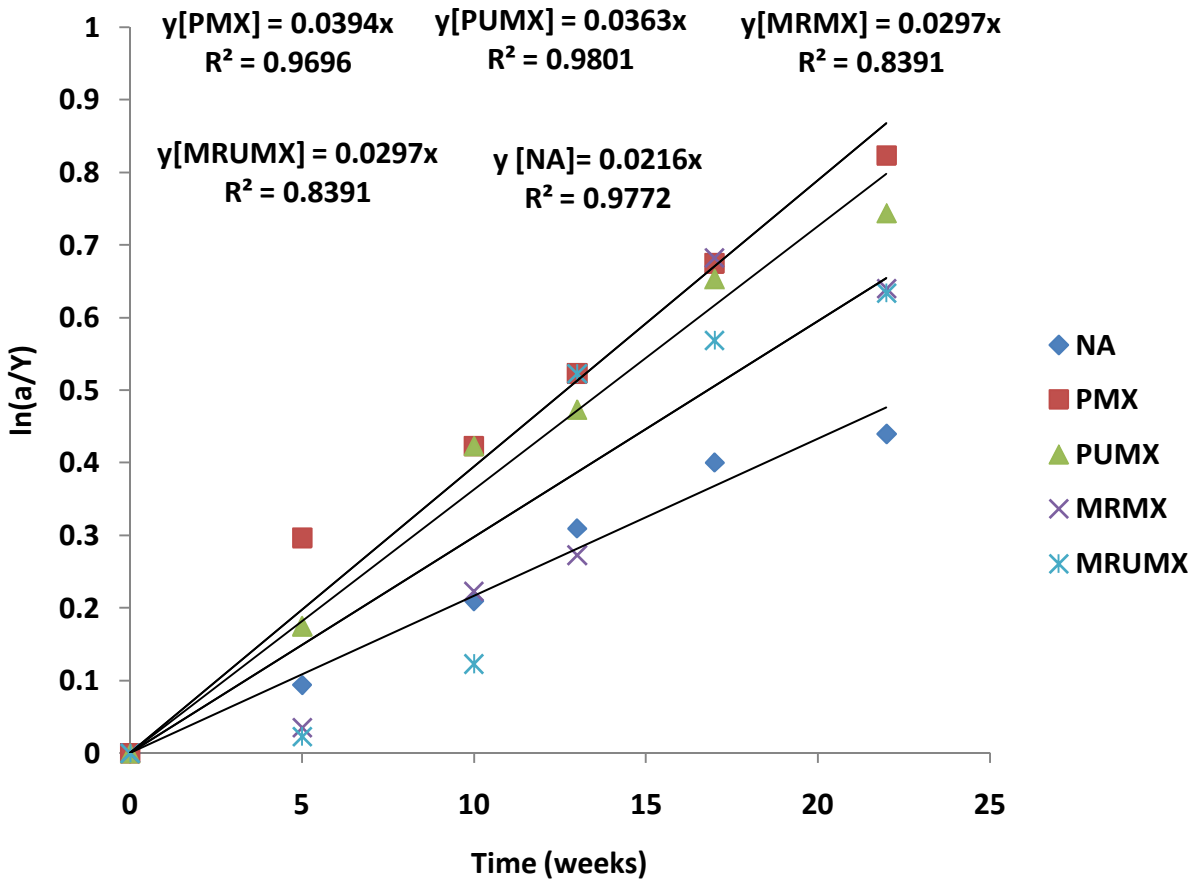


Figure 4.6: Effect of tilling on bioremediation kinetics of used engine oil contaminated soil

Table 4.19: Effect of mixing on the bioremediation kinetics.

Sample	a_E, cm^2/g	k, $week^{-1}$	% drop in TPH after 22 weeks
Control	0.428	0.022	35.59
PUMX	0.428	0.035	52.45
PMX	0.428	0.036	50.80
MRMX	0.428	0.034	47.26
MRUMX	0.428	0.035	46.93

PUMX: unmixed poultry manure treated, PMX: mixed poultry manure treated, MRMX: mixed *Moringa oleifera* treated and MRUMX: unmixed *Moringa oleifera* treated samples.

The chromatograms obtained from GC analysis for investigations into the effect of surface area and mixing on bioremediation are presented as Figures 4.7 to 4.9. The MS spectrum comparison gave the composition of oil in the soil at various times (t) tabulated in Table 4.20for the effect of mixing.

The GC chromatogram at the start of the investigation is the same as that for the studies into the effect of surface area (Figure 4.4). The result of Table 4.20 indicates that by 4 weeks of bioremediation there was a significant reduction in the C30 and above fraction of the oil for the samples treated with organic amendments. By 22 weeks, the C30 and above fractions had been completely removed for the sample treated with organic amendment (MRMX and PUMX) whereas the control sample (NA) still contained C30 and above fractions. However, the % composition has reduced from 56.78 at t = 0 weeks to 39.86 at t = 22 weeks. The result also indicated a significant reduction in C20 – C29 component for the PUMX (10.47%) than for MRMX (67.72%). Similarly, Table 4.19 indicated a higher reduction in TPH for PUMX (52.45%) than for MRMX (47.26%). Poultry manure may have performed better than *Moringa oleifera* leaves because of the antimicrobial properties of the latter.

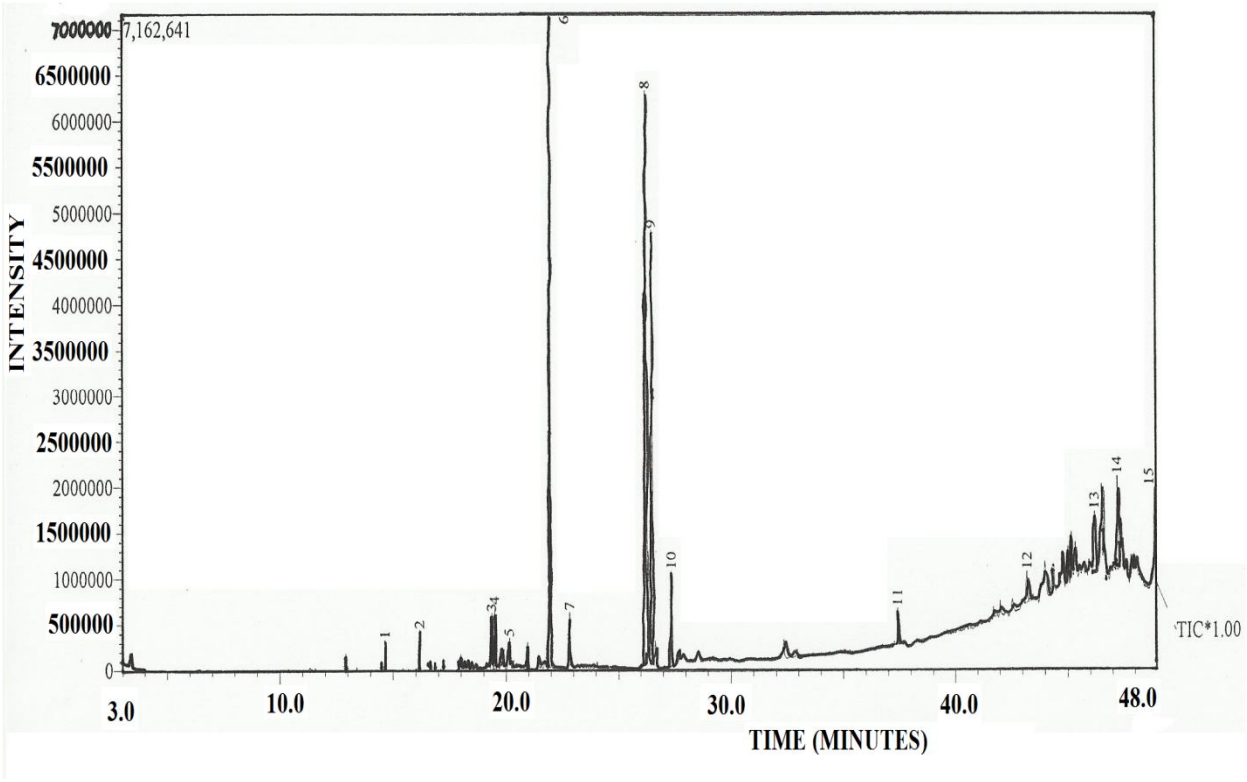


Figure 4.7: GC of extract from SAMPLE PUMX at week 22

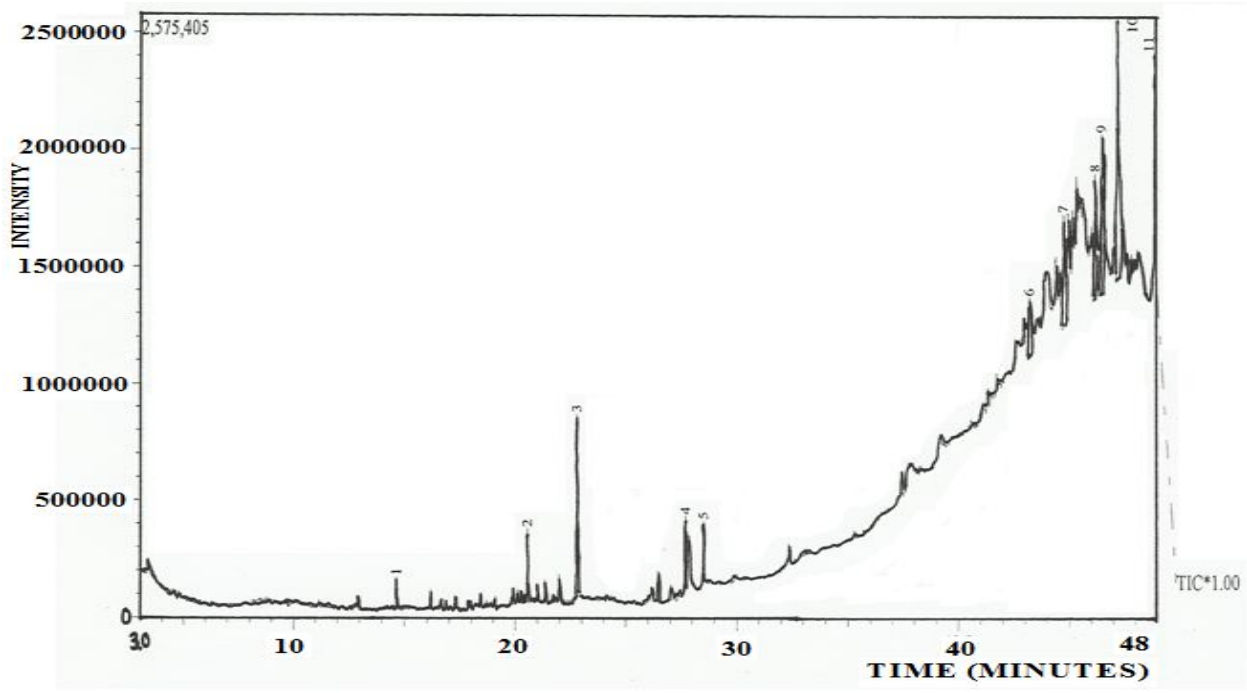


Figure 4.8: GC of extract from Sample MRMX at week 22

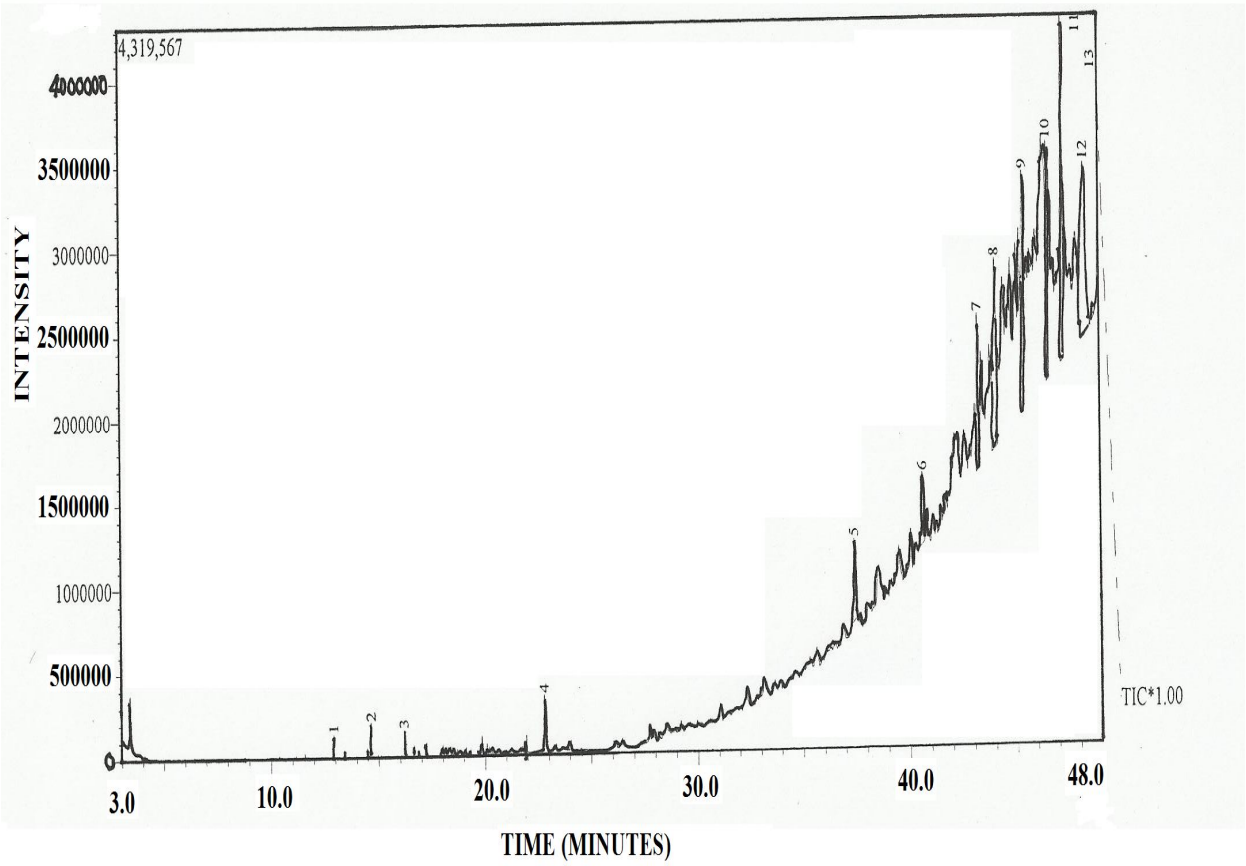


Figure 4.9: GC of extract from Sample NA at week 22

Table 4.20: Effect of mixing on the composition of UEO in bioremediation

	% COMPOSITION OF REMAINING PETROLEUM HYDROCARBON IN SOIL						
	0 weeks	4 weeks			22 weeks		
Components		**PMX**	**PUMX**	**MRMX**	**NA**	**MRMX**	**PUMX**
C9 - C19	15.80	25.62	46.31	30.35	3.07	32.28	89.54
C20 - C29	26.42	74.20	44.13	47.34	57.07	67.72	10.47
C30 and Above	56.78	0.00	8.65	9.18	39.86	0.00	0.00

PUMX: unmixed poultry manure treated, PMX: mixed poultry manure treated, MRMX: mixed *Moringa oleifera* treated, MRUMX: unmixed *Moringa oleifera* treated and NA: control samples.

4.3.1.4 Microbial isolation and identification

Tables 4.21 and 4.22 present the results of the biochemical test on the isolates from MRMX, PMX respectively. *Bacillus cereus* was the most prevalent microbe found in all three samples. Even in the isolate from MRMX in which *P. aerugenosa* was also identified, *Bacillus cereus* was found to be the dominant bacteria group. *Bacillus* is ubiquitous in nature and has been postulated to be more tolerant to high levels of hydrocarbons in soil due to their resistant endospores. *Bacillus Cereus*is an organism occurring naturally in most foods and it can cause two different forms of food poisoning, diarrhoeal and emetic, both caused by toxins produced by the bacteria. **Kebria *et al.* (2009)** reported that a strain of *bacillus* native to Tehran*Bacillus cereus*,showed great potential for *in-situ* remediation of diesel-contaminated soil in oil refinery sites.**Velázquez-Aradillas *et al.* (2011)**reported the characterization of biosurfactant produced by bacillus cereus and concluded that the biosurfactant properties of *Bacillus cereus* may have potential environmental applications.

Pseudomonas aeruginosa is a versatile bacterium that grows in soil, coastal marine habitats, and on plant and animal tissues. *P. aeruginosa* is not new in the scene of bioremediation as studies have been conducted on its use for degradation of petroleum hydrocarbon: **Das and Chandran (2011)** touched on the use of*Pseudomonas aeruginosa* for degrading sludge hydrocarbons and **Emtiazi *et al*. (2005)** reported the immobilization of *pseudomonas* isolated from a petroleum-contaminated soil for use in petroleum hydrocarbon degradation. Rhamnolipid is a biosurfactant produced by several species of *Pseudomonas*. Biosurfactants are known to lower surface tension and enhance the uptake of lipids in aqueous media.

Table 4.21: Identification of bacteria for used engine oil contaminated soil biostimulated with *moringa oleifera* leaves (sample MRMX) after 22 weeks.

Gram reaction	Shape	Indole	MR	VP	Citrate	Catalase	Coagulase	Organism
-	Rod	-	-	-	-	+	-	*Pseudomonas aerugenosa*
+	Rod	-	-	-	-	+	-	*Bacillus cereus*

Table 4.22: Identification of bacteria for used engine oil contaminated soil biostimulated with poultry manure (sample PMX) after 22 weeks.

Gram reaction	Shape	Indole	MR	VP	Citrate	Catalase	Coagulase	Organism
+	Rod	-	-	-	-	+	-	*Bacillus cereus*

4.4 EFFECT OF EARTHWORM INOCULATION OF BIOSTIMULATED USED ENGINE OIL CONTAMINATED SOIL

4.4.1 Assessment of Earthworm Survival in Biostimulated Contaminated Soil

This section discusses the survival rates of*E. eugeniae* over time at various concentrations of UEO contaminated soil prepared.

From Figures 4.10 and 4.11it can be seen that for used engine oil from motorcycle there was 100% survival of the earthworm in the 14 daystudy for both poultry manure spiked and cattle dung spiked setups for all UEO concentrations considered.

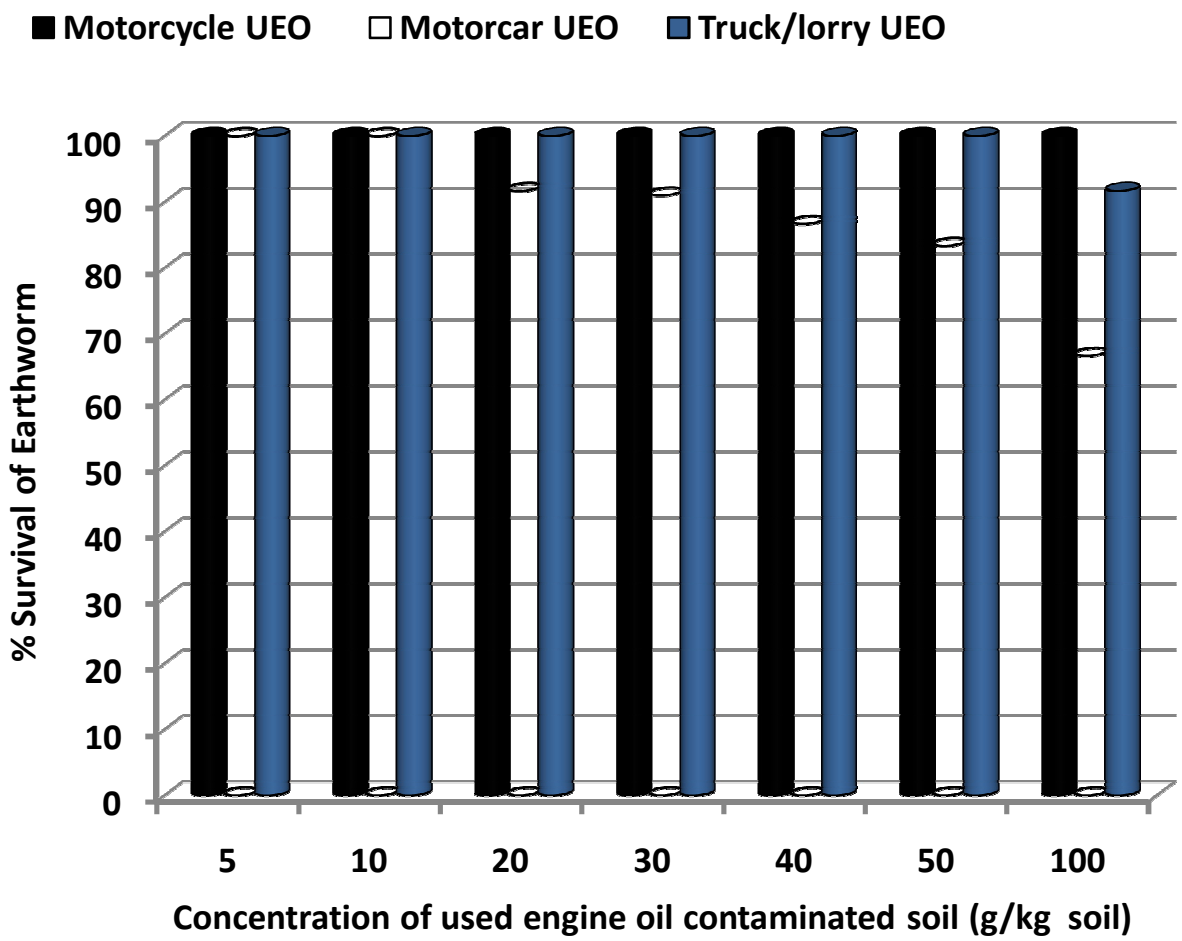


Figure 4.10: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with poultry manure

% survival was computed as the fraction of earthworm from all samples still alive at the end of the test period. Cast formation as a result of earthworm's activities was also observed at low UEO contamination levels. The observed quantity of cast was found to decrease with increase in UEO concentration.

Very similar results were obtained for used engine oil from truck/lorries save for the poultry manure spiked samples in which survival dropped to 91.67% at used engine concentration of 100g/kg soil. The results of this investigation indicate that though very high concentrations of used engine oil in soil was found not to be lethal to earthworms, in considering earthworms for

bioremediation lower UEO concentration would be preferred as this favours greater earthworm activities and cast formation.

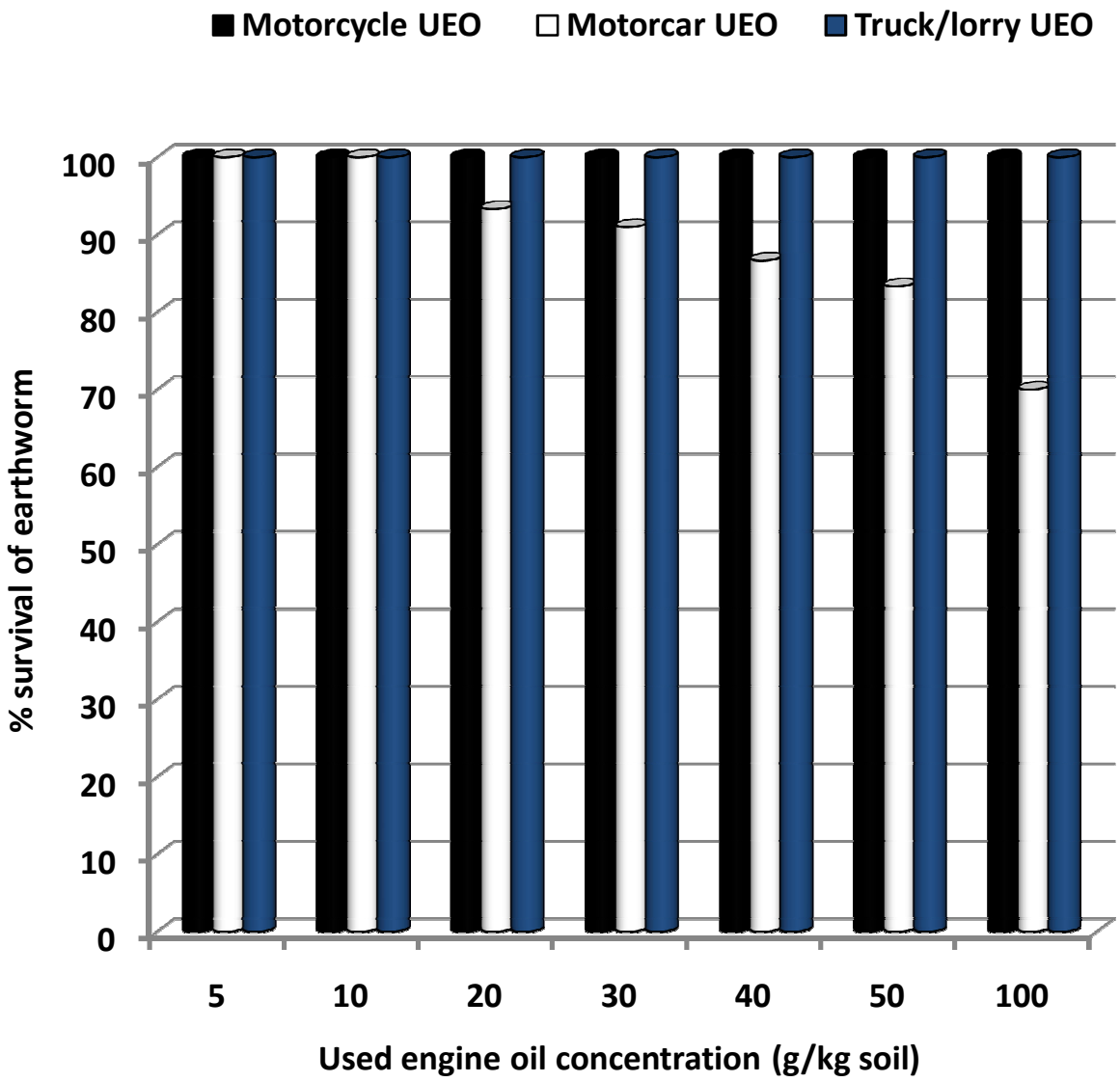


Figure 4.11: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with cattle dung

In the case of engine oil from motorcar, the % survival dropped from 100% to approximately 70% as used engine oil concentration was increased from 10 to 100g/kg soil. All control samples recorded 100% earthworm survival. Therefore the death of the worm must have been as a result of changes created by the presence of the used engine oil contamination. The survival of *Eudrilus eugeniae* in used engine oil contaminated soil was found to be higher than the survival of *Lumbricus terrestris* and *Eisenia fetida* in crude oil contaminated soil as reported by **Hanna and weaver (2002)** that no earthworms survived for 10 days when the concentration of crude oil contaminated soil was 2.5%.

Maike and Filser (2007) in their bioremediation study in which crude oil contaminated soil was inoculated with*E. fetida*, *A. chlorotica* and *L. terrestris*reported that TPH concentrations of 5000–9500 mg/kg were increasingly lethal after an incubation of 28 days. Thus recommended that earthworms may be useful remediation tools in oil contaminated soils with moderate TPH concentrations. It may therefore be inferred from the results of Figures 4.10 and 4.11, that *Eudrilus eugeniae* may be introduced into soil with high concentrations of used engine oil contamination.

The composition of Used Engine Oil has been reported to be a function of: the composition of: original crude oil, the refining process used, the additives, the efficiency and type of engine, the gasoline combustion products, and the length of time in use **(United States Department of Health and Human Services, 1997)**. Therefore, when earthworms are considered for use in the bioremediation of UEO contaminated soil, it is important to determine the tolerance of earthworm for the UEO contamination in question.

4.4.2 Effect of Earthworm on the Carbon and Nitrogen Content, Moisture Retention and Aggregate Size Distribution of Used Engine Oil Contaminated Soil

This section discusses the effect of earthworm (*E. eugeniae)* onsome soil properties thought to translate into enhanced bioremediation of contaminated soil.

4.4.2.1 Effect of Earthworm Inoculationon Soil Carbon Content

The effect of earthworm concentration on the carbon content of used engine oil contaminated soil treated with cow dung and earthworm is presented in Figure 4.12. The figure shows fluctuations (increase and decreases) in the carbon content and a general progressive decrease in the organic carbon content over the period of the study for all earthworm concentrations considered. The fluctuations (decreases and increases) in the carbon content with time may be attributed to the heterogeneity of the system (soil, earthworm, used engine oil and manure mixture)which makes it difficult for the carbon content of the sample removed to be a true average of the entire setup. The drop in organic carbon content was not proportional to the earthworm concentration and the sample with no earthworm also recorded a drop in carbon content. It may be concluded that the presence of earthworms did not significantly accelerate the drop in the organic carbon content (of the used engine oil contaminated soil) over the six weeks study period. The average drop in carbon content for all five samples was found to be 27.02% at the end of 6 weeks. The result of the investigation, howbeit short (6 weeks), is in agreement with **Yu *et al.* (2007),** that earthworm inoculation under straw application had no significant effects on soil organic carbon content in their 6 years study conducted to study the effects of straw application and earthworm inoculation on cropland soil organic carbon and labile organic carbon.

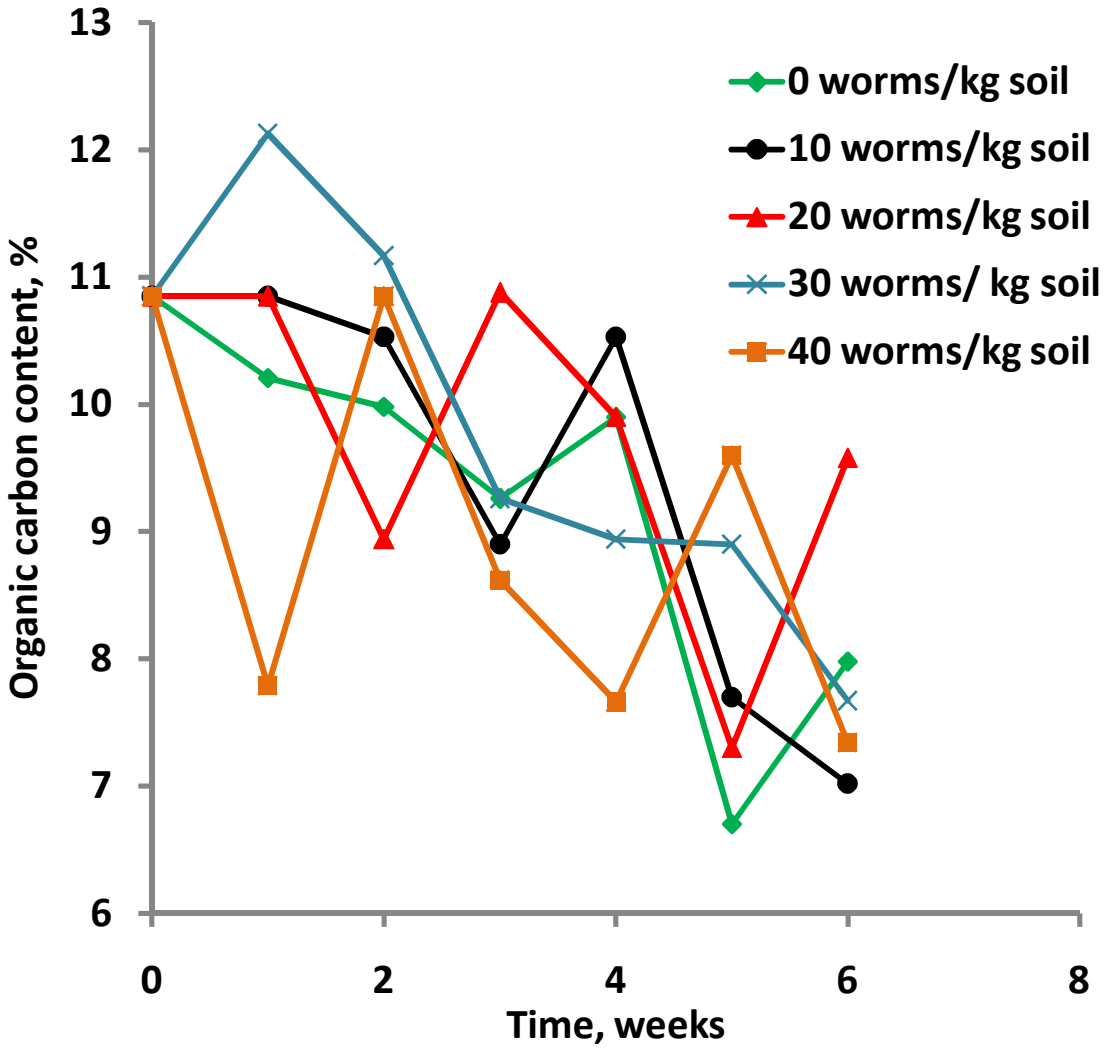


Figure 4.12: Effect of worms on organic carbon content of UEO contaminated soil

Li *et al.* (2002) also in their study using cropland soil reported that earthworms had no significant influences on total soil carbon content: which implied that there was no depletion of soil carbon and nitrogen pools in the presence of earthworms.

4.4.2.2 Effect on Earthworm Inoculation on Nitrogen Content

Figure 4.13 presents the effect of earthworm concentration on the nitrogen content of used engine oil contaminated soil over a six (6) week period. The figure shows that at the end of the study, the nitrogen content of all samples was not significantly changed. Despite the fluctuations in weekly analysis of the nitrogen contents of the samples, the composition remained fairly constant for all samples throughout the study.The observed fluctuations may be due to similar reasons advanced for fluctuation in carbon content.The average drop for all samples at the end of 6 weeks was found to be 16.46%. Similar results was reported by **Li *et al.* (2002)** that earthworms had no significant influences on nitrogen content in their study using used engine oil free soil.

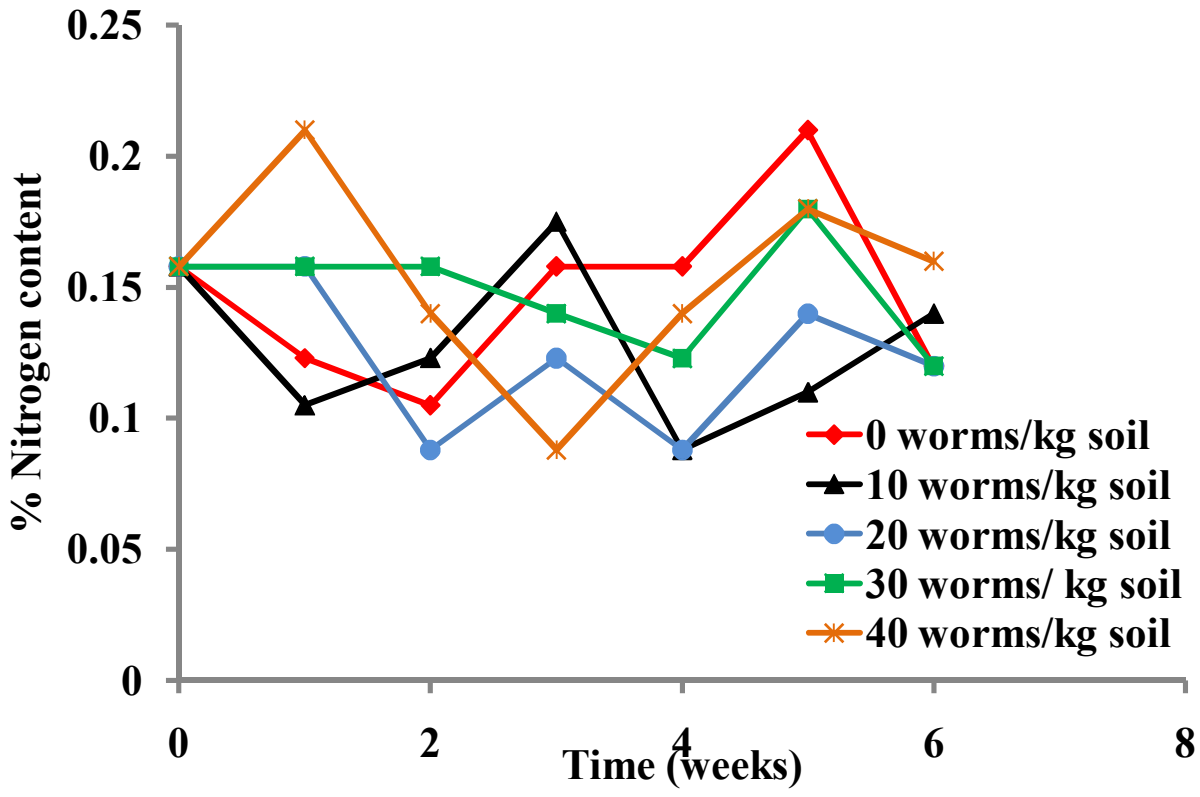


Figure 4.13: Effect of worms on nitrogen content of UEO contaminated soil

4.4.2.3 Effect of Earthworm Inoculation on the Water Holding Capacity of Used Engine Oil Contaminated Soil

Figure 4.14presents the effect of earthworm concentration on soil moisture retention. The figure shows a general increase in water holding capacity with increasing earthworm concentration (indicated by the positive slope of the of the trend line).At a confidence interval of 0.05 the effect of earthworm on soil water holding capacity was found to be significant with a *P* value of 0.025. The increase in moisture retention ability has been reported by (**Edwards and Bohlen, 1996; Schaefer and Filser, 2007**)is considered a positive development (**Hickman and Reid, 2008**) with respect to soil health.

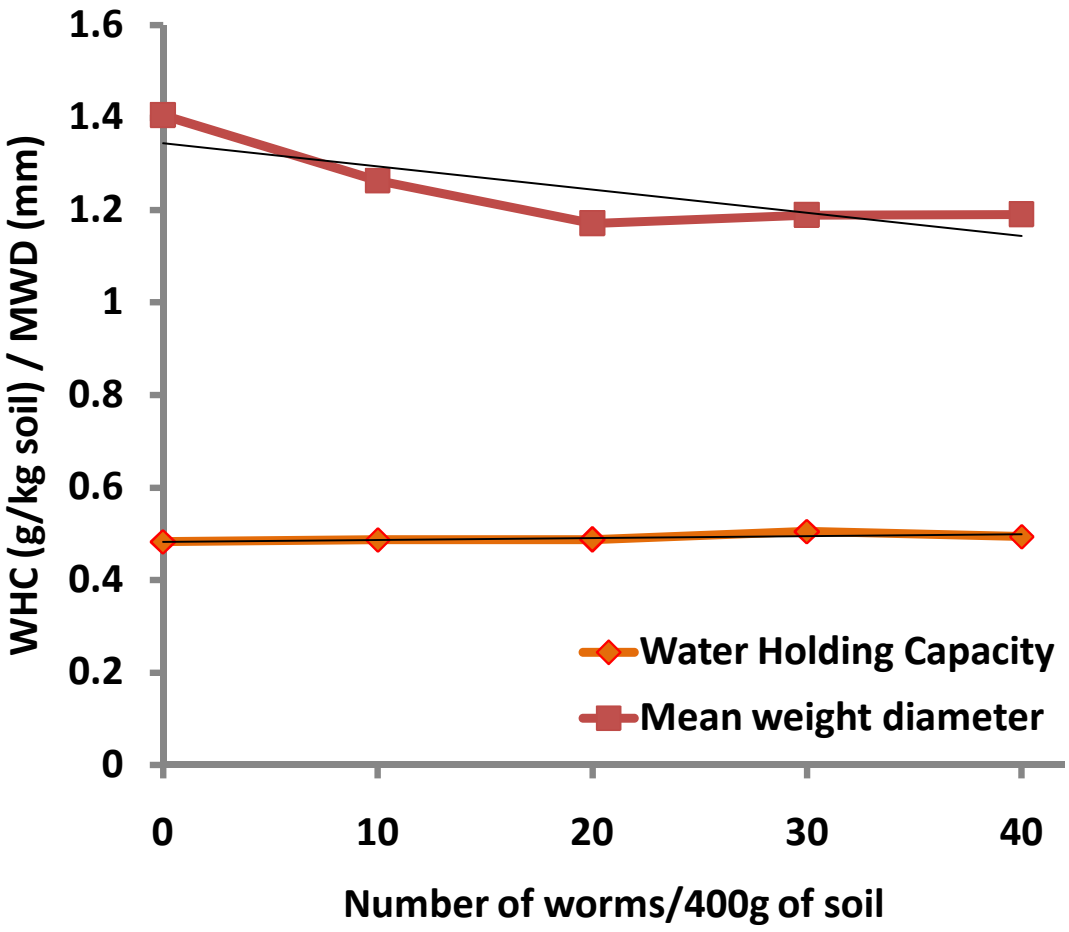


Figure 4.14: Effect of earthworm concentration on soil WHC and MWD.

4.4.2.4 Effect on Earthworm Concentration on Aggregate Size Distribution of Used Engine Oil Contaminated Soil

Figure 4.14 also shows the effect of earthworm concentration on the mean weight diameter of the soil. The mean weight diameter decreased from 1.406mm in the sample without worm to 1.191mm in the sample with the highest concentration of worms (40 worms/kg of soil) in the six weeks study. The presence of earthworms clearly has an effect on the particle size distribution, with a *P* value of 0.029 at a confidence interval of 0.050.The decrease in soil mean weight diameter evened out at earthworm concentration of 20 earthworms/400g of soil, and may be considered as optimal should the effect on particle size be the singular most important factor in earthworm assisted bioremediation of used engine oil contaminated soil.**Barois *et al.*, (1993)** hypothesized that in this reduction in particle size might exist potential for earthworm assisted release of soil associated organic contaminants, which will probably result in an increase in bioremediation rate.

4.4.2.5 Uptake of Metals by Earthworm in Simulated Used Engine Oil Contaminated Soil

Table 4.23 compares the concentration of heavy metals in the earthworms to the concentration of metals in the various concentrations of used engine oil contaminated soil. The table shows that for most of the metals detected in the worms (apart from V, Mn and Ti), the dry body burden of the worm (concentration in the dried carcasses of the earthworms) were higher than those in all contaminated soil concentrations. Only a marginal difference existed between the concentration of Fe and Ba in the earthworm and the used engine oil contaminated soil at t = 0. Based on these and should the metals in the soil be 100% bioavailable, it is expected that elimination should take place for metals with higher concentration in the worm and accumulation for the metals with lower concentrations in the worms. Fe and Ba should remain relatively constant in the worms

with time. The table also shows that the concentration of the metals for the various UEO concentrations did not vary much.

Table 4.23: Initial elemental composition of earthworm and simulated used engine oil contaminated soil.

	Worms	Concentration of used engine oil in contaminated soil (g UEO/kg soil)				
		12.5	25	50	75	100
Al	0.280	0.148	0.078	0.041	0.022	0.012
Si	0.000	32.977	31.483	31.856	30.782	30.689
K	0.000	3.369	4.216	4.307	4.482	4.008
Ca	16.214	0.446	0.478	0.448	0.479	0.602
Ti	0.527	0.857	1.121	1.067	1.198	1.180
V	0.028	0.039	0.062	0.053	0.051	0.052
Cr	0.178	0.030	0.055	0.047	0.075	0.041
Mn	0.000	0.060	0.060	0.0550	0.057	0.074
Fe	9.434	5.202	7.638	7.212	8.456	7.876
Ni	0.204	0.010	0.033	0.031	0.050	0.023
Cu	0.232	0.011	0.042	0.041	0.063	0.029
Zn	0.739	0.003	0.030	0.034	0.043	0.034

The one-compartment model of **Van Straalen *et al.* (2005**) was used to analyze the development of earthwormbody concentrations with time, and to calculate uptake and elimination rateconstants. Equation (4.4)was used for all elements.

$$C_{iworm}(t) = C_i(0)e^{-k_2 t} + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \quad (4.4)$$

In addition,

$$k_1 = \frac{a}{C_e} \quad (4.5)$$

Where: $C_{iworm(t)}$ = metal concentration in the earthworm (µg g^{-1} dry weight) at time t (days), a = flux of metals into the earthworm (mg_{metal} kg^{-1} animal day^{-1}),k_2 = elimination rate constant (day^{-1}). $C_i(0)$ is the initial body concentration (µg g^{-1}dry weight), which is measured in the animals before exposure.

Other parameters of interest include:

R^2: coefficientof correlation

C_e: external metal concentration (concentration in the soil, µg g^{-1} dry weight)

k_1: uptake rate constant (from bulk concentrations $k_1$soil, gsoil ganimal^{-1} day^{-1};

Mathematically, k_1 is defined as;

C_{SS}: calculatedsteady-state metal concentration, mg kg^{-1}

t_{SS}: calculated time taken to reach steady state, days

BAF: bioaccumulation factors (from bulk concentrations)

Figures 4.15 and 4.16 are typical examples of the kinetic model against the experimental data. The kinetic parameters generated for Zn, Cu, Ni, Cr, Ti and Mn were tabulated in Tables 4.24 – 4.26. Tables 11 – 16 in**Appendix C**shows the raw experimental kinetic data for elimination of the elements Zn, Cu, Ni and Cr from the worms as expected (as the earthworm body concentration of these were higher than the contaminated soil concentration) and accumulation of Ti and Mn were accumulated also as expected. The R^2 values obtained varied widely ranging from 0.01 to 0.99 but were very similar to those reported by **Nahmani*et al.* (2009)** and higher than those reported by **Gimbert *et al.*(2006)**.

For V_2O_5, the concentration in the worms remained relatively unchanged irrespective of UEO concentration throughout the period of the study. The value of t_{SS} for all elements considered (Tables 4.24 – 4.26) ranged from 2 to 18 days for the elements that attained steady state concentration in the worms on exposure. The 14 days study period using in the earthworm survival assessment of **Section 4.4.1** is therefore generally reasonable, but future works on earthworm survival in similar environment may consider a longer period, especially to consider the effects of those metals that did not attain steady state tissue concentration.

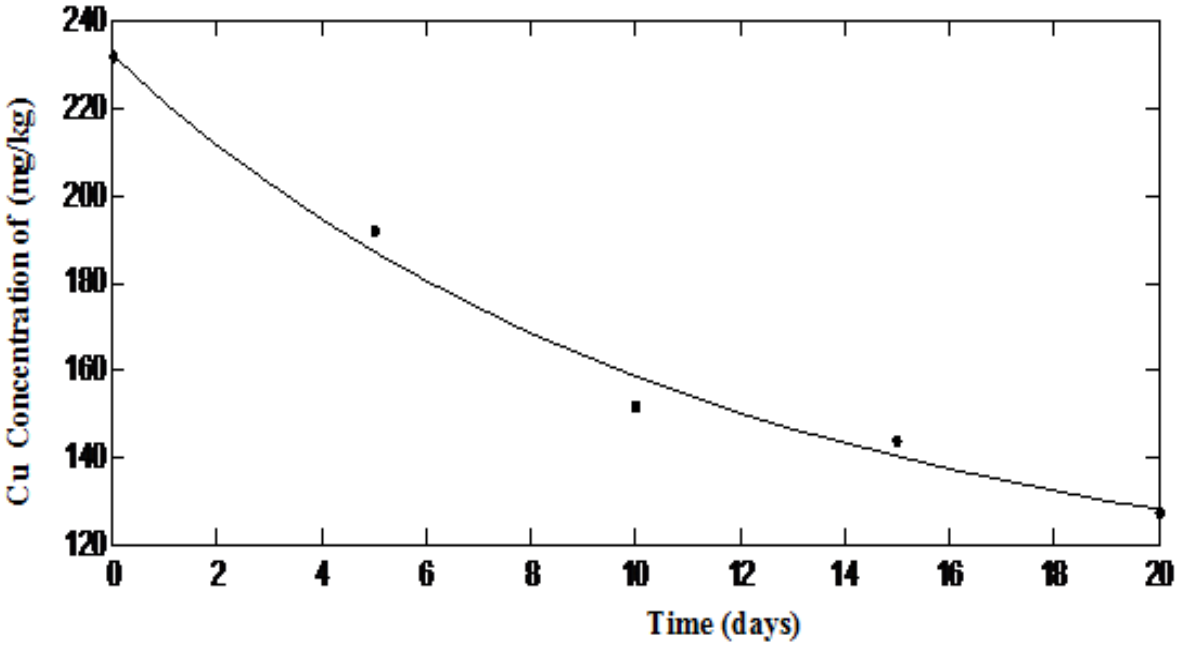


Figure 4.15: Modeling of Cu elimination kinetics for soil contaminated with 75g/kg of UEO.

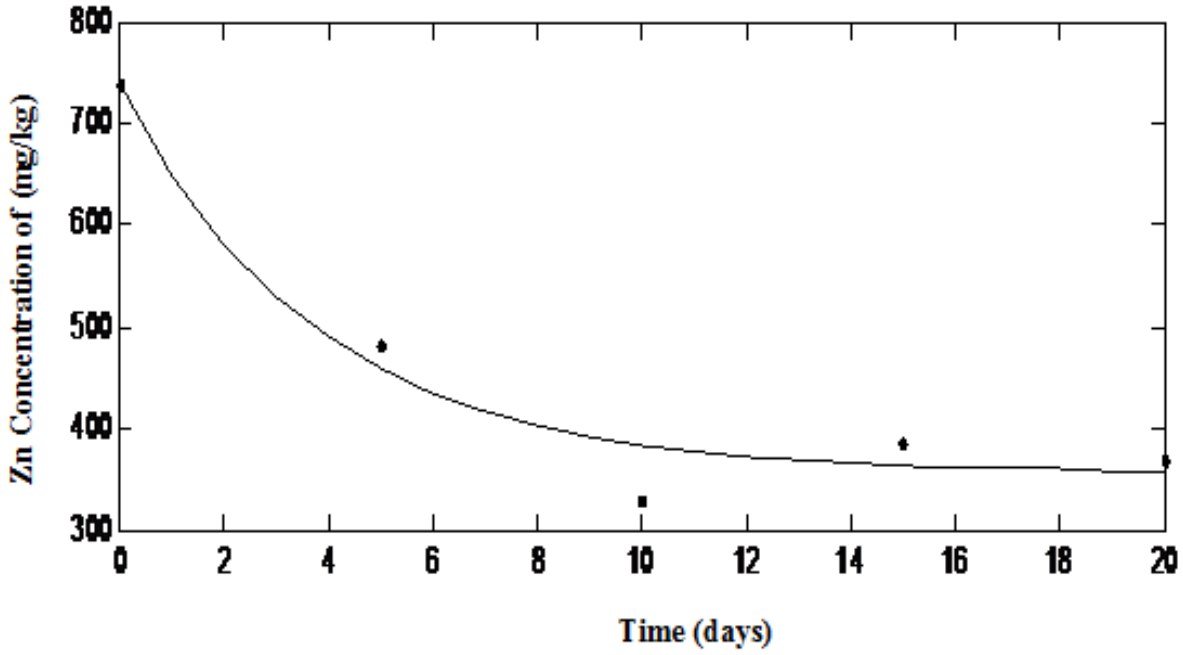


Figure 4.16: Modeling of Zn elimination kinetics for soil contaminated with 100g/kg of UEO.

Table 4.24: Elimination kinetics of Zn and Cu by the earthworm (*E. eugeniae*) exposed to simulated UEO contaminated soil.

	Zn					Cu				
	UEO concentration, g/kg soil					UEO concentration, g/kg soil				
	12.5	**25**	**50**	**75**	**100**	**12.5**	**25**	**50**	**75**	**100**
a	1268.70	1495.00	1424.30	108.45	93.82	674.77	6.50	589.91	9.46	42.93
k_2	4.40	4.40	4.20	0.28	0.26	4.12	0.08	4.22	0.09	-0.18
R^2	0.82	0.99	0.87	0.93	0.96	0.11	0.65	0.72	0.99	0.88
C_{ss}	280.00	337.50	330.00	380.00	362.50	170.00	NSS	140.00	NSS	NSS
C_e	3.21	29.72	33.73	43.37	33.73	11.18	42.33	40.73	63.10	28.75
k_1	394.87	50.31	42.22	2.50	2.78	60.34	0.15	14.48	0.15	1.49
BAF	89.74	11.43	10.05	8.85	10.55	14.64	1.99	3.43	1.70	-8.14
t_{SS} **(Days)**	2.00	2.00	2.00	18.00	18.00	2	NSS	2	NSS	NSS

NSS = steady state not attained

Table 4.25: Elimination kinetics of Ni and Cr by the earthworm (*E. eugeniae*) exposed to simulated UEO contaminated soils.

	Ni					Cr				
	UEO concentration, g/kg soil					UEO concentration, g/kg soil				
	12.50	**25.00**	**50.00**	**75.00**	**100.00**	**12.50**	**25.00**	**50.00**	**75.00**	**100.00**
a	759.62	582.38	618.20	9.36	-97.61	646.48	533.34	561.63	561.63	-22.69
k_2	4.16	4.17	4.19	0.08	-0.48	4.11	4.05	4.38	4.06	-0.13
R^2	0.01	0.49	0.37	0.82	0.60	0.01	0.27	0.20	0.68	0.44
C_{ss}	185.00	140.00	148.00	NSS	NSS	160.00	132.00	130.00	139.00	NSS
C_e	10.22	33.02	30.66	50.32	22.80	30.10	54.72	47.20	75.24	41.04
k_1	74.32	17.64	20.16	0.19	-4.28	21.48	9.75	11.90	7.47	-0.55
BAF	17.89	4.23	4.81	2.36	8.97	5.23	2.41	2.72	1.84	4.40
$t_{SS\ (Days)}$	2.00	2.00	2.00	NSS	NSS	1.00	2.00	2.00	2.00	NSS

NSS = steady state not attained

Table 4.26: Uptake kinetics of Ti and Mn by the earthworm (*E. eugeniae*) exposed to simulated UEO contaminated soils.

	Ti					Mn				
	UEO concentration, g/kg soil					UEO concentration, g/kg soil				
	12.50	**25.00**	**50.00**	**75.00**	**100.00**	**12.50**	**25.00**	**50.00**	**75.00**	**100.00**
a	90.17	248.82	-11.15	264.61	62.84	7.25	25.02	12.83	298.80	41.07
k_2	0.10	0.26	-0.04	0.36	0.08	0.04	0.30	0.28	4.45	0.53
R^2	0.82	0.64	0.66	0.51	0.79	0.97	0.78	0.99	0.91	0.84
C_{ss}	NSS	975.00	NSS	745.00	NSS	NSS	83.00	46.00	76.90	78.00
C_e	856.86	1120.50	1066.58	1198.40	1180.42	59.60	59.60	54.95	56.50	74.30
k_1	0.11	0.22	-0.01	0.22	0.05	0.12	0.42	0.23	5.29	0.55
BAF	1.15	0.87	0.27	0.62	0.64	2.97	1.42	0.84	1.19	1.05
$t_{SS\ (Days)}$	NSS	18.00	NSS	16.00	NSS	NSS	16.00	18.00	2.00	16.00

NSS = steady state not attained

4.5 EFFECT OF EARTHWORM INOCULATION ON TOTAL PETROLEUM HYDROCARBON OF SIMULATED USED ENGINE OIL CONTAMINATED SOIL

4.5.1 The Effect of Variable Initial Used Engine Oil Concentration on Bioremediation by Vermicomposting

The effect of variable concentration of UEO in soil is presented in Figure 4.17. It was in the soil with the highest initial concentration of UEO (that is TPH) that the degradation was highest a result also reported by **Schaefer (2001)**. The % drop in TPH after 42 days increased from 16.91% to 36.28% as the initial UEO concentration was increased from 5 g/kg to 20 g/kg. This may be as a result of increased metabolic activities by the microbes as more carbon is present, facilitated by earthworm activities. The seeming slight fluctuations (increase and decrease) may be attributed to the heterogeneity of the sample as earthworm activity may not be uniform throughout the sample.

Inthe heavy metals bioaccumulation study **Section 4.4.2.5**, equal number of worms were introduced into samples with variable initial UEO concentration. The drop in TPH of the samples at the end of 20 daysis shown in Figure 4.18. The figure show that increasing initial concentration of used engine oil contamination results in a higher drop in TPH (a higher rate of biodegradation): in complete agreement with the result shown in Figure 4.17. These suggest that vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil may be more effective at high initial used engine oil concentrations which fortunately is the case with most mechanic workshop soil (Table 4.9). However, this trend cannot continue indefinitely as earthworm survival reduces with increasing UEO concentration.

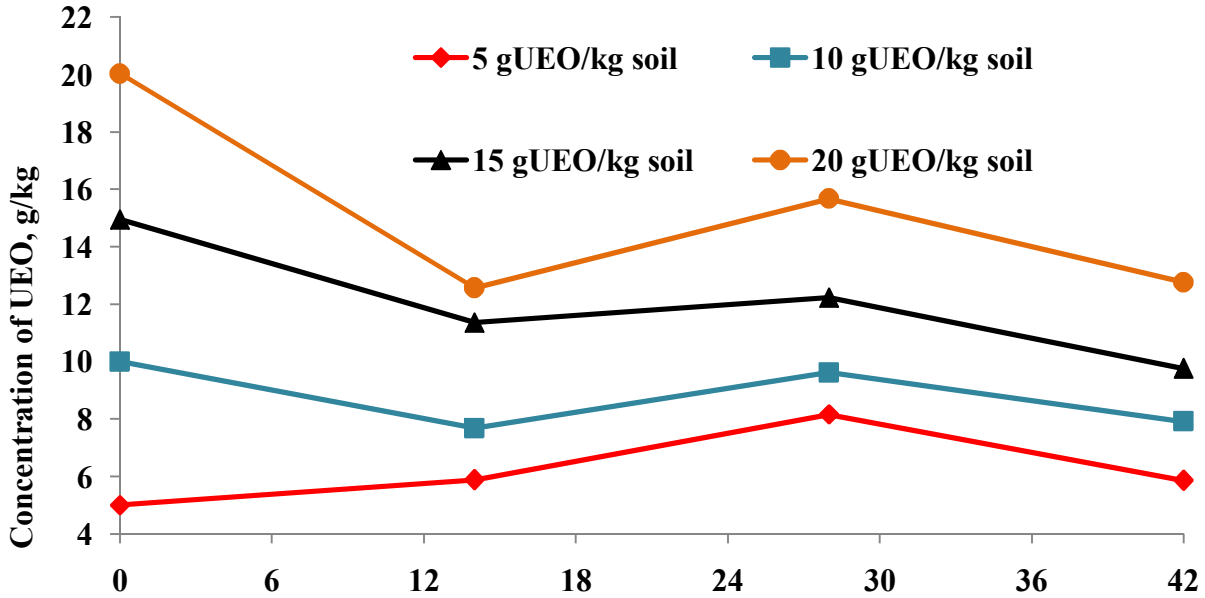


Figure 4.17: Effect of initial used engine concentration on bioremediation by vermicomposting

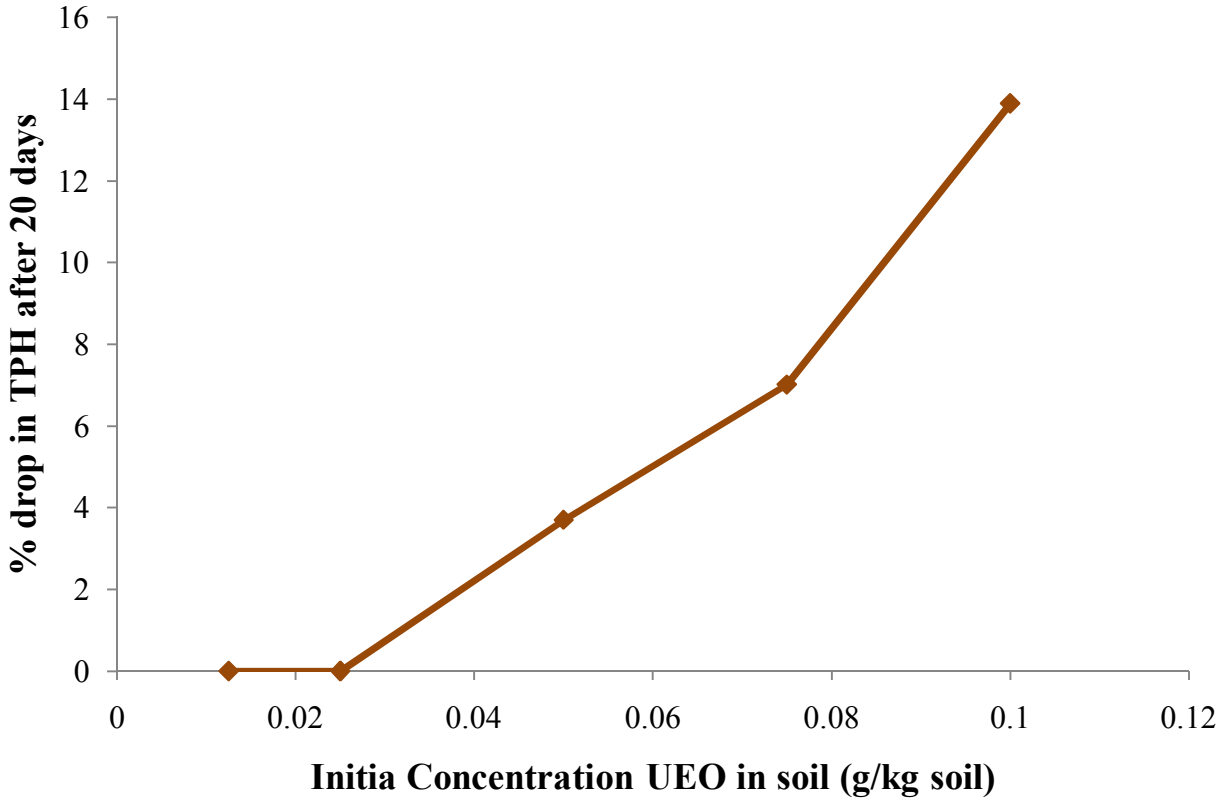


Figure 4.18: Effect of initialUEO concentration on vermi-assisted bioremediation (TPH analysis of metal uptake samples)

4.5.2 Effect of Variable Earthworm Concentration

Low initial UEO concentration

The effect of varying concentration of earthworm on the rate of bioremediation is shown in Figure 4.19. As shown in Figure 4.19, there was an initial increase in TPH for some of the samples probably as a result of the extraction of biogenic organic compounds by toluene from the organic matter **(Chaîneau *et al*., 1997)**. Samples with 0, 15 and 20 worms showed the highest drop in TPH with time. The samples with 5 and 10 earthworms had the least drop in TPH over the 42 day period considered. The percentage drop in TPH for the samples with 0, 5, 10, 15 and 20 earthworms on day 42 was computed as 31.95, 11.75, 8.00, 25.93 and 28.98% respectively. Under the condition of the study, the earthworms will eventually starve to death as the nutrient (manure) is not replenished. This implies that without addition of nutrients (in this case manure) the earthworms will eventually begin to starve to death starting with the samples with the highest concentration of worms. It is however possible that lower worm concentrationscan translate to more available food (as competition will be less) and the worms may ultimately effect the same level of bioremediation as the samples higher worm concentration (that is for systems where there is no replenishing of food) as they may survive longer. This remains to be proven.

Generally the sample without worms performed better than all the samples with worms. It can be seen that with regards to TPH drop alone (and within the range of earthworm and used engine oil concentrationsand considered), there is no significant advantage in introducing earthworms for bioremediation of used engine oil contaminated soil. It has been reported that although the action of earthworm generally increase the mineralization of soil carbon, earthworms can also decrease

mineralization of carbon by contributing to the formation of stable soil aggregates in which carbon is protected from further breakdown – this may be a possible reason for the result.

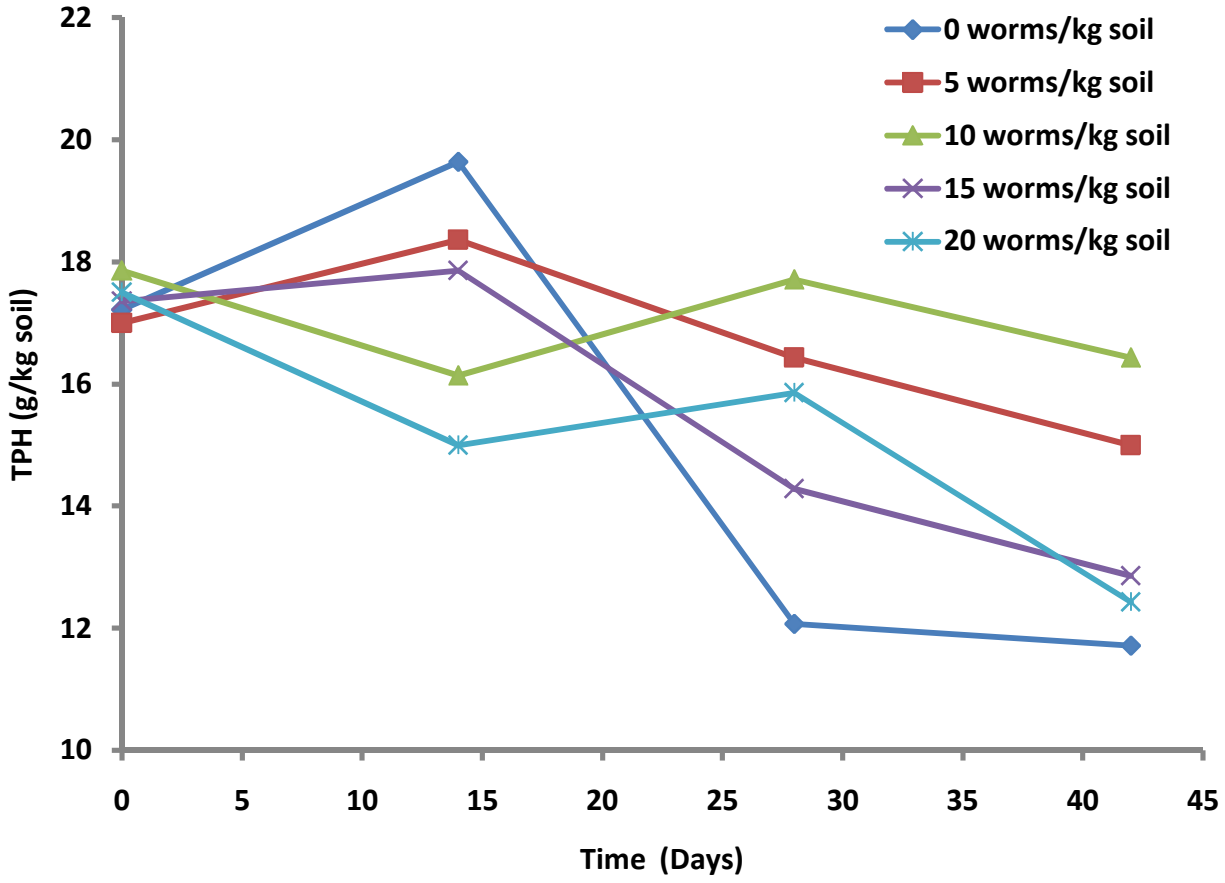


Figure 4.19: Effect of earthworm concentration on bioremediation by vermicomposting

The result of this investigation differs widely from the finding of **Maike and Filser, 2007:**TPH concentration decreased by 30–42% in samples with *E. eugeniae*, after 28 days of incubation. Also the TPH concentration of the samples without earthworms was not significantly decreased after 28 days (9–17%) when earthworm was used in the bioremediation of crude oil contaminated soil. They also concluded that addition of earthworms improved bioremediation rate more than manual tilling (in the absence of earthworms).However, **Callaham et al. (2002)**observed no difference in TPH concentrations associated with *E. fetida* or wheat

treatments when applied to petroleum contaminated soils,whilst **Stom *et al.* (2003)**, who investigated the effect of earthworm (*E. fetida*) application (n=5) to crude oil contaminated soils (25 g kg^{-1}) for 40 days with the co-application of microorganisms also observed minimal losses (~15 g kg^{-1} loss for the microbial inoculation and earthworm co-application; ~9 g kg^{-1} loss for the microbial inoculum alone, and only ~3 g kg^{-1} loss for sole earthworm treatments).Generally for the samples inoculated with worms, increase in earthworm concentration resulted in an increased rate of bioremediation. This result suggests that the kinetics of bioremediation may be dependent on the initial concentration of used engine in soil.The result of Figure 4.19 indicates that increase in earthworm number could result in an increase in the rate of bioremediation. Comparing this with the result of Figure 4.18 it may be deduced that earthworm assisted bioremediation of UEO contaminated soil is favored by both high UEO concentration and high earthworm concentration. However, a limit would naturally exist for both concentrations.

Figures 4.20 – 4.22 presents the GC analysis for effect of earthworm concentration on bioremediation. The corresponding MS resultis shown in Table 4.27. At initial time (t = 0), C30 and above fractionshad the highest proportion in the oil, being 56.78%. By 42 day, the C30 and above fraction had drop to 0% for the earthworm free samples and the earthworm treated sample (20 worms). However, the earthworm treated sample had a higher % composition of C20 -C29 when compared with the earthworm free sample. This is in line with Figure 4.19 which presents the sample with 20 worms as having a slightly higher TPH at 42 days than the earthworm free sample.

High initial UEO concentration

Analysis of the TPH of the samples of **Section 4.4.2.3** (in which earthworm concentration was varied at a fixed initial UEO concentration)showed a similar trend in TPH drop with earthworm

concentration. After 6 weeks of earthworm treatment, the sample with the highest concentration of worm recorded the highest drop in total petroleum hydrocarbon content as shown in Figure 4.23. The sample with 0, 10, 20, 30 and 40 earthworms/kg of soil recorded 28.08, 28.48, 29.19, 32.46 and 34.35% reduction in TPH respectively. This behavior is what would be expected of a catalyst: the more the active sites, the faster the reaction. Thus bioremediation using earthworms should be favored by higher earthworm concentration. The major difference between Figure 4.19 and 4.23 is that whereas for Figure 4.19 where the sample without worms recorded the highest drop in TPH after 42 days, Figure 4.23 indicates that the sample with the highest worm concentration recorded the highest drop in TPH after 35 days. This indicates that the effectiveness of earthworm assisted bioremediation of used engine oil contaminated soil is UEO concentration dependent.

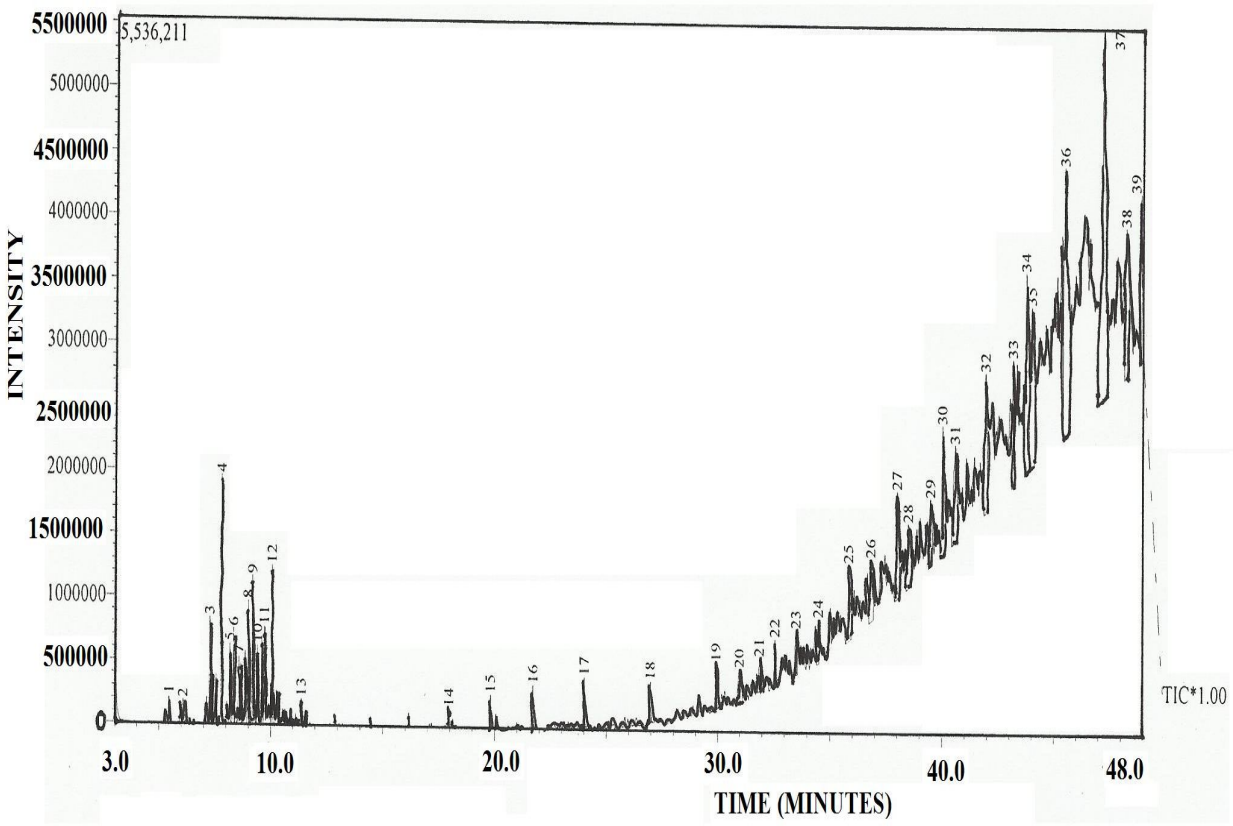


Figure 4.20: GC of extract from soil at t= 0 day

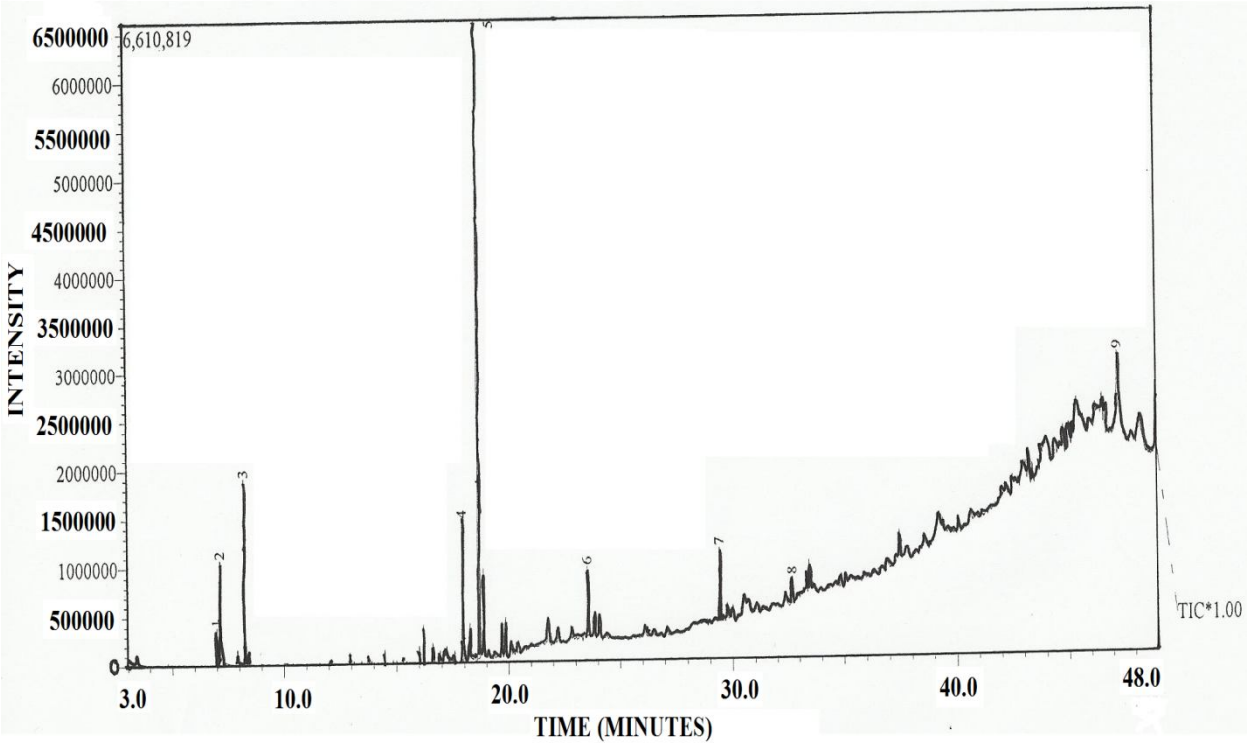


Figure 4.21: GC of extract from soil sample with 20 worms at t = 42 days

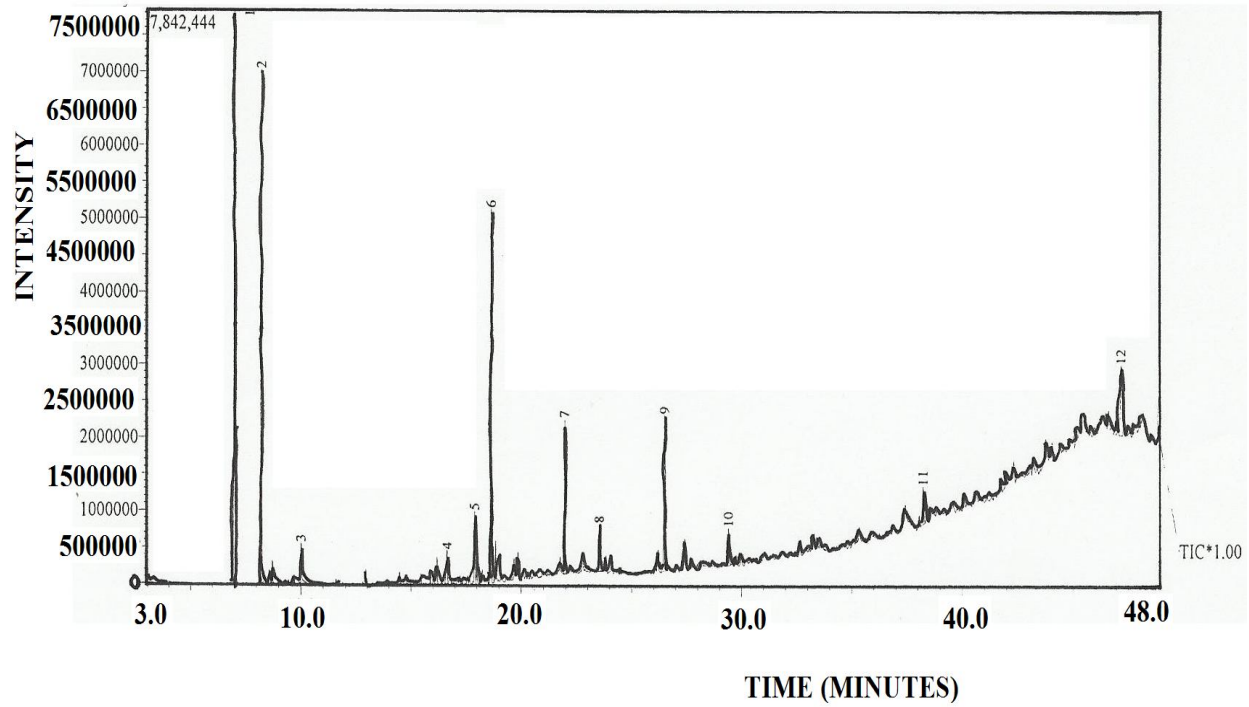


Figure 4.22: GC of extract from soil sample with no worms at t = 42 days

Table 4.27: MS analysis on the effect of earthworm concentration on the composition of UEO in bioremediation

	Composition of UEO		
	Day 0	**No worm t = 42 day**	**20 worms, t = 42 days**
C9 - C19	15.8	97.29	92.08
C20 - C29	26.42	2.71	7.91
C30 and Above	56.78	0	0

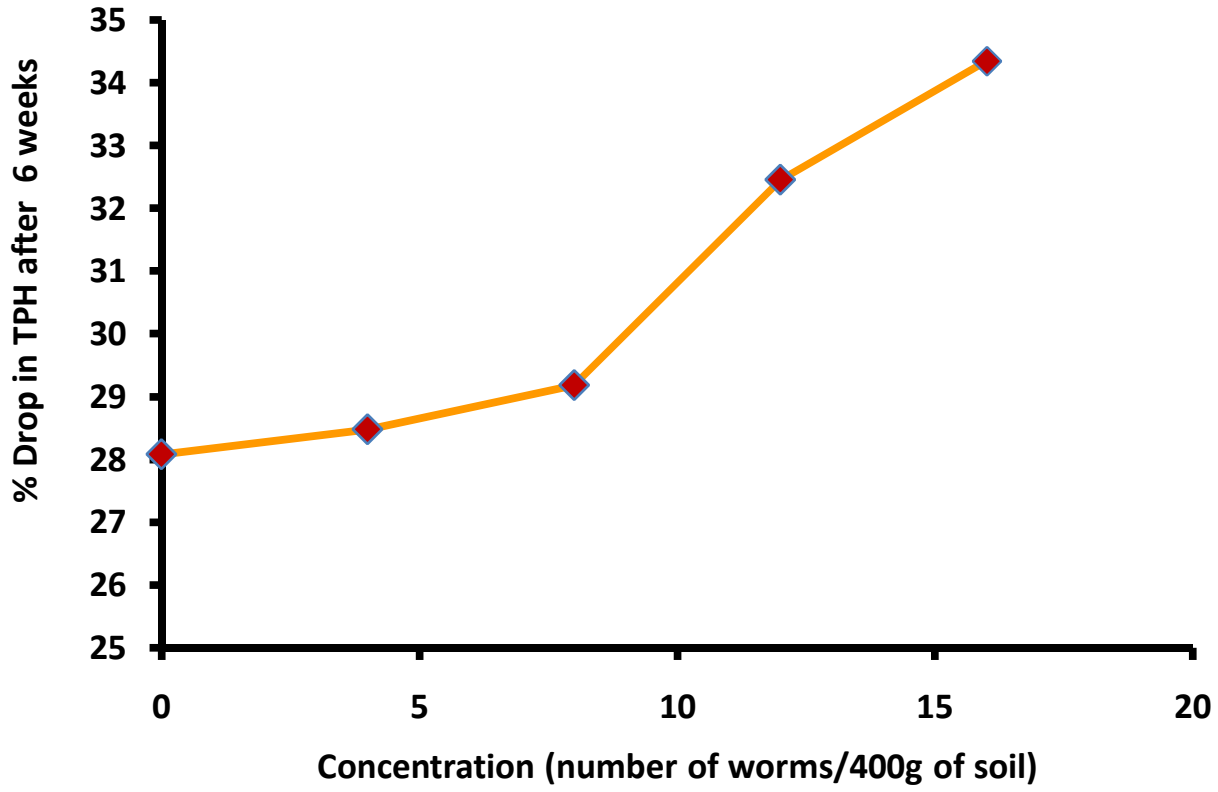


Figure 4.23: Effect of earthworm concentration on bioremediation by vermicomposting (initial TPH = 100g/kg soil – high UEO concentration)

4.5.2.1 *Effect of worm concentration on bioremediation and microbial count.*

The effect of the presence of earthworm on the microbial community is as shown in Figure 4.24. The microbial count was found to decrease and increase indiscriminately. According to **Granatstein and Bezdicek (2003)** there needs to be at least a 10-fold difference in microbial number to be meaningful because of variability in measuring organisms. Figure 4.24therefore shows that presence of earthworms had no significant effect on microbial count that all samples (untreated/earthworm treated) had similar number of bacteria at the various times and would perform the same if all else was equal. There however appears to be a general decrease in microbial count with time. The result of this investigation suggests that the effect earthworm inoculation has on bioremediation kinetics is not a direct follow up of its effect on microbial count, since earthworm treated and untreated sample recorded similar microbial counts.

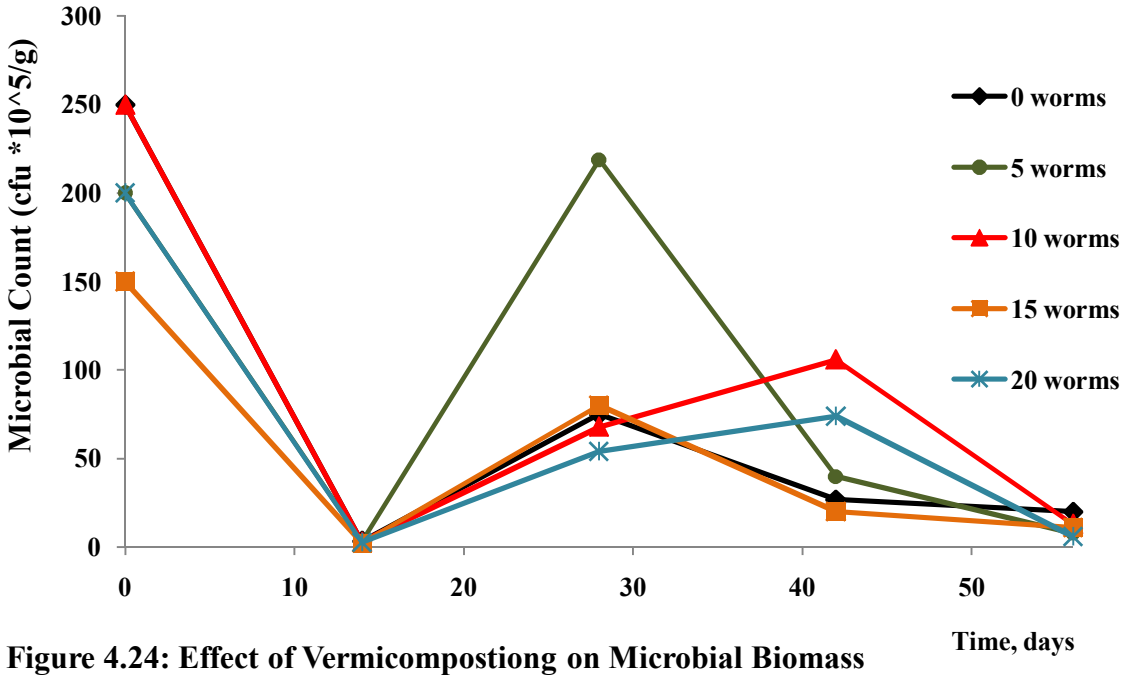


Figure 4.24: Effect of Vermicompostiong on Microbial Biomass

4.5.3 The Effect of Tilling (Mixing) and Earthworm on Bioremediation

As shown in Figure 4.25,positive drop in TPH for most of the samples began after the fifth week. At t = 7 weeks the samples with 30 earthworms/kg soil had the highest drop in TPH, which was comparable to the untilled sample (24.16% and 25% respectively**),** thereafter the TPH of this sample (with 30 earthworms/kg soil) increased probably as a result of starvation and death of the worms. The tilled (mixed) sample and the sample with 20 earthworms/kg soil indicated a slower initial drop in TPH but the mixed sample picked up after the t = 10 weeks to give the highest drop in TPH at t = 14 weeks of 36.67%. This result showed that the drop in TPH is a function of earthworm concentration with higher concentrations favoring higher rates, similar to the discussion in **Section 4.5.2**. It also shows that when earthworms are considered for use in bioremediation it is important to reintroduce nutrient (poultry manure in this case) to sustain the worms (since they cannot feed on used engine oil) and to maintain the pace of the bioremediation process. Comparison of the with-earthworm and the without-earthworms samples of both Figures 4.19 and 4.25 suggests that in term of TPH removal, the use of earthworms (*E. eugeniae*) did not offer a significant advantage (that for the UEO concentrations considered).The fluctuations in the TPH values with time may have been as a result of the heterogeneity of the samples as TPH values were not determined using the whole sample but homogenized small samples collected from different parts of the larger sample. It is important that other organic amendments as well UEO concentrations in soil (especially higher concentrations) be considered in future works as the bioremediation process is a function of a myriad of factors.

The GC chromatogram for the samples at t = 0, for the mixed sample at t = 14 weeks and for the sample with 15 worms at t = 14 weeks are presented as Figures 4.26, 4.27 and 4.28 respectively.

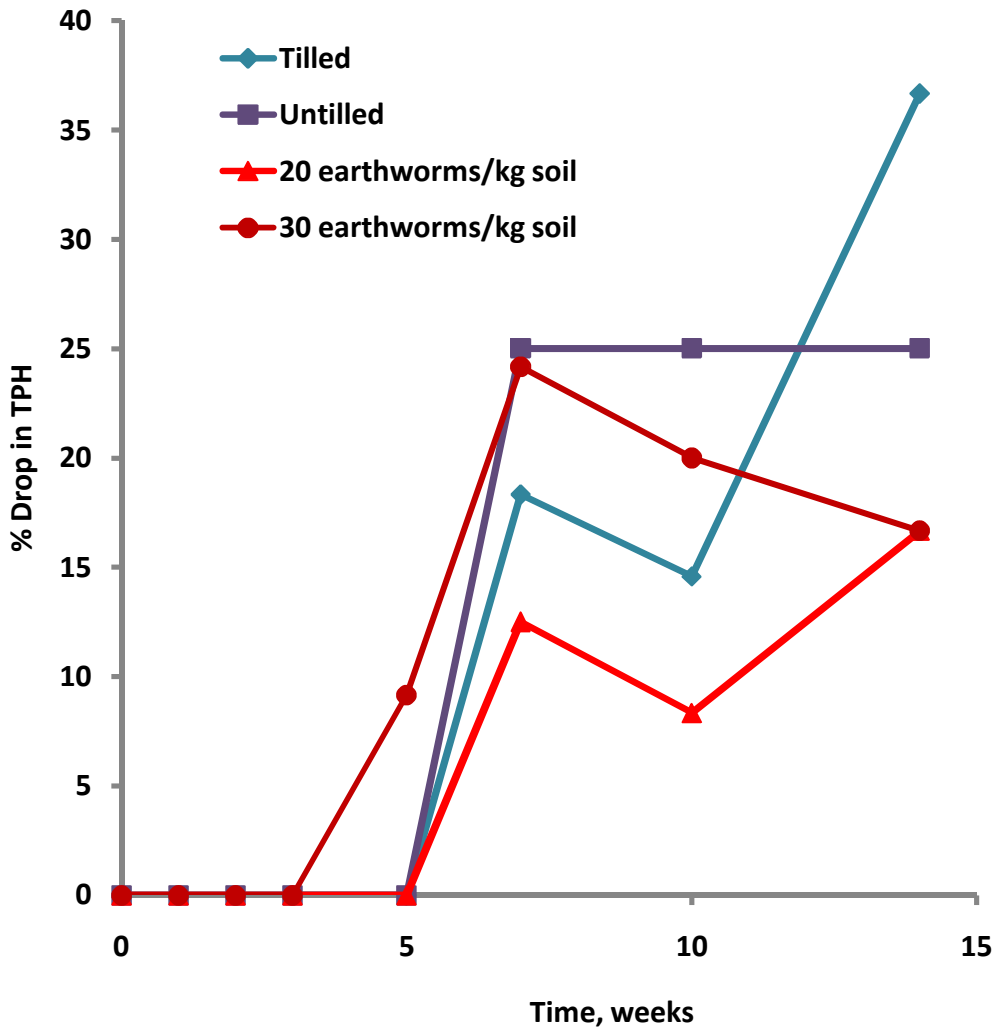


Figure 4.25: Comparison of the effect of manual mixing with the activity of earthworm on bioremediation

The MS analysis for these samples is presented in Table 4.28. Comparison of Figures 4.26, 4.27 and 4.28 show that at time t = 0 the GC of the contaminated recorded more peaks than those of 14 weeks of bioremediation for both earthworm treated and untreated (mixed) samples. Table 4.28shows that after 14 weeks of treatment all the C30 and above had been eliminated from the contaminant. The table also indicated that the earthworm treated sample recorded 100% composition of C9 – C19 whereas the mixed sample had 88.83% C9 – C19 and 11.18% C20 – C29.The TPH analysis of the samples on day 14 however indicated that the mixed sample had a

higher TPH drop (36.67%) as against the 16.67% recorded by the sample treated with 20 earthworms. Thus, higher molecular weight hydrocarbons were broken down faster in the earthworm inoculated sample.

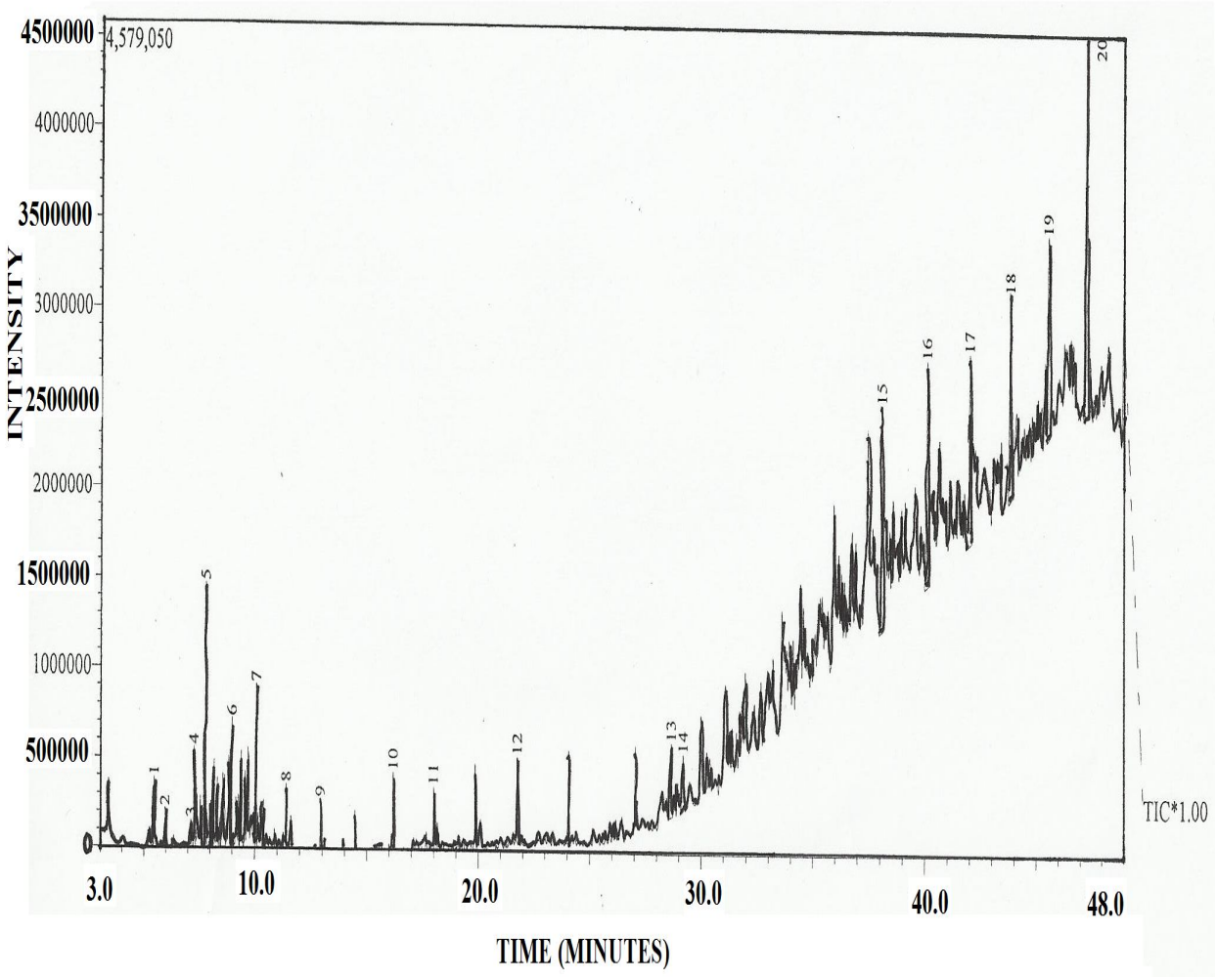


Figure 4.26: GC of extract from soil at t = 0 weeks

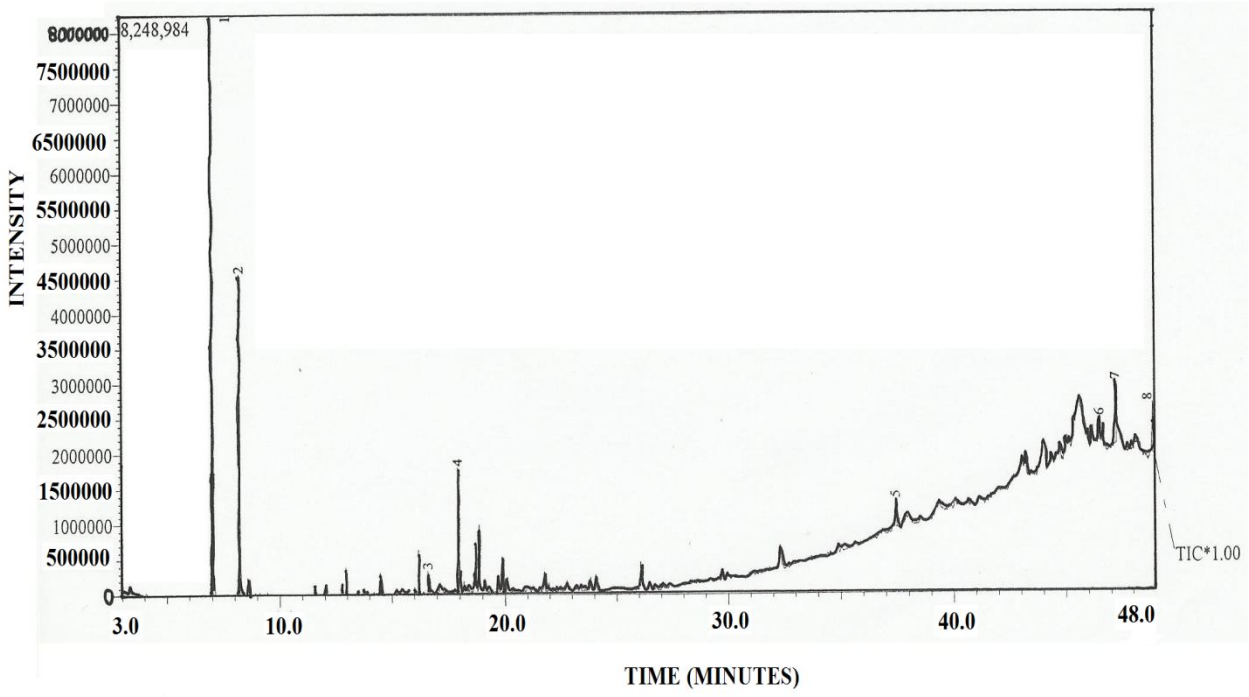


Figure 4.27: GC of extract from mixed soil sample at t = 14 weeks

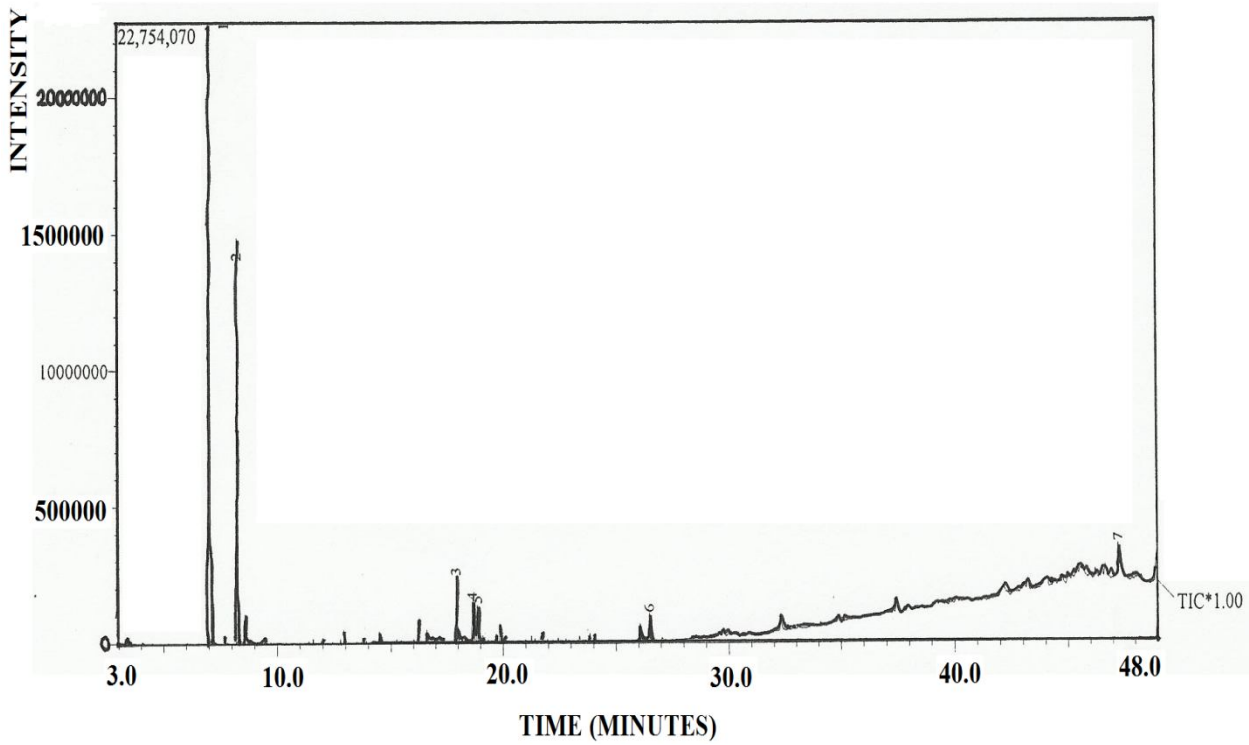


Figure 4.28: GC of extract from soil sample with 15 worms at t = 14 weeks

Table 4.28: MS analysis of the effect of earthworm to manual mixing on bioremediation of UEO contaminated soil

	Composition of UEO		
	t = 0	**15 worms, t = 14 weeks**	**Mixed, t = 14 weeks**
C9 - C19	54.58	100	88.83
C20 - 29	10.37	0	11.18
C30 and Above	35.05	0	0

4.5.3.1 Effect on microbial count

Again,as with Figure 4.24, Figure 4.29shows that there is no significant difference in microbial count between the earthworms treated and untreated (tilled and untilled) samples. All the samples showed a drop in microbial count after day 0. This drop was probably a result of the toxic effect used engine oil had on microbial community. However, the tilled sample on and after day 35 had the highest microbial count up to day 70. For the corresponding length of time (day 35 to 70) the tilled sample as shown in Figure 4.29, did not record the highest TPH drop. Therefore, the effect that the organic amendment and the presence of earthworm have on TPH reduction in bioremediation,may not be as a result of the effect they have on the microbial biomass alone.

4.5.3.2 Microbial Identification

Table 4.29 presents the result of the isolation and identification of the earthworm inoculated sample after 14 weeks of treatment. Similar to the earthworm free cases of **Section 4.3.1.4** the organism isolated was *Bacillus cereus.*

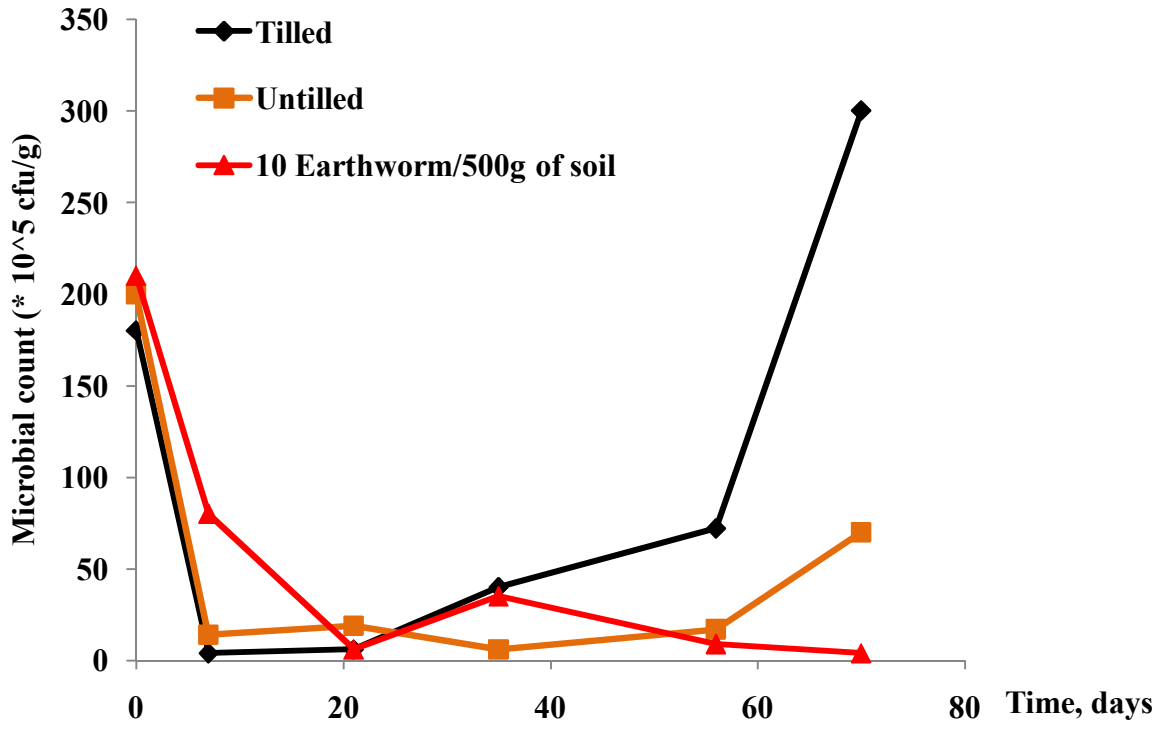


Figure 4.29: The efffect of tilling on microbial biomass

Table 4.29: Identification of bacteria for used engine oil contaminated soil biostimulated with 5g poultry manure and treated with 30 earthworms/kg soil

Gram reaction	Shape	Indole	MR	VP	Citrate	Catalase	Coagulase	Organism
+	Rod	-	-	-	-	+	-	*Bacillus cereus*

4.5.4 The Effect of Initial Organic Amendment Concentration on Bioremediation by Vermicomposting

The effect of manure concentration on bioremediation is presented in Figure 4.30.As shown in the figure, apart from the sample with 0gmanure/kg soil, all other sample indicated a positive drop in TPH at the end of 15 weeks. The sample with 10, 20, 30 and 40 g manure/kg soil recorded 15, 14.3, 23.1 and 15.4% drop in TPH respectively. These results suggest an optimum

food concentration in earthworm inoculated samples for bioremediation. That is, it seems an optimum nutrient concentration is required by earthworms in assisting bioremediation of used engine oil contaminated soil. This is in line with the report of **Schaefer *et al.* (2005)** who noted that residual TPH in the with-food system was greater than the without-food system. The TPH of the sample with zero worms remained relatively unchanged up to the week 5 after which there was a sharp increase in TPH as determined by the toluene cold extraction method. It was also observed that the worms started dying after week 5. It is therefore important when considering earthworms for bioremediation to intermittently replenish nourishment for the worms as they cannot feed on engine oil.

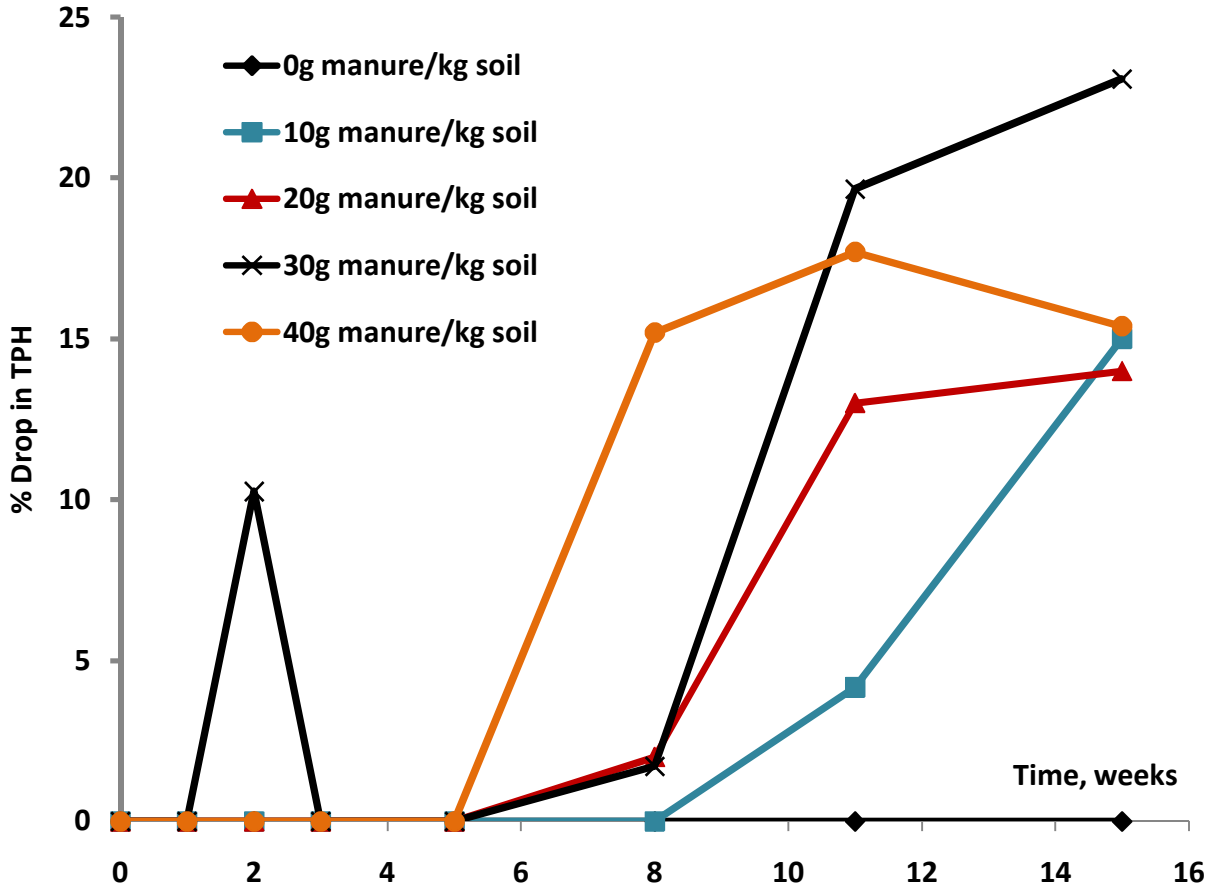


Figure 4.30 :Effect of initial manure concentration on earthworm assisted bioremediation

4.6 THE EFFECT OF EARTHWORM ACTIVITY ON THE BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOIL FROM MECHANIC WORKSHOP

Water holding capacity (WHC) and total petroleum hydrocarbon content of the auto mechanic workshop and motorcycle mechanic workshop soils are presented in Table 4.30. Table 4.31 presents the result of 35 days vermi-assisted bioremediation on the TPH and survival of the worms. For soil from both types of mechanic workshop considered, the use of earthworm inoculation proved to have an effect on the rate of degradation of petroleum hydrocarbon as shown in Table 4.31. All the samples treated with worms had higher drops in TPH than the corresponding control samples (without worms). The least effect was observed for the **MCU** samples (undiluted soil from motorcycle mechanic workshop) in which earthworm survival dropped to 0% (all the worms died) in less than 24. There was only a marginal difference in the drop in TPH recorded between the **MCU** and the control (24.27 and 24.57 respectively).

Table 4.30: Physicochemical property of soil from mechanic workshop

Sample	WHC (g water/kg soil)	TPH (g oil/kg soil)
MM	46.90	99.73
MC	50.00	150.20
DMM	42.27	42.29
DMC	49.79	76.43

MM: motorcar mechanic workshop soil, MC: motorcycle mechanic workshop soil, DMM: diluted motorcar mechanic workshop soil, DMC: diluted motorcycle mechanic workshop soil.

Table 4.31: Effect of earthworm inoculation on the bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshops

	Initial TPH, g/kg	% TPH Decrease after 35 days	% earthworm survival after 35 days
Soil from motorcar mechanic workshop			
MMUNW	75.45	34.97	
MMUW	75.45	36.35	30
MMDNW	31.61	29.50	
MMDW	31.61	39.10	80
Soil from motorcycle mechanic workshop			
MCUNW	136.36	24.57	
MCUW	136.36	24.27	0
MCDNW	69.27	28.84	
MCDW	69.27	44.18	70

MMUW: undiluted motorcar mechanic workshop soil treated with worms, MMUNW: undiluted motorcar mechanic workshop soil without worms, MMDW: diluted motorcar mechanic workshop soil treated with worms, MMDNW: diluted motorcar mechanic workshop soil treated without worms, MCUW: undiluted motorcycle mechanic workshop soil treated with worms, MCUNW: undiluted motorcycle mechanic workshop soil without worms, MCDW: diluted motorcycle mechanic workshop soil treated with worms, MCDNW: diluted motorcycle mechanic workshop soil treated without worms

4.6.1 Effect of Vermicomposting on the Total Petroleum Content of Mechanic Workshop Soil

It may also be observed that the highest drops in TPH for both mechanic workshop soil samples were recorded in the earthworm-treated dilute samples (44.18% and 39.10% for MCDW and MMDW respectively). Incidentally, these samples recorded an appreciable survival of earthworm over the duration of the study: on day 35, **MCDW** recorded 70% earthworm survival whereas **MMDW** recorded 80% survival of the earthworms.

For the undiluted soil samples (UD), it was observed in the course of the experiment that**MCUW** recorded 100% death of the worms in less than 24 hours. It was thus expected to have very similar bioremediation rate with the control (**MCUNW**) since all the worms died within 24 hours after introduction. Table 4.31 confirmed this as % drop in TPH for **MCUW** and **MCUNW** was found to be 24.26461 and 24.56509 respectively. The table also shows that **MMUW** recorded an earthworm survival rate of 30% at the end of the study (35 days) and also recorded a slightly higher TPH drop than the control (36.35 and 34.97 respectively). The death of the worms may be as a result of any or a combination of the following reasons: intolerable levels of petroleum hydrocarbon contamination, the pH of the soil, intolerable levels of heavy metal and carbon black (soot).

Figure 4.31 and 4.32presents the result of the fit of the bioremediation data obtained from the mechanic workshop soil samples (motorcar mechanic workshop soil and motorcycle mechanic workshop soil respectively) to the 1st order bioremediation kinetics. The results indicate a poor fit to the first order bioremediation kinetics (R^2 generally less than 0.9). However considering the duration of the study (only 35 days) it may be possible to obtain higher R^2 value for longer study periods.

Based on the result of this investigation the use of earthworm for the bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshop would be recommended. Apparently, the presence of earthworms effected a net positive acceleration of the bioremediation process. Since bioremediation is mass transfer limited, the presence of the worms must have resulted in the production of biosurfactant(s), capable of facilitating the availability of oil in the aqueous phase.

More research work needed be undertaken to understand the mechanism. Possible pathways include: stimulation of biosurfactant production in the microorganisms, selective increase in the population of surfactant producing microorganisms, release of aged organic contaminants from soil as a result of size reduction, interplay between earthworm gut content, microorganism and contaminated soil as earthworms takes up and excrete soil etc.

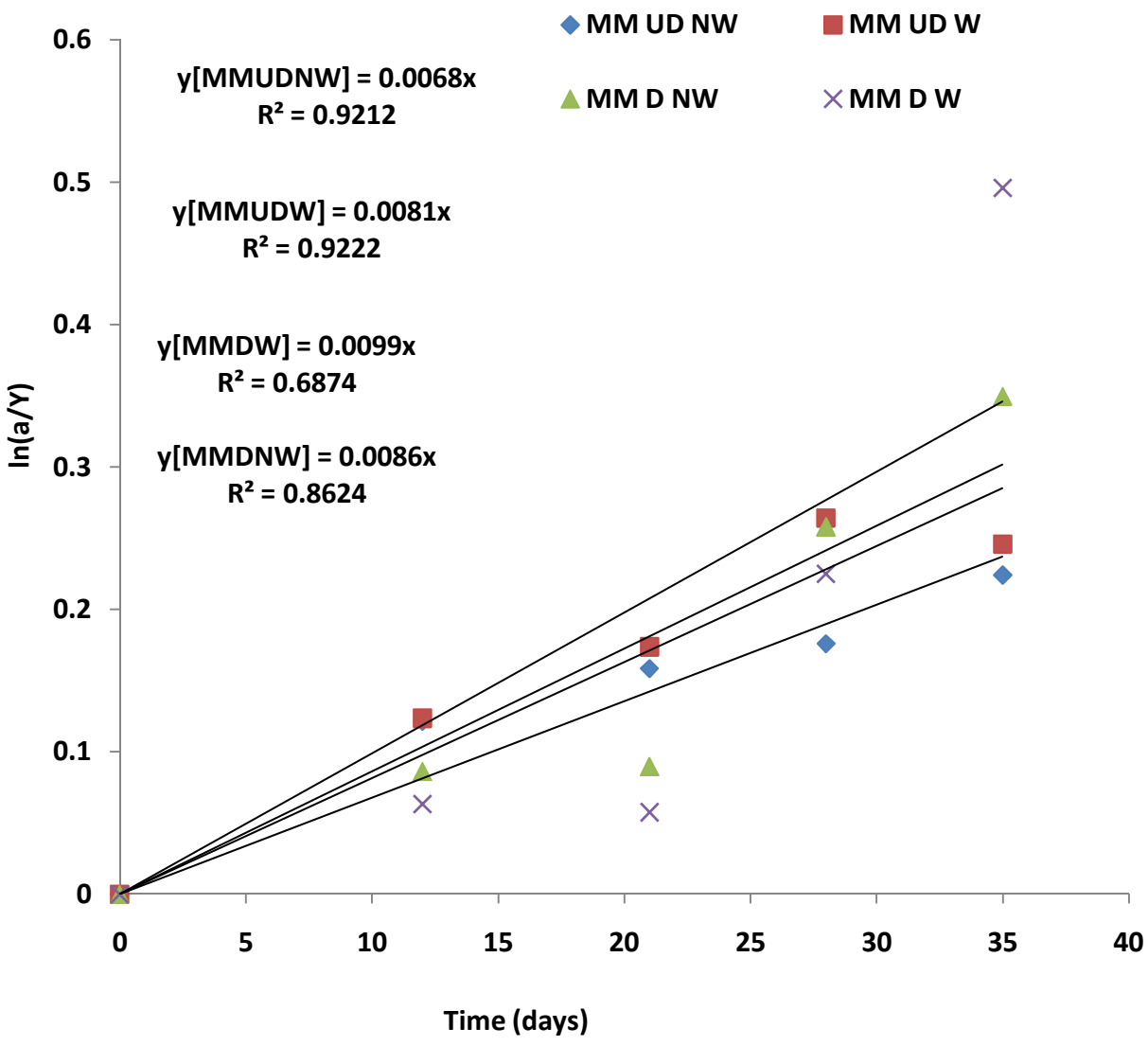


Figure 4.31: Effect of earthworm innoculation on the bioremediation kinetics of petroleum hydrocarbon contaminated soil from motorcar mechanic workshop

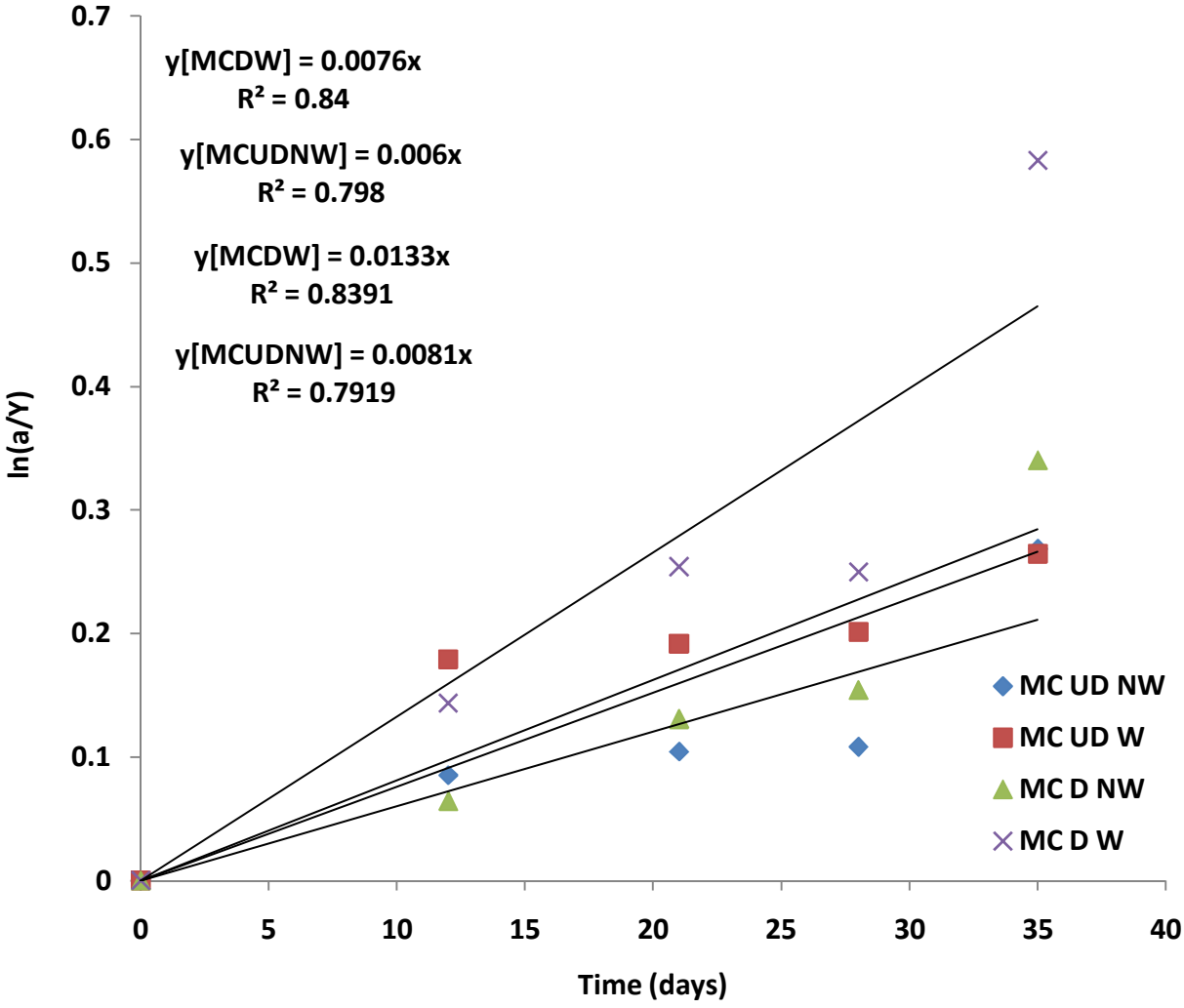


Figure 4.32: Effect of earthworm innoculation on the bioremediation kinetics of petroleum hydrocarbon contaminated soil from motorcycle mechanic workshop

Figures 4.33 and 4.34 present the result of the GC analysis of the diluted motorcycle mechanic workshop soil which recorded the highest drop in TPH while Table 4.32 presents the MS analysis for the same samples. The MS analysis of Table 4.32indicated that the C11 – C20 fraction of the contaminating petroleum hydrocarbon was most affected by the bioremediation

process, dropping from 95.47% to 92.31%. The C1 – C10 and the C20 –C30 fractions appeared to have increased. For the C1 – C10, this may be as a result of the breakdown of C11 – C20 resulting in the formation of more C1 – C10, whereas for the C20 –C30 the apparent increase in composition is most likely a result of the elimination of C11 – C20 fraction. In Table 4.31, MCDW recorded a TPH drop of 44.2% indicating that the microbial degradation of the C11 – C20 resulted in the formation of volatile organic materials which left the system or CO_2 and H_2O.

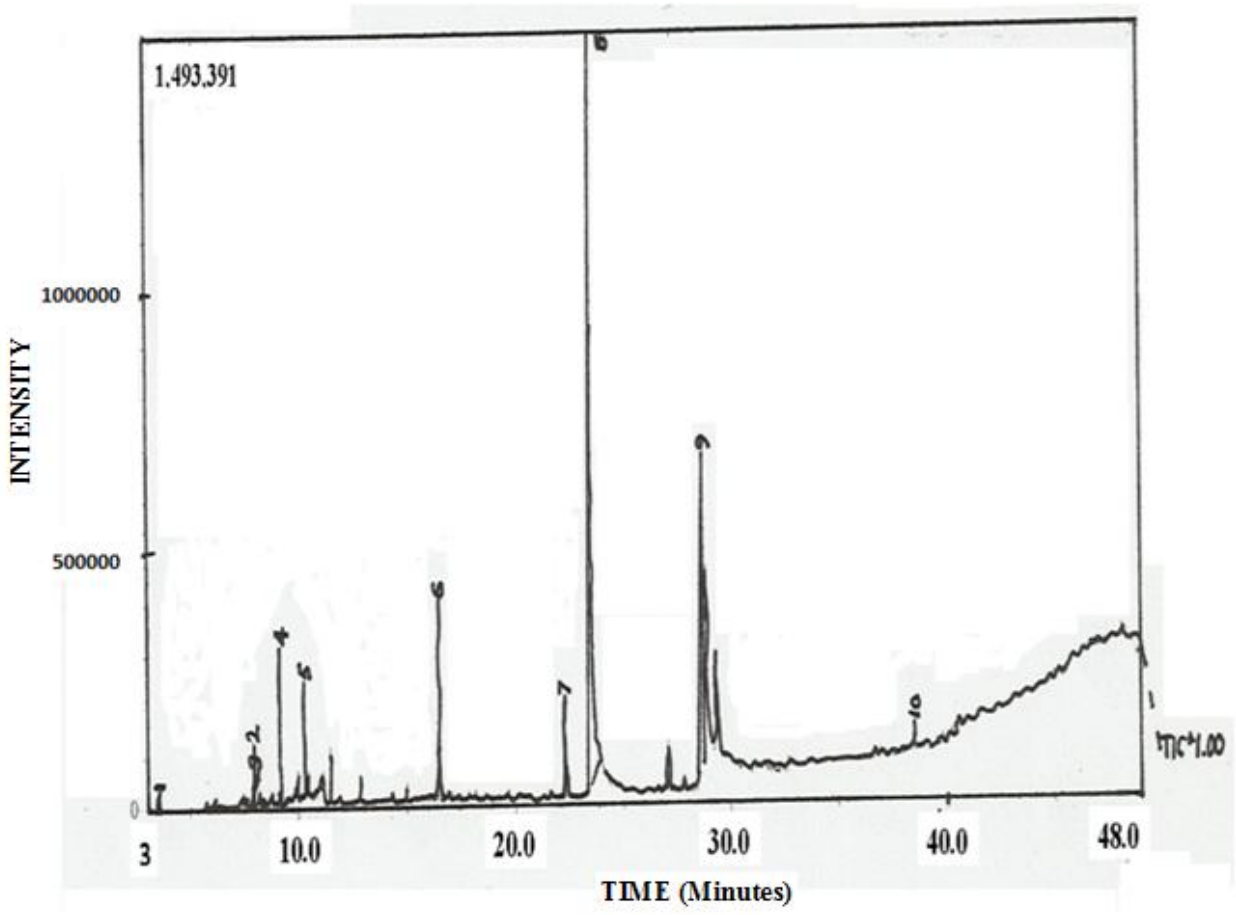


Figure 4.33: The GC chromatogram of the petroleum hydrocarbon contaminated of soil from motorcycle workshop prior to bioremediation (MCO).

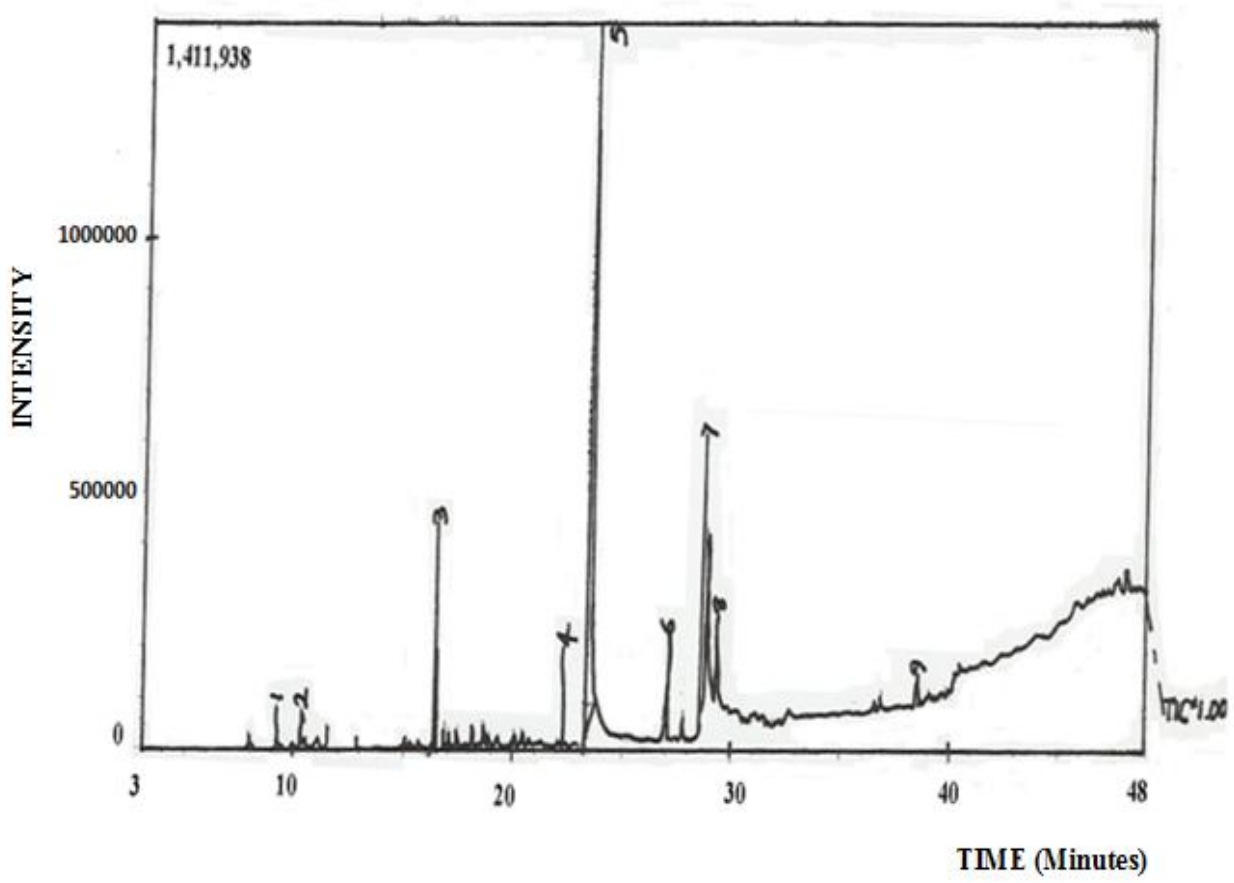


Figure 4.34: The GC chromatogram of the petroleum hydrocarbon contaminated of soil from motorcycle workshop after 35 days of vermin-assisted bioremediation (MC35).

Table 4.32: Effect of Vermin-Assisted Bioremediation on the Composition of Petroleum Hydrocarbon Content of Motorcycle Mechanic Workshop Soil.

	MCO	**MC35**
	% Composition	
C1 - C10	3.70	6.19
C11 - C20	95.47	92.31
C20 - C30	0.83	1.50

4.6.2 Effect of Vermi-Assisted Bioremediation on Microbial Community of Mechanic Workshop Soil

Table 4.33 presents the result of microbial count and identification performed on the various samples of mechanic workshop soil prior to bioremediation and after 35 days of bioremdiation.

From the table it can be seen that the microbial count of the raw motorcar and motorcycle mechanic workshop soil (MM and MC respectively) was very low. For instance at a dilution of 10^5 no microbe was enumerated in both samples. This shows the negative effect petroleum hydrocarbon contamination has on soil microbial community. After amendment with fermented cassava peels with earthworm inoculation (in some samples) microbial activity picked up as shown at the end of the 35 day study. The microbial count increased by a factor of at least 17, which is significant. In fact for MC at dilution of 10^4, the count increased by more than 300 times. This increase in microbial count however may not directly be the reason for the reduction in TPH: the samples without worms both indicate slightly higher microbial counts than the corresponding samples with worms though the TPH analysis indicated that the samples with worms recorded lower values of TPH compared to the corresponding samples without worms. Thus increased microbial count may have contributed but other factors may have also influenced the TPH of the samples. The microorganisms isolated in all samples were similar: *Bacillus sp, pseudomonas sp* and *staphylococcus sp. Staphylococcus sp.*which was not identified in the raw mechanic workshop soils now appeared in bioremediated samples. This may be from the cassava peels. All these microorganisms are known to produce biosurfactants of interest for application in petroleum hydrocarbon contaminated sites.

Table 4.33: Effect of Vermi-assisted Bioremediation on the Microbial Count and Identification of Mechanic Workshop Soil Contaminated with Petroleum Hydrocarbon.

	10^3	10^4	10^5	Organisms Isolated
MCDW	TNTC	3.18*10^5	8.15*10^5	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MCDNW	TNTC	3.34*10^5	8.7*10^6	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MMDW	TNTC	4.08*10^5	1.21*10^6	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MMDNW	TNTC	3.39*10^5	9.4*10^6	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MC (Raw)	5.0*10^3	1.0*10^3	NIL	*Pseudomonas sp*
MM (Raw)	1.19*10^4	2.0*10^4	NIL	*Bacillus sp and Pseudomonas sp*

TNTC = too numerous to count

MM (Raw): soil obtained from motorcar mechanic workshop (without organic amendment/earhworms), MC (Raw): soil obtained from motorcycle mechanic workshop (without organic amendment/earhworms) MMDW: diluted motorcar mechanic workshop soil treated with worms, MMDNW: diluted motorcar mechanic workshop soil treated without worms, MCUW: undiluted motorcycle mechanic workshop soil treated with worms, MCDW: diluted motorcycle mechanic workshop soil treated with worms, MCDNW: diluted motorcycle mechanic workshop soil treated without worms

4.6.3 Effect of Earthworm innoculation on the Carbon and Nitrogen Contents of Bioremediated Mechanic Workshop Soil

Table 4.34 presents the result of carbon and nitrogen content of the various samples of mechanic workshop after 35 days of bioremediation.Comparing the results from the diluted samples (**MMDW/MMDNW**, and **MCDW/MCDNW**) which recorded high earthworm survival in the 35 day study, the trend in the carbon/nitrogen contents followed the same pattern.

Table 4.34: Effect of Vermi-assisted bioremediation on the Carbon and Nitrogen contents of Mechanic Workshop Soil Contaminated with Petroleum Hydrocarbon.

Sample	% Nitrogen	%Carbon
MMUW	0.49	11.88
MMUNW	0.53	9.77
MMDW	0.39	4.88
MMDNW	0.53	6.19
MCUW	0.53	11.72
MCUNW	0.49	11.72
MCDW	0.42	8.14
MCDNW	0.49	9.12

MMUW: undiluted motorcar mechanic workshop soil treated with worms, MMUNW: undiluted motorcar mechanic workshop soil without worms, MMDW: diluted motorcar mechanic workshop soil treated with worms, MMDNW: diluted motorcar mechanic workshop soil treated without worms, MCUW: undiluted motorcycle mechanic workshop soil treated with worms, MCUNW: undiluted motorcycle mechanic workshop soil without worms, MCDW: diluted motorcycle mechanic workshop soil treated with worms, MCDNW: diluted motorcycle mechanic workshop soil treated without worms

The carbon and nitrogen contents were lower in the earthworm inoculated samples when compared with the earthworm free samples. Nitrogen is lost mainly through ammonia volatilization or denitrification, The process is performed primarily by heterotrophicbacteria (such as Paracoccusdenitrificans and various pseudomonads). Carbon is lost in the form of CO_2

during respiration of earthworms/microbes. The results indicate heightened loss of nitrogen and carbon in the earthworm inoculated samples. The observed trend is differs from the results of **Section 4.4.2** and the findings **Li *et al.* (2002)** that earthworms had no significant influences on nitrogen content. It is possible that the organic amendment (fermented cassava peels) as well as the nature of the soil (contaminated with petroleum hydrocarbon over a long period of time which consequently affected the microbial community) used in the investigation offered a different condition that consequently affected the rate of mineralization of nitrogen.

4.6.4 Effect of Bioremediation on Earthworm Metal Uptake and Elimination

Table 4.35presents the result of metal uptake and elimination by the worms in the 35 day study. Comparison of the metal contents of Table 4.35 with the report of **Uba *et al.,* (2009)**in which heavy metal contents of worms around dumpsites in Zaria metropolis where analyzed, indicated that Zn, Cu and Mn were present in both results. However, whereas Pb and Cd were reported by **Uba *et al.,* (2009)**, they were not detected in Table 4.35. Furthermore, the Zn, Cu and Mncontents shown in the table worms were all higher than their findings.They also reported Cd, 0.55 - 8.13mg/kg; Zn, 105.82 - 380.95mg/kg; Cu, 0.66 - 10.25mg/kg; Pb, 5.01 - 265.40mg/kg and Mn, 1.26 - 10.23mg/kg. It should however be noted that whereas they used AAS method of analysis, this work used XRF analysis based on the body weight of dried earthworm carcass.

Elemental analysis of the soil (petroleum hydrocarbon free) from which the worms were obtained indicated that its iron (Fe) content was higher than that of all mechanic workshop soil considered.The composition of all other metals was close to or lower than the values recorded in the mechanic workshop soils. The earthworms prior to introductions into the mechanic workshop

soil (REW) showed a corresponding high Fe content. As was discussed in **Section *4.4.2.*5**, the metals of interest were Zn, Cu, Cr, Ni, Ti and Mn.

Table 4.35: Earthworm metal uptake and elimination in the vermin-assisted bioremediation of mechanic workshop soil

Element	Earthworm Sample				Soil Sample				
	REW	MMUW	MMDW	MCDW	UCWS	MMUW	MMDW	MCDW	MCUW
Al	6.878	0.000	6.878	5.820	6.243	2.540	6.878	5.450	4.127
Si	10.930	4.036	12.425	16.676	25.410	22.468	33.197	31.669	29.708
S	5.240	8.680	4.320	4.720	0.376	0.560	0.000	0.744	2.580
K	8.058	11.453	7.627	6.108	3.718	4.067	2.465	3.494	2.896
Ca	8.864	17.441	8.435	6.004	4.796	8.077	1.501	1.573	3.031
Ti	0.689	0.276	0.893	0.743	1.031	0.803	0.566	0.893	0.605
V	0.030	0.0336	0.032	0.027	0.050	0.051	0.0308	0.0437	0.026
Cr	0.044	0.151	0.052	0.032	0.054	0.045	0.0226	0.047	0.099
Mn	0.085	0.070	0.147	0.132	0.093	0.077	0.058	0.108	0.148
Fe	11.106	7.518	10.576	9.712	15.666	10.873	7.276	9.52	9.569
Ni	0.035	0.110	0.039	0.030	0.039	0.028	0.008	0.033	0.035
Cu	0.066	0.104	0.058	0.036	0.061	0.064	0.013	0.053	0.058
Zn	0.321	0.2892	0.249	0.193	0.225	0.201	0.023	0.145	0.276
Ba	0.251	0.269	0.215	0.134	0.305	0.430	0.000	0.260	0.134

(REW: Earthworm at t = 0, before introduction into mechanic workshop soil, MMUW: Undiluted motorcar mechanic workshop soil, MMDW: Diluted motorcar mechanic workshop soil, MCDW: Diluted motorcycle mechanic workshop soil, MCUW: Undiluted motorcycle mechanic workshop soil, UCS: petroleum hydrocarbon free soil used in diluting the mechanic workshop soil)

Just as with the simulated used engine oil contaminated soil, there was an appreciable uptake of Ti and Mn in MMDW and MCDW that recorded high survival rate of earthworm. MMUW which recorded a 70% death rate of the worms however recorded elimination of both Ti and Mn. This may be because the unfavorable environment affected the feeding behavior of the worms leading to the elimination of these metals.

For the metals Zn, Cu, Ni and Cr which recorded elimination in the study using simulated used engine oil contaminated soil: the earthworm body concentration of Zn for all samples decreased.

Theconcentration of Zn in the soil from which the worms were obtained was also found to be higher than all mechanic workshop soils considered. This suggests that a relationship exists between the earthworm body concentration of the metal and the soil. The concentration of Cu was found to be slightly higher in MMUW when compared to the UCWS but lower than MMDW and MCDW. MMDW and MCDW after the 35 day study recorded a drop in earthworm body concentration of Cu, as was recorded in the simulation study. The anomalous behavior with MMUW may again be attributed to unfavorable environment which may have resulted in the preferential excretion of other materials by the worms, resulting in an increased Cu Concentration. A definite relationship between earthworm body concentration and the concentration in the soil cannot be seen from the concentration in Ni and Cr. Heavy metals uptake/elimination becomes important when earthworms are considered for use in selective removal of metals from contaminated soil or when the possibility of transferring harmful metals down the food chain exist. With this in mind, it is of note that the concentration of the following metals in worms introduced into the following samples increased sharply: Cr, Cu and Ni (MMUW), Mn(MMDW and MCDW).

4.7 KINETIC MODELING OF BIOREMEDIATION

Microbes are primarily responsible for the degradation of petroleum hydrocarbon in the course of bioremediation. For bioremediation to take place, oxygen (O_2) and the petroleum hydrocarbon contaminant have to dissolve in water, after which they are absorbed by the microorganism present and via metabolism break down the petroleum hydrocarbons (PH's).

Hydrocarbons differ in their susceptibility to microbial attack and generally degrade in the following order of decreasing susceptibility: n-alkanes >branched alkanes >low-molecular

weight aromatics >cyclic alkanes **(Perry, 1984)**. In breaking down the hydrocarbons, the contaminants do not just directly get converted from PH's to harmless CO_2 and O_2: the most common pathway of alkane degradation is oxidation at the terminal methyl group; alkane → alcohol → fatty acid → ketone → CO_2 and H_2O. Formation of intermediate products take place, the presence of which may or may not affect the rate of bioremediation.When environmental conditions (pH, toxicity, etc.) are corrected and microbes are acclimated, the bioremediation rate depends on the biodegradation rate of the oil (within the microbes), the supply of oxygen and nutrients, and the mass transfer of contaminants to the right environment **(Li et al., 1995; Koren *et al.,* 2003)**. This mass transfer rate of oil is in general smaller than either the oxygen transfer rate or the biodegradation rate and therefore is the controlling step of bioremediation of oil-contaminated soil due to the strong hydrophobicity and low water solubility of these compounds **(Li et al., 1995)**. Another rate-limiting step for bioremediation of polyaromatics hydrocarbon (PAH) -contaminated soil is the strong sorption between the contaminant and soil. For contamination levels in which the soil is saturated with oil, rate limitation by strong sorption between contaminants and soil would be negligible and mass transfer limitations will dominate.

From the growth rate curve of Figure 2.2, the lag phase is preceded by the phase of exponential growth in which microbial count increase sharply followed by a stationery phase then the death phase. If an assumption is made that the biodegradation starts at the stationery phase then proceeds through the death phase, then bioremediation may be likened to a deactivating catalyst process. The microbial count may in the analogy be taken to be synonymous with active sites: reduction in microbial count to deactivation of catalysts active sites.With this in mind and drawing from the types of catalyst deactivation **(Levenspiel, 1999)**, independent deactivation

would be the process under consideration. For a constant density batch-solid reactor **(Levenspiel, 1999):**

$$-r_A' = k' C_A{}^n \quad \ldots\ldots (4.6a)$$

$$-\frac{da}{dt} = k_d a^m \quad \ldots\ldots (4.6b)$$

Where

a = activity of the catalyst

k' = reaction rate constant

C_A = concentration of reactant A

k_d = deactivation co

$-r_A$'= rate of reaction

n = order of reaction

m = order of deactivation

This represents n^{th} -order reaction and m^{th}-order deactivation which is also concentration independent. For bioremediation of petroleum hydrocarbon (PH's), the reaction may be summarized as:

$$\textbf{PH's} + O_2 \rightarrow H_2O + CO_2$$

According to **(Li *et al.*, 1995)** only when the oil transfer rate is enhanced by at least 10 times does the oxygen transfer process become the rate-controlling step. Thus it may be assumed that without enhancement, the reaction takes place in the presence of excess O_2. The reaction may therefore be considered 1^{st} order with respect to PH's as the solubility and hence availability of PH's in water is very low.

In developing an expression relating the changing fluid (in this case oil) concentration with time and using time as the one independent variable throughout the run the kinetic expressions of Equation 4.6 become

i. For a nth order reaction and unit initial activity which remains constant (i.e no deactivation) the expression becomes:

$$-\frac{dC_A}{dt} = \frac{W}{V}\left(-\frac{1}{W}\frac{dN_A}{dt}\right) = \frac{W}{V}(-r'_A) = \frac{W}{V}kC_A^n = k'C_A^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.7)$$

Plugging in the 1st order kinetics:

$$(-r'_A) = \frac{dC_A}{dt} = k'C_A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.8)$$

$$ln\left(\frac{C_{Ao}}{C_A}\right) = k't \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.9)$$

This gives the simple first order kinetics also reported by Yeung *et al.* (1997)

ii. For first order kinetics and zero order deactivation

$$-\frac{dC_A}{dt} = (-r'_A) = k'C_A\mathbf{a} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.10)$$

$$-\frac{da}{dt} = k_d a^o = k_d \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.11)$$

Integrating,

$$a = 1 - k_d t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.12)$$

Substituting in Equation (4.9)

$$-\frac{dC_A}{dt} = k'C_A(1 - k_d t) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (4.13)$$

$$ln\left(\frac{C_{Ao}}{C_A}\right) = k'\left(t - \frac{k_d t^2}{2}\right) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (4.14)$$

iii. For first order kinetics and first order deactivation:

$$ln\frac{C_{Ao}}{C_A} = \frac{k'}{k_d}\left(1 - e^{-k_d t}\right) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (4.15)$$

Other kinetic models that may be considered include the shift-order kinetic model which usually characterizes enzyme-substrate reactions:

$$-r_A = \frac{k_1 C_A}{k_2 + C_A} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (4.16)$$

4.7.1 Testing of kinetic models to experimental bioremediation data

Table 4.37 presents the result of the simulation of experimental bioremediation data of **Section 4.3.1.1** with the model developed **(Equations 4.9, 4.14, 4.15 and 4.16)**. Whereas Table 4.37presents the result of the simulation of experimental bioremediation data of **Section 4.6.1.**For all the models considered as shown in Table 4.36 and 4.37, the shift order model of Equation gave the worst fit as depicted in the R^2 values which were very low (≤0.831). Next to shift order model on the worst model list is the first order kinetic model with unit catalyst activity (Equation 4.9) which was also proposed by **Yeung *et al.* (1997)**. Followed by the first order kinetics and zero order deactivation model (Equation 4.14) and finally the model with the best fit was the first order kinetics and first order deactivation model (Equation 4.16).

Table 4.36: Modeling of bioremediation kinetics

Sample	Equation (4.16)	Equation (4.9)	Equation (4.15)	Equation (4.14)
a_E = 0.514	0.4028	0.9404	nc	nc
a_E = 0.572	0.831	0.912	nc	nc
a_E = 0.335	0.1581	0.9809	0.9829	nc
a_E = 0.432	0.0293	0.9671	0.9791	nc

Table 4.37: Modeling of bioremediation kinetics

Sample	Equation (4.16)	Equation (4.9)	Equation (4.15)	Equation (4.14)
MCUDNW	nc	0.798	0.8288	0.8007
MCDW	nc	0.8391	0.8757	0.8464
MCDNW	nc	0.84	0.9026	0.8576
MMUDNW	nc	0.9212	0.9737	0.9763
MMDNW	nc	0.8624	0.9302	0.879

nc = not checked

However, though most of the R^2 for Equation (4.14) were lower that the corresponding ones obtained for Equation (4.16), it was slightly higher for MMUDNW. However, Table 4.37 shows that some of the R^2 for Equation (4.16) were less than 0.9 (for example MCDW), in which case the fits were not very good though better than the order models considered in this work. In conclusion, the 1st order reaction kinetics and 1st order deactivation kinetics (Equation 4.16) is proposed as the kinetic model for biostimulated and vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil.

Table 4.38 presents the values of the kinetics parameter k^1 and k_d for the samples shown in Table 4.37. k^1 is the 1st order chemical reaction rate constant and should have unit of per time. k^1 would be expected to vary with varying bioremediation conditions especially when mass transfer is enhanced. The deactivation constant k_d (also with per time unit) is expected to be dependent on nature and number of microbes, the concentration and nature of contaminant, the quantity and quality of organic amend and any other factor that can affect the microbial community. These may be responsible for the variation in k_d and k^1 values between similar samples (for example samples $a_E = 0.335$ and $a_E = 0.432$ on one hand and MCDW AND MCDNW on the other). It may also be observed in Table 4.39 that the k'/k_d values for all MC's are fairly constant.

Table 4.38: The empirical constants *k'* and k_d for the 1st order reaction and 1st order deactivation bioremediation model

	k_d	k'	k'/k_d
a_E = 0.335	0.05368	0.02823	0.52589
a_E = 0.432	0.03355	0.02093	0.62384
MCUDNW	0.00118	0.028256	23.946
MCDW	0.00261	0.062499	23.946
MC D NW	0.00156	0.035975	23.0611
MM UD NW	0.33163	0.074521	0.22471
MMDNW	0.00193	0.040785	21.1319

CHAPTER 5

CONCLUSIONS

This section gives the conclusions at the completion of the investigation.

The following conclusions were drawn from the results of this work.

1. Thesoil from mechanic workshops contains high levels of petroleum hydrocarbon and heavy metal worth considering for bioremediation.
2. Earthworm survival tests in used engine oil contaminated soil showed that earthworms (*E. eugeniae*) can survive in as high a contamination level of 150g UEO/kg of soil for up to 30 days. Concentration of organic amendment can affect earthworm survival in used engine oil contaminated soil.
3. Initial used engine oil concentration level affected bioremediation by vermicomposting with higher used engine oil concentrations favoring high bioremediation rates. Theuse of earthworms (*E. eugeniae*) had a significant advantage at high concentrations of used engine oil in soil.
4. Earthworm concentration affects the rate of bioremediation with higher rates favoured by higher earthworm concentrations.
5. The presence of earthworms also did not significantly affect soil microbial count.
6. Metals eliminated from earthworm when introduced into used engine oil contaminated soil of concentrations 12.5 – 100g/kg soil included Zn, Cu, Ni and Cr whereas those accumulated included Ti and Mn.
7. The most predominant bacteria in the sample treated with poultry manure and poultry manure/earthworm was *bacillus cereus*.

8. Earthworm inoculation was found to accelerate the bioremediation of mechanic workshop soil and the survival of the worms established to be an important parameter.
9. Use of earthworms (*E. eugenia*) for bioremediation of petroleum hydrocarbon potentially offers a means of accelerating the limiting step (the mass transfer resistancein bioremediation) and should be exploited for maximum benefit.
10. The kinetic model based on the 1^{st} order reaction kinetics and 1^{st} order deactivation kinetics is proposed biostimulated and vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil.

CHAPTER 6

RECOMMENDATIONS

This section gives the recommendationsat the completion of the investigation.

1. Earthworm survival in mechanic workshops soil needs to be studied as the soil composition may be different from that used in this work. Mechanicworkshop soilstend to be contaminated with other material than the used engine oil considered in this work.
2. Other sources of organic amendments, such as cattle dung,should be used for biostimulation of earthworm inoculated used engine oil contaminated soil.
3. Owing to the benefit the environment stands to gain from success in this field, a GCMS machine and spectrophotometer should be made available for timely analysis of samples.
4. Earthworm assisted bioremediation needed be studied on other sources of petroleum hydrocarbon contamination e.g. diesel.
5. Bioremediation using some other earthworm species needed be considered: this work consider only*E. eugeniae*.
6. Proper legislation should be enacted to forestall the indiscriminate dumping of used engine oil in mechanic workshops. Other remediation strategies needed be studied to address the problems of pollution already in place at mechanic workshops
7. Innovative research should be encouraged in this field: who knows, one may come up with a means to change waste to wealth.
8. Metal uptake by earthworm from soil obtained from mechanic workshops needs to be studied as the soil may contain elevated levels of metals than that considered in this work.

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APPENDICES

APPENDIX A

This section gives the details of the standard experimental procedures used in characterizing soil and manure.

F. Soil pH determination

1. 50-ml plastic beaker
2. Glass rods
3. pH meter

Procedure: weight 20gm of air-dry soil (passed over 2-mm sieve) into 50ml beaker and add 50ml of distilled water. Stir the suspension several times during the next 30 minutes with a glass rod. Let the soil suspension stand for about 30 minutes to allow most of the suspended clay to settle out from the suspension. Insert the electrodes of the pH meter into the partly settled suspension and measure the pH **(Iwuafor *et al.* 1990)**.

G. Walkley-Black Method of Soil organic carbon determination(Iwuafor *et al.* 1990)

Apparatus

1. Burettes; 50ml or 25ml
2. Conical flasks, 250ml or 500ml
3. Measuring cylinder
4. pipette

Reagents:

1. Potassium dichromate ($K_2Cr_2O_7$): 1N – dissolve 49.04g of $K_2Cr_2O_7$ in distilled water and dilute to 1 – liter.
2. Concentrated sulphuric acid (H_2SO_4) if chloride is present in the soil add Ag_2SO_4 to the acid at the rate of 15g per liter.
3. O – phosphoric acid (H_3PO_4), concentrated.
4. O – phenanthroline – ferrous complex 0.025M (ferrous). When the indicator is not available it can be prepared by dissolving 14.85g of O – phenanthroline monohydrate and 6.95g of $FeSO_4.7H_2O$ in water and diluting to 1 liter.
5. Barium diphenylamine sulphonate (0.16%). This can be used in place of O – phenanthroline – ferrous complex (0.25M) dissolve 7.425g of O – phenanthroline and 3.475g of ferrous – sulphate in distilled water and dilute to 500ml.
6. Ferrous sulphate (0.5N) – dissolve 140g of $FeSO_4.7H_2O$ in water; add 15ml conc. H_2SO_4, cool and dilute to 1 liter. Standardize this reagent daily or each time before determining organic C by titrating against 10ml 1N K_2CrO_7.

Procedure:

1. Take a representative sample and grind to pass through 0.5mm sieve.
2. Weigh out 1.00g soil and place in the 250ml flask. Use 2.00g soil if organic C is suspected to less than 1%.
3. Pipette 10ml of 1N $K_2Cr_2O_7$ solution accurately into each flask and swirl gently to disperse the soil.

4. Rapidly add 20ml conc H_2SO_4 from a measuring cylinder. Immediately swirl the flask gently until soil and reagents are mixed, then swirl more vigorously for one minute. Rotate flash again and allow to stand on a sheet of asbestos for about 30 minutes. End point is easier to observe if suspension is cold.
5. Add 100ml of distilled water after standing for 30 minutes and let cool again. Filter suspension if cloudy.
6. Add 5ml of O-phosphoric acid to sharpen the color change at the end point (optional).
7. Add 3 – 4 drops of indicator and titrate with 0.5N ferrous sulphate solution on a white background. Note: as the endpoint is approached, the solution takes on a greenish; cast and then changes to darkgreen. At this point, add the ferrous sulphate drop until the color changes sharply from blue to red (maroon color) in reflected light against a white background
8. Make the blank determination in the same way but without soil to standardize the dichromate.
9. Calculate the results according to the following formulae:

$$\%\ organicCinsoil(air-drybasis) = \frac{\left((meqK_2Cr_2O_7 - meqFeSO_4) * 0.003\ * 100 * f\right)}{gofair-drysoil}$$

Where f = correction factor = 1.33

meq = Normality of solution * ml of solution used OR

$$\%\ organicCinsoil(air-drybasis) = \frac{\left((Blanchtitre - actualtitre) * 0.3\ * m * f\right)}{gofair-drysoil}$$

Where f = correction factor = 1.33

m = concentration of FeSO4

% organic matter (OM) may be calculated thus:

$$\% OM = \%OC * 1.729$$

10. % organic matter C may also be expressed on oven-dry basis after correction for moisture content in air-dry soil.

H. Bray No. 1 Method of Soil available phosphorus determination(Iwuafor *et al.* 1990)

Apparatus

1. Centrifuge
2. Mechanical shaker
3. Centrifuge tubes
4. Spectrophotometer
5. Volumetric flask

Reagents

1. Ammonium fluoride (NH4F), 1.0N (dissolve 37g of NH4F in distilled water and make upto 1 liter. Store in polyethylene bottle.
2. Hydrochloric acid HCl, 0.5N – (dilute 20.2ml of conc HCl with distilled water and make upto 500mls.
3. Extracting solution – Add 15ml of 1.0N NH4F and 25 ml of 0.5N HCl to 460ml distilled water.

Procedure

1. Weigh 1g of air-dried soil into a 15 ml centrifuge tube.
2. Add 7ml of the extracting solution
3. Shake for 1 minute on a mechanical shaker and centrifuge the suspension at 2,000rpm for 15 minutes.
4. Decant into an acid-washed volumetric flask.
5. Determine phosphorus in the extract colorimetrically using either the ascorbic acid molybdate blue or vanadomolybdate (yellow) methods.

I. Determination of total N in soil

Regular Macro-Kjedahl Method(Iwuafor *et al.* 1990)

Apparatus

1. Macro-Kjeldahl digestion-distillation apparatus/Aluminium block digestor.
2. Macro- Kjedahl Flask, 500ml or tubes.
3. Volumetric Flasks, 100ml

Reagents

1. Concentrated sulphuric acid.
2. Catalyst – 500gm sodium sulphate (Na2SO4) or potassium sulphate (K2SO4). 50g of anhydrous cupper sulphate 0.5g of selenium powder (mix these different reagents and ground to fine powder).
3. Boric acid (2%) – dissolve 20g of boric acid in 1000cm3 volumetric flask.
4. 40% NaOH – weigh out 400g of NaOH and dissolve in 800cm3 distilled water leave to cool before making to mark in 1 litre volumetric flask.

5. 0.01M HCl – measure 0.86ml of conc. HCl and make it up in 1000cm3 volumetric flask.

Mix Indicators

Weigh out 0.2g of methyl blue into a 100cm3 volumetric flask also add 0.4g of methyl red into another 100ml volumetric flask. Dissolve both indicators with ethanol. Mix both flask in a 200cm3 volumetric flask and mix thoroughly.

Or

Methyl red – bromocresol green mixed indicator. Dissolve 0.5g of bromocresol green and 0.1g of methyl red in 100ml of 95% ethanol adjust pH to 4.5 with 0.1M of NaOH or HCl.

Procedure

1. Weigh out 2g soil into Kjedahl flask or digestion tube.
2. Add 20ml of distilled water, swirl the flask for a few minutes and allow it to stand for 30 minutes.
3. Add about 3g of catalyst and 20ml of conc H_2SO_4.
4. Heat contiously on a low heat. When the water has been removed and frothing has ceased, increase the heat until the digestion clears. Then boil the mixture for 5 hours. Regulate the heating during this boiling so that the H_2SO_4 condenses about half way up the neck of the flask or tube.
5. Allow the flask to cool and slowly with shaking add some distilled water.
6. Carefully transfer the digest into another clean flask. Retain all sand particles in the original digestion flask because sand can cause severe bumping during distillation. Wash

the sand residue with 50 ml of distilled water for about four times and transfer each portion into the same flask. Make to mark with distilled water.

7. Add 10ml H_3Bo_3 solution into a 500ml Erlenmeyer flask which is then placed under the condenser of the distillation apparatus. The end of the condenser should be at about 4 cm above the surface of the H_3Bo_3 solution.
8. Add 10ml of 40% NaOH slowly into 10ml of digest in the flask which has been attached to the distillation apparatus. Raise temperature until it boils.
9. Keep condenser cool (below 30^oC) by allowing sufficient water to flow through and regulate heat to minimize frothing and prevent suck back.
10. Collect about 50ml distillate and then stop distillation.
11. Titrate NH_3 liberated with standard HCl or H_2SO_4. Add 2 – 3 drops of indicator. The colour change at the end point is from green to pink.
12. Calculate the %N content in the soil thus
13. Run a blank similarly but without soil sample

$$\% N = \frac{(0.14 * VD * N * 100 * TV)}{wtofsoil * AD}$$

Where VD = vol. of digest

N = normality of acid

TV = titre value

AD = Aliquot of digest.

J. Determination of soil textural class(Iwuafor *et al.* 1990)

50g of 2mm sieved soil was weighed and put into a plastic beaker and 100ml of calgon was added to it and stirred with glass rod. 100ml of distilled water was then added and again stirred. The mixture was left to stand for about 30 minutes with occasional stirring though. The mixture was transferred into a 250ml plastic container and shaken on an end to end shaker for ten minutes. After this, it was transferred into 1000cm^3 measuring cylinder with the contents completely washed into the cylinder and made up to 1000cm^3 with water. After again shaking the mixture in the cylinder with a long paddle plunger, a hydrometer was used to take readings. Readings were taken for 40 seconds, 4 minutes, 2 hours, 8 hours, and 24 hours. A blank cylinder was also prepared by making 100ml of 50% calgon solution up to the 1 litre mark with water. The blank hydrometer readings were taken side by side the suspension readings. Temperatures of the suspension and the blank were taken.

After the last suspension reading was taken, the suspension was poured through a 2mm sieve and the sand grains retained on the sieve washed with water. The sand was then transferred into a beaker and dried in an oven at 105^0C. After drying the sand was weighed.

Calculations:

The corrected hydrometer readings C(g/L) are obtained by subtracting the blank reading R_L(g/L) from the hydrometer readings in the soil suspensions R(g/L) and adding 0.36g/L for every degree above 20^0C.

$$C = R - R_L + (0.36T)$$

Where T = room temperature minus20^0C.

The percentages by weight of the SILT + CLAY + SAND fractions are given by:

$$\%Clay = \frac{corrected\ 8\ hours reading - Blank}{wtofsoiltaken} * 100$$

$$\%Silt = \frac{(Corrected\ 40\ seconds reading - Blank)}{wtofsoiltaken} - \%Clay$$

%Sand = 100 − %Clay − %Silt

The sample can then be classified according to the USDA or International system of textural classification.

PARTICLE SIZE ANALYSIS ON SOIL (USAD) chart

CLASS	**% SAND**	**% SILT**	**% CLAY**
SAND	>90	< 10	< 10
LOAMY SAND	>80	< 25	< 15
SANDY LOAM	>65	< 30	< 25
SANDY CLAY LOAM	>55 – 80	< 15	< 20 – 35
SANDY CLAY	>40 – 68	< 10	< 35 – 55
LOAM	>45 – 68	< 15 – 30	< 8 – 25
CLAY LOAM	>35 - 60	< 8 – 35	< 25 – 40
SILTY CLAY LOAM	>40	< 25 – 75	< 25 - 40

APPENDIX B

Procedure for Analysis of samples using EDXRF

Mini Pal is a compact Energy dispersive X-ray spectrometer designed for the elemental analysis of a wide range of samples. The system is controlled by a PC running the dedicated Mini pal analytical software.

The Mini pal 4 version in use is PW 4030 X-ray Spectrometer, which is an energy dispersive microprocessor controlled analytical instrument designed for the detection and measurement of elements in a sample (solids, powders and liquids), from sodium to uranium.

The sample for analysis is weighted and grounded in an agate mortar and a binder (PVC dissolved in Toluene) is added to the sample, carefully mixed and pressed in a hydraulic press into a pellet.

The pellet is loaded in the sample chamber of the spectrometer and voltage (30kV maximum) and a current (1mA maximum) is applied to produce the X-ray to excite the sample for a preset time (10mins in this case). The spectrum from the sample is now analyzed to determine the concentration of the elements in the sample (**PanAnalytical user's guide, 2007**).

APPENDIX C

RAW RESULTS OF ANALYSIS

EFFECT OF EARTHWORM ON WATER HOLDING CAPACITY AND MEAN WEIGHT DIAMETER OF SOIL

Table 1: Effect of earthworm on water holding capacity of UEO contaminated soil

Earthworm concentration (number of worms/kg of soil)	WHC, g H_2O/g soil
0	0.483
10	0.487
20	0.488
30	0.505
40	0.494

Table 2: Effect of worms on aggregate size distribution

Earthworm concentration (number of worms/kg of soil)	Mean weight diameter
0	1.406
10	1.264
20	1.172
30	1.190
40	1.191

Table 3: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with poultry manure

	% survival of 10 earthworms (*E. eugeniae*)		
g UEO/kg soil	**Motorcycle UEO**	**Motorcar UEO**	**Truck/lorry UEO**
5	100.00	100.00	100.00
10	100.00	100.00	100.00
20	100.00	91.67	100.00
30	100.00	90.99	100.00
40	100.00	86.67	100.00
50	100.00	83.33	100.00
100	100.00	66.67	91.67

Table 4: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with cattle dung

	% survival of 10 earthworms (*E. eugeniae*)		
g UEO/kg soil	**Motorcycle UEO**	**Motorcar UEO**	**Truck/lorry UEO**
5	100.00	100.00	100.00
10	100.00	100.00	100.00
20	100.00	93.33	100.00
30	100.00	90.99	100.00
40	100.00	86.67	100.00
50	100.00	83.33	100.00
100	100.00	70.00	100.00

INVESTIGATION INTO THE EFFECT OF INITIAL USED ENGINE OIL CONCENTRATION ON BIOREMEDIATION

Table 5: Initial pH of samples

Sample/manure	0g/kg	5g/kg	10g/kg	15g/kg	20g/kg
pH	6.7	6.8	6.89	6.81	6.85

Table 6: Experimental Data on the investigation into the effect of initial concentration of used engine oil in soil (Concentration versus time data)

Time, Days	5g/kg	10g/kg	15g/kg	20g/kg
0	5.01476	10.0049	14.9466	20.03364
14	5.886824	7.6794	11.36145	12.57265
28	8.16388	9.61732	12.23351	15.67332
42	5.8626	7.92164	9.762664	12.76644

INVESTIGATION INTO THE EFFECT OF EARTHWORM CONCENTRATION ON BIOREMEDIATION

Each sample contains: 1000g wet soil, 20g UEO, 6gm poultry manure.
Initial pH of samples = 6.9

Table 7: Effect of variable earthworm concentration on bioremediation

Time, Days	TPH (g UEO/kg soil)				
	0 worms	5 worms	10 worms	15 worms	20 worms
0	17.21429	17	17.85714	17.35714	17.5
14	19.64286	18.35714	16.14286	17.85714	15
28	12.07143	16.42857	17.71429	14.28571	15.85714
42	11.71429	15	16.42857	12.85714	12.42857

Table 8: Effect of worm concentration on microbial count

Time, Days	0 worms	5 worms	10 worms	15 worms	20 worms
	Microbial count * 10^(5) cfu/g				
0	250	200	250	150	200
14	3.2	3	3.1	2.3	2.8
28	75	219	68	80	54
42	27	40	106	20	74
56	20	8	13	11	6

VERMI-ASSISTED BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOIL FROM MECHANIC WORKSHOP

Table 9: Effect of vermin-assisted bioremediation on motorcar mechanic workshop soil

	0 day	12 days	21 days	28 days	35 days
MMUNW	75.45	66.81	64.39	63.28	60.31
MMUW	75.45	59.13	63.42	57.95	59.03
MMDNW	31.61	29.00	28.90	24.42	22.29
MMDW	31.61	27.33	29.84	25.24	19.25

Table 10: Effect of vermin-assisted bioremediation on motorcycle mechanic workshop soil

	0 day	12 days	21 days	28 days	35 days
MCUNW	136.36	125.22	122.86	122.37	104.24
MCUW	136.36	114.00	112.54	111.50	104.65
MCDNW	69.27	64.96	60.78	59.37	49.29
MCDW	69.27	60.00	53.73	45.71	38.67

Table 11: Earthworm heavy metal uptake and elimination from soil contaminated with 12.5g/kg of UEO

Time (days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1	1.42	1.6	1.4
V_2O_5	0.05	0.06	0.06	0.06	0.06
Cr_2O_3	0.26	0.17	0.13	0.45	0.17
NiO	0.26	0.16	0.11	0.5	0.16
CuO	0.29	0.18	0.1	0.39	0.15
ZnO	0.92	0.3	0.28	0.56	0.28
MnO	0	0.05	0.094	0.1	0.15

Table 12: Earthworm heavy metal uptake and elimination from soil contaminated with 25g/kg of UEO

Time(days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1.3	1.9	1.3	1.71
V_2O_5	0.05	0.07	0.04	0.04	0.07
Cr_2O_3	0.26	0.14	0.26	0.15	0.22
NiO	0.26	0.16	0.24	0.14	0.17
CuO	0.29	0.19	0.25	0.13	0.15
ZnO	0.92	0.41	0.47	0.4	0.41
MnO	0	0.078	0.16	0.09	0.12

Table 13: Earthworm heavy metal uptake and elimination from soil contaminated with 50g/kg of UEO

Time(days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1	1.2	1	1.4
V_2O_5	0.05	0.04	0.04	0.04	0.05
Cr_2O_3	0.26	0.15	0.16	0.14	0.3
NiO	0.26	0.17	0.15	0.17	0.26
CuO	0.29	0.16	0.15	0.16	0.23
ZnO	0.92	0.29	0.39	0.53	0.46
MnO	0	0.05	0.063	0.06	0.07

Table 14: Earthworm heavy metal uptake and elimination from soil contaminated with 75g/kg of UEO

Time (days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1.1	1.5	1.2	1.1
V_2O_5	0.05	0.06	0.07	0.05	0.08
Cr_2O_3	0.26	0.18	0.19	0.22	0.22
NiO	0.26	0.22	0.19	0.21	0.16
CuO	0.29	0.24	0.19	0.18	0.16
ZnO	0.92	0.55	0.59	0.46	0.45
MnO	0	0.11	0.08	0.086	0.11

Table 15: Earthworm heavy metal uptake and elimination from soil contaminated with 100g/kg of UEO

Time (days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	0.94	1.2	1.1	1.2
V_2O_5	0.05		0.02	0.04	0.03
Cr_2O_3	0.26	0.24	0.28	0.22	0.22
NiO	0.26	0.25	0.33	0.24	0.18
CuO	0.29	0.25	0.28	0.25	0.17
ZnO	0.92	0.6	0.41	0.48	0.46
MnO	0	0.1	0.14	0.12	0.08

Table 16: Metal uptake and elimination by earthworm in mechanic workshop soil.

Oxide	REWEW	MUDWEW	MMDWEW	MCDWEW	UCSWS	MMUWS	MMDWS	MCDWS	MCUWS
					% Composition				
Al2O3	13		13	11	11.8	4.8	13	10.3	7.8
SiO2	23.4	8.64	26.6	35.7	54.4	48.1	71.07	67.8	63.6
P2O5	10.4	15	10.4	10.8	0.6	0.91	0.38	0.44	0.81
SO3	13.1	21.7	10.8	11.8	0.94	1.4	0	1.86	6.45
Cl	2.03	3.16	1.96	1.63		0.33	0.242	0.083	0.44
K2O	9.71	13.8	9.19	7.36	4.48	4.9	2.97	4.21	3.49
CaO	12.4	24.4	11.8	8.4	6.71	11.3	2.1	2.2	4.24
TiO2	1.15	0.46	1.49	1.24	1.72	1.34	0.944	1.49	1.01
V2O5	0.053	0.06	0.057	0.048	0.089	0.091	0.055	0.078	0.047
Cr2O3	0.064	0.22	0.076	0.047	0.079	0.066	0.033	0.068	0.144
MnO	0.11	0.091	0.19	0.17	0.12	0.1	0.075	0.14	0.191
Fe2O3	12.35	8.36	11.76	10.8	17.42	12.09	8.091	10.59	10.64
NiO	0.045	0.14	0.049	0.038	0.05	0.036	0.0098	0.042	0.044
CuO	0.083	0.13	0.073	0.045	0.076	0.08	0.016	0.066	0.073
ZnO	0.4	0.36	0.31	0.24	0.28	0.25	0.028	0.18	0.343
Ga2O3			0.02	0.03	0	0.02		0.026	
Br	0.62		0.51	0.43					0.22
ZrO2		2					0.2		
Rb20						14			
Rh207					0.65				
BaO	0.28	0.3	0.24	0.15	0.34	0.48		0.29	0.15
MoO3							0.57		
Re2O7	0.05	0.3		0.06	0.06	0.06	0.067	0.14	0.3
PtO2		0.3							
IrO2	0.4				0.21				
Au			0.45				0.09		

APPENDIC D

Pictorial representations of major equipment use in the course of this investigation



Figure A: Equipment for elemental analysis using XRF, MiniPal PW4025, Philips Analytical.



Figure B: MiniPal PW4025, Philips Analytical.



Figure C: GCMS equipment (GCMS-QP2010 Plus Shimadzu, Japan).



Figure D: Spectrophotometer (B. Bran Scientific and Instrument Company, 722 – 2000 20D spectrometer).



Figure E: Sieve shaker

Sample of Result of XRF Analysis of Motorcycle Mechanic Workshop Soil (MC5)

05-Nov-2010 14:38:51

Sample results

Sample Ident

SAMPLE MC 5			

Application	<Standardless>
Sequences	1 of 1
Measurement time	05-Nov-2010 14:34:30
Position	2

Channel	Al	Si	S	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu
Compound	Al2O3	SiO2	SO3	K2O	CaO	TiO2	V2O5	Cr2O3	MnO	Fe2O3	NiO	CuO
Conc Unit	12%	61.5%	3.5%	3.86%	1.53%	0.046%	0.068%	0.16%	12.24%	0.029%	0.029%	0.14%

Channel	Zn	Rb	Sr	Y	Zr	Ru	Ba	Eu	Pb
Compound	ZnO	Rb2O	SrO	Y2O3	ZrO2	RuO2	BaO	Eu2O3	PbO
Conc Unit	0.286%	0.042%	0.038%	0.049%	0.446%	0.75%	0.1%	0.08%	0.12%

Sample of Result of XRF Analysis of Motorcar Mechanic Workshop Soil (MM8)

05-Nov-2010 14:30:49 Page 1

Sample results

Sample Ident			
SAMPLE MM8			

Application	<Standardless>
Sequences	1 of 1
Measurement time	05-Nov-2010 14:28:15
Position	6

Channel	Al	Si	S	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu
Compound	Al2O3	SiO2	SO3	K2O	CaO	TiO2	V2O5	Cr2O3	MnO	Fe2O3	NiO	CuO
Conc Unit	12%	53.4%	0.83%	3.37%	8.75%	1.54%	0.06%	0.071%	0.11%	17.60%	0.024%	0.13%

Channel	Zn	Rb	Sr	Y	Zr	Ru	Ba	Re	Pb	Th
Compound	ZnO	Rb2O	SrO	Y2O3	ZrO2	RuO2	BaO	Re2O7	PbO	ThO2
Conc Unit	0.26%	0.06%	0.053%	0.049%	0.429%	0.90%	0.1%	0.04%	0.23%	0.12%

Sample of Result of XRF Analysis of Earthworms (20g/kg UEO Contaminated Soil on Day 5), in Metal Uptake/Elimination Study

07-May-2011 16:25:38

Sample results

Sample Ident			
20C5			

Application	<Standardless>
Sequences	1 of 1
Measurement time	27-May-2011 16:19:3
Position	12

Channel Compound	Al2O3	SiO2	P2O5	SO3	Cl	K2O	CaO	TiO2	V2O5	Cr2O3	MnO	Fe2O3
Conc Unit	16%	14.4%	11.6%	16.1%	2.71%	9.85%	15.1%	1.0%	0.04%	0.15%	0.05%	10.4%

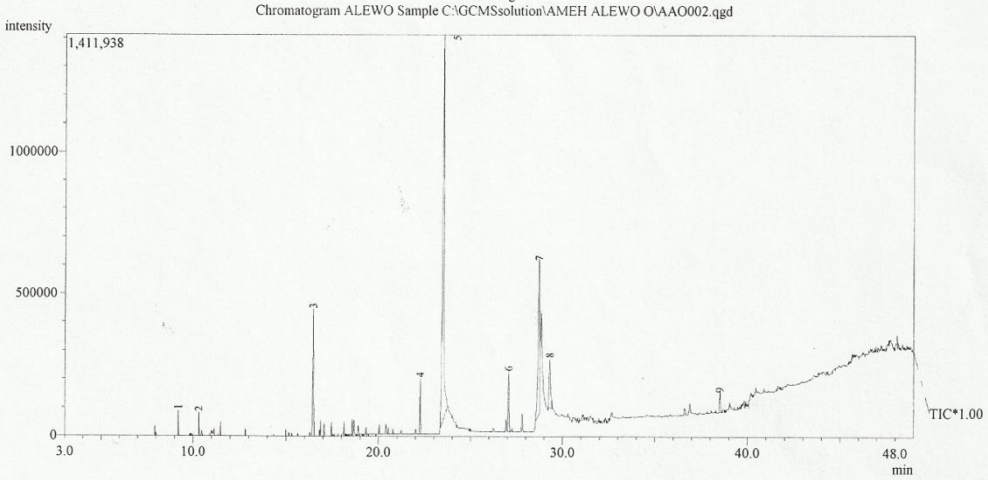
Channel Compound	NiO	CuO	ZnO	Br	BaO	Re2O7
Conc Unit	0.17%	0.16%	0.29%	1.1%	0.3%	0.71%

GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA
GCMS ANALYSIS

AMEH ALEWO O (SAMPLE-MC35W)

Chromatogram
Chromatogram ALEWO Sample C:\GCMSsolution\AMEH ALEWO O\AAO002.qgd



Method

[Comment]

===== Analytical Line 1 =====

[AOC-20i]

# of Rinses with Presolvent	:5
# of Rinses with Solvent(post)	:5
# of Rinses with Sample	:3
Plunger Speed(Suction)	:High
Viscosity Comp. Time	:0.2 sec
Plunger Speed(Injection)	:High
Syringe Insertion Speed	:High
Injection Mode	:Normal
Pumping Times	:5
Inj. Port Dwell Time	:0.3 sec
Terminal Air Gap	:No
Plunger Washing Speed	:High
Washing Volume	:8uL
Syringe Suction Position	:0.0 mm
Syringe Injection Position	:0.0 mm
Use 3 Solvent Vial	:1 vial

[GC-2010]

Column Oven Temp.	:60.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:100.2 kPa
Total Flow	:6.2 mL/min
Column Flow	:1.61 mL/min
Linear Velocity	:46.3 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:1.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	60.0	5.00
20.00	140.0	0.00
5.00	200.0	5.00

4.00 280.0 5.00

< Ready Check Heat Unit >
Column Oven : Yes
SPL2 : Yes
MS : Yes
< Ready Check Detector(FTD) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
SPL2 Carrier : Yes
SPL2 Purge : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait :No
Equilibrium Time :3.0 min

[GC Program]

[GCMS-QP2010 Plus]
IonSourceTemp :200.00 °C
Interface Temp. :250.00 °C
Solvent Cut Time :2.50 min
Detector Gain Mode :Relative
Detector Gain :0.00 kV
Threshold :8000

[MS Table]
--Group 1 - Event 1--
Start Time :3.00min
End Time :49.00min
ACQ Mode :Scan
Event Time :0.50sec
Scan Speed :1428
Start m/z :40.00
End m/z :700.00

Sample Inlet Unit :GC

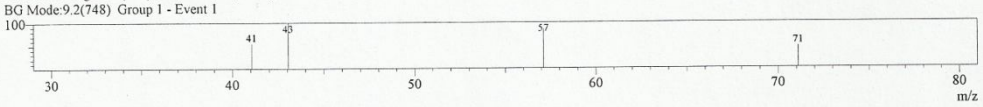
[MS Program]
Use MS Program :OFF

Peak Report TIC

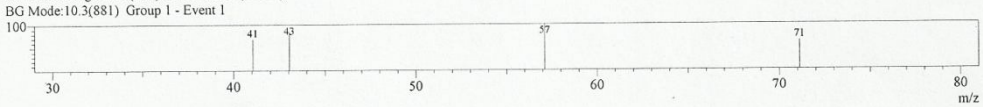
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2	10.304	10.275	10.342	142105	0.88	82651	2.61	1.72		
3	16.459	16.392	16.542	1449966	8.97	445279	14.08	3.26		
4	22.261	22.217	22.317	521713	3.23	195517	6.18	2.67		
5	23.504	23.333	23.742	8210420	50.79	1352224	42.75	6.07		
6	27.063	26.992	27.142	719721	4.45	207610	6.56	3.47		
7	28.709	28.517	28.775	3817164	23.61	546389	17.27	6.99		
8	29.301	29.217	29.408	923895	5.71	172626	5.46	5.35		
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Spectrum

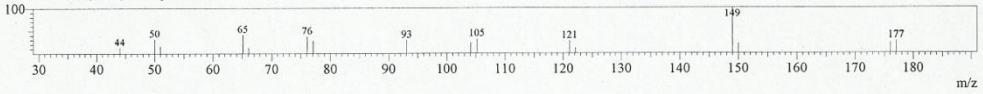
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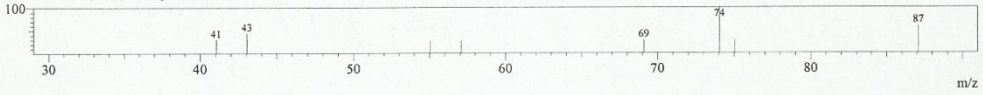
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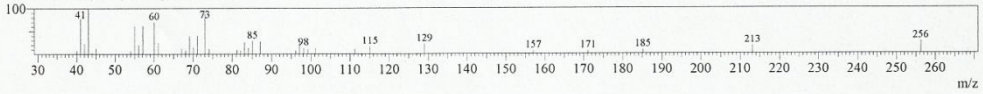
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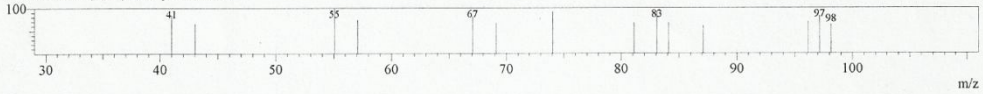
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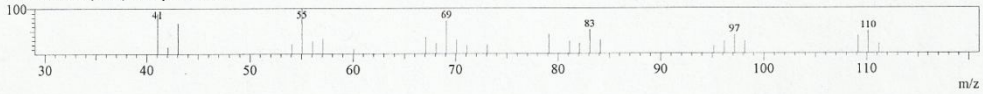
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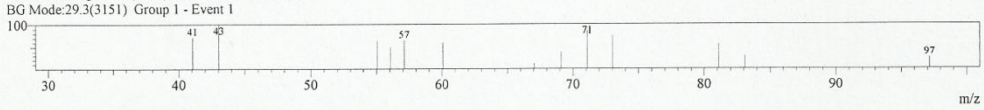
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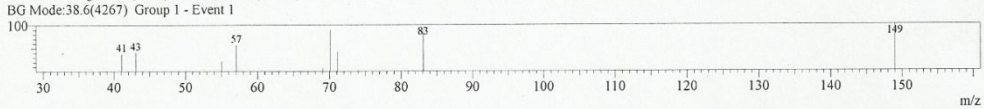
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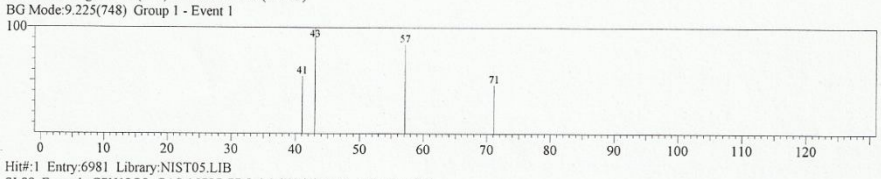
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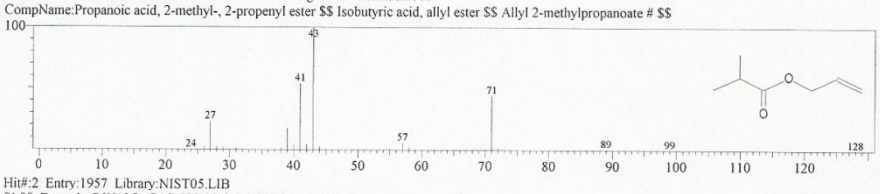
Spectrum Comparison

Library

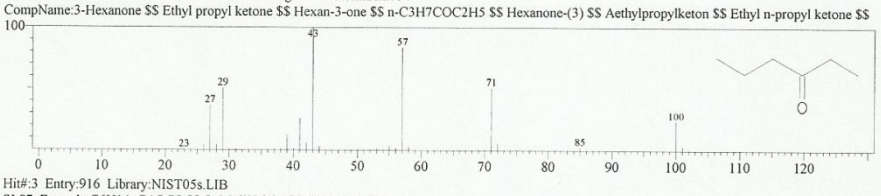
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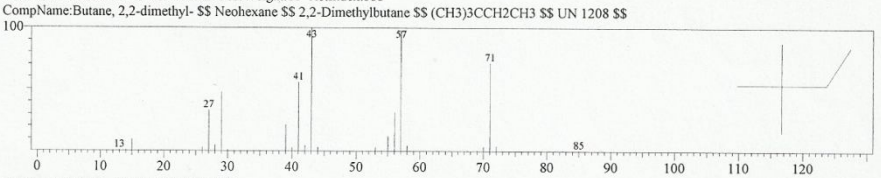
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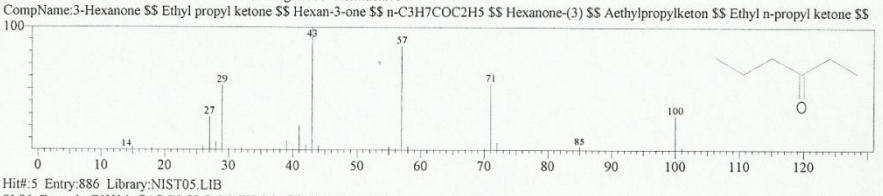
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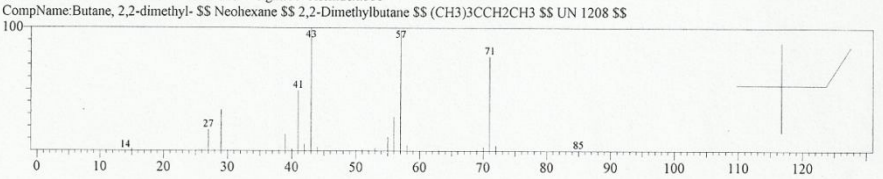
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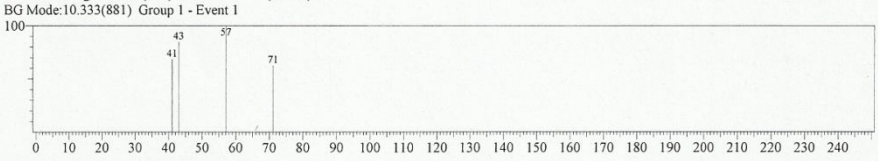
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SI:87 Formula:C6H12O CAS:589-38-8 MolWeight:100 RetIndex:754
CompName:3-Hexanone $$ Ethyl propyl ketone $$ Hexan-3-one $$ n-C3H7COC2H5 $$ Hexanone-(3) $$ Aethylpropylketon $$ Ethyl n-propyl ketone $$



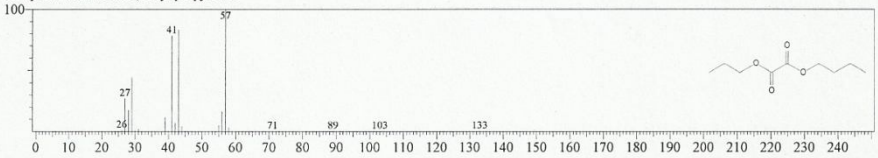
Hit#:5 Entry:886 Library:NIST05.LIB
SI:86 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



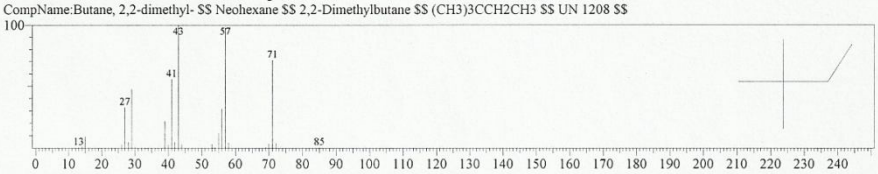
<< Target >>
Line#:2 R.Time:10.300(Scan#:877) MassPeaks:4
RawMode:Single 10.300(877) BasePeak:57.10(23526)
BG Mode:10.333(881) Group 1 - Event 1



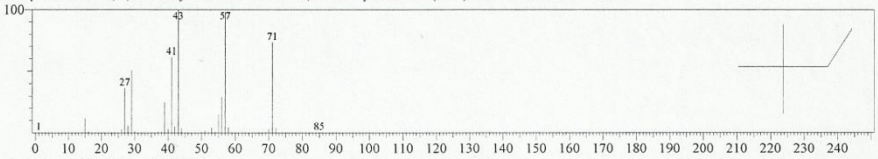
Hit#:1 Entry:34044 Library:NIST05.LIB
SI:88 Formula:C9H16O4 CAS:0-00-0 MolWeight:188 RetIndex:1250
CompName:Oxalic acid, butyl propyl ester



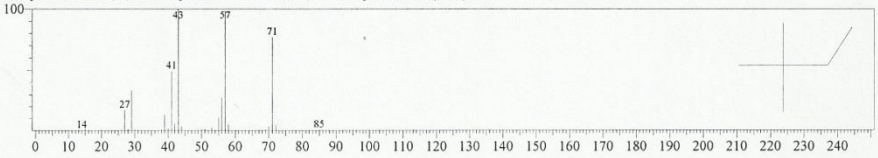
Hit#:2 Entry:916 Library:NIST05s.LIB
SI:88 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



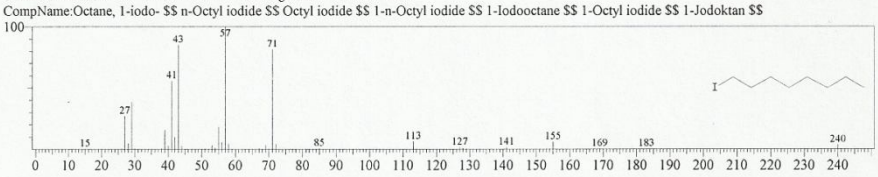
Hit#:3 Entry:915 Library:NIST05s.LIB
SI:87 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



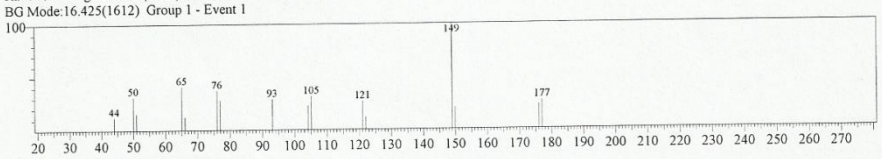
Hit#:4 Entry:886 Library:NIST05.LIB
SI:87 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



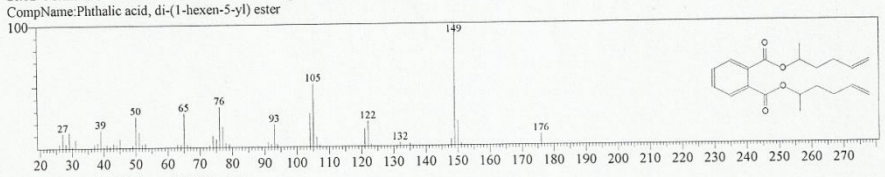
Hit#:5 Entry:20129 Library:NIST05s.LIB
SI:87 Formula:C8H17I CAS:629-27-6 MolWeight:240 RetIndex:1231
CompName:Octane, 1-iodo- $$ n-Octyl iodide $$ Octyl iodide $$ 1-n-Octyl iodide $$ 1-Iodooctane $$ 1-Octyl iodide $$ 1-Jodoktan $$



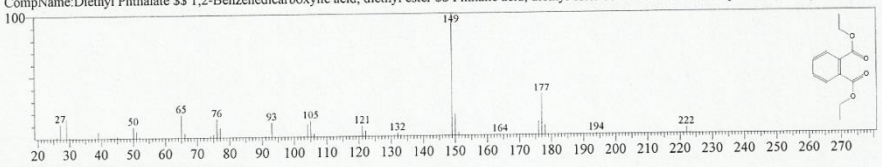
<< Target >>
Line#:3 R.Time:16.458(Scan#:1616) MassPeaks:16
RawMode:Single 16.458(1616) BasePeak:149.00(70201)
BG Mode:16.425(1612) Group 1 - Event 1



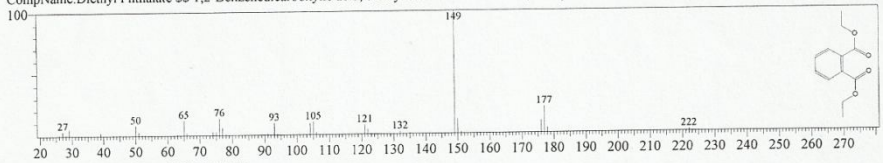
Hit#:1 Entry:117551 Library:NIST05.LIB
SI:82 Formula:C20H26O4 CAS:0-00-0 MolWeight:330 RetIndex:2286
CompName:Phthalic acid, di-(1-hexen-5-yl) ester



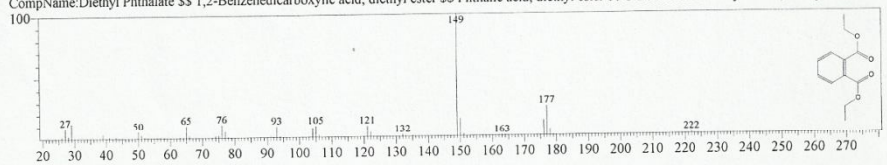
Hit#:2 Entry:18578 Library:NIST05s.LIB
SI:81 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:1639
CompName:Diethyl Phthalate $$ 1,2-Benzenedicarboxylic acid, diethyl ester $$ Phthalic acid, diethyl ester $$ o-Benzenedicarboxylic acid, diethyl ester $$



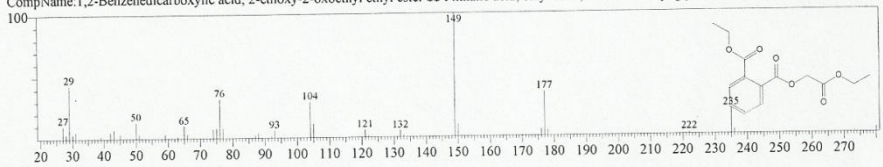
Hit#:3 Entry:18579 Library:NIST05s.LIB
SI:79 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:1639
CompName:Diethyl Phthalate $$ 1,2-Benzenedicarboxylic acid, diethyl ester $$ Phthalic acid, diethyl ester $$ o-Benzenedicarboxylic acid, diethyl ester $$



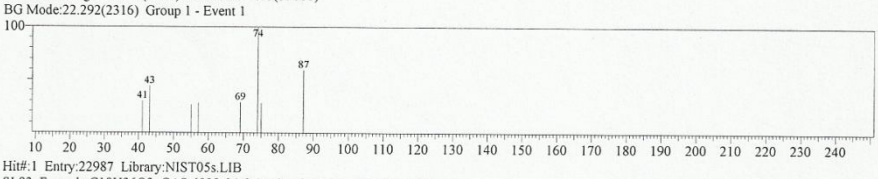
Hit#:4 Entry:18580 Library:NIST05s.LIB
SI:79 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:1639
CompName:Diethyl Phthalate $$ 1,2-Benzenedicarboxylic acid, diethyl ester $$ Phthalic acid, diethyl ester $$ o-Benzenedicarboxylic acid, diethyl ester $$



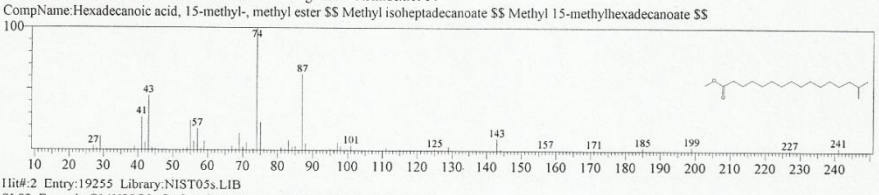
Hit#:5 Entry:22735 Library:NIST05s.LIB
SI:77 Formula:C14H16O6 CAS:84-72-0 MolWeight:280 RetIndex:2005
CompName:1,2-Benzenedicarboxylic acid, 2-ethoxy-2-oxoethyl ethyl ester $$ Phthalic acid, ethyl ester, ester with ethyl glycolate $$ Carbethoxymethyl ethyl



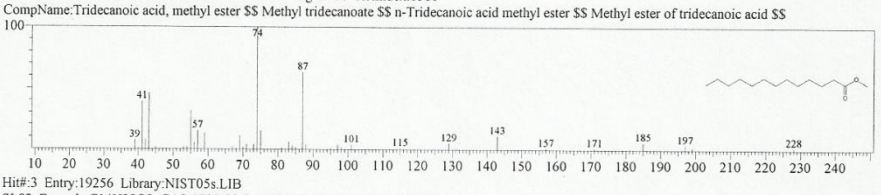
<< Target >>
Line#:4 R.Time:22.258(Scan#:2312) MassPeaks:9
RawMode:Single 22.258(2312) BasePeak:74.05(39686)
BG Mode:22.292(2316) Group 1 - Event 1



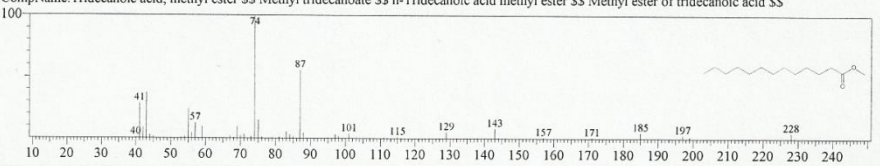
Hit#:1 Entry:22987 Library:NIST05s.LIB
SI:83 Formula:C18H36O2 CAS:6929-04-0 MolWeight:284 RetIndex:1914
CompName:Hexadecanoic acid, 15-methyl-, methyl ester $$ Methyl isoheptadecanoate $$ Methyl 15-methylhexadecanoate $$



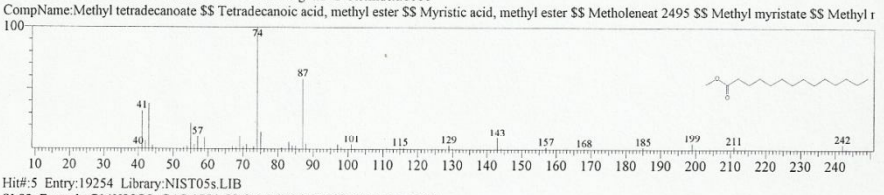
Hit#:2 Entry:19255 Library:NIST05s.LIB
SI:82 Formula:C14H28O2 CAS:1731-88-0 MolWeight:228 RetIndex:1580
CompName:Tridecanoic acid, methyl ester $$ Methyl tridecanoate $$ n-Tridecanoic acid methyl ester $$ Methyl ester of tridecanoic acid $$



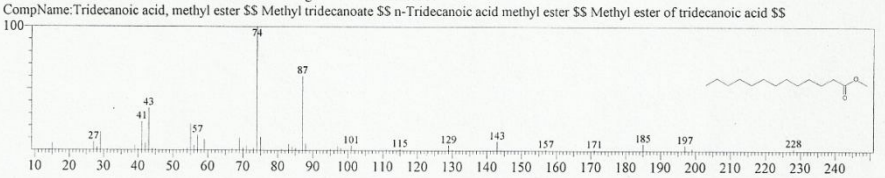
Hit#:3 Entry:19256 Library:NIST05s.LIB
SI:82 Formula:C14H28O2 CAS:1731-88-0 MolWeight:228 RetIndex:1580
CompName:Tridecanoic acid, methyl ester $$ Methyl tridecanoate $$ n-Tridecanoic acid methyl ester $$ Methyl ester of tridecanoic acid $$



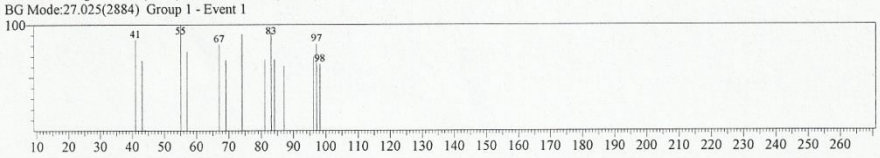
Hit#:4 Entry:20373 Library:NIST05s.LIB
SI:82 Formula:C15H30O2 CAS:124-10-7 MolWeight:242 RetIndex:1680
CompName:Methyl tetradecanoate $$ Tetradecanoic acid, methyl ester $$ Myristic acid, methyl ester $$ Metholeneat 2495 $$ Methyl myristate $$ Methyl r



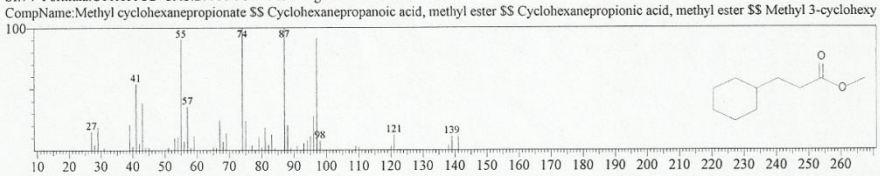
Hit#:5 Entry:19254 Library:NIST05s.LIB
SI:82 Formula:C14H28O2 CAS:1731-88-0 MolWeight:228 RetIndex:1580
CompName:Tridecanoic acid, methyl ester $$ Methyl tridecanoate $$ n-Tridecanoic acid methyl ester $$ Methyl ester of tridecanoic acid $$



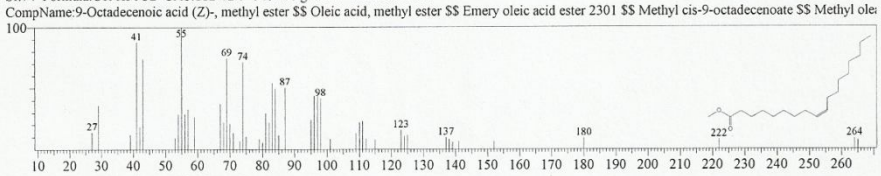
<< Target >>
Line#:6 R.Time:27.067(Scan#:2889) MassPeaks:15
RawMode:Single 27.067(2889) BasePeak:55.05(15231)
BG Mode:27.025(2884) Group 1 - Event 1



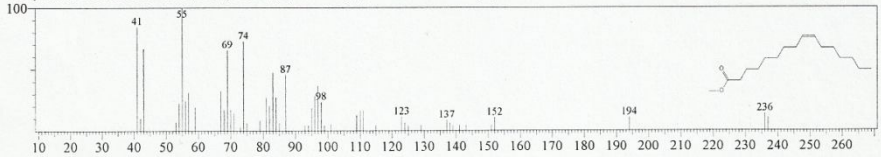
Hit#:1 Entry:24819 Library:NIST05.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



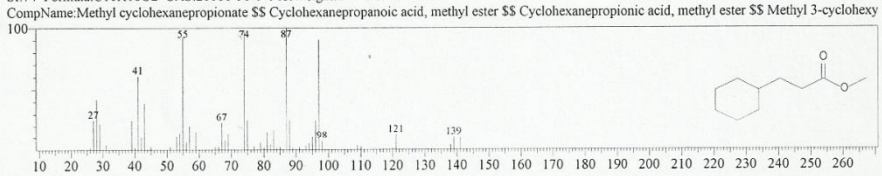
Hit#:2 Entry:98788 Library:NIST05.LIB
SI:74 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl ole



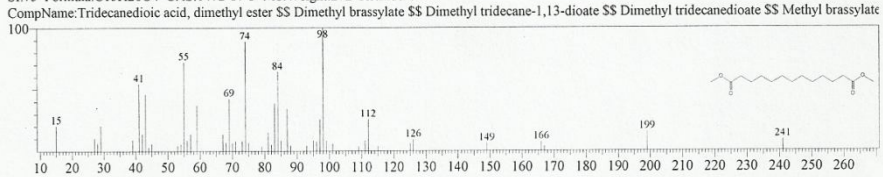
Hit#:3 Entry:22069 Library:NIST05s.LIB
SI:74 Formula:C17H32O2 CAS:1120-25-8 MolWeight:268 RetIndex:1886
CompName:9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate $$ Methyl palmitoleinate $$ Palmitoleic acid, methyl ester $$ Methyl (9Z)-9-he



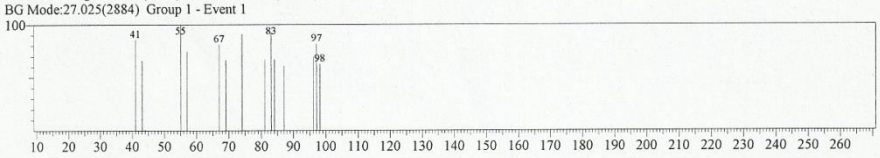
Hit#:4 Entry:11955 Library:NIST05s.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



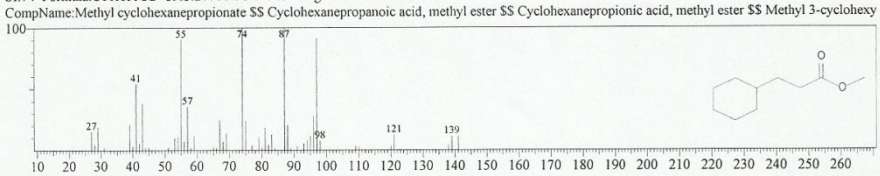
Hit#:5 Entry:22294 Library:NIST05s.LIB
SI:73 Formula:C15H28O4 CAS:1472-87-3 MolWeight:272 RetIndex:1847
CompName:Tridecanedioic acid, dimethyl ester $$ Dimethyl brassylate $$ Dimethyl tridecane-1,13-dioate $$ Dimethyl tridecanedioate $$ Methyl brassylate



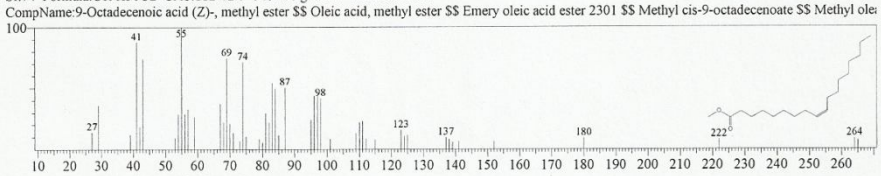
<< Target >>
Line#:6 R.Time:27.067(Scan#:2889) MassPeaks:15
RawMode:Single 27.067(2889) BasePeak:55.05(15231)
BG Mode:27.025(2884) Group 1 - Event 1



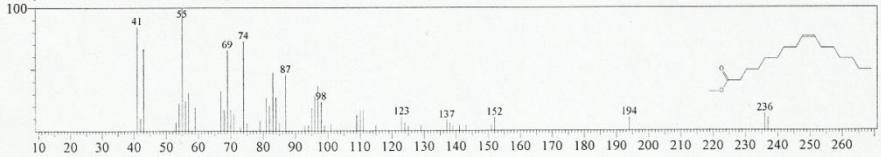
Hit#:1 Entry:24819 Library:NIST05.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



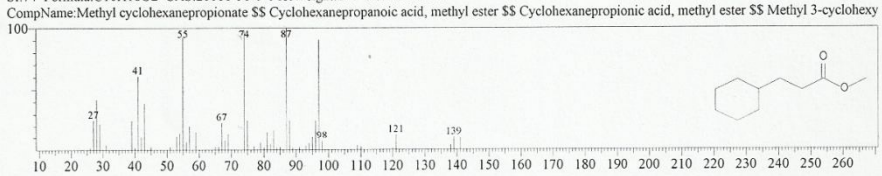
Hit#:2 Entry:98788 Library:NIST05.LIB
SI:74 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl ole



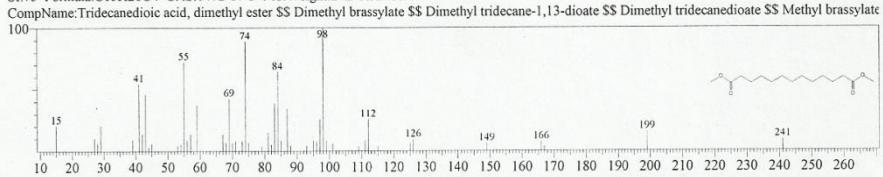
Hit#:3 Entry:22069 Library:NIST05s.LIB
SI:74 Formula:C17H32O2 CAS:1120-25-8 MolWeight:268 RetIndex:1886
CompName:9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate $$ Methyl palmitoleinate $$ Palmitoleic acid, methyl ester $$ Methyl (9Z)-9-he



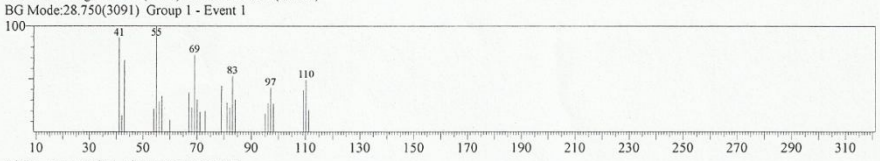
Hit#:4 Entry:11955 Library:NIST05s.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



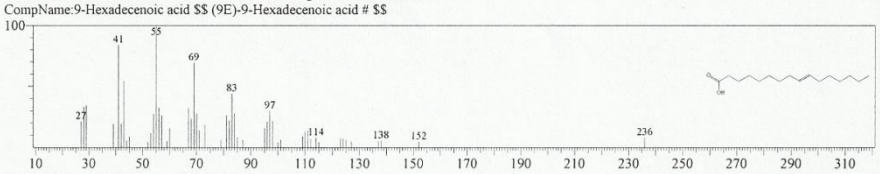
Hit#:5 Entry:22294 Library:NIST05s.LIB
SI:73 Formula:C15H28O4 CAS:1472-87-3 MolWeight:272 RetIndex:1847
CompName:Tridecanedioic acid, dimethyl ester $$ Dimethyl brassylate $$ Dimethyl tridecane-1,13-dioate $$ Dimethyl tridecanedioate $$ Methyl brassylate



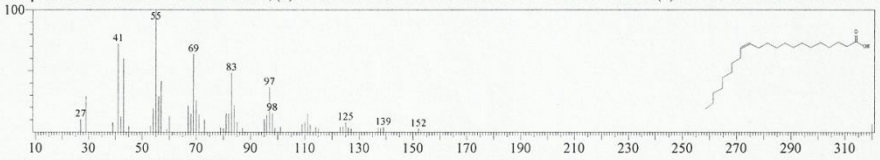
<< Target >>
Line#:7 R.Time:28.717(Scan#:3087) MassPeaks:27
RawMode:Single 28.717(3087) BasePeak:55.05(20756)
BG Mode:28.750(3091) Group 1 - Event 1



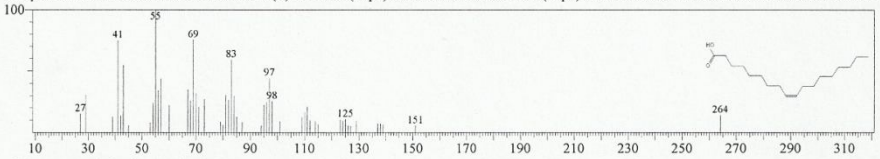
Hit#:1 Entry:73685 Library:NIST05.LIB
SI:88 Formula:C16H30O2 CAS:2091-29-4 MolWeight:254 RetIndex:1976
CompName:9-Hexadecenoic acid $$ (9E)-9-Hexadecenoic acid # $$



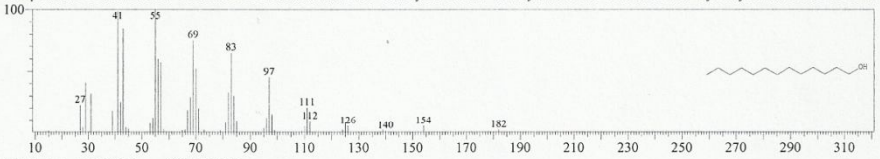
Hit#:2 Entry:121691 Library:NIST05.LIB
SI:87 Formula:C22H42O2 CAS:112-86-7 MolWeight:338 RetIndex:2572
CompName:Erucic acid $$ 13-Docosenoic acid, (Z)- $$ .delta.13-cis-Docosenoic acid $$ cis-13-Docosenoic acid $$ (Z)-13-Docosenoic acid $$ Prifrac 299



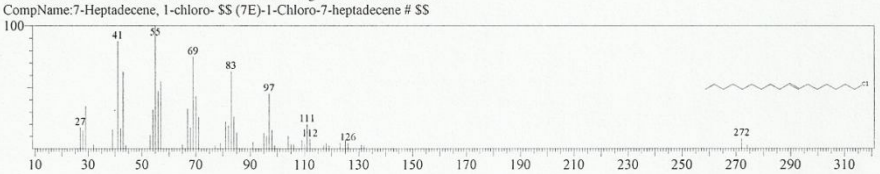
Hit#:3 Entry:90577 Library:NIST05.LIB
SI:87 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:2175
CompName:Oleic Acid $$ 9-Octadecenoic acid (Z)- $$ .delta.(Sup9)-cis-Oleic acid $$ cis-.delta.(Sup9)-Octadecenoic acid $$ cis-Oleic Acid $$ cis-9-Octad



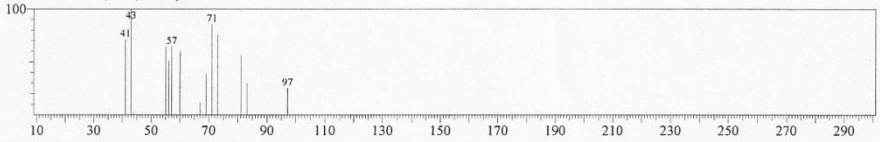
Hit#:4 Entry:41278 Library:NIST05.LIB
SI:86 Formula:C13H28O CAS:112-70-9 MolWeight:200 RetIndex:1556
CompName:1-Tridecanol $$ n-Tridecan-1-ol $$ n-Tridecanol $$ n-Tridecyl alcohol $$ Tridecyl alcohol $$ Tridecanol $$ 1-Hydroxytridecane $$ C13 Linea



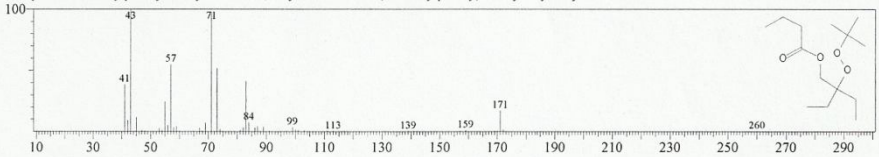
Hit#:5 Entry:84726 Library:NIST05.LIB
SI:86 Formula:C17H33Cl CAS:56554-78-0 MolWeight:272 RetIndex:1944
CompName:7-Heptadecene, 1-chloro- $$ (7E)-1-Chloro-7-heptadecene # $$



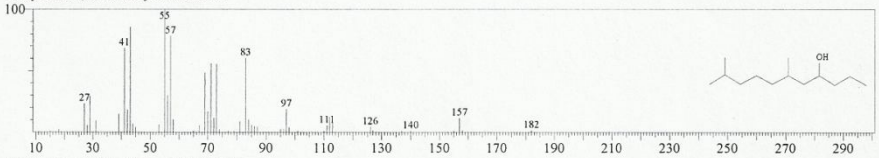
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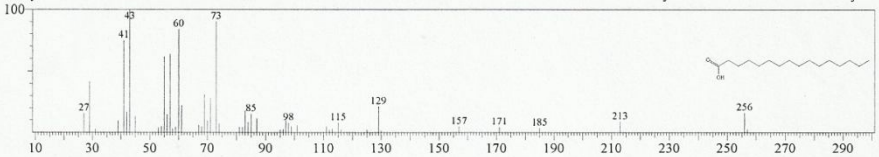
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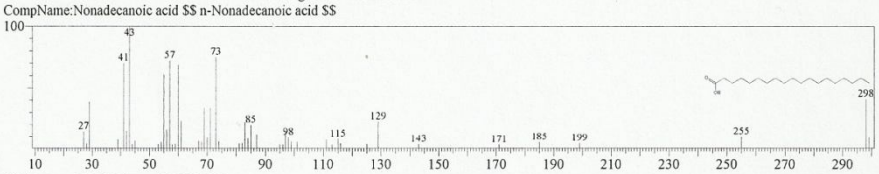
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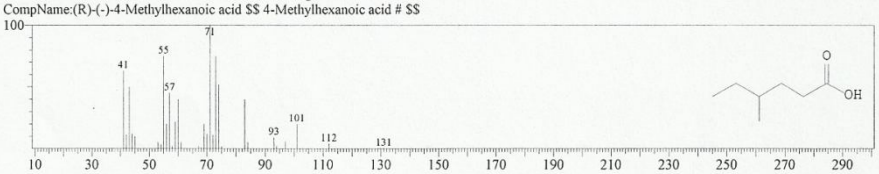
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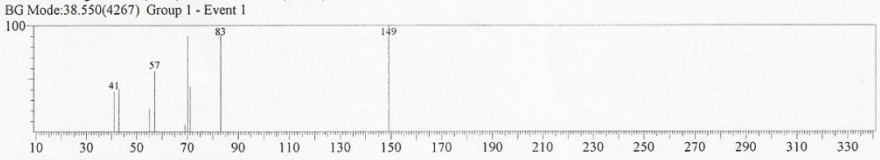
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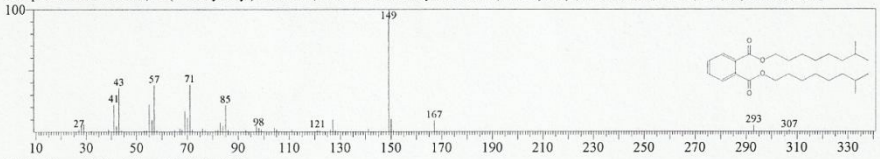
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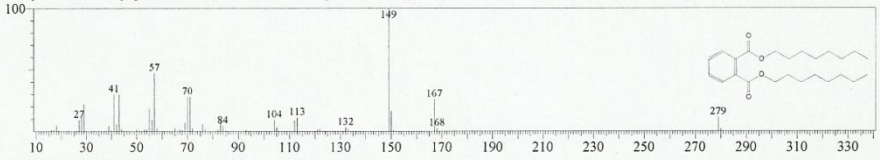
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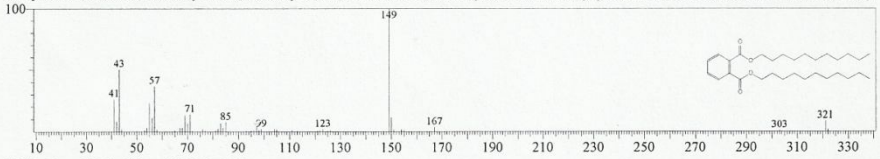
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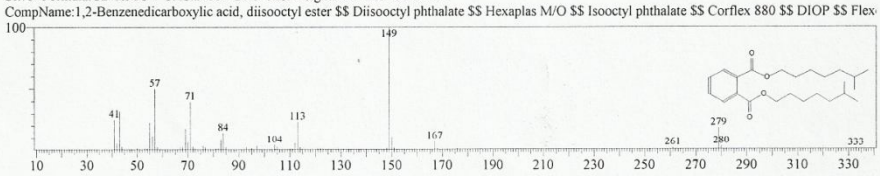
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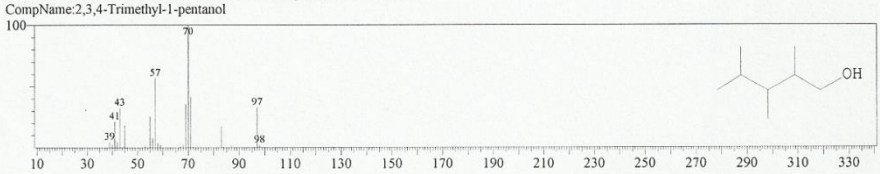
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SI:73 Formula:C24H38O4 CAS:27554-26-3 MolWeight:390 RetIndex:2704
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Hit#:5 Entry:7895 Library:NIST05.LIB
SI:72 Formula:C8H18O CAS:6570-88-3 MolWeight:130 RetIndex:867
CompName:2,3,4-Trimethyl-1-pentanol



CHAPTER 1

INTRODUCTION

Thenumber of roadway, railway, air and water vehicles has always been on the increase. Consequently, the quantity of engineoil and other engine fluids (cooling liquids,brake oils, gearbox oils, etc.) used is expected to increase**(Baladincz*et al.*, 2008)**.The lubricating oils used by vehicle engines have to be replaced at least every 20,000 km**(www.daewoobus.com)**. The used oil is either dumped or re-refined and reused.

The term, used engine oil (UEO), refers to any lubricating oil that has served its service purposes in a vehicle and withdrawn from the designed area of application and considered no longer fit for the initial intended purpose. These used lubricants contain a lot of toxic and carcinogenic substances that can cause detrimental effects to the ecosystem. Basically lubricating oils are produced by some refining processes of crude oil. Additives are usually added to the lubricating oil to boost some of the oils properties like viscosity, thermal and oxidation stability. These additives when releasedinto the environment have toxic effect to humans and animals**(Abdullahi and Kawo, 2006)**.

The toxic and carcinogenic components of UEO may also be formed as a result of the thermal decomposition of lubricating materials upon processing, transportation, exploitation and storage. Oxidation of lubricating oil hydrocarbons at the point of application is accompanied by release of free radicals that transform to peroxides, which condense and polymerizeto produce per acids, nephtenic acids, etc. It is widely agreed that toxicity of lubricating oils have significant effects on germination, growth and yield of crops**(Abdullahi and Kawo, 2006)**.

Since the advent of oil exploration, the Nigeria environment has been heavily contaminated with hydrocarbon pollutants, which enters the environment through several routes. Pollution from spent engine oil is more widespread than crude oil pollution especially in urban centers where there are automobiles and generators using this petroleum product to reduce the friction between moving surfaces of engine**(Odjegbaand Sadiq, 2002)**. The presence of these pollutants in the terrestrial and aquatic environments constitutes public health and socio-economic hazards. In Nigeria, about 20 million gallons of waste engine oil are generated annually from mechanic workshops and dischargedcarelessly into the environment **(Adegoroye, 1997)**, out of which only one liter is enoughto contaminate one million gallons of freshwater. Apart from this, UEO renders the environmentunsightly and constitutes a potential threat tohumans, animals and vegetation. Several components of the oil, e.g. solventsand detergents added during the blending process, aliphatic hydrocarbon and polyaromatic hydrocarbons (PAHs) distilled from crude oil, andmetals from engine wear are either toxic in themselves orcan combine with products of combustion to generate carcinogens and endocrine disrupters.

Environmental problems resulting from oil pollution havebeen a major research issue in Nigeria over the last decadeowing to their effect on agriculture, economy, and societyat large. The economic growththat Nigeria has enjoyed as a result of oil revenue hasits drawbacks through exposure of the population in oilproducingareas to environmental contamination, largelydue to the increase in the movement of oil. Spillage of crude oil on soils hinders plant growthand productivity. The retarded plant growthby oil spillage usually results from insufficient aerationcaused by air displacement from the pore spaces betweenthe soil particles by the crude oil. Insufficientaeration also causes root stress, which consequentlyreduces plant growth, and

alwaysresults in leaf chlorosis. Low levelof chlorophyll in leaves of plants grown in soils treatedwith spent engine oil has been reported (**Adenipekun *et al.*, 2008**).

Biological degradation of petroleum hydrocarbon pollutantsand petrochemicals has been reported worldwide. Several researchers have looked at the remediation of oil contaminated soils.**Deshpande *et al.*(1999)** reported the use of surfactants in remediation of oil contaminated soils,**Marja *et al.*(2002)** reported the phytoremediation of soil contaminated with diesel and**Szewczyk and D1ugon´ski (2009)** reported the degradation of Pentachlorophenol and spent engine oil by *Mucor ramosissimus*, etc. In Nigeria, reports on the degradation of usedengine oil have not been widespread.**Adelowo *et al.* (2006)**reported thebio-degradation of used engine oil by locally isolated speciesof *Pseudomonas fragi* and *Achromobacter aerogenes*, while **Adenipekun (2008)** reported the bioremediation of engine oil contaminated soil using a Nigerian white-rot fungus**.**

When environmental conditions (pH, toxicity, etc.) are correctedand microbes are acclimated, the bioremediationrate depends on chemical kinetics within the microbe,the supply of oxygen and nutrients, and the masstransfer of contaminants to the right environment**(Li et al., 1995; Koren *et al.*, 2003)**.This mass transfer rate of oil is in general smallerthan either the oxygen transfer rate or the biodegradation rate and therefore is the controlling step of bioremediation of oil-contaminated soil due to the strong hydrophobicity and low water solubility of these compounds. The engineer's major task is to optimize the environmentalconditions for bioremediation**(Li et al., 1995)**. Another rate-limiting step for bioremediation of polyaromatics hydrocarbon (PAH) -contaminated soil is the strong sorption between the contaminant and

soil.Thus, any factor that works to minimize the effects of these resistances should hasten the bioremediation process.

In spite of the numerous ways in which the activities of earthworm promotes soil health (for example burrowing, soil ingestion, cast formation etc which theoretically is thought capable of influencing bioremediation) relatively few research have been undertaken to study the effects of earthworm on petroleum hydrocarbons contamination removal from soil **(Hickman and Reid, 2008).Hickman and Reid (2008)** enumeratedsome of the effects of earthworm activities which theoretically can enhance bioremediation. These effects include microorganism promotion and dispersal, improved soil structure, fragmented soil particles and increased surface area, organic matter turnover, increased water holding capacity, enhanced nutrient content and availability (with increased C:N ratio), improved O_2 and gas diffusion, increased porosity, increased pH etc.**Maike and Filser (2007)** studied the biodegradation of crude oil contaminated soil using earthworm and reported that earthworms can trigger bioremediation and may be applied in the bioremediation of crude oil contaminated soil of low to moderate concentrations. **Philip *et al.*(1998)**reported that earthworms are sensitive indicators of soil quality and may be used to evaluate the quality of remediated oily soils.

1.3 NULL HYPOTHESIS

The use of earthworm in the bioremediation of used engine oil contaminated soil will not contribute to the rehabilitation of the soil.

1.4 PROBLEM STATEMENT

Environmental (soil, water and air) contamination is a phenomenological problem worldwide. Bioremediation as a cleanup option,though cost effective and environment friendly, suffers the

disadvantage of being relatively very slow – thought to be mass transfer limiting. Furthermore, in spite of the numerous ways in which the activities of earthworm promotes soil health (which theoretically is thought capable of influencing bioremediation), relatively few research efforts have been undertaken to study the use of earthworm as a biocatalyst in the bioremediation of petroleum hydrocarbon contaminated soil.

1.3 JUSTIFICATION

The justifications for this work include:

Used engine oil contamination is wide spread in Nigeria and constitutes a serious environmental hazard.Bioremediation is considered to be cheap (**Aalok *et al., 2008)*** and therefore inviting as a treatment option. Finally, success in the work will provide a faster means of bioremediation using indigenous organisms.

1.4 AIM AND OBJECTIVES

This work is aimed at studying the use of earthworm in enhancing the bioremediation of engine oil contaminated soil.

The set objectives include:

5. To carry out investigation into the effect of earthworm and organic amendments on biodegradation of used engine oil contaminated soil (simulated contamination and contaminated soil from mechanic workshops).
6. To fit the bioremediation data to established bioremediation kinetic equations.
7. To investigate the effect of vermicomposting on microbial biomass.
8. To investigate the effect of vermicomposting on used engine oil contaminated soil moisture retention, metal uptake/elimination, particle sizeand carbon/nitrogen contents.

1.5 RESEARCH LIMITATION

This research was limited by the unavailability of the appropriate standards for total petroleum hydrocarbon (TPH) determination using gas chromatograph as well as the appropriate crucible for elemental analysis of the used engine oil samples.

1.6 SCOPE

7. Testing of soil samples collected from various mechanic workshops,where used engine oil is usually dumped. Physical and chemical properties will be investigated.
8. Preparation and analysis and of organic materials for use as amendment.
9. Investigations into the effect of earthworms on metal uptake (bioaccumulation in earthworm), moisture retention in soil (water holding capacity), soil carbon and nitrogen contents and aggregate size distribution in the bioremediation of used engine oil contaminated soil.
10. Investigationsinto the use of earthworm in the bioremediation of soil samples in which the microorganisms have been stimulated. Parameters to be investigated include the effect of earthworm concentration and used engine oil concentration.
11. Analysis and interpretation of data and testing of data with models established in the literature.
12. Studies on vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshop.

CHAPTER 2

REVIEW OF LITERATURE

The quality of life on Earth is linked inextricably to the overall quality of the environment. In early times, it was believed that there was an unlimited abundance of land and resources; today, however, the carelessness and negligence in the use of these natural endowments have resulted in the degradation of the environment which in turn affects life negatively. The problems associated with contaminated sites now assume increasing prominence in many countries. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today. The problem is worldwide, and the estimated number of contaminated sites is significant **(Cairney, 1993)**. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use.

This chapter gives an extensive review of work on remediation with emphasis on bioremediation and earthworm assisted bioremediation of petroleum hydrocarbon. It covers the theories and hypothesis of bioremediation.

2.1 BIOREMEDIATION

The conventional techniques used for remediation has been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create

significant risks in the excavation, handling, and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. The cap and contain method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability.

A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents.

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result.Because bioremediation

seems to be a good alternative to conventional clean-up technologies research in this field is rapidly increasing.

Bioremediation has been used at a number of sites worldwidewith varying degrees of success. Techniques are improving as greater knowledge and experience are gained, and there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Unfortunately, the principles, techniques, advantages, and disadvantages of bioremediation are not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators**(Vidali, 2001)**.

2.1.1 Principles of Bioremediation

Environmental biotechnology is not a new field; composting and wastewater treatments are familiar examples of old environmental biotechnologies. However, recent studies in molecular biology and ecology offer opportunities for more efficient biological processes. Notable accomplishments of these studies include the clean-up of polluted water and land areas. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities **(Mueller *et al.*, 1996)**.

By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are

transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation we have a process known as bioaugmentation**(Vidali, 2001)**.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

Like other technologies, bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies.

Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions **(Colberg and Young, 1995)** may permit microbial organisms to degrade

otherwise recalcitrant molecules. Alist of some contaminants potentially suitable for bioremediationis shown in Table 2.1.

Table 2.1: Some contaminants potentially suitable for bioremediation

Class of contaminants	Examples	Aerobic	Anaerobic	Potential sources
Chlorinated solvents	Trichloromethylene		+	Drycleaners
	Perchloroethylene			Chemical manufacture
Polychlorinated biphenyls	4-Chlorobiphenyl		+	Electrical manufacturing
	4.4-Dichlorobiphenyl			Power station, Railway yards
Chlorinated phenol	Pentachlorophenol		+	Timber treatment, landfills
BTEX	Benzene Toluene Ethylbenzene Xylene	+	+	Oil production and storage, Gas work sites, Airports, Paint manufacture, Port facilities, Railway yard, Chemical manufacture
Polyaromatic hydrocarbon (PAHs)	Naphthalene Antracene Flourene Pyrene Benzo(a)pyrene	+		Oil production and storage, Gas work sites, Coke plants, Engine works, Landfills, Tar production and storage, Boiler ash, Dump sites, Power stations
Pesticides	Atrazine Carbaryl Carbofuran Coumphos Diazinon Glycophosphate Parathion Propham 2,4-D	+	+	Agriculture, Timber treatment plants, Pesticides, Pesticides manufacture, Recreational areas, Landfills.

Source: Vidali, 2001

2.1.2 Factors of Bioremediation

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environmental factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

2.1.2.1 Microbial Population for Bioremediation Processes

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are an energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. We can subdivide these microorganisms into the following groups:

Aerobic (in the presence of oxygen). Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

Bacillus is a genus of gram-positive rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species can be obligate aerobes or facultative anaerobes, and test positive for the enzyme catalase **(Tumbull, 1990)**. Ubiquitous in nature, *Bacillus* includes both free

living and pathogenic species.Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods.

Few studies have been reported on the roles of *Bacillus spp.* in hydrocarbon bioremediation; although there are several reports on bioremediation of pollutants by the action of *Bacillus spp*. occurring in extreme environments (**Khan and Rizvi, 2011).Ijah and Antai (2003)** reported *Bacillus spp*. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples (30 and 40% crude oil). It has been postulated that *Bacillus spp*. are more tolerant to high levels of hydrocarbons in soil due to their resistant endospores.

Singh and Lin (2008) reported that *Bacillus pumilus* achieved 86.94% of diesel degradation in 2 weeks. **Kebria *et al.* (2009)** reported that a strain of *bacillus* native to Tehran*Bacillus Cereus*,showed great potential for in situ remediation of diesel-contaminated soil in oil refinery sites. *Bacillus cereus*is an organism occurring naturally in most foods and it can cause two differentforms of food poisoning, diarrhoeal and emetic, both caused by toxins produced by the bacteria.**Khan and Rizvi (2011)** cultured and isolated microorganisms from mechanic workshops and reported that the isolate showing maximum oil degrading ability was gram positive *bacillus spp*.

Pseudomonas is a genus of gammaproteobacteria, belonging to the family Pseudomonadaceae containing 191 validly described species. *Pseudomonas aeruginosa* is a versatile Gram-negative bacterium that grows in soil, coastal marine habitats, and on plant and animal tissues.In their review**Das and Chandran (2011)**touched on the use of*Pseudomonas aeruginosa* for degrading sludge hydrocarbons. **Emtiazi *et al*. (2005)** reported the immobilization of *pseudomonas* isolated from a petroleum-contaminated soil for use in petroleum hydrocarbon degradation.

Anaerobic(in the absence of oxygen): Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform.

Ligninolytic fungi: Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs.

Methylotrophs (Aerobic bacteria that grow utilizing methane for carbon and energy): The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatics trichloroethylene and 1,2-dichloroethane **(Allard and Neilson, 1997)**.

For degradation it is necessary that bacteria and the contaminants be in contact. This is not easilyachieved, as neither the microbes nor contaminants are uniformly spread in the soil. Some bacteriaare mobile and exhibit a chemotactic response, sensing the contaminant and moving toward it. Othermicrobes such as fungi grow in a filamentous form toward the contaminant. It is possible to enhancethe mobilization of the contaminant utilizing some surfactants such as sodium dodecyl sulphate (SDS).

Cometabolism is used to describe the transformation of an organiccompound by a microorganism that is unableto use the substrate as a source of energy. Metabolites or transformation products fromcometabolism by one organism can typicallybe used as an energy source by another.

2.1.2.2 Environmental Factors

Nutrients:Although microorganisms are present in contaminated soil, they cannot necessarily be there in thenumbers required for bioremediation of the site. Their growth and activity must be stimulated.Biostimulation usually involves the addition of nutrients and oxygen to help in the growth of indigenous microorganisms.These nutrients are the basic building blocks of life and allow microbes to create the necessaryenzymes to break down the contaminants. These nutrients include nitrogen, phosphorous, and carbon.

Carbon is the most basic element of living forms and is needed in greater quantities than otherelements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells. Phosphorous and sulfur make up 70% of the remainders. The carbon/nitrogen and carbon/phosphorous ratios required for microbial growthis 10:1and 30:1 respectively.

Environmental requirements:Optimum environmental conditions for the degradation of contaminants are reported in Table 2.2.Microbial growth and activity are readily affected by pH, temperature, and moisture. Although microorganisms have been also isolated in extreme conditions, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions. If the soil has too much acid it ispossible to raise the pH by adding lime. Temperature affects biochemical reactions rates, and the rates of many of them double for each 10 °C rise in temperature. Above a certain temperature, however, the cells die. Plastic covering can be used to enhance solar warming in late spring, summer, and autumn. Available water is essential for all the living organisms and irrigation is needed to achieve the optimal moisture level. The amount of available oxygen will determine whether the system is aerobic or anaerobic. Hydrocarbons are readily degraded under aerobic conditions, whereas chlorurate compounds are

degraded only in anaerobic ones. To increase the oxygen amount in the soil it is possible to till or sparge air. In some cases, hydrogen peroxide or magnesium peroxide can be introduced in the environment. Soil structure controls the effective delivery of air, water, and nutrients. To improve soil structure, materials such as gypsum or organic matter can be applied. Low soil permeability can impede movement of water, nutrients, and oxygen; hence, soils with low permeability may not be appropriate for *insitu* clean-up techniques**(Vidali, 2001)**.

Table 2.2: Environmental conditions affecting degradation.

Parameters	Condition required for microbial activity	Optimum value for an oil degradation
Soil moisture	25-28 % of water holding capacity	30-90%
Soil pH	5.5- 8.8	6.5 – 8.0
Oxygen content	Aerobic, minimum air-filled pore space of 10%	10 – 40%
Nutrient content	N and p for microbial growth	C:N:P=100:10:1
Temperature (^{o}C)	15-45	20-30
Contaminants	Not too toxic	Hydrocarbon 5-10% of dry weight of soil
Heavy metals	Total content 2000ppm	700ppm
Type of soil	Low clay or silt content	

Source: (Vidali, 2001)

2.1.3 Bioremediation Strategies

Different techniques are employed depending on the degree of saturation and aeration of an area. *In situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. *Ex situ* techniques are those that are applied to soil and groundwater which

has been removed from the site via excavation (soil) or pumping (water). *Bioaugmentation* techniques involve the addition of microorganisms with the ability to degrade pollutants.

2.1.3.1 In situ Bioremediation

These techniques are generally the most desirable options due to lower cost and less disturbance since they provide the treatment without excavation and transport of contaminants. *In situ*treatment is limited by the depth of the soil that can be effectively treated. In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases. The most important land treatments are:

Bioventing: Bioventing is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.

In situ biodegradation: Thisinvolves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment.

Biosparging: Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

Bioaugmentation: Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit:

c. Nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and
d. Most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

2.1.3.2 Ex situ Bioremediation

These techniques involve the excavation or removal of contaminated soil from ground.

Landfarming: Thisis a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attentionas a disposal alternative.

Composting: This technique involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials support the development of a rich microbial population and elevated temperature characteristic of composting.

Biopiles: Biopilesare a hybrid of landfarming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms**(Von Fahnestock*et al.*, 1998)**.

Bioreactors:Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil-bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction

(e.g., vacuum extraction) before being placed in a bioreactor. Table 2.3 shows the advantages and disadvantages of bioremediation methods.

2.1.3.3 Advantages of Bioremediation

Bioremediation enjoys the following advantages:

6. 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 increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.

7. 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.

8. 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.

9. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.

Table 2.3: Summary of bioremediation strategies

Technology	Examples	Benefits	Limitations	Factors to consider
In situ	*In situ* bioremediation Biosparging Bioventing Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	Biodegradative abilities of indigenous microorganisms Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Landfarming Composting Biopiles	Cost efficient Low cost Can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation	See above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhanced mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relative high cost capital Relatively high operating cost	See above Bioaugmentation Toxicity of amendments Toxicity concentrations of contaminants

Source: (Vidali, 2001)

10. Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

2.1.3.4 Disadvantages of Bioremediation

Bioremediation as a cleanup option suffers the following disadvantages:

8. Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptibleto rapid and complete degradation.

9. There are some concerns that the products of biodegradation may be more persistent or toxic thanthe parent compound.

10. Biological processes are often highly specific. Important site factors required for success includethe presence of metabolically capable microbial populations, suitable environmental growth conditions,and appropriate levels of nutrients and contaminants.

11. It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.

12. Research is needed to develop and engineer bioremediation technologies that are appropriate forsites with complex mixtures of contaminants that are not evenly dispersed in the environment.

13. Bioremediation often takes longer than other treatment options, such as excavation and removalof soil or incineration.

14. Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation: there is no accepted definition of “clean”, evaluating performance of bioremediation is difficult,and there are no acceptable endpoints for bioremediation treatments.

2.1.4 Phytoremediation

Although the application of microbial biotechnology has been successful with petroleum-based constituents, microbial digestion has met limited success for widespread residual organic and metals pollutants. Vegetation- based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. In natural ecosystems, plants act as filters and metabolize substances generated by nature. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water **(Raskin and Ensley, 2000)**. The term "phytoremediation" is relatively new, coined in 1991. Its potential for encouraging the biodegradation of organic contaminants is promising and requires further research.Phytoremediationtechniques are classified as: phytoextraction, phytotransformation, phytostabilization, phytodegradation, rhizofiltration(based on the contaminant fate).

2.2 EARTHWORM

Earthworms are perhaps the most important soil organisms in terms of their influence on organic matter breakdown, soil structural development, and nutrient cycling, especially in productive ecosystems. Aristotle called them the "intestines of the earth" and the eminent nineteenth century biologist, Charles Darwin, spent many years observing their major influence on the formation of humus and transport of soil **(Darwin, 1881)**.

2.2.1 Earthworm Biology and Classification

Earthworms are terrestrial annelids with bilateral symmetry and corresponding external and internal segmentation. They have a thinly pigmented cuticle bearing setae on all segments except the first two. All earthworms are hermaphroditic. Their gonads are situated in specific segments,

which vary among different taxa. When sexually mature, they develop a swollen area of the epidermis called a clitellum. This region produces a cocoon in which one or more eggs are deposited and then the cocoon is passed over the anterior segments and deposited in or on the soil. The young develop within the cocoon and newly hatched worms resemble adults. The time to hatching and reproductive maturity varies widely among different earthworm species and is influenced by environmental factors. Earthworms are classified within the phylum Annelida and the class Oligochaeta, which consists of as many as 36 families worldwide. About two-thirds of oligochaete families comprise aquatic or semi-aquatic worms, and the remaining families comprise mostly or exclusively terrestrial worms or earthworms. There are over 3500 known earthworm species and it is estimated that the global total may be twice that number. Distinct taxonomic groups have arisen on every continent except Antarctica, and some groups are now distributed throughout the world**(Partrick, 2002)**. Figure 2.1 is a pictorial representation of *Eudrilus Eugenia* an earthworm species commonly found in Northern Nigeria.

2.2.2 Earthworm Ecology

2.2.2.1 Ecological groups

Earthworm species can be grouped according to behavioral, morphological or physiological adaptations that enable them to partition available resources in the soil. The three main life history strategies are termed epi, anecic, and endogeic **(Edward and Bohlen, 1996 and Lee 1985)**.



Figure 2.1: Earthworm (*Eudrilus eugeniae*)

Epigeic worms feed on plant litter, dwell on the soil surface or within the litter layer, tend to be heavily pigmented, and are small- to medium-sized. Anecic worms feed on plant litter and soil, live in nearly vertical permanent burrows, are dorsally pigmented, and large. Endogeic species are soil-feeders, are not heavily pigmented, form extensive horizontal burrow systems, and range in size from small to large. Endogeic worms have been further divided into polyhumic, mesohumic, and oligohumic groups, which are separated, respectively, by the descending importance of organic rich mineral soil in their diet and increasing size. Earthworm species do not always fall clearly into these three main categories and may even exhibit traits of different groups at different life stages or under different environmental conditions.

2.2.2.2 Earthworm communities

Earthworm communities generally consist of from one to six species. The relative abundance and species composition of earthworm communities depend upon soil type, topography, and vegetation, and is also influenced by land use history and earthworm biogeography. Earthworms account for the majority of animal biomass in soil in a wide range of productive ecosystems, from temperate grasslands, pastures and forests to tropical pastures, savannas and rainforests, and many temperate and tropical agroecosystems. They generally do not occur in deserts and arid grasslands or in extreme alpine or boreal habitats. Earthworms are often absent from strongly acidic forest soils with poor litter quality, such as some northern coniferous forests. Many earthworm communities consist of invasive exotic species. In North America, where approximately 100 native earthworm species have been described, at least 45 exotic species have been introduced. Lumbricid earthworms of European origin dominate many North American agroecosystems. A worldwide survey of earthworms in tropical regions reported that a total of 51 exotic and 151 native species commonly occur in tropical agroecosystems **(Fragoso *et al.*, 1999)**.

2.2.3 Effects of Earthworms on Soil Properties

The influence of a given earthworm species on soil properties depends upon that species' life history strategy. For example, the large vertical burrows of anecic earthworms, such as the common nightcrawler *Lumbricus terrestris*, can facilitate preferential flow of water through the soil profile, increasing the transport of water, nutrients, and agricultural chemicals into deeper soil layers **(Subler *et al.*, 1997 and Edward *et al.*, 1992)**. Epigeic species facilitate the breakdown and mineralization of surface litter, whereas anecic species incorporate surface litter deeper into the soil profile. Anecic species also bring soil from deeper soil horizons to the surface, which over a long period of time can change the mineralogy of surface soil. Endogeic species feed on fragmented organic matter and mix it thoroughly through the surface mineral soil.

2.2.3.1 Effects on organic matter breakdown and nutrient cycling

Earthworm activity accelerates the decomposition of plant litter, increases rates of nutrient transformation and plant nutrient uptake, improves soil aggregation and porosity, and enhances water infiltration and solute transport. Earthworms can consume and incorporate large amounts of organic matter into soil (Table 2.4). Such mixing is largely responsible for the formation of mull soils in which surface organic horizons are thoroughly mixed with underlying mineral soil.

Earthworms are a major influence on nutrient cycling in many ecosystems. Although they generally increase the mineralization of soil carbon, earthworms can also decrease mineralization of carbon by contributing to the formation of stable soil aggregates in which carbon is protected from further breakdown.

Table 2.4: Amount of organic matter ingested or incorporated into soil by earthworm populations in different environments

Ecosystem	**Location**	**Type of organic matter**	**Amount consumed or incorporated kgha^{-1}**
Alfalfa field	United States	Alfalfa residue	1,220
Maize field	United States	Maize residue	840
Mixed forest	England	Canopy tree leaves	3,000
Oak forest	Japan	Oak leaves	1,071
Orchard	England	Apple leaves	2,000
Savannah	Ivory coast	Total organic matter	1,300
Tallgrass prairie	United States	Total organic matter	740-8,980

Source: (Patrick, 2002)

Direct fluxes of nutrients through earthworm biomass can be considerable. For example, the turnover of nitrogen (N) through earthworm tissues can be up to 150 kgNha^{-1} yr^{-1}**(Lee, 1985)**. Earthworm casts contain elevated amounts of inorganic N relative to surrounding soil. As a consequence, earthworms can greatly enhance the mineralization of N and can stimulate other N transformations such as denitrification. By increasing bypass flow of infiltrating water, earthworm burrows can increase the amount of N and other nutrients leaching from the soil

profile. Alternatively earthworms can reduce the amount of nutrients lost in surface runoff by increasing rates of water infiltration into the soil **(Subler *et al.*, 1997 and Sharpley *et al.*, 1979)**.

2.2.3.2 Effects on physical properties of soil

The effect of earthworms on soil structure results from the net outcome of their feeding and burrowing activities. Earthworms ingest soil particles and organic matter, mixing these two fractions together and egesting them as surface or subsurface casts. Once egested, soil in casts can be eroded due to impact of rainfall or can form stable soil aggregates through a variety of stabilizing mechanisms. Earthworms generally improve the aeration and porosity of soil through formation of burrows and by increasing the proportion of large aggregates in the soil, and their effects are especially important in poorly structured or reclaimed soil.

By increasing rates of water infiltration, earthworms can reduce the amount of surface runoff. Alternatively, earthworms can increase erosion by removing the protective cover of surface litter, increasing surface sealing and depositing surface casts, which can be carried downslope during heavy rains. Some tropical species actually increase soil bulk density and decrease infiltration by producing compact soil casts. Overall, the effects of earthworms on soil structure are considered to improve soil fertility.

2.2.3.3 Effects on plant growth

The majority of studies examining the influence of earthworms on plant growth have reported that earthworms stimulate plant growth, although some studies have reported no effect or even a negative effect of earthworms on plant growth.

Earthworms have been shown to increase production of shoots and grain in a variety of field trials and greenhouse experiments. Introduction of earthworms into reclaimed polders and pastures resulted in large increases in forage quantity and quality **(Hoogerkamp*et al.*, 1983)**. Beneficial effects of earthworms on plant growth may be due to increased nutrient and water availability, improved soil structure, stimulation of microorganisms or formation of microbial products that enhance plant growth, or possibly through direct production of plant growth promoting substances.

2.2.3.4 Undesirable Effects of Earthworms

Despite the documented and putative beneficial effects of earthworms on nutrient dynamics, soil structure, and fertility, some aspects of earthworm activities are considered undesirable **(Edward and Bohlen, 1996)**. These include removing and burying surface residues that would otherwise protect soil surfaces from erosion; producing fresh casts that increase erosion and surface sealing; depositing castings on the surface of lawns and golf greens or irrigation ditches where they are a nuisance, or in pastures where they interfere with haying operations; dispersing weed seeds in gardens and agricultural fields; transmitting plant or animal pathogens; increasing losses of soil nitrogen through leaching and denitrification; and increasing soil carbon loss through enhanced microbial respiration. It is the net outcome of their positive and negative effects that determines whether earthworms are to be considered pests or benefactors in any given situation, and they are generally considered to be of great benefit to soil properties and fertility.

2.2.4 Earthworms and Soil Management

2.2.4.1 Earthworms and soil reclamation

Introduction of appropriate earthworm species or encouraging natural population through the addition of suitable amendments can increase the rate of soil improvement and genesis of soil structure of reclaimed land. Introduction of European earthworms into pasture in New Zealand and Australia, as well as in Dutch polders, greatly facilitated improvements in soil structure and plant productivity **(Hoogerkamp*et al.*, 1983)**. There has also been some success in introducing earthworms into reclaimed mining sites and in reclaimed peat with beneficial effects on soil structural development, nutrient cycling, and productivity **(Edward and Bohlen, 1996)**.

Earthworms can be used to assess toxicity in terrestrial systems and the survival rate of theworms, or changes in other parameters such as biomass, can be used to calculate an LC_{50} value(lethal concentration to 50% of the population) for test chemicals spiked into soil. This type ofinformation can be useful in predicting the likely toxicological effect of oily drill cuttings on thesoil environment and may be useful for calculating application rates for fluids with differenttoxicological characteristics if the oily cuttings are to be land farmed**(Getliff *et al*., 2002)**.

Studies have also been conducted on the use of earthworms in the remediation of spent drilling fluidcontaminated soil. When drill cuttings and other organic materials are fed to earthworms such as*Eisenia foetida* the oil content of the cuttings is rapidly degraded and the mixture converted into ahigh quality soil-like compost that can be used as a soil improver or fertilizer **(*Getliff et al*., 2002)**. In addition, treatment of crude oil contaminated soil was also indicative of

a significant decrease in the TPH concentration in treatments with earthworms, compared with the samples without worms**(Maike and Filser, 2007)**.

2.2.4.2 Influence of agricultural practices on earthworm populations

Earthworms can be affected favorably or adversely by different agricultural practices. Whereas heavy cultivation is detrimental to earthworm populations, reduced and notillage agricultural practices promote the growth of earthworm populations. Organic amendments, such as animal or green manures, also stimulate the growth of earthworm populations. Inorganic fertilizers may benefit earthworm populations by increasing the production of crop residues, but their effects are not as great as the effects of organic fertilizers. Long-term application of inorganic fertilizers may adversely affect earthworm populations due to soil acidification or other changes in the soil environment. Liming can benefit earthworm populations in some instances.

2.2.4.3 Effects of pesticides and soil pollutants on earthworms

Pesticides have widely varying effects on earthworm populations, with many chemicals having little or no toxicity toward earthworms and others exhibiting acute toxicity **(Edward and Bohlen, 1992)**. In general, the carbamate insecticides and soil fumigants are very or highly toxic to earthworms. Herbicides in general show low toxicity toward worms, although there are some exceptions. Organochlorine and organophosphorous insecticides have varying levels of toxicity. Soil contamination with organic pollutants, heavy metals, and acid precipitation can be detrimental to earthworm populations. Most toxic organic soil pollutants are highly toxic to earthworms. Heavy metals vary in their toxicity toward earthworms. In addition to acute negative effects of some heavy metals on earthworms, sublethal heavy metal concentrations can cause declines in earthworm growth and reproduction. Heavy metals can accumulate in earthworm tissues,which is potentially problematic for movement of heavy metals into higher

trophic levels, because of the wide array of animals that prey on earthworms. Earthworm species vary in their tolerance of acid soil conditions but some reports have noted a decline in earthworm populations in response to large amounts of acid deposition.

2.2.5 *Eudrilus Eugeniae*

The earthworm species, *Eudrilus eugeniae* commonly referred to as the West African night crawler, occurs all over the world but mostly in West African regions (**Shagoti 1985; Segun 1998**). It is largely found in West Africa from Ghana to Nigeria to West Cameroun and Gabon. It grows well at a temperature of more than 25^{o}C but best at 30^{o}C (**Viljoen and Reinecke, 1992**), attaining maximum weight, length and number of segments in about 15 to 20 weeks (**Rodriguez and Lapiere, 1992**). It ranges in size from about 10cm in length to huge specimens of over 12cm and size may depend on habitat (**Segun, 1998**). The total number of segments in *Eudrilus eugeniae* varies from about 80 to over 100 with the location of a thick cylindrical collar – the clitellum between segments 13 – 20 (**Pirrone, 1985**). It has a purple sheen and the posterior segments are evenly tapered to a point (**Blackburn, 1989**).

2.2.6 Vermicomposting

Vermicomposting is biooxidation and stabilization of organic material involving the joint action of earthworms and microorganisms in which negligible organic waste resources are converted into nutrient rich plant growth media i.e. vermicompost. Studies reveal that epigeics seem well fitted for such kind of operations, because of their surface activity, their ability to colonize organic material quickly, and in the process to convert it into useful compost and their ability to minimize malodour formation. However, involvement of earthworms in composting process

decreases the time of stabilization of the waste and produces an organic pool with energy reserves as vermicompost. The composting biology and ecology of earthworms in waste resources are controlled by several abiotic and biotic factors: temperature, moisture, pH, substrate, feed and its palatability, particle size, earthworm density and fecundity, which directly or indirectly influence the composting potential of the composting species. Several earthworm species e.g. *Eisenia fetida, Eisenia andrei, Eudrilus eugeniae, Perionyx excavatus*have been identified as potential candidates for managing organic waste resources. World-wide spread *E. fetida*was and still remains a favoured earthworm species for vermicomposting operations due to their wide range of tolerance for environmental variables. However, in tropical and sub-tropical conditions earthworm: *P. excavatus, P. sansibaricus,* and*E. eugeniae*appeared as best vermicomposting species. The life cycle parameters of above tropical species has been investigated in respect to their environmental requirement like temperature, moisture, fecundity and density pressure, pH, but influence of culture material or substrate quality on their life cycle parameters is less considered. Although, animal dung is recognized as a suitable earthworm culture media, other organic waste materials from agriculture and industry have also proved successful. The chemical structure of a given culture media for large scale vermiculture practices is always important. Apart from having high concentrations of nutrients, plant wastes contain important secondary metabolites e.g. polyphenols, which directly or indirectly influence the composting potential as well as growth patterns of given composting species during vermicomposting practices. The impact of such kind of wastes on life cycle parameters of composting worms must be of a research priority**(Suthar, 2007)**.

2.2.7 Effect of Earthworm Activities on Soil Propertiesand Bioremediation

When soil becomes contaminated, thecontaminants become entrapped and sorbed onto and into soilmineral and organic matter fractions rendering them inaccessible and thus unavailable for microbial interaction. Earthwormactivity has the potential to release these residues and could thus potentially enhance bioremediation performance.

Both single strains and consortia of microorganisms added to contaminated soils usually produce little added value in terms of degradative ability over the soil's indigenous microorganisms, which are often only limited in their contaminant degradation capacities due to the soil's suboptimal status. Promotion of the indigenous microorganisms is thus a primary aim and would occur via optimization and enhancement of both abiotic and biotic conditions; something that earthworms could enhance.

2.2.7.1 Abiotic Effects

In bioremediation, it is often necessary to enhance and maintain moisture, oxygen and nutrient levels, whilst also ensuring they can be homogenously dissipated. Whilst time consuming, labour intensive and expensive methods can be utilized to aid in the optimization of these variables, there might exist a relatively low input, low technological tool available to undertake this work: as earthworms move throughout the soil environment, their resulting burrows (burrowing being species specific) act as input points of, and preferential pathways for, water and particle movement **(Shipitalo and Le Bayon, 2004; Kretzshmar, 2004; Dominguez, 2004)**, and nutrient flow and aeration **(Dominguez 2004)**.

As earthworms ingest and digest soil, a further positive effect upon the soil environment occurs; that of the mechanical breakdown of soil particles **(Edwards and Bohlen, 1996; Bohlen et al., 2004; Kersante et al., 2006)**.It could be hypothesized that this action might assist in the release

of soil associated (aged) organic contaminants **(Barois et al., 1993)**. Suchactivities have also been shown to increase soil organic matter surface area **(Edwards and Bohlen, 1996)**, whichwill have an effect upon the fraction of material exposed for microbial interaction **(Dominguez, 2004)**, potentially resulting in increased contaminant degradation. These aspects are worthy of serious research and consideration with respect to increasing degradation potential and offsetting the residual concentration problem, which often limits final bioremediation success **(Hickman and Reid, 2008)**.

In cast formation, it is known that there are many subsequent positive effects upon the improvement of the soil environment and soil structure. These include increased soil porosity **(Bolan and Baskaran, 1996; Shipitalo and Le Bayon, 2004)**, increased oxygenation, moisture retention **(Edwards and Bohlen,1996; Schaefer and Filser, 2007)**, improved soil fertility via accelerated organic matter decomposition, and improvement of the availability of nutrients **(Edwards and Bohlen, 1996; Lavelle et al., 2004**). Thedeposition of these casts upon the burrow walls, within burrows or on the soil surface can significantly differ in chemical and physical composition to the surrounding soil in terms of altered C:N ratios and higher pH **(Bolan and Baskaran, 1996; Edwards and Bohlen, 1996; Brown and Doube, 2004; Shipitalo and Le Bayon, 2004)**. There could thus be potential for the use of earthworms casts (either directly applied to contaminated soils, or being produced as earthworms reside within contaminated soils) to firstly improve chemical conditions to aid bioremediation and, secondly, improve the overall soil condition. Some of these effects upon the soil environment have been investigated within soil/land rehabilitation **(Lowe and Butt, 2003; Butt et al., 2004)**, specifically the increases in soil fertility and structure, leading to increased soil 'health'. Thus, not only might soils benefit from earthworm input in terms of reduced contaminant concentrations, but also

from improved soil 'health'; both aspects being fully in line with the recently proposed **EU Soil Framework Directive (2006)**.

2.2.7.2 BioticEffects

Itis known that earthworms interact with soil microorganisms (fungi, bacteria and actinomycetes) on three broad spatial scales (burrow linings, casts and earthworm gut or intestine) **(Brown and Doube, 2004)**. For example, mucus and cast deposition on burrow walls, and other organic carbon sources transferred through the burrow systems promote the increase and distribution of microorganisms in earthworm burrows **(Farenhorst *et al.*, 2001)**, whilst mucus, urine and glucose can result in increases in microbial biomass **(Scheu,1987)**. This is of relevance because an increase in microbial biomass is linked to increased microbial catabolic activity **(Meharg, 1996)**. Higher microbial numbers, diversity and activity are also known to be related to passage of microorganisms through the earthworm gut, as well as the promotion therein, and the 'awakening' of dormant gut flora **(Brown *et al.*, 2000; Brown and Doube, 2004)**. Importantly, the increased gut associated microflora are then excreted throughout the media within earthworm casts and via microbial adheration to earthworm skin **(Edwards and Bohlen, 1996)**, whilst the transit and dispersal mechanisms associated with the water flow (**Kretzshmar, 2004)** also help to further dissipate microorganisms. Such actions are clearly relevant to any bioremediation methodology desiring the spatial incorporation of microorganisms capable of organic contaminant degradation. Earthworm casts are therefore generally much richer in microbial numbers in comparison to the initially ingested material. Forexample, **Scheu (1987)** reported an increase of 90% in respiration rate infresh casts, whilst, **Teotia *et al.* (1950)** reported bacterial counts of32 million/g compared to 6–9 million/g for the surrounding soil. This isdue not only to the aforementioned intestinal promotion of microorganismsbut also to the inherently high soil

organic matter levels,resulting in further microbial activity and proliferation (**Brown andDoube, 2004)**. Dynamics and succession of microorganisms withinearthwormcasts is complex, and relies heavily upon many factors suchas type of food ingested, gut passage time and ingested and inherent gutmicroorganisms. Further discussion upon these factors, numbers ofmicroorganisms in earthwormcasts, and the effect of earthworms upon microorganisms on the meso and macroscale, are further discussed by **Edwards and Bohlen (1996)**, and **Brown and Doube (2004)**. It has also been noted that water soluble **(Edwards and Bohlen, 1996)** low molecular weight **(Barrois and Lavelle, 1986; Lavelle *et al*., 1993)** organic compounds such as enzymatic fluid and mucus based solutions are added to earthworm gut contents during digestion, **(Brown and Doube, 2004)**, which also subsequently stimulate microbial activity both in the gut and in the egested casts **(Edwards and Bohlen, 1996)**. It is known that pre-exposure, or pre-induction of microorganisms to contaminants, or groups of contaminants, can result in subsequent increased degradation rates **(Carmichael and Pfaender, 1997; Reid *et al*., 2002)**. Therefore, these intestinal organic compounds might help 'prime' the microorganisms in the gut to break down more complex organic compounds in the ingested soil. Earthworm casts have had a strong presence within this particular approach, and relevantly, the application of earthworms within waste management involves the digestion of a wide range of organic wastes **(Edwards and Bohlen,1996)** into vermicast/compost. Vermidigestion is an area rapidly increasing in interest and research due to the use of the nutrient and microbially rich casts as plant growth media, plant pest and disease suppressant and itsusewithin horticulture **(Edwards and Arancon, 2004)**. Such amaterialmaywell have a placewithin remediation, andwill be discussed subsequently. Pertinent reviews of earthworm feeding ecology **(Curry and Schmidt, 2007)** and vermidigestion, and the

use of earthworm casts have been undertaken elsewhere **(Edwards and Bohlen, 1996; Dominguez, 2004; Edwards and Arancon, 2004)**.

Intheory, it is the earthworm's combined mechanical activity upon the soil and subsequent promotion of microorganisms that would be of most benefit to remediation efforts, as opposed to uptake/accumulation of organic contaminants. It has been shown that earthworm body burdens of organic contaminants can typically be low **(Kelsey and Alexander, 1997; White *et al*., 1999; Gevao *et al*., 2001)** and is unlikely to prove to be a worthwhile tool in bioremediation. Table 2.5summarizes the discussed positive earthworm actions upon the soil environment.

Table 2.5: The effect of earthworm activities on the soil environment

Burrowing/ Movement	Burrow creation	Soil ingestion, Digestion and excretion	Earthworm casts
Improved soil structure	Improved O_2 and gas diffusion	Breakdown of particles	Fragmented soil particles and increased surface area
Increased porosity		Release of organic residue	Increased water holding capacity
Release of nutrient	Improved moisture drainage and dispersal	Microorganism dispersal and transport	Higher SOM
Organic matter turnover	Improved nutrient movement	Organic matter turnover	Enhanced nutrient content and availability, with increased C:N ratio
Improved fertility	Microorganism promotion and dispersal	Addition of high energy water soluble organic compounds	Increased pH
Microorganism promotion and dispersal		Microorganism promotion	Microorganism promotion

(*Source:* Hickman and Reid, 2008).

In addition to the effects of earthworms upon microbial biotic effects, a number of studies have highlighted direct earthworm biotic effects in the form of feeding behaviors upon contaminant fate. For example, whilst **Ma *et al.* (1995)** noted that leaf litter food made only minimal difference in polycyclic aromatic hydrocarbons (PAH) loss extents, they did note that bioaccumulation of these PAHs was greatly enhanced via food limitation, thus it is a possibility that earthworms increase their oral intake of soil particles when driven by hunger stress. When investigating total petroleum hydrocarbons (TPH) losses in with-earthworm systems that either had or had not received food, **Schaefer *et al.* (2005)** noted that residual TPH in the with-food systems was greater, however, these were not significant values. These results suggest that if

earthworms were applied to suboptimal contaminated soils lacking in organic matter, a greater degree of biological interaction might ensue resulting in greater extents of loss. In further support of this, **Haimi *et al.* (1992)** stated that the lower the organic matter content, the higher the bioaccumulation, which obviously implies increased soil association. This result was confirmed by **Peters *et al.* (2007)** who noted significantly greater uptake of p,p-DDE from soil than from compost by earthworms (*E. fetida* and *L. rubellus*).

2.2.7.3 Earthworm and bioremediation of petroleum and crude oil hydrocarbons

Whilst a relatively large amount of research has been undertaken investigating the effects of earthworms upon agrochemicals, less has been undertaken upon the effects on petroleum hydrocarbons (petroleum and crude oil hydrocarbons) **(Hickman and Reid, 2008)**.

In contrast to the agrochemical studies, many of the subsequent studies are descriptive investigations as opposed to mechanistic ones. However, to highlight the increased interest of earthworm inclusion in bioremediation for hydrocarbons, a recent paper elucidated upon the co-application of compost and earthworms for bioremediation **(Ceccanti *et al.*, 2006)**: the work investigated biochemical processes, including microbial enzyme activity and carbon dioxide evolution, which took place during bioremediation treatment of oil refinery sludge with (1) a mixture of microorganisms, enzymes and nutrients (2) compost only, and (3) compost with earthworms (E. fetida; 10 per kg soil) for 3 months. Their aim was to use these treatments and variables to stimulate soil microbial biomass and therefore degradation ability to explain both microbial activity and the intensity of microbial metabolism. They concluded that the reduction in TPH of the oil refinery sludge was greatest in the treatment with earthworms present (reduction of 50.3%), followed by compost only (reduction of 39.5%), highlighting the

usefulness of the co-application of compost and earthworms. Importantly, they stated that the result could be due to earthworm action and/or soil microbial stimulation via compost addition and earthworm casts. An earlier study **(Schaefer, 2001)** had already concluded that increased microbial catabolic activity due to *E. fetida* presence was responsible for the loss of 91% (1074 mg kg^{-1} to 96 mg kg^{-1}) of crude oil contamination when present for 56 days. Thiswas in comparison to the without-earthworm treatments, which showed no significant difference in concentrations over the same period. Interestingly, it was in the soils with the highest initial concentration that degradationwas fastest.Such results are not always the rule, for example, **Callaham *et al.* (2002)** observed no difference in TPH concentrations associated with *E. fetida* or wheat treatments when applied to petroleum contaminated soils, whilst **Stom *et al.* (2003)**, who investigated the effect of earthworm (*E. fetida*) application (n=5) to crude oil contaminated soils (25 g kg^{-1}) for 40 days with the co-application of microorganisms also observed minimal losses (~15 g kg^{-1} loss for the microbial inoculation and earthworm co-application; ~9 g kg^{-1} loss for the microbial inoculum alone, and only ~3 g kg^{-1} loss for earthworm only treatments). However, in the treatments with earthworms, it was noted that soil structure and porosity were improved, thus facilitating oil losses via the introduced microorganisms. However, little methodological data was provided, thus, full understanding of the underlying mechanisms cannot be discussed. **Schaefer *et al.* (2005)** investigated the effects of differing earthworm species (10 worms/1 kg treatment) (*L. terrestris, Allolobophora chlorotica* and *E. fetida*) on the microbial community within a crude oil contaminated soil (10,000 mg kg−1 TPH) for 28 days. This is one of the few studies that have been undertaken with a view to specifically utilising earthworms for bioremediation of organic contaminants. It was noted that both respiration and concentration of microbial biomass was significantly enhanced, with observed changes not only in microbial

numbers and activity but of taxonomic groups, whilst TPH concentrations were significantly reduced (TPH reduction of 30–42% in samples with *L. terrestris*, 31–37% in samples with *E. fetida* and of 17–18% in samples with *A. chlorotica* with an average control TPH concentration decrease of a non-significant 9–17% between test start and test end). Whilst positive earthworm usefulness within bioremediation was noted, there were differences in results between earthworm species due to ecological behavioural differences, thus reinforcing the importance of choosing species suited to specific environmental conditions. **Schaefer and Filser (2007)** further reported upon their earlierwork **(Schaefer *et al.*, 2005)**, which utilized the three differing earthworm species to effect TPH losses in crude oil contaminated soils. Interestingly, they noted that there was more degradation products in the with-earthworm soils, indicating that degradation of the longer chain aliphatics (C29–C36) had occurred, thus resulting in an increase of shorter chain aliphatics (C19–C29). Such an observation was reported by **Prince *et al.* (2007)**, who stated that within a complex mixed gasoline mixture; larger n-alkanes were more readily degraded than the smaller n-alkanes. Further to this, **Schaefer and Filser (2007)** introduced organic additives (coffee grains, horticultural waste (compost) and brewery mash) (as 10% mass) in combination with earthworms (*L. terrestris*) to assess microbial crude oil (5000 mg kg−1) degradation over 28 days. Whilst increased microbial respiration was observed in all treatments, only significant TPH degradationwas noted in the earthworm only treatments (decrease of 29%) and the mixed brewery mash treatment with earthworms (19%), whilst the brewery mash without earthworms resulted in a decrease of 35%. Other additives had no significant effects upon TPH degradation. It was noted by the authors that the earthworms did not interact or feed on the additives, thus minimizing the co-application potential. They concluded that the microorganisms preferred the more easily degradable organic additives as opposed to the more complex hydrocarbons.

Considering that compost proves to be useful in bioremediation procedures and its use as a remediation amendment in combination with earthworms has been demonstrated by **Ceccanti *et al.* (2006)**, it was surprising that the other additives did not determine any significant TPH decreases. **Tomoko *et al.* (2005)** also investigated the addition of the earthworm (*E. fetida*) and varying organic wastes to an oil contaminated soil and found that earthworms alone significantlydecreased oil contents in comparison to the control. Earthworms used to remediate hydrocarbon contaminated drilling fluids and cuttings has been investigated **(Getliff *et al.*, 2002)**. However, as opposed to direct earthworm inclusion to hydrocarbon impacted soils to effect remediation, the process described by **Getliff *et al.* (2002)** involves adding the drilling wastes to other greenwastes, altering the C:N ratio and the moisture content and adding the mixture to worm beds as feed. Worm beds were mounds 88 m×3 m covered with polypropylene-backed felt mat, with moisture and aeration capabilities. The drilling materials/greenwasteswere applied to the mounds once a week at an average depth of 15–30 mm. The worms (*E. fetida*) integrate with the applied material and consume it within 5–7 days. Interestingly, the digested material (wormcast,or vermicast/compost) can be harvested and packaged for fertilizer or as a soil improver. Over an experimental period of 45 days and at application of 30% w/w (drill cuttings/organic material), 50% w/w or 70% w/w, significant TPH reductions were observed. At 30% application, TPH decreased from 1900 mg/kg to less than 60 mg/kg in 45 days, whilst at 50% application TPH decreased from 2100 mg/kg to below detection limit and at 70% application, TPH decreased from 20,000 mg/kg to 1500 mg/kg. However at a tested application of 100% drill cuttings, the earthworms did not associate with the material and thus degradation was minimal. The difficulty in the removal of recalcitrant hydrocarbons and the lack of biodegradability by inherent microorganisms of high molecular weight compounds is known

(Atlas, 1995; Alexander, 2000). This aspect of bioremediation is often a final limiting factor. **Martin-Gil *et al.* (2007)** has recently investigated the use of earthworms (*E. fetida*) and vermicomposting in the treatment of the high molecular weight hydrocarbons asphaltens, from the Prestige oil spill. Green waste (cow bed and potato peelings) (total mass 80%) was added to heavy fuel oil (20%), which then underwent a composting process. Subsequent to the composting process, earthworms were added at a density of 330 g/m^2in a treatment vessel $0.40\times0.50\times0.12$ m^3 for 6 months. Definite values of asphaltens reduction were not given, but they did state that their results revealed that the microorganisms living in either the earthworm intestines or vermicompost substrate can possibly mineralize and thus eliminate asphaltens.

2.2.8 Earthworms and Heavy Metal Uptake in Contaminated Soil

Within the field of ecological risk assessment, it is widely recognized that earthworms are an important component of the soilbiota. For this reason, much work has focused on metal accumulationand toxicity to earthworms.Earthworms have been reported as being able to accumulate heavy metals, from soil, in their bodies, complex the metals with other compounds thereby making them less toxic**(Jain and Singh, 2004)**.It is also supposed that the activities of earthworms increase the mobility and bioavailability of heavy metals in soils **(Wen *et al.*, 2004)**.Earthworms constitute more than 90% of the invertebrate biomass in soils **(Ireland, 1983)** and are suggested to be an appropriate tool to predict bioaccumulation in the terrestrial food chain **(Rhett *et al.*, 1988; Hinton and Veiga, 2002)**. Several studies have shown that earthworms accumulate trace metals such as Cu, Pb and Zn **(Ma, 1982; Ireland, 1983; Morgan and Morgan, 1988; Bengtsson and Transvik 1989)**.

Heavymetal accumulation in earthworm tissue has been reported to be dependent not only on the concentration of the substrate they consume, but also on ecological and species-specific physiological properties of different earthworms, such as efficiency of detoxification mechanisms, gut morphology, quantity of metal-binding ligands, consumption rates of the food material and feeding behavior **(Ma, 1982; Morgan and Morgan, 1992, 1999)**.

Furthermore, previous studies on metal uptake by earthworms show that the magnitude of accumulation and steady-state tissue metal concentrations are a function of either total or ‘‘available’’ metal concentration in the soil. The available metal concentrations are controlled by soil parameters such as pH, organic matter content, clay content, cation exchange capacity and the concentration of Fe- and Al-oxyhydroxides**(Nahmani *et al.*, 2009)**.The uptake of lead by earthworm showed a linear increase with time **(Davies*et al.*, 2003)**.
However, surprisingly little is known about the kinetics of uptakeand excretion of metals by earthworms**(Nahmani *et al.*, 2009)**. The majority of availabledata focus on Cd, Cu, Pb and Zn **(Spurgeon and Hopkin, 1999; Conder *et al.*, 2002; Vijver *et al.*, 2005)** though data exist for otherelements such as Mn, Cs, I **(Sheppard *et al.*, 1997**), As, Cr, Ni**(Peijnenburg *et al.*, 1999)** and Co **(Crossley *et al.*, 1995)** as well.
Various researchers have studied and derived models that predict metal uptake and excretion kinetics by earthworms**(Nahmani *et al.*, 2007)**. Notable amongst these are;

3. **Spurgeon and Hopkin (1999)**:

$$C_{iworm}(t) = C_i(0) + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \ldots \ldots (2.1)$$

$$C_{iworm}(t) = C_i(0) + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) - \frac{a}{k_2}\left(1 - e^{-k_2(t_c - t)}\right) \quad \ldots\ldots (2.2)$$

Where: t_c= time at which animals were transferred to clean soil (days)

Equation (2.1) holds for $t < t_c$: (accumulation phase)

Equation (2.2) holds for $t > t_c$: (elimination phase)

4. **Van Straalen *et al.*, 2005**

A one-compartment model was used to analyze the development of earthwormbody concentrations with time, and to calculate uptake and elimination rate constants. Equation (2.3) was used for essential elements and Equation (2.4) for non-essential.For non-essentialmetals this initial metal load may be excreted when the earthworm istransferred to the test soil.

$$C_{iworm}(t) = C_i(0) + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \quad \ldots\ldots (2.3)$$

$$C_{iworm}(t) = C_i(0)e^{-k_2 t} + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \quad \ldots\ldots (2.4)$$

Where: $C_{iworm(t)}$ = metal concentration in the earthworm (µg g^{-1} dry weight) at time t (days), a = flux of metals into the earthworm (mg_{metal} kg^{-1} animal day^{-1}),k_2 = elimination rate constant (day^{-1}). $C_i(0)$ is the initial body concentration (µg g^{-1}dry weight), which is measured in the animals before exposure.

2.3 SOIL

Soils are made up of four basic components: minerals, air, water, and organic matter. In most soils, minerals represent around 45% of the total volume, water and air about 25% each, and organic matter from 2% to 5%. The mineral portion consists of three distinct particle sizes classified as sand, silt, or clay. Sand is the largest particle that can be considered soil. Sand is largely the mineral quartz, though other minerals are also present. Quartz contains no plant nutrients, and sand cannot hold nutrients, they leach out easily with rainfall. Silt particles are much smaller than sand, but like sand, silt is mostly quartz. The smallest of all the soil particles is clay. Clays are quite different from sand or silt, and most types of clay contain appreciable amounts of plant nutrients. Clay has a large surface area resulting from the plate-like shape of the individual particles. Sandy soils are less productive than silts, while soils containing clay are the most productive and use fertilizers most effectively. *Soil texture* refers to the relative proportions of sand, silt, and clay. A loam soil contains these three types of soil particles in roughly equal proportions. A sandy loam is a mixture containing a larger amount of sand and a smaller amount of clay, while a clay loam contains a larger amount of clay and a smaller amount of sand. Another soil characteristic -soil structure - is distinct from soil texture. *Structure* refers to the clumping together or, aggregation, of sand, silt, and clay particles into larger secondary clusters.

Goodstructure is apparent when the soil crumbles easily in the hand. This is an indication that the sand, silt, and clay particles are aggregated into granules or crumbs. Both texture and structure determine pore space for air and water circulation, erosion resistance, looseness, ease of tillage, and root penetration. While texture is related to the minerals in the soil and does not change with agricultural activities, structure can be improved or destroyed readily by choice and timing of farm practices.

Largequantities ofpollutants have continuously been introduced intoecosystems as a consequence of urbanization andindustrial processes. Metals are persistent pollutants thatcan be biomagnified in the food chains, becomingincreasingly dangerous to human beings and wildlife.Therefore, assessing the concentrations of pollutants indifferent components of the ecosystem has become animportant task in preventing risk to natural life and publichealth. Heavy metals enter into the environment mainlyvia three routes namely: (i) deposition of atmosphericparticulate, (ii) disposal of metal enriched sewagesludges and sewage effluents and (iii) by-products frommetal mining process. Soil is one of the repositories foranthropogenic wastes. Biochemical processes canmobilize them to pollute water supplies and impact foodchains. Heavy metals such as Cu, Cr, Cd, Ni, and Pb arepotential soil and water pollutants. Globally, the problemof environmental pollution due to heavy metals hasbegun to cause concern in most large cities since thismay lead to geoaccumulation, bioaccumulation andbiomagnifications in ecosystems. The Nigerian soil quality standard by the environmental regulatory body is presented in Table 2.6.

Table 2.6: Soil Quality Standards

Parameters	**Guideline value**	
	(mg/kg dry weight)	**% composition**
Arsenic	20	0.00200
Barium	400	0.04000
Cadmium	3	0.00030
Chromium (Cr^{+6})	100	0.01000
Cobalt	50	0.00500
Copper	100	0.01000
Lead	164	0.01640
Mercury	4	0.00040
Molybdenum	40	0.00400
Nickel	70	0.00700
Tin	50	0.00500

Zinc	421	0.04210
Benzene	0.1	0.00001
Toluene	0.1	0.00001
Xylene	0.1	0.00001
Styrene	0.1	0.00001
Hexane	0.5	0.00005
Heptanes	0.5	0.00005
Flourine	100	0.00100
Cyanide	5	0.00050
Phenol	10	0.00100

Source: National Environmental Regulation (2009).

2.3.1 The Importance of Soil Organisms

An acre of living topsoil contains approximately 900 pounds of earthworms, 2,400 pounds of fungi, 1,500 pounds of bacteria, 133 pounds of protozoa, 890 pounds of arthropods and algae, and even small mammals in some cases**(Preston, 2004)**. Therefore, the soil can be viewed as a living community rather than an inert body. Soil organic matter also contains dead organisms, plant-matter, and other organic materials in various phases of decomposition. Humus, the dark-colored organic material in the final stages of decomposition, is relatively stable. Both organic matter and humus serve as reservoirs of plant nutrients; they also help to build soil structure and provide other benefits. The type of healthy living soil required to support humans now and far into the future will be balanced in nutrients high in humus, with a broad diversity of soil organisms. It will produce healthy plants with minimal weed, disease, and insect pressure. Nativeprairies and forests soils are tilled by soil organisms. The soils are covered with a layer of plant litter and/or growing plants throughout the year. Beneath the surface litter, a rich complexity of soil organisms decompose plant residue and dead roots, then release their stored nutrients slowly over time. In fact, topsoil is the most biologically diverse part of the earth. Soil-dwelling organisms release bound-up minerals, converting them into plant-available forms that

are then taken up by the plants growing on the site. The organisms recycle nutrients again and again with the death and decay of each new generation of plants. There are many different types of creatures that live on or in the topsoil. Each has a role to play. While a great variety of organisms contribute to soil fertility, earthworms, arthropods, and the various microorganisms merit particular attention.

Type specimens of bacterialstrains used in bioremediation exist in various repositoriesor are commerciallyavailable and are usually covered by patent rights. Eachof these strains may yield dramatic results in vitro forspecific target compounds. However, the overall successof such strains in treating a wide range of contaminants*in situ* remains limited. The reintroduction of indigenousmicroorganisms isolated from a contaminated site afterculturing seems to be a highly effective bioremediationmethod, especially when microbial growth is supplementedby oxygen and fertilizers. Thus, bioremediationis normally achieved by stimulating theindigenous microbiota (naturally occurring microorganisms).Stimulation is achieved by the addition ofgrowth substrates, nutrients, terminal electron acceptor,electron donors, or some combination therein, resultingin an increase in contaminant biodegradation and biotransformation**(Yehuda, 2002)**.

2.4 PETROLEUM HYDROCARBON AND BIOREMEDIATION

Bioremediation, a process that utilizes the capability of microorganismto degrade toxic waste, is emerging as a promising technology for thetreatment of soil and groundwater contamination. The technology is very effectivein dealing with petroleum hydrocarbon contamination. Some microorganisms can decompose or transform the chemical substancespresent in petroleum and petroleum derivatives. A single microorganism can degrade only certain types of petroleumcompounds, but a mixed population – microbial community enables a higher levelof

degradation. Moreover, some substances can be decomposed only by co-metabolism**(Jelena *et al.*, 2009)**.

Given favorable environmental conditions, all naturalorganic compounds degrade. Petroleumis, in one sense, a natural product, resulting from theanaerobic conversion of biomass under high temperatureand pressure. Most of its components are subject tobiodegradation, but at relatively slow rates. Petroleumhydrocarbons can be divided into four classes: saturates,aromatics, asphaltenes (phenols, fatty acids, ketones,esters, porphyrins), and resins (pyridines, quinolines,carbazoles, sulfoxides, amides).Hydrocarbons differ in their susceptibility to microbial attack and generally degrade in the following order of decreasing susceptibility: n-alkanes >branched alkanes >low-molecularweightaromatics >cyclic alkanes **(Perry, 1984)**.Most common pathway of alkane degradation isoxidation at the terminal methyl group; alkane →alcohol → fatty acid → ketone → CO_2 and H_2O.

Microorganisms are capable of aerobicallymetabolizing single-ring aromatic hydrocarbonsubiquitous in the subsurface. PAHs with two or three rings such asnaphthalene, anthracene, and phenanthrene aredegraded at reasonable rates when O_2 is present. PAHs with four rings such as chrysene, pyrene,and pentacyclic compounds are highly persistent and are considered recalcitrant. The ease with which microbes degrade organic compounds is presented in Table 2.7.The biodegradation of hydrocarbons is generally an aerobic process.As high as 20% of all soil microbes (bacteria, fungiand yeast) are capable of degrading aliphatichydrocarbons.

Table 2.7: Ease of biodegradability of some organic compounds.

Hydrocarbons	**Ease of degradation**

Simple hydrocarbons, C1 – C15	Very easy
Alcohols, phenols, amines	Very easy
Acids, esters, amides	Very easy
Hydrocarbons, C12 – C20	Moderately easy
Ethers, monochlorinated hydrocarbons	Moderately easy
Hydrocarbons, >C20	Moderately difficult
Multichlorinated hydrocarbons	Moderately difficult
PAHs, PCBs, pesticides	Very difficult

Petroleum-based products are the major source ofenergy for industry and daily life. Petroleum is also theraw material for many chemical products, such asplastics, paints, and cosmetics. The transport of petroleumacross the world is frequent and the amounts ofpetroleum stocks in developed countries are enormous.Consequently, the potential for oil spills is significant.

The volume of spills usually exceeds the inherent remediationcapacity for any given environment, resulting ina significant ecological impact. The rate of biodegradation depends on both physicochemical and biological variables.The ability of hydrophobic organic compounds to be solubilized and transported into the immediate vicinity of bacterial cells capable of metabolizing them is potentially the rate-limiting step in bioremediation.

2.5 LUBRICATION AND ENGINE OILS

Lubrication is simply the use of a material to improve the smoothness of movement by reducing friction. The immediate result is reduced wear and reduced heat generation.

Lubricating oils are used for the majority of applications. They may be classified according to their viscosities and any special properties imparted to them by additives. Oils whose base stocks are derived primarily from crude oil refining are called mineral or petroleum oils. Petroleum oils may be further classified as being paraffinic or naphthenic based on the types of hydrocarbons comprising the base stock. Oils that have been manufactured by chemical synthesis such as polymerization are called synthetic oils. Additivesmay be blended into the base stock to impart special properties to the finished product**(Baladincz*et al.*, 2008)**. Some common lubricant additives are given in Table 2.8.

Pure mineral oil has good lubricating properties, but it is the additives in the oil that give it its characteristics and enhance its lubricating properties to enable it to meet modern requirements.Different oils and different materials and loading conditions can create vastly different coefficients of friction that can affect performance and longevity of an engine and other mechanical components. A few basic key functions of a motor oil is to reduce friction under all extremes of operating condition, prevent corrosion of internal engine components and provide for cooling via transfer of heat.

Table 2.8: Common lubricant additives

Additives	Compound Used	Function
Extreme Pressure (EP)	Sulphur-Phosphorus compounds	Increase load carrying ability
Antioxidant	Hindered Phenols, Amines	Prolonged oil life by slowing oxidation
Antifoam	Silicones	Control foaming in forcefeed systems
Anticorrosion	Zinc dithiophosphate (ZDDP)	Foam anticorrosion film
Detergent or Dispersant	Calcium sulphonates	Prevent deposition of carbon
Alkalinity	Calcium carbonate, Magnesium Sulphonates	Neutralize acidic engine byproducts.
Antiwear	Zinc dithiophophates (ZDDP)	Reduce wear.
Pour Point Depressant	Methacrylate polymers	Lower pour point
VI Improver	Organic Polymers	Improve IV

Source: Naval Ship's Technical Manual, 1999.

Oxidation is the most important form of chemical breakdown of a motor oil and itsadditives. The chemicals in a motor oil are continuously reacting with oxygen inside anengine. The effects of oxidation due to this reaction as well as the by-products ofcombustion produce very acidic compounds inside an engine. These acidic compoundslead to corrosion of internal engine components, changes in oil viscosity, formation of varnish,sludge and other insoluble oxidation products that can cause performance and durabilitydegradation of engines over a period of time. The products of oxidation are lessstable than the original base hydrocarbon molecular structure and as they continue to beattacked by these acidic compounds can produce varnish and sludge.

The environmental problem caused by the high quantity of used lubricating oil is currently attempted to be resolved simultaneously from multiple sides. On one hand, it is an important criteria to ensure the biodegradability of modern engine oils and other industrial oils.Onthe other hand, increase of the oil replacement period also constitutes one of the main objectives of research and development activity. Apart from these, several modern processeswere also developed for the effective recycling of used engine oils and other industrial oils**(Baladincz*et al.*, 2008)**.

2.5.1 Properties of used engine oil

Used engine oil is of variable composition depending on the composition ofthe original oil and the degree of degradation. Used engine oils contain contaminating materials due to wearing and combustion, and their chemical composition also differs from that of new oils due to oxidation and other chemical reactions. Most important contaminants materials in used engine oils are as detailed below.

2.5.1.1 Combustion products

Water: In the case of a perfect process, engine fuel combusts into carbon monoxide and water. In normal operating conditions the steam vents through the exhaust system, though after cold-start a part of the water may get also into engine oil and aid in sludge formation at the bottom of oil sump.

Carbon black: In diesel engines, carbon black also forms as a product of combustion, a part of which gets into engine oil.

Engine fuel: a part of the non-combusted engine fuel accumulates also in engine oil.

2.5.1.2 Contaminations originating from wearing

Metals: majority of metal particles coming from the cylinder wall and from the piston get into the engine oil. Such particles may include: iron (400-800 mg/kg), chromium (30-50 mg/kg), copper (2-5mg/kg), magnesium (150-400 mg/kg), nickel (2-7 mg/kg), calcium (600-2000mg/kg), aluminium (300-800 mg/kg), arsenic (0-5 mg/kg) etc. Other parts of the metals originated from additives that are originally in engine oil (for example zincdialkyl-dithio-phosphates)**(Baladincz*et al.*, 2008)**.

Dust: Dust and other solid contaminating materials (mainly silicates) get into engine oil through air filter and promote sludge formation.

2.5.1.3 Oxidation products

Lubricantsalso contain oxidation products forms as a result due to the heat load exercised in normal operating conditions within engine space. Theyform complexes with metals and are basically organic acids.

2.5.1.4 Halogenised hydrocarbons

Totalhalogenic content of used engine oils is between 500-1000 mg/kg in general. These may include substances such as PCB's (polychlorinated Biphenyls).

Other contaminats may include polycyclicaromatic hydrocarbons (PCAH's) and glycols.

Due to the contaminating materials present in relatively high amount, certain analytical and application properties of used engine oils change as well. Its extent is also affected by the composition of engine fuel (for example: the vegetable oil fatty acid methyl ester content of

diesel gas oils). Properties such as viscosity (of used engine oils), significantly decreases with the increase of engine fuel concentration.

2.5.2 Used Oil and the Environment

Most of the concern about used oil relates to poor management practices some of which are:

e. Throwing oil in the garbage: This may result in groundwater contamination if it goes to landfills with inadequate liners.

f. Storm sewer disposal: This may create oil sheens that have negative effects on biota and may cause clogging of lines and pumps.

g. Road oiling: This may kill plants and grasses in the immediate vicinity of use and contaminate groundwater through the release of petroleum hydrocarbons.

h. Burning as fuel in small commercial boilers: This is generally safe except in the case of high contaminant levels or inadequate pollution control devices.

2.6 CELL GROWTH KINETICS IN A BATCH REACTOR

When living cells are added to a solution of essential nutrients at a suitable temperature and pH the cells grow. For unicellular organisms, increase in biomass (mass of living matter) is accompanied by increase in the number of cells present.

Associated with cell growth are two other processes: uptake of some material from the cell's environment and release of metabolic end products into the surroundings.

In a typical batch reactor the number of living cells varies with time, as shown in Figure 2.2. After a lag phase, where no increase in cell number is evident, a period of rapid growth ensues, during which the cell number increases exponentially with time. Since cell multiplication cannot

continue indefinitely in a closed vessel, a stationary phase follows the period of exponential growth during which the population achieves its maximum size. Eventually a decline in cell numbers occurs during the death phase.Each phase is of importance in design: to minimize the length of the lag phase, maximize the rate and length of the exponential phase, to slow down the onset of transition to stationary phase.

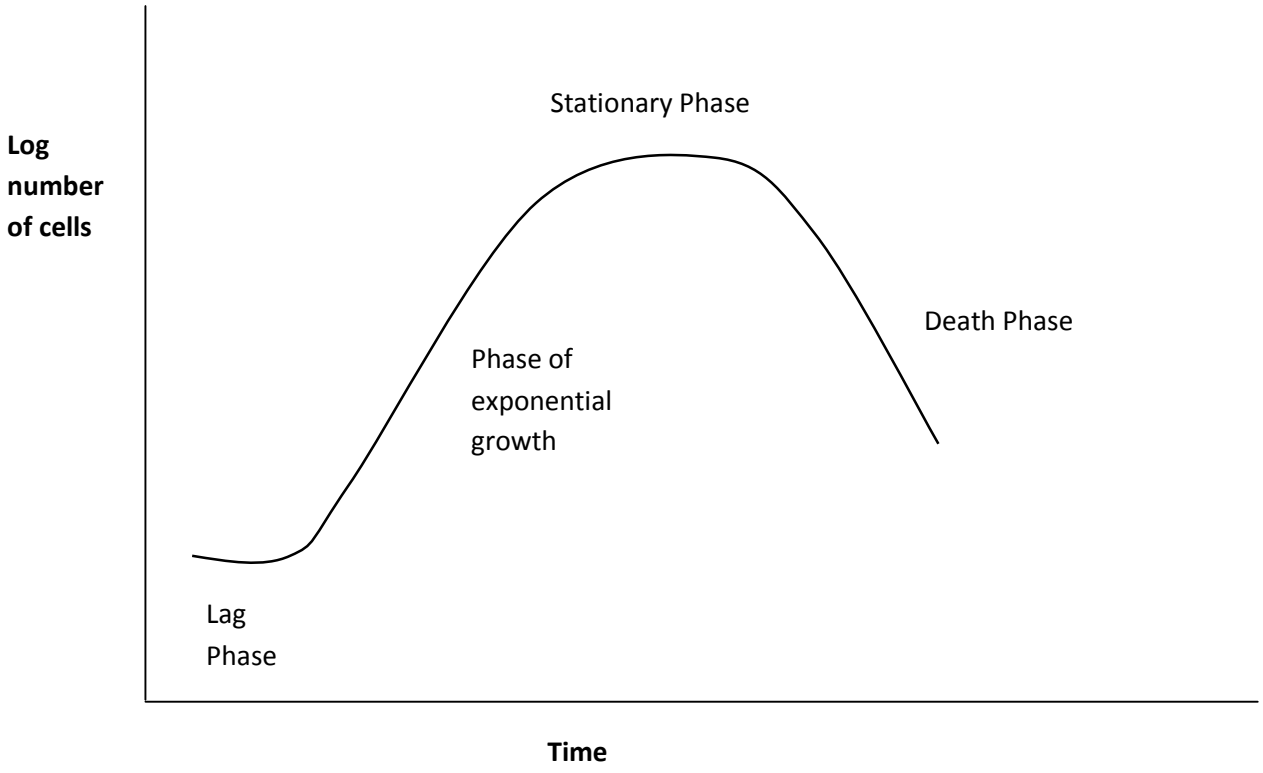


Figure 2.2: Typical growth curve for batch cell cultivation

The length of the lag phase depends on changes in nutrient composition. The shock of a switch to a new environment has effects on the living cells: the cells have to adapt to the new environment necessarily resulting in a time interval of insignificant cell division rate. Multiple lag phases may

sometimes be observed when the medium contains multiple carbon sources. This phenomenon is known as diauxic growth and is caused by a shift in metabolic patterns in the midst of growth.

At the end of the lag phase, the population of microorganisms is well adjusted to the environment. The cells can then multiply rapidly. The equation

$$\frac{dx}{dt} = \mu x \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (2.6)$$

with $x = x_o$ at $t = t_{lag}$

describes the increase in cell numbers during this period and

$$x = x_o e^{\mu(t-t_{lag})} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots .. (2.6)$$

$t > t_{lag}$

Thus only μ is required to characterize the population during exponential batch growth. Useful growth kinetic data may be obtained from batch experiments confined to the experimental growth phase.

During the stationary phase, some cells are dividing while others die. Often the dead cells lyse (break open) and the carbohydrates, amino acids and other components freed from the lysed cells are then used as nutrients by the remaining living members of the population. Eventually due to nutrient depletion and toxic product buildup, the population cannot sustain itself and the death phase sets in.

Usually death of the population is assumed to follow an exponential decay:

$$x = x_s e^{-k_d t} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots .. (2.7)$$

Where t denotes the time elapsed after the onset of the death phase.

Various kinetic models have been proposed to account for the various transient growth situations of cell population. They are generally grouped as structured and unstructured growth kinetic models. A structured model attempts to explicitly describe intracellularprocesses in both a structural and physiological sense, and thus offersthe most realistic representation of a cell. Structured models are usuallysingle-cell models that look in some detail at individual processes and reactions.However, whereas cell behavior to varying growth conditions is notthoroughly understood, a structured model is not applicable to most of celllines, for example to CHO cells. Therefore, the majority of the kinetic modelsin literature are categorized as unstructured: they rely on the global relationshipsbetween cell growth and environmental properties of the culture,and neglect intracellular processes.

2.7 KINETICS OF BIOREMEDIATION OF USED ENGINE OIL CONTAMINATED SOIL

Biological processes may be classified as fermentations, elementary physiological processes and the action of living entities. Fermentations are reactions wherein a raw organic feed is converted into a product by the action of microbes or by the action of enzymes. The key distinction between the two types of fermentation is that in enzyme fermentation the catalytic agent, the enzyme, does not reproduce itself, but acts as an ordinary chemical, while in microbial fermentation the catalytic agent, the cell or microbe, reproduces itself. Within the cell, it is the enzyme which catalyses the reaction, just as in enzyme fermentation (**Levenspiel, 1999**). Bioremediation may thus be classified as microbial fermentation.

Bioremediation of used engine oil contaminated soil by microorganisms has been shown to be a function of several parameters some of which include the pH, nature of soil, moisture content, nature of bacteria consortium, temperature, quantity and nature of organic amendment, presence of heavy metals etc.

The first order bioremediation kinetics has been shown to describe the change in TPH with time in the process of bioremediation**(Yeung *et al.*, 1997; Adesodun and Mbagwu, 2008)**:

$$\boldsymbol{y = ae^{-kt}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(\mathbf{2.8})$$

In Equation (2.8), *y* is the residual hydrocarbon content in soil(g kg^{-1}),

a is the initial hydrocarbon content in soil(g kg^{-1}),

k is the biodegradation rate constant(day^{-1}),and

t is time (day).

Half life was computed as

$$\boldsymbol{Halflife} = \frac{\mathbf{ln(2)}}{\boldsymbol{k}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(\mathbf{2.9})$$

The factors involved in bioremediation among other include: the nature and type of organisms, moisture content, temperature, degree of agitation, degree of aeration, nature, type and concentration of organic amendment. Since more than one phase is usually present, the movement of material from phase to phase must be considered in the rate equation. Thus the rate expression in general will incorporate mass transfer terms in addition to the usual chemical kinetics term **(Levenspiel, 1999)**. This implies that the mass transfer terms and the usual

chemical kinetics terms are incorporated in *k*. Examples of frequencies of mixing that have been used in bioremediation studies to improve aeration include: twice a month **(Bagherzadeh-Namazi *et al*,. 2008)**, twice a week **(Margesin *et al.*, 2000)**, every alternative day **(Vasudevan and Rajaram, 2001)** and every day **(Maike and Filser, 2007)**.

2.8 ENVIRONMENTAL BIOTECHNOLOGY AND THE CHEMICAL ENGINEER

Over the past few decades large quantities of industrial pollutants have been discharged into the environment. Thosestructurally related to natural compounds, are readily degraded or removed by microorganisms found in soil and water. However, there is also an increasing number of novel industrial compounds rarely found in nature. These xenobiotic compounds are usually removed slowly and tend to accumulate in the environment. They can accumulate and cause severe environmental problems due to their high degree of toxicity.

The development of alternative, economical and reliable biological treatments becomes increasingly important because of the problems associated with pollutant treatment by conventional methods, such as incineration or landfills. Although natural microorganisms collectively exhibit remarkable evolutionary capabilities to adapt to a wide range of chemicals, natural evolution occurs at a relatively slow rate, particularly when the acquisition of multiple catalytic activities is necessary. In these cases, the acceleration of these events via genetic engineering/processing engineering is helpful since the desirable traits can be carefully designed and controlled. The drive toward this goal represents the essence of environmental biotechnology**(Chen *et al*., 2005)**.

Environmental biotechnology deals with the utilization of microorganisms to improve environmental quality. Although the field of environmental biotechnology has been around for decades, starting with the use of activated sludge and anaerobic digestion in the early 20th century by civil engineers, the introduction of new technologies from modern microbiology and molecular biology has enabled engineers and scientists to tackle the more contemporary environmental problems such as detoxification of hazardous chemicals. Chemical engineers are uniquely poised to contribute in this emerging area since many of the potential solutions require a combined perspective from modern biology and process engineering, two areas where chemical engineers excel**(Chen *et al.*, 2005)**.

For example, the realization of environmental biotechnology into practical solutions requires the implementation of process design, which is the foundation of the chemical engineering discipline. The same is also true at the cellular level, where the functions of cells are determined primarily by networks of specific catalytic reactions. The nature and activities of these networks are dictated by the genetic information, thereby defining the ways in which engineers can influence cellular functions and metabolic capabilities toward the design of improved biocatalysts for environmental remediation**(Bailey, 1991)**. Although, superficially these strategies seem distant from traditional chemical engineering, a deeper inspection reveals that the design of biological catalysts, based on defined techniques from biochemistry and biology is indeed parallel to our understanding of chemical kinetics, transport, separation, and control. In addition, advances in genomics and proteomics are providing opportunities to predict, in a quantitative manner, the potential manipulations necessary. Even though chemical engineers are well prepared to contribute new research directions in environmental biotechnology, only a few are working in this area today. Fortunately, the number of chemical engineers showing interest is

growing every year and with the recent research emphasis on biotechnology, it is easy to envision that many others will join this exciting research area in the near future **(Chen *et al*., 2005)**.

2.9 SAFETY CONSIDERATIONS

The safety measures required for this research work include:

5 Handling of used engine oil contaminated soil: Used engine oils are thought to contain carcinogenic substances as such would require being prevented from coming into contact with the skin. Laboratory coat, hand gloves, and safety goggles must be worn. To prevent inhalation of soil particles, nose mask should be worn.

6 Laboratory coat, hand gloves, and safety goggles must be worn. There should be provision for a large quantity of water for washing in case of an accident.

7 Handling of poultry manure: the area around the experimental rig should be well ventilated to take care of the foul smell from the manure.

8 Care should be exercised in handling toluene and dichloromethane. Appropriate nose mask is recommended.

CHAPTER 3

MATERIALS AND METHODS

This chapter gives the description of equipment and instruments used in the course of this investigation. Details of the experimental proceduresemployed are also presented.

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3.1 EQUIPMENT

The major equipment used in the course of the study were:

14. pH meter (Kent EIL 7055)
15. Oven (Tamson, Heraeus T500)
16. Gas Chromatograph (GC) (GCMS-QP2010 Plus Shimadzu, Japan)
17. Spectrophotometer (B. Bran Scientific and Instrument Company, 722 – 2000 20D spectrometer)
18. X-ray fluorescence (MiniPal PW4025, Philips Analytical)
19. Weighing balance (Berkel, max wt 15kg)
20. Analytical balance (Adam PW 184)
21. Vacuum pump (Edwards, ES 100)
22. Shaker (*Tamson,* Holland)
23. Sieve shake GRAVQUICK (A/S) EFL2 MK3
24. Colony counter (Stuart Scientific Colony Counter, Model No: R000102672)
25. Microscope (Olympus, Model 280317, max mag x 1000)
26. Thermometer (0 – 100^{o}C)

3.2 APPARATUS

21. Hand trowel
22. Evaporating dishes
23. Sieves
24. Glass funnel
25. Muslin cloth
26. Plastic containers with tight fitting lids (2liters).
27. Spatula
28. Measuring cylinders (5, 10, 20 and 250mls)
29. Syringes (2, 5 and 10mls)
30. Sample bottles
31. Filter papers (Whatman No 42)
32. Plastic containers of various cross-sectional areas (70 – 250cm^2)
33. Stop watch (Hanhatl mechanical analog stopwatch)
34. Glass rod
35. Sieves
36. Bent glass rod
37. Flat bottom flask (50, 250 and 500mls)
38. Bijou bottle
39. Pipettes
40. Glass rod

3.3 MATERIALS

The major materials used were:

24. Earthworms
25. Cattle dung (from National Animal Production and Research Institute (NAPRI), Ahmadu Bello University, Zaria)
26. Poultry manure (from National Animal Production and Research Institute (NAPRI), Ahmadu Bello University, Zaria)
27. Toluene (Analytical grade, BDH Chemicals Ltd England)
28. Dichloromethane (Analytical grade, BDH Chemicals Ltd England)
29. Nutrient Agar (Oxoid England procured from HADDIS, Samaru)
30. MacConkey Agar (Oxoid England procured from HADDIS, Samaru)
31. Calcium carbonate (Analytical grade, BDH Chemicals Ltd England)
32. Chromic acid (Analytical grade, BDH Chemicals Ltd England)
33. Deionized and distilled water
34. Soil (petroleum hydrocarbon contaminated and uncontaminated soil samples)
35. Normal saline
36. Plate count agar (Oxoid England procured from HADDIS, Samaru)
37. Citrated human plasma (from Microbiology Department A.B.U. Zaria)
38. Hydrogen peroxide (BDH, from HADDIS, Samaru)
39. Simmon's citrate agar (Oxoid England procured from HADDIS, Samaru)
40. Peptone water (Oxoid England procured from HADDIS, Samaru)
41. Kovac's reagent (Oxoid England procured from HADDIS, Samaru)

42. Methyl-red VOGES-PROSKAUER Broth(Oxoid England procured from HADDIS, Samaru)
43. Alpha naphthol (BDH Chemicals Ltd England, from HADDIS, Samaru)
44. Ethanol (Analytical grade, BDH Chemicals Ltd England)
45. Potassium hydroxide(Analytical grade, BDH Chemicals Ltd England)
46. Filter paper

3.4 COLLECTION OF SOIL SAMPLES, MANURE, *MORINGA* LEAVES, CASSAVA PEELS AND EARTHWORMS

Soil samples from mechanic workshops were collected and analyzed to confirm that they were trulycontaminated with petroleum hydrocarbon. This section presents details of the investigation.

3.4.1 Enumeration of Mechanic Workshops

A tour was made round Zaria town, Nigeria (Latitude: 11° 4' 0 N, Longitude: 7° 42' 0 E) and mechanic workshops were enumerated namely: motorcar (MM), motorcycle (MC) and trucks/lorries (HD). The workshop managers were interviewed for vital information like the age of the workshop, number of times the site was sand filled, if any, and if UEO was usually dumped on the soil.The area coveredis shown on the Google Earth image presented in Figure 3.1.

3.4.1.1 Collection of Soil Samples

Based on the spread and the number of the various types of workshops along the enumeration route, soil samples were collected from MC, MM and HD so as to give a fair representation of what obtains in the soil in mechanic workshops in Zaria. In sample collection, soil was collected

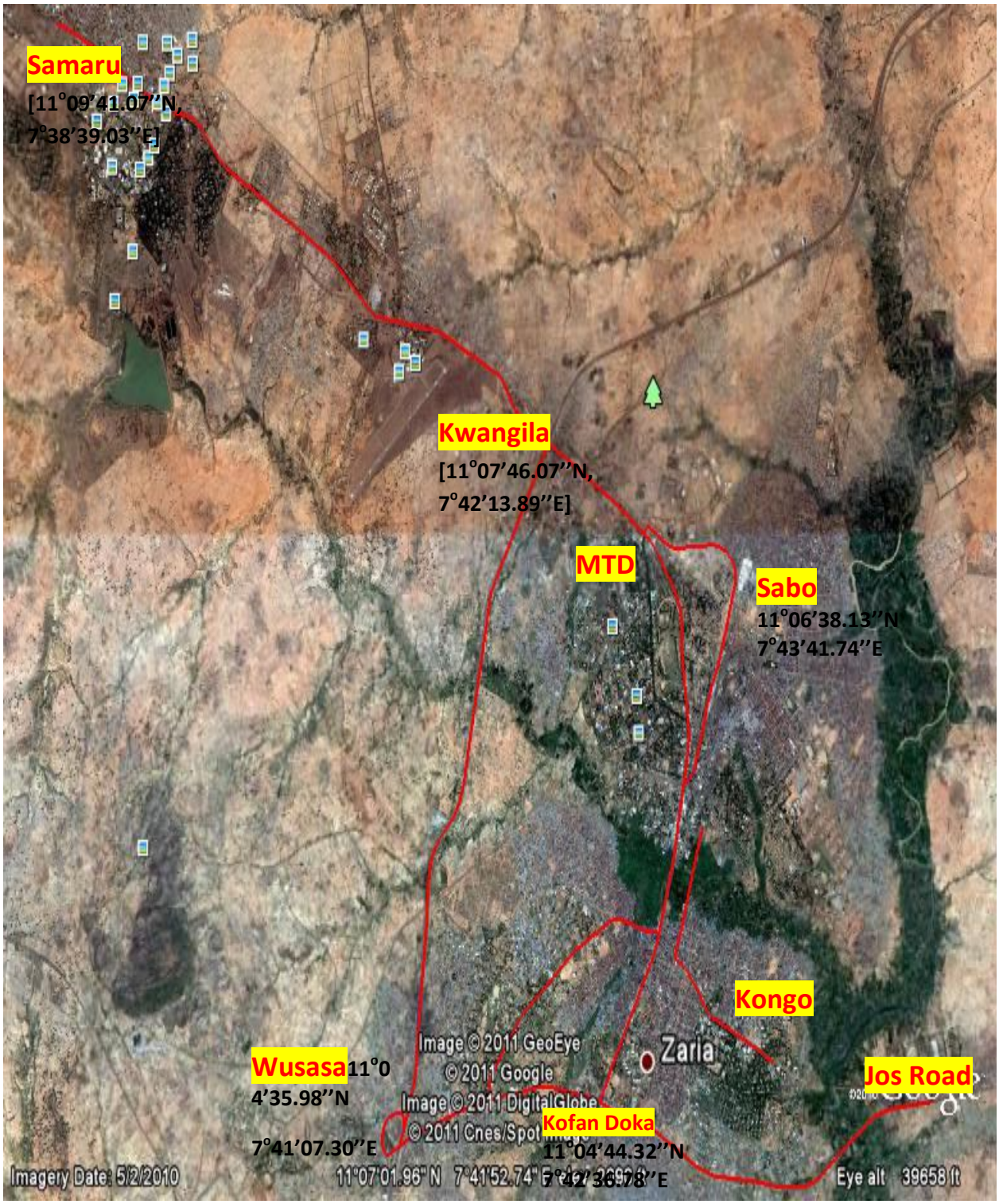


Figure 3.1: Google Earth map of Zaria (Latitude: 11° 4' 0 N, Longitude: 7° 42' 0 E) showing the area covered during the enumeration of mechanic workshops

from the centre of the workshop using a hand trowel to dig to a depth of six (6) centimeters (that is the top soil, where the concentration of the contaminants is expected to be high). Samples were collected into cleansterilized plastic containers.

Used engine oil uncontaminated soil (UEO free soil) was collected from the farmland of Ahmadu Bello University, Zaria in clean plastic containers.

From six (6) motorcar mechanic workshops, twelve (12) liters of usedengine oil were collected (two (2) liters per mechanic workshop) and kept in cleaned plastic containers (4 liter cans). This was also done for used engine oil from motorcycle and truck/lorries (diesel engine vehicles)mechanic workshops.

Earthworm was collected following the recommendation by **Uba *et al.*(2009)**that earthworm may be easily obtained in Zaria by digging into soil.Adult earthworms(with clitellum) ranging in weight from 0.3 to 0.5 gram each, were collected by digging into the earth at Samaru Extension, Zaria, Nigeria. Samples of the worms were submitted to the Department of Biological Sciences, Landmark University, Kwara State, Nigeriafor identification. The remainder was used for study within 72 hours after collection.

Poultry manure and cattle dung were collected from the National Animal Production and Research Institute (NAPRI), Ahmadu Bello University (ABU), Zaria. Leaves of *Moringa oleifera* where procured from Samaru village, Zaria and identified in the Department of Biological Sciences, ABU, Zaria. Fresh cassava tubers were procured from Samaru market and peeled. The cassava peels was soaked in water in a closed plastic container for 1 week to allow for elimination of cyanide that might be present. The fermented peels were then air dried, crushed and sieved (≤5mm).

3.4.2 Analysis of Soil and Organic Amendments

The following analyses were performed on all the soil samples obtained from the mechanic workshop.

3.4.2.1 Elemental Composition

The determination of the elementalanalysis of all samples was performed using an ED-XRF machine the MiniPal PW4025 (Philips Analytical). The Procedure for Analysis of samples using EDXRF is presented in **Appendix B**.

3.4.2.2 Soil Texture

Soil textural classification of contaminated and uncontaminated soil samples was done using the hydrometer method **(Iwuafor *et al.* 1990)**described in **Appendix A**

3.4.2.3 Microbial Count

Microbial count was determined using the plate count method **(Talaro and Talaro, 2002)**. Serially diluted samples (0.1 millilters) were plated on nutrient agar mediumsupplemented with 50µg/ml nystatin to suppress the growth of fungi. Triplicate plates were incubated at 30°C for 24 hours before the colonies were counted.

3.4.2.4 Total Petroleum Hydrocarbon (TPH)

The TPH of the soil samples was determined using the toluene cold extraction method **(Adesodun and Mbagwu, 2008)**. 5gm of soil from mechanic workshop was placed in a 200 milliliters beaker into which was added 50 milliliters of toluene. The mixture was manually stirred continuously for 30 minutes, left to stand in a fume cupboard for 2 hours and afterwards

filtered using Whatman No 42 filter paper. The residue (soil) was allowed to dry in an oven at 50^{o}C. The TPH was computed as:

$$TPH\ (g/kg) = \frac{(initial\ weight\ of\ soil - weight\ of\ soil\ after\ solvent\ extraction)\ in\ g}{Initial\ weigh\ of\ soil\ in\ kg} \ldots\ldots\ldots 3.1$$

3.4.2.5 Carbon,Nitrogen and Phosphorus Content

The carbon, nitrogen and phosphorus contents of the soil samples were analysed using Walkley-Black **(Iwuafor *et al.* 1990)**, Kjedahl **(Iwuafor *et al.* 1990)**and Bray No 1 **(Iwuafor *et al.* 1990)** methods respectively. Details of the procedure are shown in **Appendix A**. The materials used as organic amendment (manures,cassava peels and *moringa oleifera* leaves)were also analyzed for carbon, nitrogen and phosphorus contents following the same procedure as those used for the soil samples.

3.4.2.6 Soil pH Determination

Ten grams of sieved (≤ five millimeters) and air-dried soil was weighed into a 50milliliters capacity beaker and 25milliliters of distilled water added. The content was stirred manually for 30 minutes with a glass rod and left to stand for an 1 hour. The electrode of the pH meter (Kent EIL 7055) was then inserted and the pH read.

3.4.2.7 Determination of Water Holding Capacity (WHC) and Moisture Content

35g of soil was placed in percolation tube (to give 15 cm of soil) and compacted by gentle tapping. Water was added until the water level stood at about 5 cm above the soil level. The tube was covered and left to stand for 2 days. After this period, the top half-inch of soil was discarded and wet soil was weighed into a pre-weighed evaporating dish. The sample was placed in oven at

110^{o}C for 24 hours. It was then removed and weighed to obtain bone dry weight of the sample. Percent water holding capacity was calculated as **(Srivastava and Thakur, 2006):**

$$\boldsymbol{waterholdingcapacity(WHC)} = \frac{\boldsymbol{wetweight(g) - bonedryweight(g)}}{\boldsymbol{bonedryweight(g)}} \ldots\ldots\ldots\ldots\ldots\ldots \mathbf{3.2}$$

For soil moisture content determination, a watch glass was weighed (W1). About10g of soil was placed on the watch glass and the combined weight of glass and soil recorded (W2). The sample (watch glass and soil) was placed in an oven at 105^{o}C and left for 48 hours. After the 48 hours drying time the weight of the sample (watch glass and dry soil) was recorded (W3). The moisture content of the soil was computed as:

$$\boldsymbol{moistute\ content(MOC)} = \frac{\boldsymbol{W2 - W3}}{\boldsymbol{W3 - W1}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{3.3}$$

3.4.2.8 Elemental Analysis of Simulated Used Engine Oil Contaminated Soil

Five different concentrations (12.5, 25, 50, 75, 100g/kg) of used engine oil contaminated soil were prepared and thoroughly mixed. Elemental analysis was then conducted on each sample using EDXRF.

3.5 BIOREMEDIATION USING ORGANIC AMENDMENTS

3.5.1 Soil Contaminated in the Laboratory

The uncontaminated soil samples were contaminated with 10% (wt/wt) used engine oil. As was done with the soil sample from mechanic workshop, weighted soil samples in different sized

containers were biostimulated using 10% (wt/wt) cow dung. Control samples were also prepared. Allsamples were maintained at 60% water holding capacity and at room temperature.

To make soil up to 60% water holding capacity Wo grams of water (calculated using Equation 3.3) was added to Ws grams of soil.

$$\boldsymbol{Wo = (0.6 * (WHC) - MOC) * Ws \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.3.3}$$

Where, WHC = water holding capacity

MOC = moisture content

At intervals of time (twice a week), all samples were tilled and makeup water added. The TPH and pH of the samples were determined every two weeks, using the toluene cold extraction method as described by **(Adesodun and Mbagwu, 2008)** and pH meter (Kent EIL 7055) respectively.For the TPH determination,soil sample (5g) was weighed into a 50 milliliterscapacity flask and 10 milliliters of toluene (AnaLar grade) was added. After shaking for 30 minutes on a shaker **(***Tamson,* Holland**)** and allowing to stand for an hour in an air tight sample bottle, the supernatant was extracted using a syringe and its absorbance measured at four hundred and twenty nanometers (420nm) using spectrophotometer (B. Bran Scienticific and Instrument Company). The TPH of the soil with time was estimated using a calibration curve prepared by recording the absorbance of supernatants obtained by mixing five (5) grams of UEO contaminated soil obtained from soil with known concentration of UEO contaminat (1 to 20grams UEO/kg of soil) with 10ml of toluene.

3.5.2 Soil from Mechanic Workshop

Weighted soil samples in containers of different cross-sectional-areas were mixed with 10% (wt/wt) manure and the moisture content maintained at 60% water holding capacity. The manure considered for use was cow dung. A control sample without manure (but with all other parameters – quantity of soil, moisture content and geometry of container - kept constant) was also prepared. The moisture content was maintained at 60% water holding capacity and TPH followed with time as in **Section 3.5.1**.

3.5.3 Effect of Surface Area

Five hundred grams of the uncontaminated soil was weighted into six (6) containers of different cross-sections to give surface area to weight of soil ratio of 0.143, 0.147, 0.243, 0.278, 0.349 and 0.428cm^2/g. The soil was made up to 60% water holding capacity and thoroughly mixed. Fifty grams of used engine oil was added to each container and mixed. The organic material used as amendment was *Moringa oleifera* leaves. Twenty five grams each of dry pulverized *Moringa oleifera* leaves and poultry manure was then added and again thoroughly mixed. All samples were maintained at room temperature (≈ 29^oC) and moisture content of 60% water holding capacity by weighing twice a week and adding make up deionized water when necessary. The TPH of each sample was followed with time using gravimetric method. This involved collecting soil and drying at room temperature for 72 hours. Ten milliliters of toluene was then mixed with five grams(5g) of the dried soil sample, covered and left to stand for 24 hours. The supernatant was extracted using a syringe and 10 milliliters of fresh toluene was added. After 24 hours the supernatant was again removed and the soil sample placed in an oven at 60^oC to remove any remaining toluene. The weight of soil (M_1) was then determined. The TPH was calculated as:

$$TPH(g/kg) = \frac{5 - M_1}{5} * 1000 \quad \ldots\ldots \quad 3.3$$

The composition of the used engine oil was determined by mixing two grams of dry contaminated soil (at time, t = 0) with 5milliliters of dichloromethane and analyzing the supernatant after settling using GC-MS (GCMS-QP2010 Plus Shimadzu, Japan).The initial column oven temperature was kept at 60^{o}C for five minutes and then raised by 20^{o}C per minute to 140^{o}C and then at 5^{o}C per minute to 200^{o}C. It was held at 200^{o}C for 5 minutes and then raised by 4^{o}C per minute to 280^{o}C. The total run time was 49 minutes.

GC-MS analysis was conducted after 21 weeks of bioremediationfor the sample with optimum rate.

3.5.4 Effect of Mixing

Five hundred grams of soil was placed in a plastic container. The ratio of the exposed surface area to the weight of soil was 0.428cm^2 per gram of soil. The soil was made up to 60% water holding capacity and 50 grams of used engine oil added. The mixture was thoroughly mixed with 50 grams of poultry manure. Two samples labeled PUMX and PMX were prepared, both in duplicates. All samples were maintained at 60% water holding capacity for twenty two (22) weeks. After the initial mixing of the content of the container (on day 0) PUMX samples remained unmixed throughout the period of the study (22 weeks). For PMX samples, the mixture was manually mixed every three days (twice a week) for 5min/day. The initial TPH of all samples was determined and this was repeated at intervals of time during the period of the study

so as to follow the progress of biodegradation with time. The procedure was repeated using pulverized *Moringa oleifera* leaves as the organic amendment. Themixed and unmixed samples were called MRMX and MRUMX respectively. A control sample (NA) was prepared with similar quantities of used engine oil, soil and water but with no organic amendment for both set of experiment (i.e. with poultry manure and *M. oleifera*). All samples were covered with muslin cloth and incubated in the open laboratory condition (≈29^{o}C).

TheTPH of all samples were determined as described in **Section 3.5.3**. Thecompositions of the used engine oil contaminant wasalso determined using GCMS. Samples analyzed using GCMS were NA (at t = 0 weeks and 22 weeks) and PUMX and MRMX (at t = 22 weeks).

3.7 EFFECT OF EARTHWORM INTRODUCTION INTO USED ENGINE OIL CONTAMINATED SOIL

This section presents the procedure used in investigating the effect of the use of earthworms for bioremediation. Adult earthworms (with clitellum) were used for all investigations involving worms. The average weight of the worm was found to 0.4g. Investigations were first carried out using simulated used engine oil contaminated soil to generate information on the most important parameters to consider in earthworm (*E. eugeniae*) assisted bioremediation of petroleum hydrocarbon contaminated soil. Secondly, the effect of earthworm inoculation on the bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshops was investigated.

3.7.1 Earthworms Survival Assessment

Used engine oil is thought to contain some toxic organic compounds and heavy metals **(Obidike, 1985)**. As a result, an initial investigation was conducted to investigatethe used-engine-oil concentrations tolerable to earthworm. The test substrates were prepared according to the OECD guidelines for testing of chemicals **(OECD, 1984)**. The soil sample collected was sieved (≤5mm) to remove coarse stones and to ensure homogeneity. 0.5kilograms of soil was weighed into plastic test containers. The soil was made up to 60% water holding capacity using deionised water. The soil samples were contaminated with various concentrations of the motorcycle UEO (5, 10, 20, 30, 40, 50 and 100 per kg of soil). The mixture was thoroughly mixed manually. Used engine oil was collected from six (6) different motorcycle mechanic workshops, so six replicas were prepared. 5grams of poultry manure was then added to the soil and thoroughly mixed. After mixing, 1gram of the manurewas littered on the surface. Worms were kept on moist filter paper for three hours before being placed in test containers so they can void their gut contents. They were then rinsed,quickly killed and dried for elemental analysis. Tenearthworms (*E. eugeniae*) each were introduced into each test container and the weight of each test container recorded. Adult earthwormswith clitellum,were considered having an average weight of 0.47grams. At intervals of time (after every 2 days), the samples were examined. The number of living earthworms in each sample was recorded and the dead worms removed. The setup used for investigations involving earthworm inoculation is shown in Figure 3.2.

The moisture content of 60% water holding capacity was maintained throughout the test period by weighing, to determine moisture loss, and adding make up deionised water. The test containers were covered with muslin cloth firmly held in place to prevent the worms escaping.

Testing was done in continuous light (to ensure that worms remain in the test medium throughout the duration of test).The samples were monitored for a total of 14 days. This procedure was repeated using cow dung in place of poultry manure. The above procedure was repeated for used engine oil collected from motorcar and diesel engine vehicles. Control samples, without engine oil was also prepared for each run.

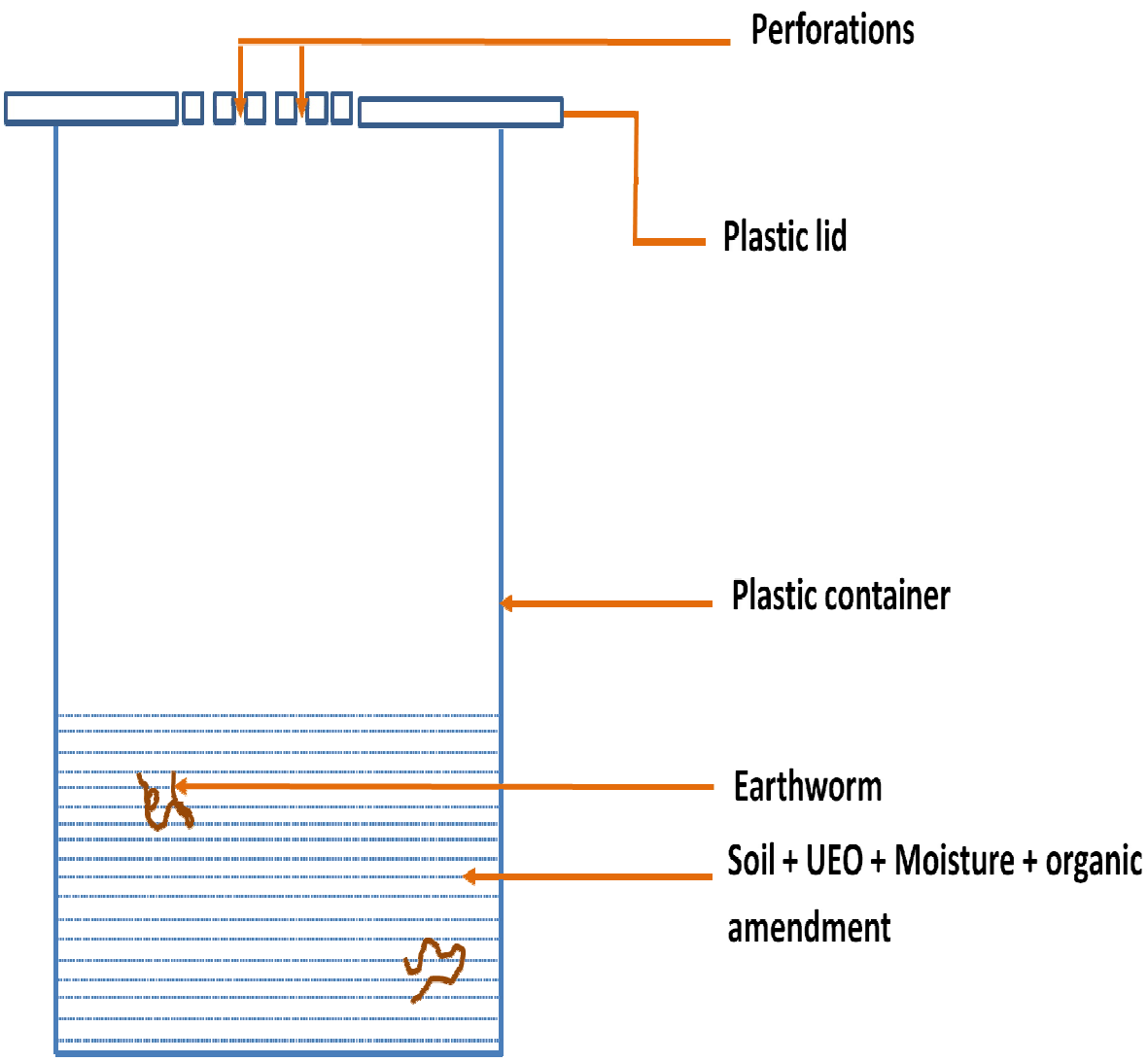


Figure 3.2: Setup used for earthworm inoculation studies

3.6.2 Effect of Earthworm on the Carbon and Nitrogen Content, Moisture Retention and Aggregate Size Distribution of Used Engine Oil Contaminated Soil

Five samples were prepared each containing 40grams of used engine oil in 400grams of soil made up to 60% water holding capacity. The samples were kept in plastic containers covered with muslin cloth. 10grams of cow dung was added to each and the mixture thoroughly homogenized. The soil samples were labeled 0W, 4W, 8W, 12W, and 16W according to the number of worms the contained (i.e. 0, 4, 8, 12 and 16 worms respectively). All samples were prepared in duplicates. Samples were monitored for 6 weeks. The moisture content was maintained at 60% water holding capacity by weighing every day to determine the weight loss and adding makeup deionized water.

3.6.2.1 Carbon Nitrogen Content

Starting from the day the samples were prepared, 10g of the contaminated soil was collected from each sample and the carbon and nitrogen contents determined using the Walkley-Black and the Macro-Kjedahl methods respectively. This was repeated every seven (7) days for six weeks.

3.6.2.2 Water Holding Capacity and Aggregate Size Distribution

At the end of six weeks, the water holding capacity and the aggregate size distribution of each sample was determined. The water holding capacity was determined as described in **Section 3.4.2.7.**In the determination of aggregate size distribution, 300grams of dried bulk soil was weighted and transferred into the nest of sieves consisting sieves of diameters (D_i) 2.00, 0.66, 0.50, 0.25 and 0.05millimeters. The nest of sieves was placed on a mechanical sieve shaker (GRAVQUICK (A/S) EFL2 MK3) and shaken for 60 seconds after which the weight of soil

retained in each was determined. Aggregate size distribution was calculated as proportion of soil retained in each sieve**(Hajabbasi and Hemmat, 2000)**using:

$$\boldsymbol{MWD} = \sum(\boldsymbol{P_i D_i}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.\ \mathbf{3.4}$$

Where,

$$\boldsymbol{P_i} = \frac{\boldsymbol{weight\ of\ soil\ retained\ on\ sieve(g)}}{\boldsymbol{total\ weight\ of\ soil\ taken(g)}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.\ \mathbf{3.5}$$

3.6.3 Effect of UEO Concentration on Bioremediation

1 kilogram of soil contaminated with 5g UEO was mixed with 5grams of poultry manure. 1gram of the manure was littered on the surface **(Schaefer and Juliane, 2007)**. The moisture content of the soil was made up to 60% water holding capacity using deionized water. The soil, manure, UEO and water were thoroughly mixed manually in a plastic test container. This was repeated using other concentrations of UEO contaminated soil (10, 15 and 20g UEO/kg soil). 10 earthworms were introduced into each sample. The test containers were covered with muslin cloth to prevent worms escaping. The total petroleum hydrocarbon (TPH) of the soil (g/kg) was determined every two weeks for a total of 6 weeks using the toluene cold extraction method as described by **Adesodun and Mbagwu (2008).**

3.6.4 Effect of Worm Concentration on Bioremediation and Microbial Count

1 kilogram of soil was spiked with 6grams of poultry manure, contaminated with 20grams of UEO and 5 earthworms were introduced. Other samples were also produced but with varying

numbers of earthworms introduced (0, 10, 15 and 20). At intervals of two (2) weeks, microcosms were prepared from each sample by mixing 5g of soil collected from four different areas of the spiked soil (so as to obtain a composite sample) for isolation and enumeration of bacteria and determination of TPH. TPH was determined using the toluene cold extraction method. The microbial count of the samples at the start of the experiment and after every two week was determined using the plate count method **(Talaro and Talaro, 2002)**. The culture media used was nutrient agar. Serially diluted samples (0.1 ml) were plated on nutrient agar medium (Oxoid) supplemented with 50µg/ml nystatin to suppress the growth of fungi. Triplicate plates were incubated at 30°C for 24 hours before the colonies were counted. Samples were incubated for 42 days.

GCMS analysis was conducted at t = 0 (for sample without worms) and at t = 42 days (for sample without worm and for sample with 20 worms).

3.6.5 Effect of Mixing/Tilling Versus Earthworm Assisted Bioremediation

This investigation was aimed at comparing the effect of the activities of earthworm with manual tilling/mixing in the process of bioremediation. Four samples were prepared in which 5grams of UEO was added to 500grams of soil and thoroughly mixed in plastic test containers. 5grams of poultry manure was added and again thoroughly mixed manually. Water was added to 60% water holding capacity and the content of the container was again thoroughly mixed. 15 earthworms were added to one test container and 10 earthworms to another.One of the remaining two was labeled MX (mixed) as the contents of the container were regularly mixed while the other was labeled UMX (unmixed) as it was unstirred throughout the test period, save for the initial mixing when the content of the test container was thoroughly mixed. The test containers where covered with muslin cloth to allow air in-flow and preventing earthworms from escaping.

At intervals of two (2) weeks, microcosms were prepared from each sample by mixing 5 grams of soil collected from four different areas for isolation and enumeration of bacteria and determination of TPH. The process lasted 14 weeks and all samples where produced in duplicates.GCMS analysis was conducted at time t = 0 weeks and at t = 14 weeks (for mixed sample and sample with 15 worms).

3.6.6 The Effect of Manure Concentration on Bioremediation

Five initial concentration of manure were considered for this investigation namely 0, 1, 2, 3 and4% manure (%wt/wt manure per soil). Each test sample contained 500grams of soil and 7.5grams of UEO. They contents of the containers where thoroughly mixed and made up to 60% water holding capacity using de-ionized water. Poultry manure was added and thoroughly mixed. 10 earthworms where then introduced into each test container apart from the one having 4% manure. At intervals of two weeks a 5grams microcosm was prepared and analyzed for TPH using the toluene old extraction method. All samples where produced in duplicates.

3.6.7 Heavy Metals Uptake and Elimination in Earthworm in Used Engine Oil Contaminated Soil

The procedure followed was adopted from the **OECD 317 (2010)**. Five different concentrations of used engine oil contaminated soil were prepared using 40, 30, 20, 10 and 5grams of used engine oil in 400grams of uncontaminated soil. The mixtures were kept in clean plastic containers. The soil was made up to 60% water holding capacity and spiked with 10grams of cow dung. Twenty (20) earthworms were added to each sample and on days 0, 5, 10, 15 and 20 five earthworms were removed and prepared for XRF analysis. Earthworms were introduced

without prior conditioning in the laboratory. In preparing the worms for analysis, on the set days, the worms were removed from the soil and placed on wet filter papers so as to void their guts for a period of 24 hours. Theywere then quickly killed by freezing at 0^{o}C. The dead worms were then sun dried for four (4) hours before being transferred to an oven at 60^{o}C for 12 hours. Elemental analysis of the carcasses of the worms was then conducted using XRF. The soil samples were maintained at 60% water holding capacity throughout the test period by weighing everyday and adding make up deionized water when necessary.

Elemental analysis of the various used engine oil contaminated soil before earthworm introduction (at time zero) was determined by drying 5grams of each soil sample in open air (26 – 31^{o}C) for 72 hours and analyzing using EDXRF. The soil samples were similarly analyzed, at the end of the investigation (day 20).All samples were prepared in duplicates.

At the end of the investigation, the % drop in TPH for all samples was determined using the toluene cold extraction method.

The Procedure for Analysis of samples using EDXRF is presented in **APPENDIX B**.

3.6.8 Effect of Earthworm on the Bioremediation of Mechanic Workshop Soil

This investigation was conducted using petroleum hydrocarbon contaminated soil from a motorcycle and motorcar mechanic workshop.Large particles were removed from the soil samples. The TPH and water holding capacities of the soil samples were determined using Equation 3.1 and 3.2 respectively.GCMS analysis was also conducted on the dichloromethane extract obtained using the freshly collected mechanic workshop soil samples. 500g of soil from each source was weighted and placed in clean plastic containers. Deionised water was then added to make up 60% water holding capacity and thoroughly mixed manually. 50gm of

fermented cassava peels (prepared as reported in **Section 3.4.1**) was added and again thoroughly mixed. A second sample was similarly prepared. The TPH of the mixture was determined using the toluene cold extraction method. 10 earthwormswas added to one of the samples. Both samples were maintained at 60% water holding capacity by recording the drop in weight every other day and adding makeup deionized water. All samples were prepared in duplicates.A control sample was also prepared containing neither worms nor the fermented cassava peels but it was also maintained at 60% water holding capacity. The TPH of all samples was followed using the toluene cold extraction method. Microbial count and identification were also determined at set intervals of time.

The TPH of the sample from each type of mechanic workshop soil considered was reduced by diluting with uncontaminated soil from a farmland (reported in **Section 3.4.1**). The TPH and water holding capacities of these mixtures were determined. Two samples were also prepared (as was done for the undiluted), one with organic amendment alone and the other with organic amendment and worms. A control sample was also prepared containing neither cassava peels nor worms. All samples were prepared in duplicates and maintained at 60% water holding capacity. The TPH of all samples was followed using the toluene cold extraction method. Microbial count and identification were also determined at set intervals of time. At the end of the investigation (after 35 days) GCMS analysis was conducted on the earthworm inoculated diluted motorcycle mechanic workshop soil sample.

Elemental analysis using EDXRF analysis of soil samples at the beginning and end of the experiments (after 35 days of inoculation) was also conducted on the samples in which the worms were still alive).

3.6.9 Total Bacterial Count and Characterization of Bacterial Flora

Samples considered for this investigation included PMX, MRMX (of **Section 3.5.4**)and at 22 weeks of bioremediation and the samples with 30 earthworms/kg of soil amended with poultry manure (of **Section 3.6.5**)at 14 weeks of bioremediation, also samples from Section 3.6.8.

Twenty five (25) grams of soil sample was mixed with 225mls of normal saline (NaCl + H_2O) (1:10). Ten fold dilution was then prepared, that is 1ml of the diluted sample (stock) was transferred into 9ml of sterile normal saline (10^1) using a sterile pipette, this was repeated to obtain 10^2, 10^3, 10^4 and 10^5 dilutions respectively. Using a sterile pipette, 0.1milliliters of 10^5dilution was aseptically transferred into sterile plate count agar (PCA) plates in duplicates.

With the aid of a sterile bend glass rod, the inoculum was spread on the surface of the culture medium (PCA). The inoculated plates were incubated at 37^{o}C for 24 hours. The numbers of bacterial colonies were counted by using a colony counter and recorded as colony forming units per gram (cfu/g).

Total colony forming unit per gram was computed using Equation 3.6:

$$\boldsymbol{Colony\ forming\ per\ gram} = \frac{\boldsymbol{Number\ of\ colonies\ counted} * \boldsymbol{Dilution\ factor}}{\boldsymbol{volume\ of\ inoculum}} \ldots\ldots\ldots\ldots \mathbf{3.6}$$

The isolates were Gram stained and observed under the microscope to determine the gram reaction, morphology and the presence of spores. The organisms were identified and characterized. Gram-reaction, motility, shape and color of colony, catalase and oxidase activities, were checked.

Colonies to be identified were picked from the nutrient agar plates and maintained on slants of the same medium. These were subsequently used for various biochemical tests which include motility test, coagulase, catalase, citrate utilization, methyl red voges proskaner and indole tests.

Motility test:The motility medium was inoculated by making a fine stab with a straight wire containing the isolates to a depth of 1-2 centimeters short of the bottom of the tube. The inoculated medium was then incubated at $37^{o}C$ for 24 – 48 hours. However, for motile organisms, the line of inoculation was not defined and the rest of the medium was somewhat cloudy. For non motile organisms, growth was restricted to the line of inoculation, which remained sharply defined, the rest of the medium remaining clear.

Coagulase test: Two drops of physiological saline about 2centimeterswere placed on a clean glass slide that was divided into two with grease pencil. A colony was carefully emulsified in each drop of saline after which a drop of citrated human plasma was added on one side and mixed. The side was held up and tilted back and forth for 1 minute.Clumpingof cells of bacterial suspension with plasma was regarded as a positive coagulase test.

Catalase test:**A** drop of 3% hydrogen peroxide was placed on a glass slide, a bit of growth from the slant was removed and placed on the drop of hydrogen peroxide. Bumbling and frothing indicated a positive catalase test.

Citrate utilization:A freshly prepared slant of Simmon's citrate agar was inoculated and put in a bijou bottle and incubated for 48 – 72 hours. The development of a deep blue color was recorded as a positive reaction.

Indole test:Each test organism was grown in 5ml peptone water for 24 hours after which Kovac's reagent was added (about 4 drops) and shaken. The development of a red color in the reagent layer above the broth within one minute was recorded as positive reaction.

Methyl-red VOGES-PROSKAUER (MR – VP) test:MR – VP broth was inoculated with the isolated colony and incubated at 37^{o}C for least 24 – 48 hours, after which 1ml of the broth was transferred into a bijou bottle, 2 – 3 drops of the methyl red was added to this quantity. A red color on addition of the indicator signifies a positive methyl red test. A yellow coloration signifies a negative result. 15 drops of 5% alphanaphthol in ethanol and 5 drops of 40% potassium hydroxide (KOH) were added to the rest of the broth in the original tube.The cap of the tube was loosened and placed in a sloppy position. The development of a red color starting from the liquid-air interphase, within one hour indicates VP positive test.Nocolor change signifies a negative VP test.

CHAPTER 4

RESULTS AND DISCUSSIONS

In this chapter, the results of the various investigations carried out in Chapter Threeare presented and discussed.

4.3 SOIL ANALYSIS

4.1.2 Analysis of Soil from Mechanic workshops

In this section the investigation into pollution in mechanic workshops is presented and discussed.

4.1.2.1 Enumeration of Mechanic Workshop for Assessment of Petroleum Hydrocarbon Contamination.

The data obtained from the enumeration of auto mechanic workshop is presented in Table 4.1.As shown in the table, a total of 129 mechanic workshops sites (where used engine oil isusually withdrawn and dumped into the soil) where identified. The shaded boxes indicate the type of mechanic workshop selected for soil analysis and the location.

Based on the distribution in Table 4.1, soil samples were collected from nine (9) motorcar mechanic workshops, nine (9) motorcycle mechanic workshops and seven (7) truck/lorry mechanic workshops. 3 motorcycle mechanic workshops were selected between Samaru and Kwagila, three (3) in Sabo and three (3) from Wusasa to Jos road. In the case of motorcar workshops, 3 workshops were selected between Samaru and Kwagila, 3 in Sabo and three (3) from Wusasa to Jos road andfor the trucks/lorries mechanic workshops one (1) was selected along Jos road and 6 between Wusasa and Kwagilla.

Table 4.2 presents the information obtained from the managers of the workshops on interview: The location of the various mechanic workshops is given as well as the age, possible sources of soil petroleum hydrocarbon contamination and number of times the workshop had been refilled since inception.

Table 4.1: Identification and enumeration of mechanic workshops in and around Zaria.

Location		DISTANCE (km)	MM	MC	HD
FROM	**TO**				
NUGA Gate	**Kwagilla**	7.59	19	5	1
Kwagilla	**Wusasa Bridge**	7.59	6	0	7
Wusasa	**Kofa-Doka**	3.58	10	7	0
Kofa-Doka	**MTD**	5.25	1	1	0
MTD	**Kwagilla**	1.47	8	2	0
MTD-thru Sabo	**PZ**	3.36	4	22	0
Kofa-Doka	**Jos road**	4.76	8	6	2
Shagalinku Junction	**Kongo Junction**	2.95	5	5	0
PZ	**KONGO**	2.80	6	4	0
TOTAL			67	52	10

MM is Motor mechanic workshop, MC is Motorcycle mechanic workshop, HD is Truck/lorry mechanic workshop.

Table 4.2: Description of mechanic workshops from which soil samples were obtained.

Designation	Location	Age of workshop	Petroleum hydrocarbon dumped on soil	Refilling	Comments
MC1	Bassawa road	11 years	Petrol, grease, engine oil	No refill	
MC2	Dogon Iche garage, Samaru	10yrs	"	No refill	
MC3	Bala's garage, Bassawa Rd, Samaru	28yrs	Petrol, grease, engine oil	No refill	Works generators – gasoline and diesel
MC4	Amo's garage, NTC roundabout, Sabo.	10yrs	Petrol, grease, engine oil	No refill	
MC5	Umar Farouk Garage, Aminu Rd, Tudun Wada	6yrs	Petrol, grease, engine oil	1 refill at inception	
MC6	Emma's workshop, Crescent str. Sabo	6 yrs	Petrol, grease, engine oil	No refill	
MC7	Bakin Makabarta, Jos Rd	6 yrs	Petrol, grease, engine oil	No refill	
MC8	Ban Zau Zau, Jos Rd	15yrs		Refills: marshy area	
MC9	Mikailu workshop, Jos Rd	2 yrs		No refill	
MM1	Olu motor mechanic garage, Danraka str. Samaru	27yrs	ATF, gear oil, grease, engine oil, petrol	No refill	
MM2	Oga Jimoh garage along Bassawa Rd, Palladan	38yrs	ATF, gear oil, grease, engine oil, petrol	No refill	
MM3	Kan Dutse workshop, Hanwa	25yrs	"	No refill	
MM4	Adaka Mugani Garage, Jos Rd.	22yrs	ATF, gear oil, grease, engine oil, petrol	No refill	
MM5	Mustpha garage, Jos Rd	5yrs	"	No refill	
MM6	Master Engineering workshop, Jos Rd	18 yrs	"	No refill	

Table 5.2 continued

MM7	Amita mechanic workshop, Wusasa Rd	4 yrs	"	No refill	
MM8	Ganuwa garage, Kofa Dokar, Wusasa Rd	20yrs	"	No refill	UEO is poured on the ground when there are no buyers
MM9	Alhaji Aminu Abdulazeez workshop, Dan Magaji	20 yrs	"	No refill	
HD1	Anchau filling station, Gwugi, Jos Rd.	13yrs	Engine oil, gear oil, diesel, hydraulic, grease	No refill	
HD2	Madarkachi Engineering Workshop , Jos Rd (tractor workshop)	18yrs	Gear, diesel, engine oil	No refill	
HD3	Two brother mechanic Engineering Workshop, Dan Magaji	7 yrs	"	No refill	
HD4	Tipper garage, Dan Magaji	3yrs	"	No refill	UEO is used in the tipping system
HD5	Nwansika mechanical construction company, Zaria-Kaduna Express way	35 yrs	Penetrating oil, gear oil, engine oil, hydraulic	No refill	
HD6	Dan Mallam garage, Zaria-Kaduna express way	30yrs	"	No refill	
HD7	Garage Alhaji Saleh Turu, Dan Magaji	15 yrs	"	No refill	
			"		

MC 1 - 9: 1st to 9th motorcycle mechanic workshop, MM 1 -9: 1st to 9th motorcar mechanic workshop, HD 1 – 7: 1st to 7th truck/lorry mechanic workshop

4.1.2.2 Elemental Composition of Soil Samples

The elemental analysis of the soil samples collected from the various mechanic workshops are presented in Table 4.3, 4.4 and 4.5. In Table 4.6, the mechanic workshop soil samples were grouped by age, X, of the mechanic workshop in years. The average composition was computed for the various age categories.The concentration of heavy metals in the soil samples are generally higher than typical safe limit levels for leafy or root vegetables as well as for gardens and children contact **(http://www.*al-labs-eastern.com*, 2011)**. The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be naturally degraded like organic pollutants and have the tendency to persist in the ecosystem or accumulated in different parts of the food chain **(Igwe *et al*., 2005 and Smejkalova *et al*., 2003)**. Thus when UEO contaminated soil is considered for remediation, the heavy metal content becomes an issue. The oxides of certain elements such as Re, Eu, V,and K did not show any appreciable change. Using Two-Way ANOVA, at $\alpha = 0.05$, the *P*-values for effect of age and type of mechanic workshop on the increase in heavy metal content (from Ti through to Nb in Table 4.3) were found not to be significant (0.12 and 0.23 respectively).

The heavy metal contents of the soils from mechanic workshops generally were found to be above the optimum heavy metal content (see Table 2.2) recommended for bioremediation. It is thus important to take the heavy metal content of these soils into consideration when bioremediation is envisaged.

Table 2.6 presents the Nigeria's soil quality regulatory limits for chemicals, pharmaceuticals, soaps and detergents sectors (National Environmental Regulation, 2009).

Table 4.3: Elemental analysis of UEO contaminated soil from motorcar mechanic workshops.

	MM1	MM2	MM3	MM4	MM5	MM6	MM7	MM8	MM9
					% Composition				
Al_2O_3	11.00	12.00	10.00	14.00	11.00	0.00	15.00	12.00	0.00
As_2O_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BaO	0.25	0.34	0.27	0.10	0.17	0.17	0.10	0.10	0.24
CaO	8.11	4.08	6.93	2.64	5.69	4.17	3.91	8.75	4.93
Cr_2O_3	0.075	0.10	0.07	0.06	0.06	0.06	0.09	0.07	0.07
CuO	0.22	0.22	0.17	0.13	0.15	0.17	0.17	0.13	0.19
Eu_2O_3	0.00	0.00	0.07	0.10	0.00	0.00	0.00	0.00	0.09
Fe_2O_3	14.82	19.42	12.50	14.05	11.80	11.20	21.58	17.60	13.2
K_2O	3.74	3.95	5.20	3.49	4.95	5.77	2.29	3.37	5.06
MnO	0.11	0.19	0.12	0.09	0.16	0.10	0.16	0.11	0.15
Nb_2O_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NiO	0.02	0.03	0.02	0.02	0.03	0.03	0.03	0.02	0.00
OsO_4	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
PbO	0.4	0.94	0.41	0.14	0.12	0.22	0.35	0.23	0.29
Rb_2O	0.07	0.09	0.08	0.04	0.07	0.08	0.05	0.06	0.09
Re_2O_7	0.00	0.00	0.05	0.01	0.03	0.02	0.01	0.04	0.00
RuO_2	0.96	1.20	0.95	0.61	0.87	0.90	0.68	0.90	0.92
SiO_2	56.50	52.80	59.10	61.60	60.70	72.90	51.30	53.40	70.2
SO_3	1.00	1.60	0.96	0.88	1.50	1.40	1.10	0.83	1.30
SrO	0.09	0.07	0.07	0.03	0.07	0.06	0.04	0.05	0.06
ThO_2	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.12	0.15
TiO_2	1.75	1.84	1.75	1.46	1.81	1.74	1.80	1.54	1.90
V_2O_5	0.055	0.06	0.04	0.05	0.05	0.05	0.08	0.06	0.06
Y_2O_3	0.06	0.06	0.05	0.04	0.06	0.06	0.05	0.05	0.06
Yb_2O_3	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00
ZnO	0.35	0.68	0.24	0.16	0.21	0.25	0.20	0.26	0.38
ZrO_2	0.56	0.54	0.59	0.47	0.64	0.61	0.63	0.43	0.71

MM 1 -9: 1st to 9th motorcar mechanic workshop

Table 4.4: Elemental analysis of UEO contaminated soil from motorcycle mechanic workshops.

	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8	MC9
	% Composition								
Al_2O_3	12.00	0.00	11.00	13.00	12.00	9.70	12.00	15.00	12.00
As_2O_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BaO	0.10	0.24	0.10	0.10	0.10	0.20	0.20	0.21	0.23
CaO	6.29	6.93	3.61	2.79	3.86	8.35	4.18	6.65	5.15
Cr_2O_3	0.08	0.12	0.08	0.08	0.07	0.10	0.11	0.06	0.08
CuO	0.11	0.17	0.18	0.16	0.14	0.18	0.21	0.10	0.13
Eu_2O_3	0.00	0.00	0.1	0.00	0.08	0.00	0.00	0.00	0.06
Fe_2O_3	11.50	17.20	17.82	18.21	12.24	21.90	21.89	13.30	23.27
K_2O	5.50	4.77	3.51	4.19	3.22	3.93	4.15	5.14	5.50
MnO	0.15	0.19	0.15	0.13	0.16	0.19	0.21	0.14	0.17
Nb_2O_5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
NiO	0.06	0.09	0.13	0.04	0.03	0.05	0.05	0.04	0.05
OsO_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PbO	0.09	0.19	0.20	0.33	0.12	2.11	0.16	0.00	0.14
Rb_2O	0.10	0.15	0.08	0.14	0.04	0.12	0.13	0.12	0.17
Re_2O_7	0.00	0.09	0.02	0.00	0.00	0.00	0.00	0.02	0.00
RuO_2	1.74	2.77	1.30	1.76	0.75	2.30	2.11	1.48	1.90
SiO_2	56.50	61.90	57.4	54.70	61.50	46.4	50.40	53.60	47.50
SO_3	2.30	1.60	1.50	0.10	3.50	1.00	1.00	1.60	0.60
SrO	0.11	0.15	0.06	0.09	0.04	0.15	0.11	0.14	0.14
ThO_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TiO_2	1.69	1.64	1.29	1.99	1.53	1.60	1.69	1.50	1.55
V_2O_5	0.05	0.05	0.06	0.08	0.05	0.06	0.07	0.04	0.05
Y_2O_3	0.10	0.15	0.08	0.12	0.05	0.14	0.12	0.10	0.12
Yb_2O_3	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00
ZnO	0.33	0.41	0.23	0.40	0.29	0.50	0.47	0.30	0.43
ZrO_2	0.96	1.18	0.74	1.03	0.45	1.03	1.02	0.81	0.75

MC 1 - 9: 1st to 9th motorcycle mechanic workshop

Table 4.5: Elemental analysis of UEO contaminated soil from truck/lorry mechanic workshops.

	HD1	HD2	HD3	HD4	HD5	HD6	HD7
	% Composition						
Al_2O_3	0.00	0.00	0.00	10.00	13.00	0.00	12.00
As_2O_3	0.00	0.00	0.01	0.00	0.00	0.00	0.00
BaO	0.25	0.42	0.20	0.23	0.17	0.43	0.30
CaO	3.45	6.99	2.42	3.69	4.90	5.56	4.21
Cr_2O_3	0.13	0.09	0.08	0.10	0.09	0.11	0.13
CuO	0.17	0.29	0.32	0.19	0.21	0.625	0.20
Eu_2O_3	0.00	0.10	0.00	0.10	0.00	0.00	0.10
Fe_2O_3	17.80	20.30	10.50	22.67	18.83	21.1	22.42
K_2O	6.90	7.28	5.71	3.49	2.67	5.96	3.74
MnO	0.11	0.21	0.09	0.19	0.11	0.2	0.12
Nb_2O_5	0.00	0.00	0.00	0.00	0.00	0.084	0.00
NiO	0.05	0.04	0.05	0.04	0.03	0.077	0.02
OsO_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PbO	0.19	0.32	0.20	0.42	0.17	0.62	0.28
Rb_2O	0.23	0.00	0.19	0.14	0.08	0.21	0.11
Re_2O_7	0.03	0.04	0.03	0.03	0.03	0.07	0.00
RuO_2	3.13	2.99	2.05	2.20	1.56	3.14	1.50
SiO_2	62.60	55.80	74.20	51.90	55.00	55.4	50.50
SO_3	0.60	1.20	0.80	0.60	0.89	1.9	1.80
SrO	0.12	0.17	0.10	0.10	0.06	0.15	0.09
ThO_2	0.20	0.00	0.00	0.00	0.00	0.00	0.00
TiO_2	2.27	2.13	1.83	1.85	1.38	2.13	1.56
V_2O_5	0.09	0.07	0.06	0.05	0.06	0.075	0.06
Y_2O_3	0.18	0.21	0.12	0.13	0.09	0.17	0.09
Yb_2O_3	0.00	0.00	0.00	0.00	0.01	0.00	0.03
ZnO	0.21	0.41	0.18	0.34	0.25	0.49	0.37
ZrO_2	1.29	0.95	0.90	1.15	0.80	1.49	0.75

HD 1 – 7: 1st to 7th truck/lorry mechanic workshop

Table 4.6: Elemental Analysis of used engine oil contaminated soil from mechanic workshops

	MC			MM			HD		
	% Composition								
Elements									
	X≤10	**10<X≤20**	**20<X**	**X≤10**	**10<X≤20**	**X<20**	**X≤10**	**10<X≤20**	**20<X**
Al	5.175	7.143	5.820	6.878	2.116	6.217	2.646	1.587	3.439
S	0.520	0.780	0.600	0.520	0.472	0.444	0.280	0.360	0.560
Ba	0.161	0.143	0.090	0.125	0.152	0.215	0.197	0.215	0.269
Ca	3.724	4.625	2.580	3.431	4.253	3.889	2.187	2.616	3.738
Cr	0.062	0.048	0.055	0.055	0.048	0.055	0.062	0.062	0.068
Cu	0.136	0.080	0.080	0.128	0.128	0.152	0.208	0.136	0.336
Eu	0.009	0.000	0.043	0.000	0.013	0.017	0.022	0.022	0.000
Fe	13.361	8.665	12.453	11.663	9.783	10.622	11.593	10.573	13.955
K	3.560	4.415	2.913	3.004	3.925	3.402	3.818	3.718	3.585
Mn	0.139	0.116	0.116	0.124	0.093	0.101	0.108	0.085	0.124
Nb	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014
Ni	0.039	0.039	0.102	0.024	0.016	0.016	0.031	0.0236	0.039
Os	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pb	0.473	0.046	0.186	0.223	0.232	0.436	0.288	0.186	0.371
Rb	0.112	0.101	0.074	0.055	0.064	0.064	0.156	0.0828	0.129
Re	0.015	0.008	0.015	0.015	0.015	0.015	0.023	0.015	0.038
Ru	1.666	1.390	1.122	0.673	0.786	0.803	1.839	1.649	2.029
Si	25.097	25.714	26.811	26.158	30.595	26.858	29.451	19.726	25.784
Sr	0.093	0.110	0.051	0.042	0.051	0.051	0.085	0.085	0.093
Th	0.000	0.000	0.000	0.272	0.0791	0.000	0.000	0.044	0.000
Ti	1.001	0.959	0.773	1.085	1.037	1.019	1.103	0.892	1.0546
V	0.033	0.0224	0.0336	0.034	0.034	0.028	0.028	0.034	0.039
Y	0.094	0.079	0.063	0.047	0.047	0.047	0.102	0.094	0.102
Yb	0.009	0.000	0.000	0.000	0.000	0.009	0.000	0.009	0.009
Zn	0.337	0.249	0.185	0.169	0.241	0.289	0.209	0.201	0.297
Zr	0.674	0.659	0.548	0.474	0.429	0.340	0.755	0.555	0.851

(**X:** the age of the workshop (in years)

Comparison of Tables 4.6 and 2.6 indicated that apart from As, Cd, Co, Hg and Mo which fell below detection level, all other metals in the mechanic workshop soil were found to be in excess of the Nigerian recommended limits. The Al_2O_3 content of some of the soil samples fell below

detection (MM6, MC2, HD1 etc). This is unexpected as Al is a normally a component of soil mineral. This observed absence of Al may be as a result of alteration of the composition of the top soil by physical or chemical process resulting from activities in the mechanic workshop (for example land filling, battery-acid leaching, etc).

4.1.1.3 Other Physicochemical Properties of Soil from Mechanic Workshop

Table 4.7 presents other physicochemical properties of contaminated soil from mechanic workshop.

Soil textural class:Soil textural analysis indicated that 11.1% of the soil samples for MC were found to be sandy, 55.6% loamy sand and 33.3% were sandy loams. 85.7% of the soil samples for MM were found to be sandy while the remaining 14.3% was found to be loamy sand while 88.9% of the soil samples for HD were found to be sandy and the remaining 11.1% was found to be loamy sand.These suggest that there is a relationship between the mechanic workshop type and the texture of the soil. A greater percentage of the soil samples collected from MM and HD (85.7 and 88.9% respectively) were sandy. The sandy nature of the oil contaminated soils is in agreement with the findings of **Al-Sanad *et al.* (1995)** and **Ijimdiya (2010)** that oil contamination leads to a decrease in fine fractions. The most probable reason for this could be the fact that the oil affected the physic-chemical characteristics of the soil particularly the fine fractions. The observed soil textural class may however be the nature of the soil in and around the workshops even before contamination.There was no direct relationship between the textural class of soil sample and the age of the workshop.

Table 4.7: Physicochemical properties and microbial count of soil from mechanic workshops.

Designation	% C	% N	%P	C/N ratio (g/g)	TPH (g/kg)	Soil Textural class	Microbial count, cfu/kg ($*10^5$)
MC1	6.80	0.16	0.30	43.04	165.80	Loamy Sand	125.00
MC2	6.00	0.28	0.37	21.43	90.91	Loamy Sand	140.00
MC3	6.00	0.18	0.26	34.29	193.09	Loamy Sand	150.00
MC4	3.49	0.16	0.88	22.09	111.67	Sandy Loam	120.00
MC5	6.58	0.14	0.46	47.00	183.36	Sand	145.00
MC6	11.85	0.14	0.28	84.64	111.12	Sandy Loam	105.00
MC7	8.48	0.23	0.23	37.19	93.92	Loamy Sand	150.00
MC8	8.08	0.12	0.37	65.69	145.62	Sandy Loam	130.00
MC9	9.04	0.16	0.21	57.21	77.87	Loamy Sand	135.00
MM1	5.44	0.18	0.35	31.09	96.38	Loamy Sand	143.00
MM2	10.81	0.14	0.42	77.21	137.74	Sand	142.00
MM3	4.45	0.16	0.33	28.17	101.60	Sand	145.00
MM4	5.00	0.12	0.25	40.65	70.31	Sand	144.00
MM5	6.00	0.14	0.30	42.86	103.07	Sand	143.00
MM6	4.65	0.16	0.28	29.43	63.80	Sand	135.00
MM7	5.79	0.12	0.23	47.07	89.78	Sand	130.00
MM8	5.40	0.11	0.18	51.43	74.41	Sand	120.00
MM9	6.38	0.19	0.40	33.06	133.41	Sand	120.00
HD1	8.52	0.18	0.18	48.69	84.19	Sand	135.00
HD2	9.04	0.12	0.18	73.50	99.56	Sand	110.00
HD3	6.38	0.11	0.33	60.76	92.30	Loamy Sand	80.00
HD4	5.20	0.11	0.23	49.52	130.34	Sand	140.00
HD5	4.69	0.12	0.19	38.13	84.50	Sand	143.00
HD6	11.53	0.16	0.19	72.98	120.62	Sand	120.00
HD7	10.29	0.12	0.29	83.66	127.99	Sand	140.00

MC 1 - 9: 1st to 9th motorcycle mechanic workshop, MM 1 -9: 1st to 9th motorcar mechanic workshop, HD 1 – 7: 1st to 7th truck/lorry mechanic workshop

Earthworms generally prefer loamy soils **(Delate *et al.*, 2003)** therefore, in the bioremediation of UEO contaminated soil from mechanic workshops using earthworms, the contaminated soil may have to be amended with appropriate quantities of loam or clay soil so as to make the soil better suited for earthworm survival.

All samples irrespective of mechanic workshop type recorded similar microbial counts falling within the range 80 x 10^5 cfu/g – 150 x 10^5 cfu/g.

4.1.1.4 Microbial count

The mean values of the microbial count from the various types of mechanic workshops are presented in Table 4.8. According to **Granatstein and Bezdicek (2003)** there needs to be at least a 10-fold difference in microbial number for changes in microbial count to be considered meaningful because of variability in measuring organisms. Table 4.8 therefore shows that MM, MC and HD have similar number of bacteria and would perform the same if all else was equal.

Table 4.8: Microbial count of UEO contaminated soil from mechanic workshops.

	MC	MM	HD
Microbial count, cfu/g * 10^5	133.33± 15.00	135.78± 10.17	124.00 ± 22.88

MM: motor mechanic workshop, MC: motorcycle mechanic workshop, HD: truck/lorry mechanic workshop

Healthy soils will have a Microbe count of 500-800 million per gram of soil, whereas soils that are near sterile conditions will have a count of 4 million microbes per gram**(http://www.microlifefertilizer.com/resource.htm)**. The mean values of the microbial counts of Table 4.8 indicate that the soils in mechanic workshops are a far cry from being healthy, having bacteria counts 30 times less than the values considered healthy.

4.1.1.5 Total Petroleum Hydrocarbon

The mean values for TPH for the various types of mechanic workshops are presented in Table 4.9. The TPH of all soil samples from mechanic workshops were found to be high: the value

exceeds the safe limit of 500 mg/kg set by the Nigeria Ministry of the Environment and therefore, needs intervention for public safety and environmental health**(Abdulsalam *et al*., 2011).** It can be seen that MC has the highest TPH. This may be as a result of (1) the soil type: loamy soils have a higher holding capacity than sandy soil as such oil will not drain through easily: textural classification of the soil from the mechanic workshops showed that the soil samples from MM and HD workshops were predominantly sandy whereas the MC were mostly loamy sand or sandy loam, (2) that the UEO from motorcycles finds less use compared to those from motorcars and trucks/lorries and are thus wholly dumped oil the soil.

Table 4.9: Total petroleum hydrocarbon of UEO contaminated soil from mechanic workshops.

	MC	MM	HD	UCS
TPH, g/kg	130.37 ± 42.71	96.72 ± 26.03	105.64 ± 20.23	0.00±0.00

MM: motor mechanic workshop, MC: motorcycle mechanic workshop, HD: truck/lorry mechanic workshop UCS: uncontaminated soil

4.1.1.6 Carbon/Nitrogen Ratio

The mean values for carbon/nitrogen ratio for soil samples collected from mechanic workshop (MC, MM and HD) and an uncontaminated soil sample (UCS) is presented in Table 4.10.

The resultsshow that all contaminated soil samples had a C/N at least three times greater than that of the uncontaminated soil sample suggesting that the activities at the mechanic workshop must have increased the carbon content of the soil. The table also shows that the soil samples are grossly deficient in Nitrogen hence the large C/N ratio. It has been reported that a high C/N ratio

stimulates increased microbial activity and that growth in microbial population is limited by lack of nitrogen **(Sullivan, 2004)**. Therefore, C/N of the soil samples is not unconnected with the effect of the oil contamination on microbial count herein reported: HD which has the highest C/N ratio of 61.03 and also the least microbial count 124.00 (Table 4.8).

Table 4.10: The carbon/nitrogen ratio of soil samples from mechanic workshop

	MC	MM	HD	UCS
C:N ratio	45.84 ± 20.64	42.330 ± 15.43	61.03 ± 16.43	12.00 ± 0.00

MM: motor mechanic workshop, MC: motorcycle mechanic workshop, HD: truck/lorry mechanic workshop

4.1.4 Analysis of Uncontaminated Soil Sample

In this section,the result of the analysis of soil sample from a farmland (largely expected to be free of petroleum hydrocarbon contamination) is presented and discussed.

4.1.2.1 Elemental Analysis

The elemental analysis of the petroleum hydrocarbon-uncontaminated soil considered is presented in Table 4.11. Comparison of Tables 4.6 and 4.11 shows that irrespective of type of mechanic workshop (MM, MC or HD) there is a general increase in the % concentration of heavy metal in the soil samples when compared to the values for uncontaminated soil sample (UCS). Comparison with Table 2.6 showed that most of the regulated metals were within safe limits. It could be noted that Pb, Hg and As fell below detection, while Cr and Cu contents of the soil were higher than the set limits in Table 2.6. It is important to note that EDXRF analysis

gives the net elemental composition as distinguished from AAS which gives a better indication of the fraction of the metal bioavailable.

Table 4.11: Elemental analysis of petroleum hydrocarbon-free Soil

Elements	% composition
Al	6.349
Ba	0.000
Ca	0.293
Cr	0.021
Cu	0.064
Eu	0.022
Fe	3.291
K	3.469
Mn	0.046
Nb	0.000
Ni	0.008
Os	0.015
Pb	0.000
Rb	0.046
Re	0.023
Ru	0.423
S	0.084
Si	35.500
Sr	0.025
Th	0.000
Ti	0.929
V	0.028
Y	0.031
Yb	0.000
Zn	0.008
Zr	0.281

4.1.2.2 Physicochemical properties of uncontaminated soil sample

Other physicochemical properties of the uncontaminated soil sample used are presented in Table 4.12. The soil textural class was determined to be silt-loam. This texture favors earthworm

(*Eudrilus eugeniae*) habitation **(Oboh *et al.*, 2007).**The corresponding water holding capacity of the soil was found to be 0.41gwater/g dry soil. The pH indicated the soil was very slightly acidic.

Table 4.12: Physicochemical properties of uncontaminated soil sample.

Properties	Soil
% Nitrogen	0.11
% Carbon	1.26
% Phosphorus	1.68
pH	6.7
Textural class	Silt Loam
WHC (g/g bone dry soil)	0.41

4.1.5 Analysis of SimulatedUsed Engine Oil Contaminated Soil

In this section, the result of the analysis of simulated used engine contaminated soil is presented and discussed.

4.1.5.1 Elemental Analysis

Table 4.13 shows that the concentration of used engine oil did not significantly affect the elemental composition of the mixture as contamination level was increased from 25 to 100 gUEO/kg soil.

Table 4.13: Elemental Analysis of simulated used engine oil contaminated soil.

	Used Engine Concentration (g UEO/kg soil)				
	12.5	**25**	**50**	**75**	**100**
Al_2O_3	14.4	11.6	11.6	11	13.1
SiO_2	70.6	67.4	68.2	65.9	65.7
P_2O_5	0.67	0.43			0.41
SO_3	0.2	0.26	0.2	0.2	0.71
K_2O	4.06	5.08	5.19	5.4	4.83
CaO	0.558	0.599	0.561	0.6	0.754
TiO_2	1.43	1.87	1.78	2	1.97
V_2O_5	0.07	0.11	0.095	0.091	0.093
Cr_2O_3	0.044	0.08	0.069	0.11	0.06
MnO	0.077	0.077	0.071	0.073	0.096
Fe_2O_3	7.444	10.93	10.32	12.1	11.27
NiO	0.013	0.042	0.039	0.064	0.029
CuO	0.014	0.053	0.051	0.079	0.036
ZnO	0.004	0.037	0.042	0.054	0.042
Ga_2O_3	0.006	0.04		0.04	0.039
MoO_3	0		0.2	0.6	
Rh_2O_7	0.11	0.8	0.79	1.1	0.46
BaO		0.37	0.43	0.52	0.29
Re_2O_7		0.1	0.1	0.1	0.16
PtO_2		0.04		0.02	
IrO_2	0.13		0.24		
Au	0.11				

(the blank spaces signify 'not detected')

Comparison of Tables 4.13 and 4.6 shows that the metal content of soils from mechanic workshops were higher than the simulated contaminated soil. This may suggests that the elevated metals contamination contents of soil obtained from mechanic workshop may be as a result of continuous dumping of used engine oil over a period of time or may be as a result of other activities taking place in the mechanic workshop.

4.2 CHARACTERIZATION OF ORGANIC AMENDMENT AGENTS AND IDENTIFICATION OF EARTHWORM SPECIES USED

8.2.1 Analysis of Organic Amendment Agents

The moringa leaves were identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria, as *moringa oleifera* of the family *moringaleae*.The carbon, nitrogen and phosphorus contents of the various materials poultry manure, cow dung, *moringa oleifera* leaves and cassava peels are presented in Table 4.14. The properties of the manures (poultry manure and cow dung) were found to be consistent with values obtained from literature **(Marsh and Campling, 1970 and Amanullah, 2007)**. Nitrogen content of *moringa oleifera* (3.59%) leaves fell within the range 3.064 – 4.608% reported by **Jongrungruangchok *et al.*, (2010)**.The composition of the cassava peels is similar to reported literature values **(Yoonan and Kongkiattikajorn, 2004)**.

Table 4.14: Carbon, Nitrogen and phosphorus content of materials used as organic amendment.

Properties	Cow dung	Poultry manure	*M. oliefera* leaves	Fermented Cassava peels
% Nitrogen	1.14	2.98	3.59	0.74
% Carbon	56.65	45.58	37.67	48.84
% Phosphorus	1.87	1.90	1.85	0.07

4.2.2 Identification of Earthworms Used

The worms collected for this work were identified in the Department of Biological Sciences, Landmark University, Kwara State, Nigeria as *Eudrilus eugeniae*.

4.3 EFFECT OF ORGANIC AMENDMENT ON THE PROPERTIES OF USED ENGINE OIL CONTAMINATED SOIL

4.3.1 Bioremediation Using Manure

In this section, investigation into the effect of organic amendment on the degradation of petroleum hydrocarbon in mechanic workshop contaminated soil and soil from laboratory simulated contamination is presented and discussed.

4.3.1.1 Biostimulation Using Cattle Dung

The Total Petroleum Hydrocarbon (TPH) versus time data is presented in the linearized first order kinetic form inFigures 4.1and 4.2. The first order kinetic model is given as:

$$\boldsymbol{Y = ae^{-kt}} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \quad \mathbf{(4.1)}$$

On linearization this gives:

$$\boldsymbol{ln(Y) = ln(a) - kt} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \quad \mathbf{(4.2)}$$

or,

$$\boldsymbol{ln\left(\frac{a}{Y}\right) = kt} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \quad \mathbf{(4.3)}$$

Where:

t = bioremediation time (weeks)

Y = the TPH (g/kg) at various t

a = the initial TPH (g/kg)

The first order kinetics as stated in **Section 2.7** has been reported to give good fits to bioremediation kinetics. The R^2 values for the liberalized first order model generally > 0.9 indicated a good fit. The *k* values, as shown in Tables 4.15 and 4.16were found to increase with increase in surface area of soil directly open to air/weight of soil ratio (a_E) as the only parameter that was varied for the pair of samples shown in both table was the surface a_E.Increase in surface area translates to increased aeration of the soil or generally, reduction in the mass transfer resistance, which is the limiting step in bioremediation. Comparison of Tables 4.15 and 4.16showsthat the rate of bioremediation are seemingly higher for the soil samples obtained from mechanic workshop than for those contaminated in the laboratory. This was probably as a result of better acclimatization of the microbes as they had been in contact with used engine oil for a longer period (the mechanic workshop had been in existence for 10 years) than the soil samples contaminated in the laboratory.This acclimatization would result in the reduction or possible elimination of the lag phase (see Figure 2.2). However, since the a_E considered for the mechanic workshop soil were not the same with those used for the soil contaminated in the laboratory, a definite comparison cannot be made.

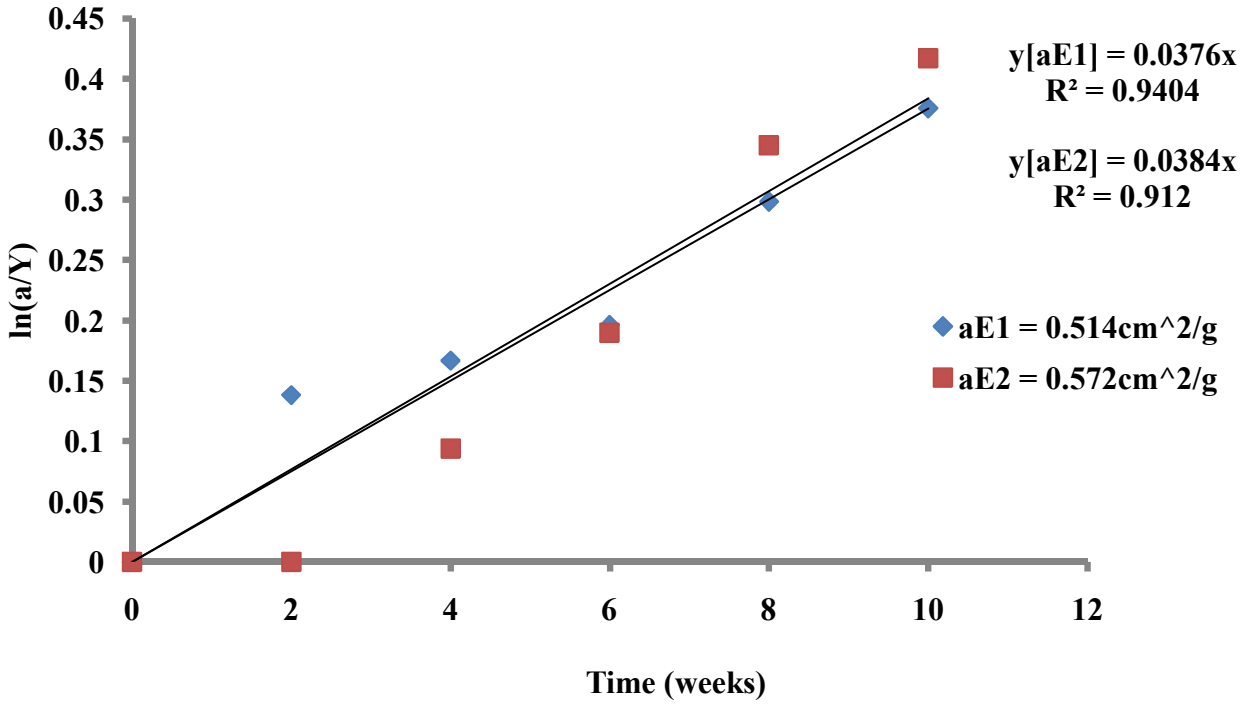


Figure 4.1: Bioremediation of UEO contaminated soil (from mechanic workshop) by biostimulating with cattle dung as a function of surface area

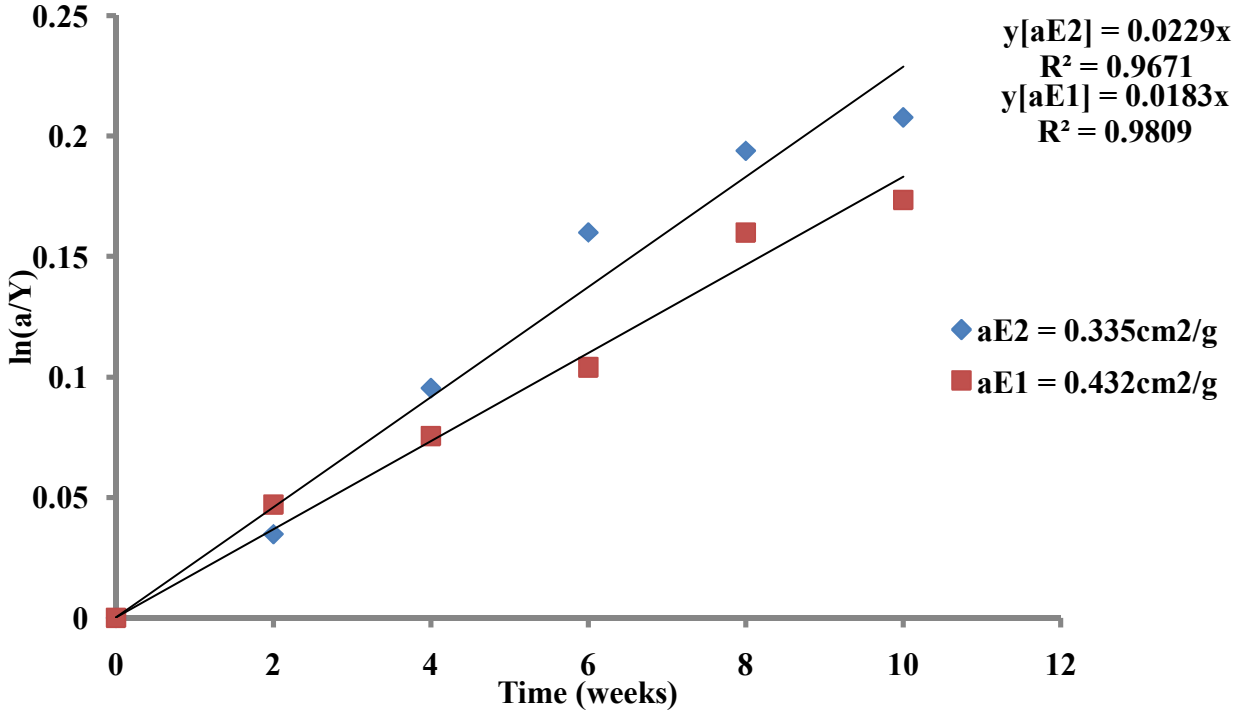


Figure 4.2: Bioremediation of UEO contaminated soil (laboratory simulated contaminated soil) by biostimulating with cattle dung as a function of surface area

Table 4.15: Effect of biostimulation (using cow dung) on bioremediation of UEO contaminated soil (contaminated soil obtained from mechanic workshop).

Biostimulant	*k*, week^{-1}	a_E, cm^2/g	R^2
Cow dung	0.0384	0.572	0.912
Cow dung	0.0376	0.514	0.9404

Table 4.16: Effect of biostimulation (using cow dung) on bioremediation of UEO contaminated soil (soil contaminated in the laboratory).

Biostimulant	*k*, week^{-1}	a_E, cm^2/g	R^2
Cow dung	0.0229	0.423	0.9671
Cow dung	0.0183	0.335	0.9809

4.3.1.2 Effect of Surface Area on bioremediation kinetics

Figure 4.3shows the results of the investigation into the effect of surface area on the rate of bioremediation. They are presented as a linearized form of the first order kinetics.The R^2 values for all samples indicate a good fit, being generally high (>0.959). The *k* values for the various surface areas (a_E) are presented in Table 4.17. The table shows that *k* increases with increasing a_E with a corresponding general increase in the drop in TPH value after 21 weeks also shown in Table 4.17. As a_E increased from 0.143 to 0.243cm^2/g, *k* increased from 0.023 to 0.033 per week. However with further increase in a_E, *k* remained fairly constant.This is suggestive of an optimum

a_E for bioremediation systems.The increase in *k* with increasing a_E may probably be due to greater availability of O_2 required (improved aeration) for aerobic degradation and release of biodegradationproducts, for example volatiles and CO_2 which may be undesirable for bioremediation. Since the major rate determining step in the bioremediation process is mass transfer (solubility of petroleum hydrocarbon in water), it follows that aeration is an important factor in bioremediation. This probably is as a result of enhanced biosurfactant production with improved aeration, in agreement with **Saharan *et al.*(2011)** who reported that aeration is an important parameter to be optimized in the production of biosurfactants. Microorganisms are known to produce biosurfactants which are substance that can reduce surface tension and facilitates the solubilization of oil in water thereby resulting in improved mass transfer.

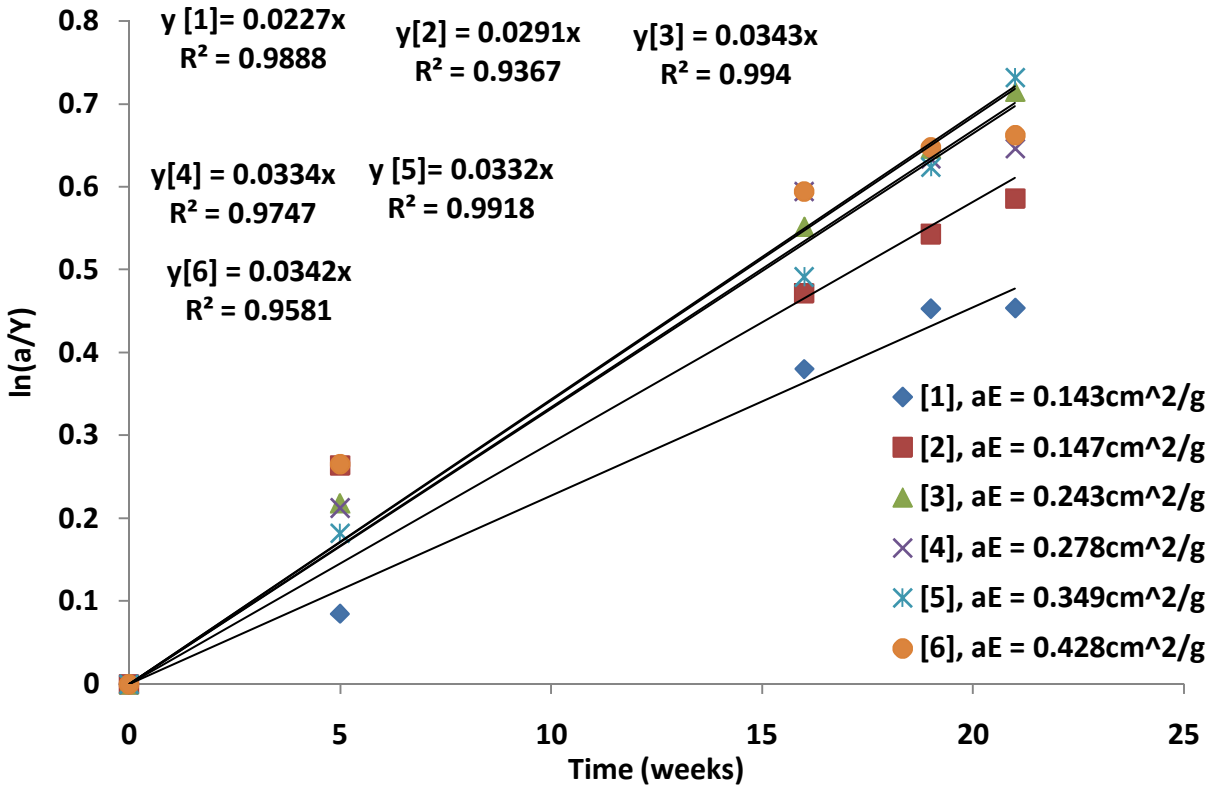


Figure 4.3: Effect of surface area on bioremediation kinetics of used oil contaminated soil

For the effect of surface area, the chromatogram (Figures 4.4 and 4.5) and the MS analysis presented are for the sample with the largest surface area 0.428cm^2/g at time 0 and 21 weeks (at the start and finish of the investigation). The TPH result of Table 4.17 for Sample 6 indicated a 48.44% drop and the GC-MS result of Figures 4.4 - 4.5 and Table 4.18 showed that by 21 weeks of bioremediation, a lot of peaks (corresponding to hydrocarbons) had disappeared from the sample and that all the hydrocarbons constituting C30 and above had been degraded whereas, the C9 – C19 and C19 – C29 fraction increased. Similar observation was reported by **Prince *et al.* (2007)**, who stated that within a complex mixed gasoline mixture; larger n-alkanes were more readily degraded than the smaller n-alkanes.

Table 4.17: Effect of surface area on the bioremediation kinetics.

Sample	a_E, cm^2/g	k, $week^{-1}$	% drop in TPH after 21 weeks
1	0.143	0.023	36.44
2	0.147	0.029	44.33
3	0.243	0.034	51.06
4	0.278	0.033	47.56
5	0.349	0.033	51.87
6	0.428	0.034	48.44

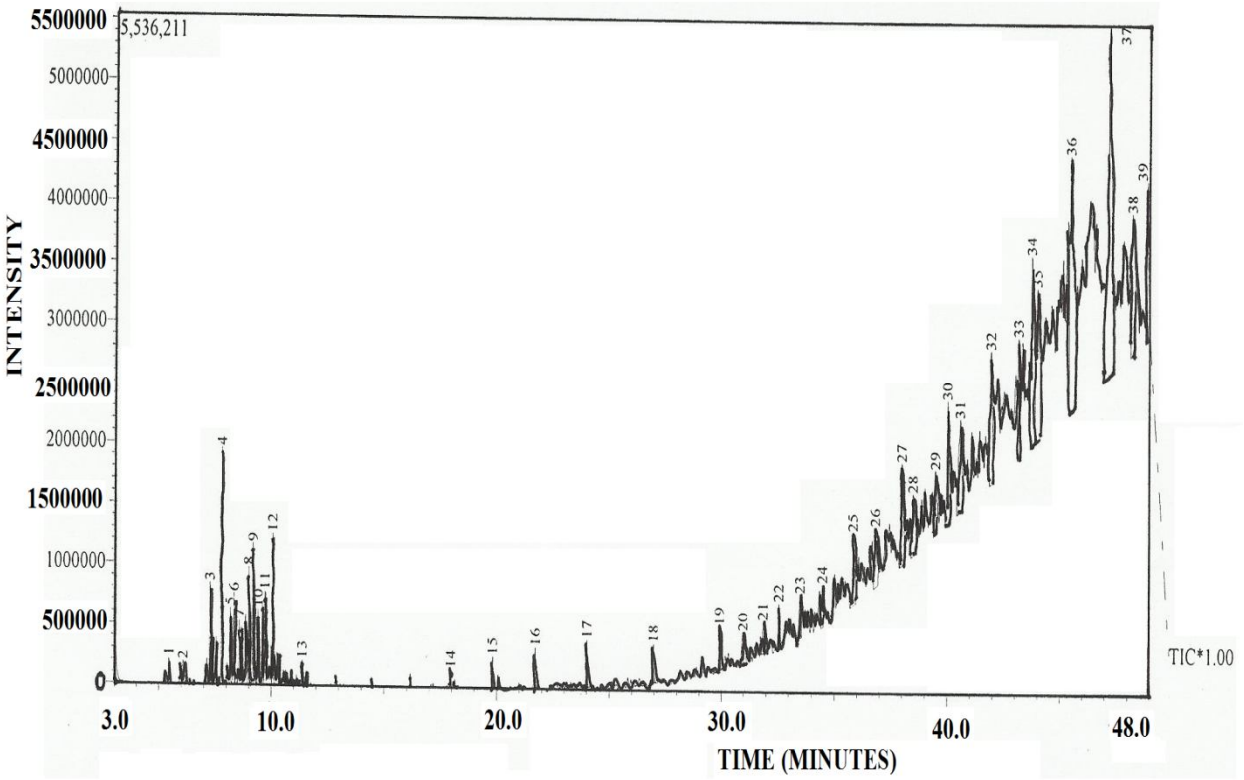


Figure 4.4: GC of extract from used engine oil contaminated soil at t = week 0

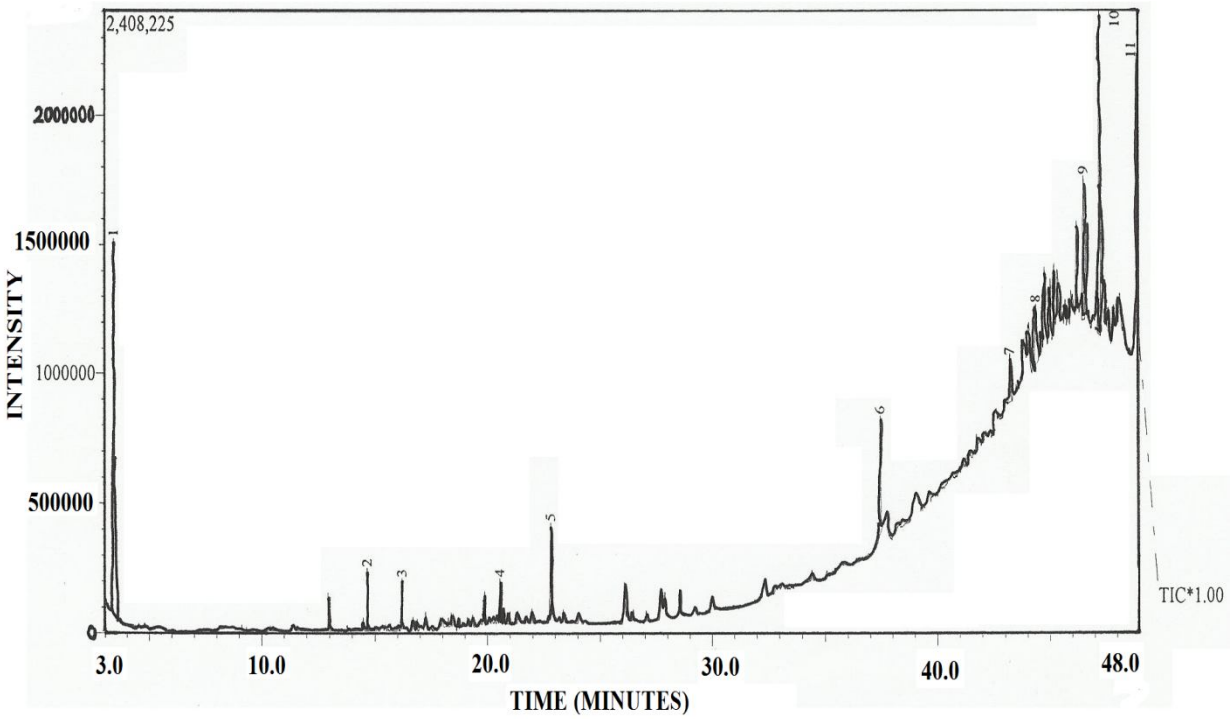


Figure 4.5: GC of extract from sample 6 (area = 0.428cm^2/g) at week 21

Table 4.18: Composition of used engine oil contaminated soil for sample with surface area of 0.428g/cm^2.

	Composition of UEO	
	0 weeks	21 weeks
Components		**Sample6**
C9 - C19	15.8	60.37
C20 - C29	26.42	39.62
C30 and Above	56.78	0

4.3.1.3 Effect of Mixing (agitation) on the rate of bioremediation

Figure 4.6 presents the result of the investigation into the effect of manual tilling on the bioremediation kinetics. The graph is also plotted in the form of the linearized first order kinetics. The values of *k* and % TPH drop after 22 weeks, as a function of degree of mixing, are shown in Table 4.19. The R^2 values for the samples amended with poultry manure (>0.977) were higher than those amended with *Moringa oleifera* leaves (<0.880). This may be attributed to the initial slow rate of the samples amended with*Moringa oleifera*leaves (the initial slow rate being probably as a result of the antimicrobial properties of *Moringa* leaves).

It is worthy of note that in spite of the antimicrobial properties of *Moringa oleifera* leaves **(Dahot, 1998; Chen, 2009)**, it was able to effect a higher degree of TPH removal than the control sample. Comparison of the *k* values and the drop in TPH values after 22 weeks indicated that mixing every three days (for 5min/day) had no significant effect on TPH removal. For poultry manure as well as *Moringa oleifera* leaves amended processes, both the mixed and unmixed samples had very similar *k* and TPH values. These results are in agreement with the findings of **Maike and Filser (2007)**. Though generally agreed that biosurfactant production

increases with stirring and aeration, instances have been reported where agitation has no or a negative influence on biosurfactant production. For example *N. erythropolis* and *A. calcoaceticus* produce less biosurfactant due to the increase ofshear stress (**Saharan *et al.*, 2011**).

The frequency of mixing used in this investigation was every three days (twice a week) for 5min/day. Examples of frequencies of mixing that have been used in bioremediation studies to improve aeration include: twice a month **(Bagherzadeh-Namazi *et al.*, 2008)**, twice a week **(Margesin *et al.*, 2000)**, every alternative day **(Vasudevan and Rajaram, 2001)** and every day **(Maike and Filser, 2007)**.

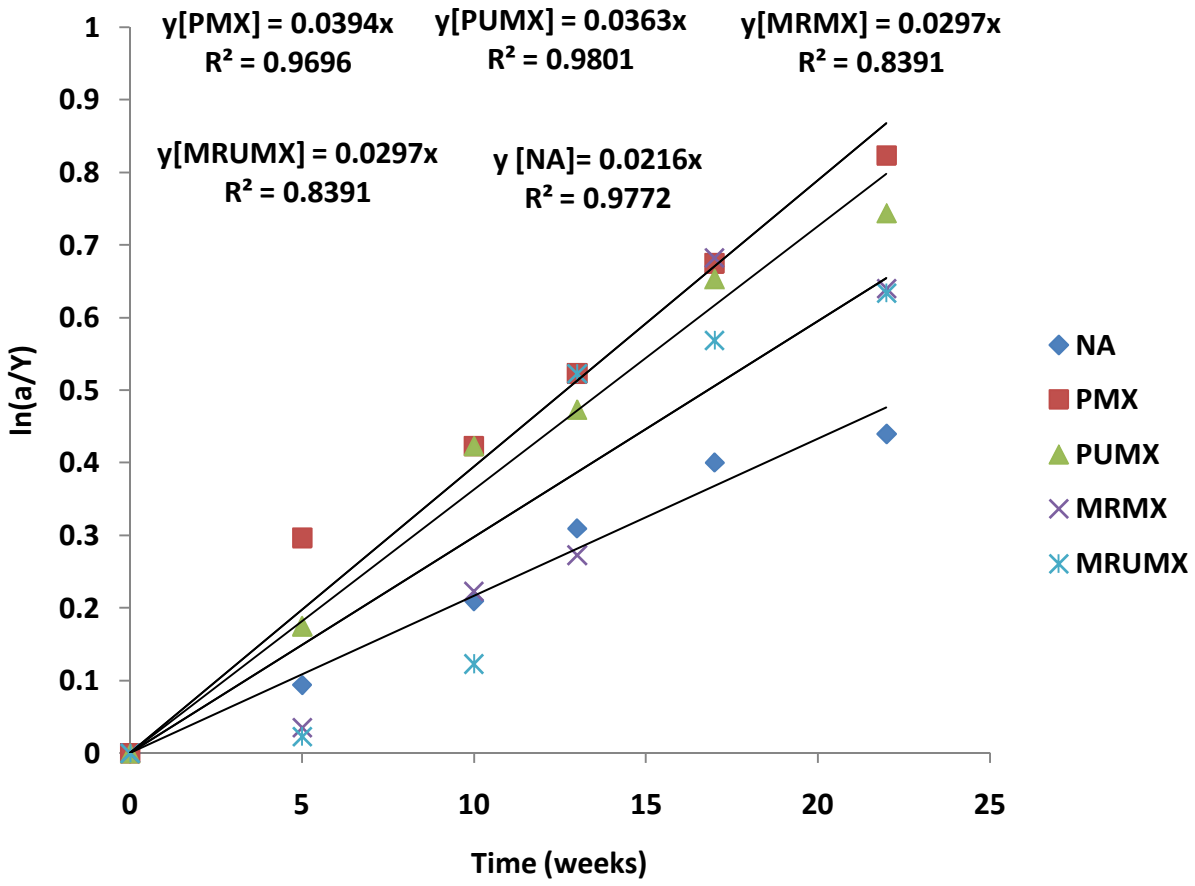


Figure 4.6: Effect of tilling on bioremediation kinetics of used engine oil contaminated soil

Table 4.19: Effect of mixing on the bioremediation kinetics.

Sample	a_E, cm^2/g	k, $week^{-1}$	% drop in TPH after 22 weeks
Control	0.428	0.022	35.59
PUMX	0.428	0.035	52.45
PMX	0.428	0.036	50.80
MRMX	0.428	0.034	47.26
MRUMX	0.428	0.035	46.93

PUMX: unmixed poultry manure treated, PMX: mixed poultry manure treated, MRMX: mixed *Moringa oleifera* treated and MRUMX: unmixed *Moringa oleifera* treated samples.

The chromatograms obtained from GC analysis for investigations into the effect of surface area and mixing on bioremediation are presented as Figures 4.7 to 4.9. The MS spectrum comparison gave the composition of oil in the soil at various times (t) tabulated in Table 4.20for the effect of mixing.

The GC chromatogram at the start of the investigation is the same as that for the studies into the effect of surface area (Figure 4.4). The result of Table 4.20 indicates that by 4 weeks of bioremediation there was a significant reduction in the C30 and above fraction of the oil for the samples treated with organic amendments. By 22 weeks, the C30 and above fractions had been completely removed for the sample treated with organic amendment (MRMX and PUMX) whereas the control sample (NA) still contained C30 and above fractions. However, the % composition has reduced from 56.78 at t = 0 weeks to 39.86 at t = 22 weeks. The result also indicated a significant reduction in C20 – C29 component for the PUMX (10.47%) than for MRMX (67.72%). Similarly, Table 4.19 indicated a higher reduction in TPH for PUMX (52.45%) than for MRMX (47.26%). Poultry manure may have performed better than *Moringa oleifera* leaves because of the antimicrobial properties of the latter.

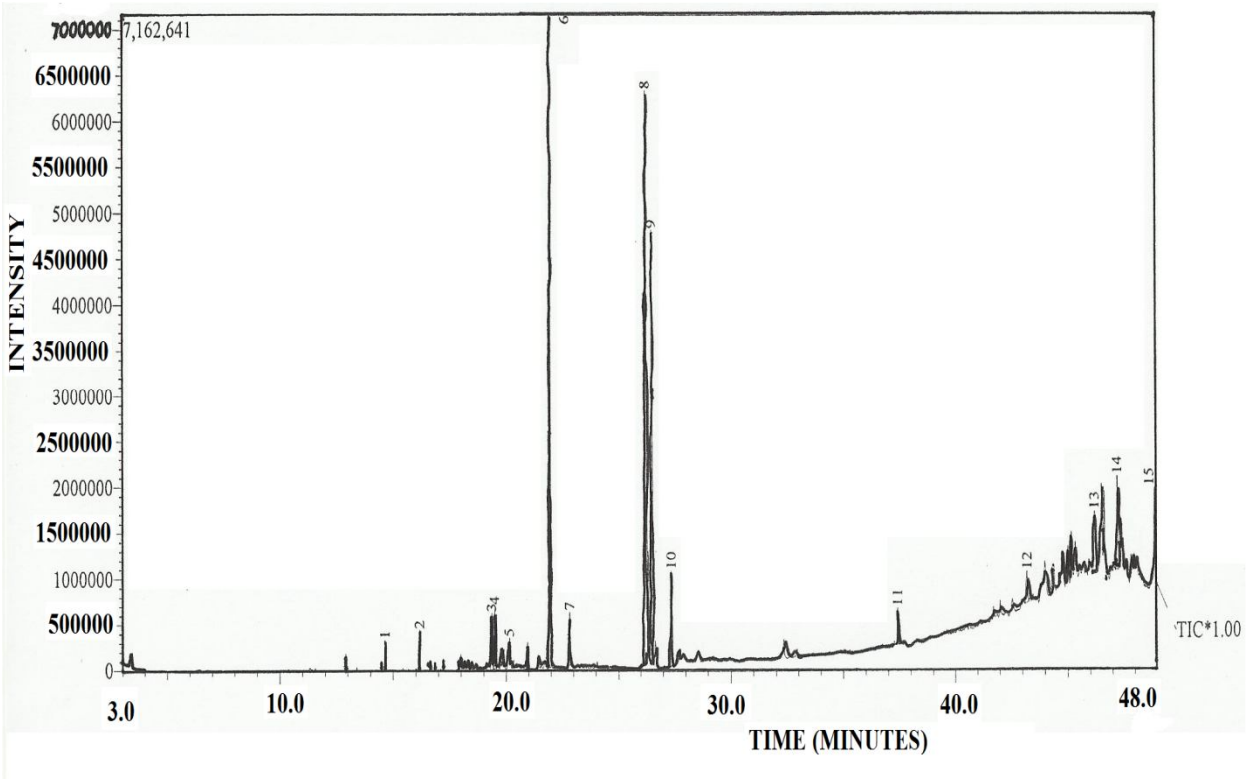


Figure 4.7: GC of extract from SAMPLE PUMX at week 22

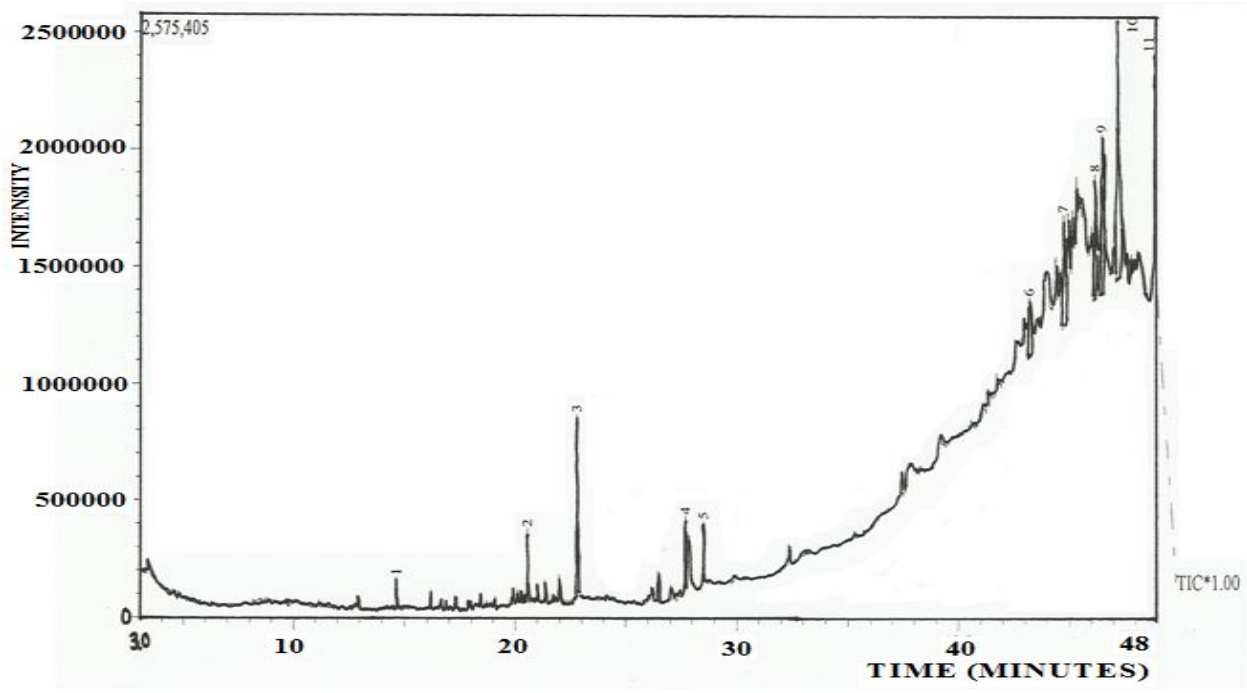


Figure 4.8: GC of extract from Sample MRMX at week 22

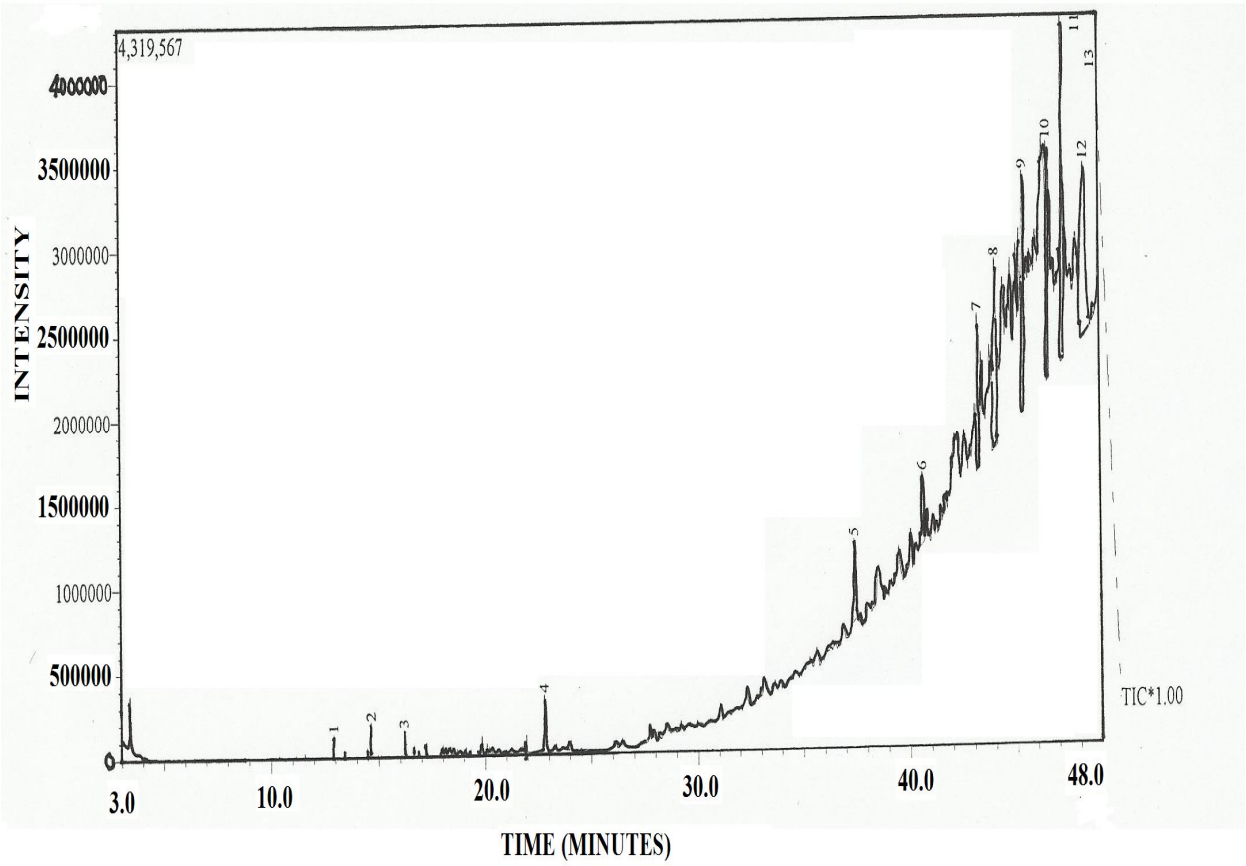


Figure 4.9: GC of extract from Sample NA at week 22

Table 4.20: Effect of mixing on the composition of UEO in bioremediation

	% COMPOSITION OF REMAINING PETROLEUM HYDROCARBON IN SOIL						
	0 weeks	4 weeks			22 weeks		
Components		**PMX**	**PUMX**	**MRMX**	**NA**	**MRMX**	**PUMX**
C9 - C19	15.80	25.62	46.31	30.35	3.07	32.28	89.54
C20 - C29	26.42	74.20	44.13	47.34	57.07	67.72	10.47
C30 and Above	56.78	0.00	8.65	9.18	39.86	0.00	0.00

PUMX: unmixed poultry manure treated, PMX: mixed poultry manure treated, MRMX: mixed *Moringa oleifera* treated, MRUMX: unmixed *Moringa oleifera* treated and NA: control samples.

4.3.1.4 Microbial isolation and identification

Tables 4.21 and 4.22 present the results of the biochemical test on the isolates from MRMX, PMX respectively. *Bacillus cereus* was the most prevalent microbe found in all three samples. Even in the isolate from MRMX in which *P. aerugenosa* was also identified, *Bacillus cereus* was found to be the dominant bacteria group. *Bacillus* is ubiquitous in nature and has been postulated to be more tolerant to high levels of hydrocarbons in soil due to their resistant endospores. *Bacillus Cereus*is an organism occurring naturally in most foods and it can cause two different forms of food poisoning, diarrhoeal and emetic, both caused by toxins produced by the bacteria. **Kebria *et al.* (2009)** reported that a strain of *bacillus* native to Tehran*Bacillus cereus*,showed great potential for *in-situ* remediation of diesel-contaminated soil in oil refinery sites.**Velázquez-Aradillas *et al.* (2011)**reported the characterization of biosurfactant produced by bacillus cereus and concluded that the biosurfactant properties of *Bacillus cereus* may have potential environmental applications.

Pseudomonas aeruginosa is a versatile bacterium that grows in soil, coastal marine habitats, and on plant and animal tissues. *P. aeruginosa* is not new in the scene of bioremediation as studies have been conducted on its use for degradation of petroleum hydrocarbon: **Das and Chandran (2011)** touched on the use of*Pseudomonas aeruginosa* for degrading sludge hydrocarbons and **Emtiazi *et al*. (2005)** reported the immobilization of *pseudomonas* isolated from a petroleum-contaminated soil for use in petroleum hydrocarbon degradation. Rhamnolipid is a biosurfactant produced by several species of *Pseudomonas*. Biosurfactants are known to lower surface tension and enhance the uptake of lipids in aqueous media.

Table 4.21: Identification of bacteria for used engine oil contaminated soil biostimulated with *moringa oleifera* leaves (sample MRMX) after 22 weeks.

Gram reaction	Shape	Indole	MR	VP	Citrate	Catalase	Coagulase	Organism
-	Rod	-	-	-	-	+	-	*Pseudomonas aerugenosa*
+	Rod	-	-	-	-	+	-	*Bacillus cereus*

Table 4.22: Identification of bacteria for used engine oil contaminated soil biostimulated with poultry manure (sample PMX) after 22 weeks.

Gram reaction	Shape	Indole	MR	VP	Citrate	Catalase	Coagulase	Organism
+	Rod	-	-	-	-	+	-	*Bacillus cereus*

4.4 EFFECT OF EARTHWORM INOCULATION OF BIOSTIMULATED USED ENGINE OIL CONTAMINATED SOIL

4.4.1 Assessment of Earthworm Survival in Biostimulated Contaminated Soil

This section discusses the survival rates of*E. eugeniae* over time at various concentrations of UEO contaminated soil prepared.

From Figures 4.10 and 4.11it can be seen that for used engine oil from motorcycle there was 100% survival of the earthworm in the 14 daystudy for both poultry manure spiked and cattle dung spiked setups for all UEO concentrations considered.

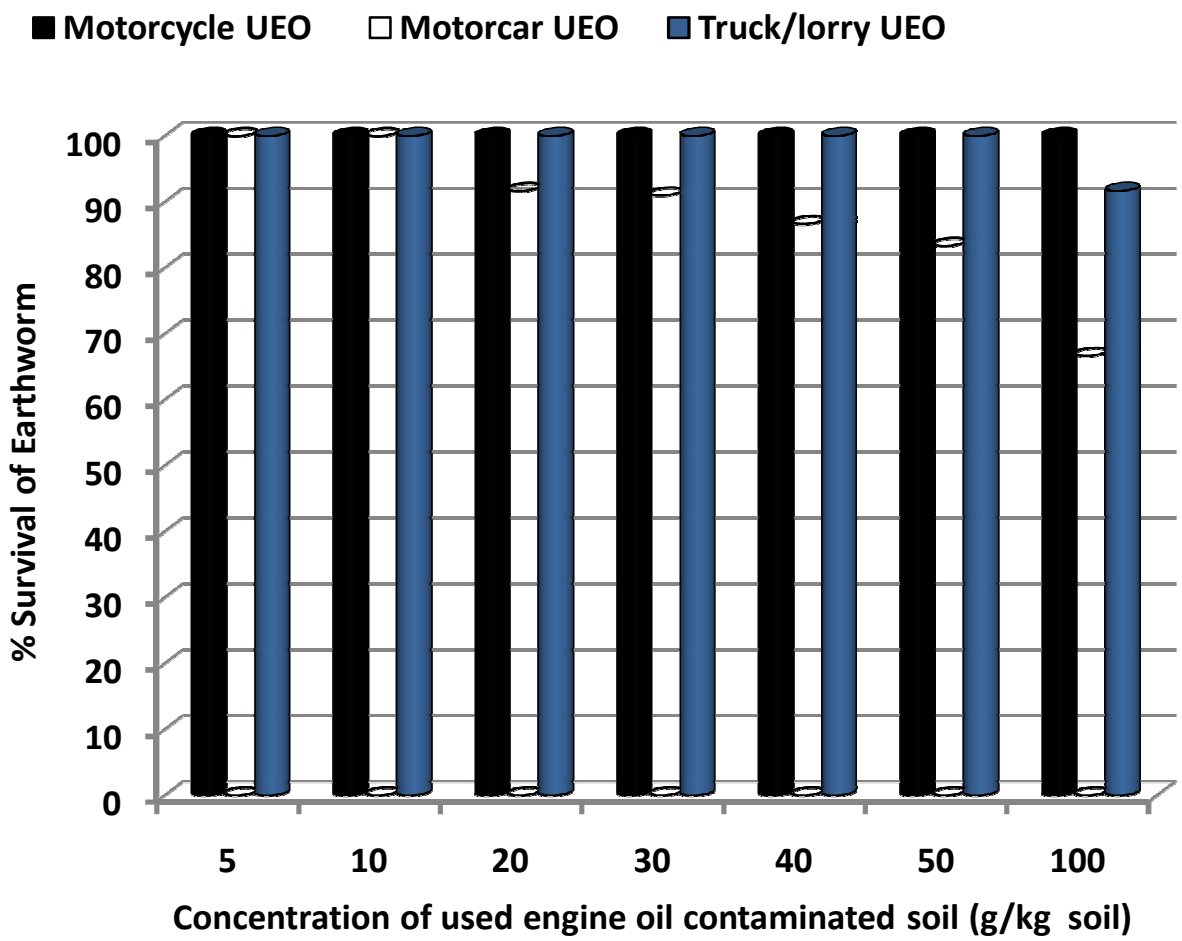


Figure 4.10: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with poultry manure

% survival was computed as the fraction of earthworm from all samples still alive at the end of the test period. Cast formation as a result of earthworm's activities was also observed at low UEO contamination levels. The observed quantity of cast was found to decrease with increase in UEO concentration.

Very similar results were obtained for used engine oil from truck/lorries save for the poultry manure spiked samples in which survival dropped to 91.67% at used engine concentration of 100g/kg soil. The results of this investigation indicate that though very high concentrations of used engine oil in soil was found not to be lethal to earthworms, in considering earthworms for

bioremediation lower UEO concentration would be preferred as this favours greater earthworm activities and cast formation.

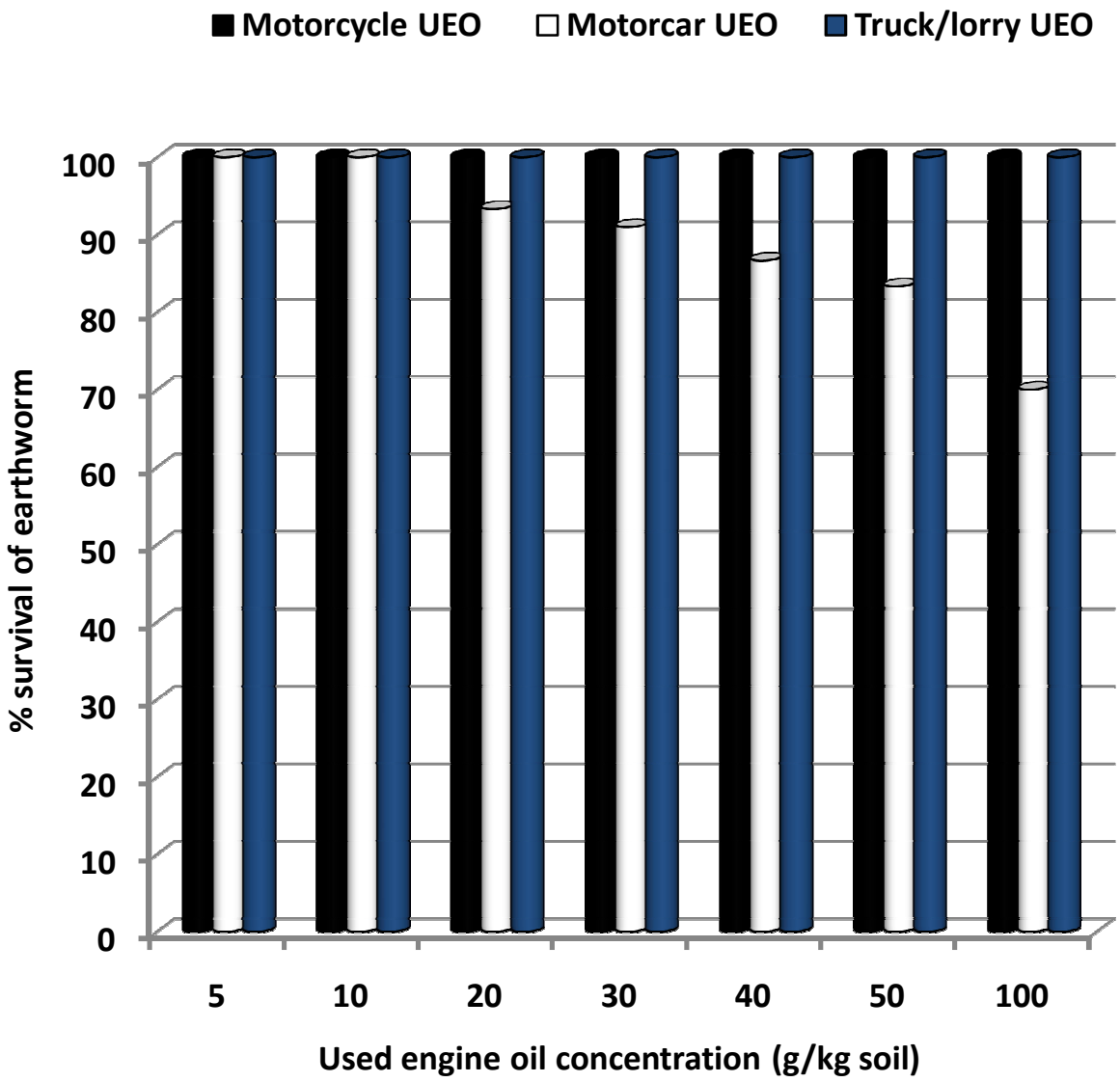


Figure 4.11: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with cattle dung

In the case of engine oil from motorcar, the % survival dropped from 100% to approximately 70% as used engine oil concentration was increased from 10 to 100g/kg soil. All control samples recorded 100% earthworm survival. Therefore the death of the worm must have been as a result of changes created by the presence of the used engine oil contamination. The survival of *Eudrilus eugeniae* in used engine oil contaminated soil was found to be higher than the survival of *Lumbricus terrestris* and *Eisenia fetida* in crude oil contaminated soil as reported by **Hanna and weaver (2002)** that no earthworms survived for 10 days when the concentration of crude oil contaminated soil was 2.5%.

Maike and Filser (2007) in their bioremediation study in which crude oil contaminated soil was inoculated with*E. fetida*, *A. chlorotica* and *L. terrestris*reported that TPH concentrations of 5000–9500 mg/kg were increasingly lethal after an incubation of 28 days. Thus recommended that earthworms may be useful remediation tools in oil contaminated soils with moderate TPH concentrations. It may therefore be inferred from the results of Figures 4.10 and 4.11, that *Eudrilus eugeniae* may be introduced into soil with high concentrations of used engine oil contamination.

The composition of Used Engine Oil has been reported to be a function of: the composition of: original crude oil, the refining process used, the additives, the efficiency and type of engine, the gasoline combustion products, and the length of time in use **(United States Department of Health and Human Services, 1997)**. Therefore, when earthworms are considered for use in the bioremediation of UEO contaminated soil, it is important to determine the tolerance of earthworm for the UEO contamination in question.

4.5.2 Effect of Earthworm on the Carbon and Nitrogen Content, Moisture Retention and Aggregate Size Distribution of Used Engine Oil Contaminated Soil

This section discusses the effect of earthworm (*E. eugeniae)* onsome soil properties thought to translate into enhanced bioremediation of contaminated soil.

4.4.2.1 Effect of Earthworm Inoculationon Soil Carbon Content

The effect of earthworm concentration on the carbon content of used engine oil contaminated soil treated with cow dung and earthworm is presented in Figure 4.12. The figure shows fluctuations (increase and decreases) in the carbon content and a general progressive decrease in the organic carbon content over the period of the study for all earthworm concentrations considered. The fluctuations (decreases and increases) in the carbon content with time may be attributed to the heterogeneity of the system (soil, earthworm, used engine oil and manure mixture)which makes it difficult for the carbon content of the sample removed to be a true average of the entire setup. The drop in organic carbon content was not proportional to the earthworm concentration and the sample with no earthworm also recorded a drop in carbon content. It may be concluded that the presence of earthworms did not significantly accelerate the drop in the organic carbon content (of the used engine oil contaminated soil) over the six weeks study period. The average drop in carbon content for all five samples was found to be 27.02% at the end of 6 weeks. The result of the investigation, howbeit short (6 weeks), is in agreement with **Yu *et al.* (2007),** that earthworm inoculation under straw application had no significant effects on soil organic carbon content in their 6 years study conducted to study the effects of straw application and earthworm inoculation on cropland soil organic carbon and labile organic carbon.

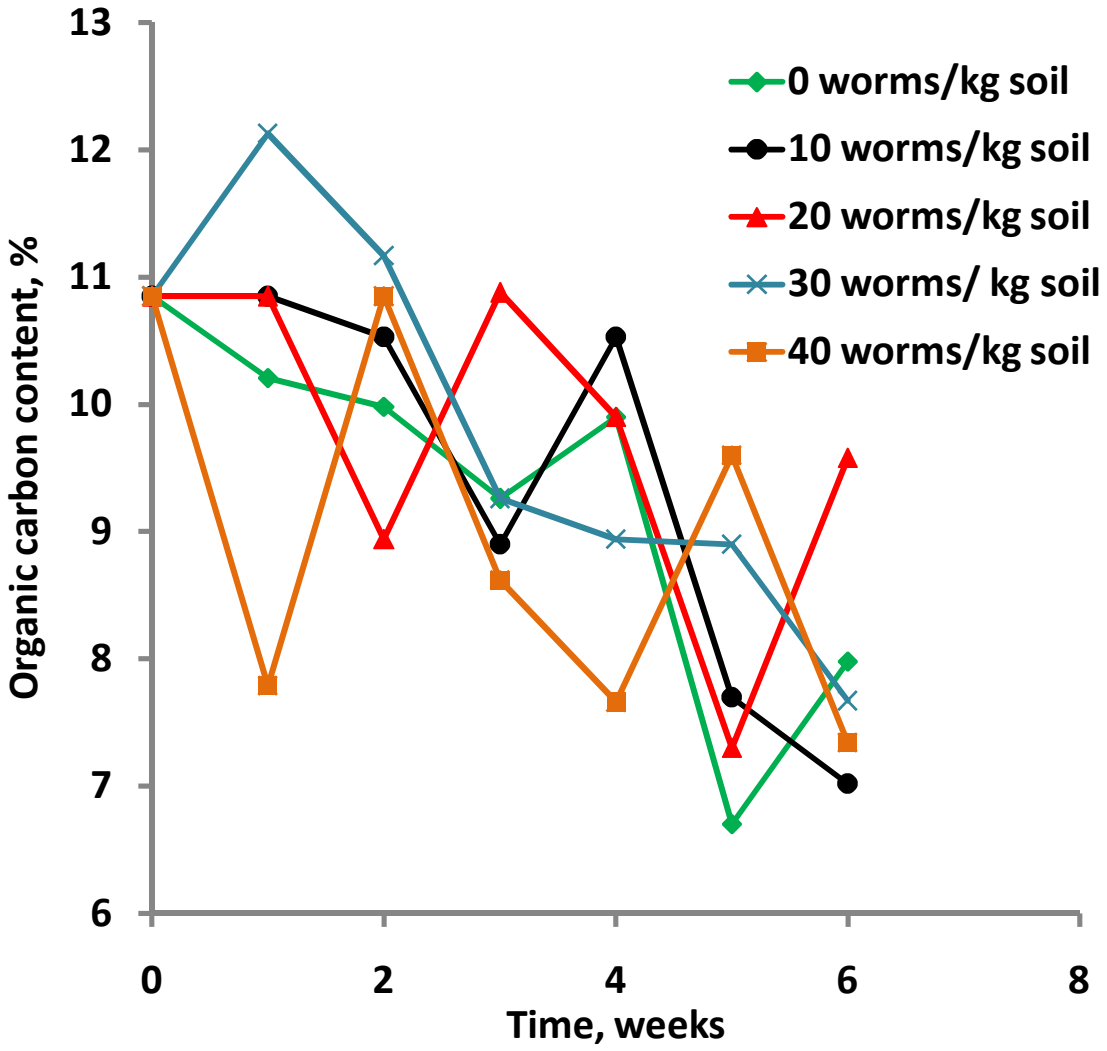


Figure 4.12: Effect of worms on organic carbon content of UEO contaminated soil

Li *et al.* (2002) also in their study using cropland soil reported that earthworms had no significant influences on total soil carbon content: which implied that there was no depletion of soil carbon and nitrogen pools in the presence of earthworms.

4.4.2.2 Effect on Earthworm Inoculation on Nitrogen Content

Figure 4.13 presents the effect of earthworm concentration on the nitrogen content of used engine oil contaminated soil over a six (6) week period. The figure shows that at the end of the study, the nitrogen content of all samples was not significantly changed. Despite the fluctuations in weekly analysis of the nitrogen contents of the samples, the composition remained fairly constant for all samples throughout the study.The observed fluctuations may be due to similar reasons advanced for fluctuation in carbon content.The average drop for all samples at the end of 6 weeks was found to be 16.46%. Similar results was reported by **Li *et al.* (2002)** that earthworms had no significant influences on nitrogen content in their study using used engine oil free soil.

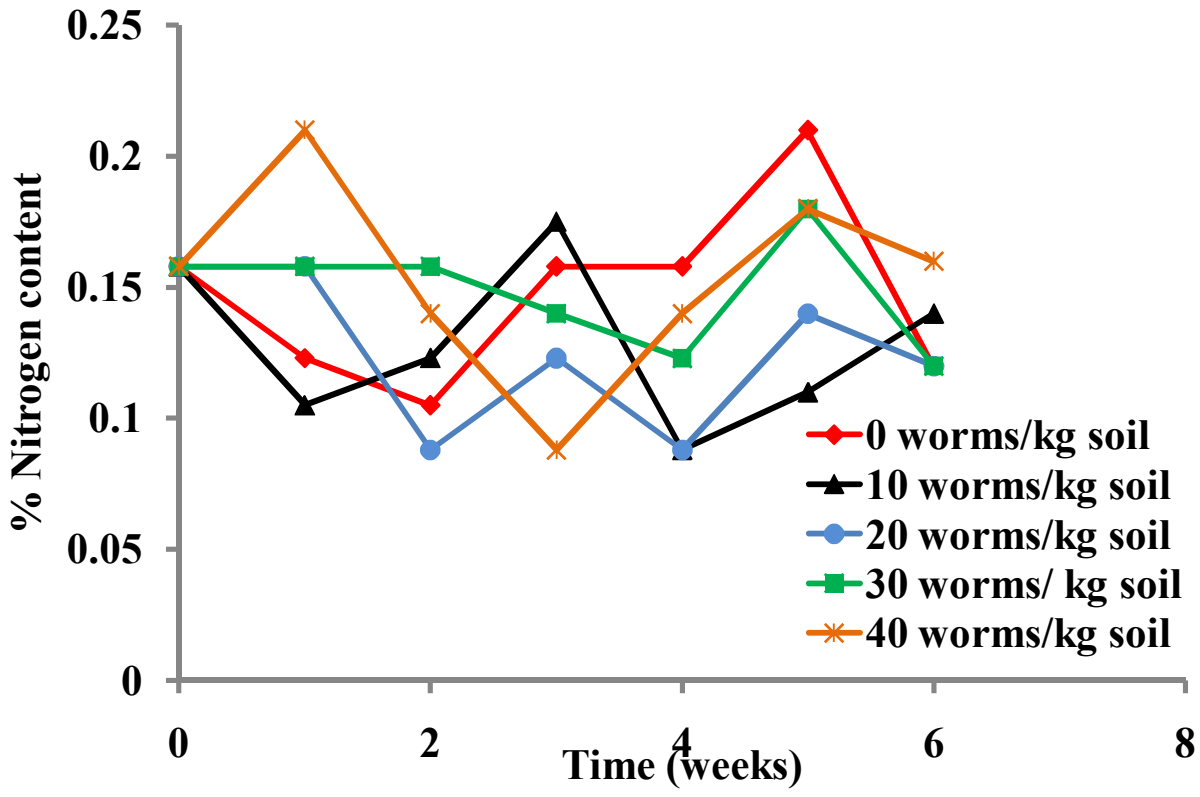


Figure 4.13: Effect of worms on nitrogen content of UEO contaminated soil

4.4.2.3 Effect of Earthworm Inoculation on the Water Holding Capacity of Used Engine Oil Contaminated Soil

Figure 4.14presents the effect of earthworm concentration on soil moisture retention. The figure shows a general increase in water holding capacity with increasing earthworm concentration (indicated by the positive slope of the of the trend line).At a confidence interval of 0.05 the effect of earthworm on soil water holding capacity was found to be significant with a *P* value of 0.025. The increase in moisture retention ability has been reported by (**Edwards and Bohlen, 1996; Schaefer and Filser, 2007**)is considered a positive development (**Hickman and Reid, 2008**) with respect to soil health.

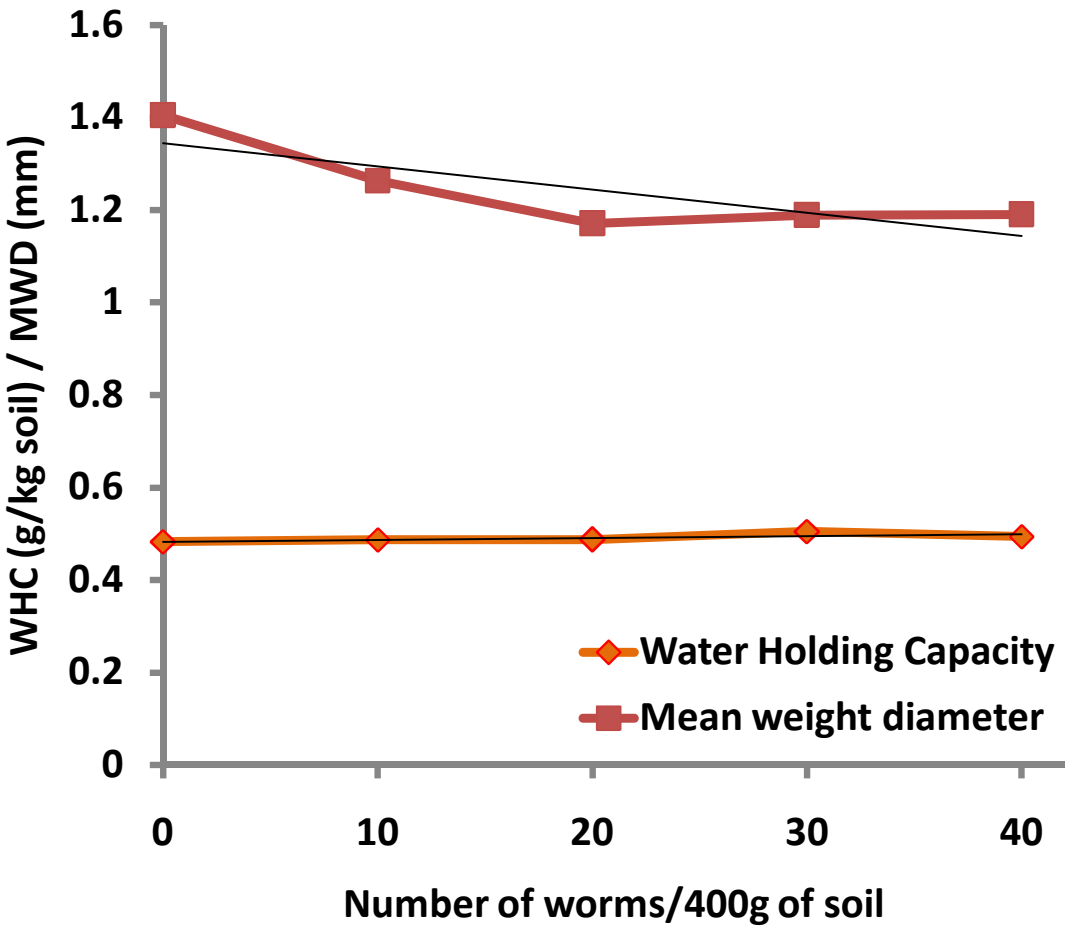


Figure 4.14: Effect of earthworm concentration on soil WHC and MWD.

4.4.2.4 Effect on Earthworm Concentration on Aggregate Size Distribution of Used Engine Oil Contaminated Soil

Figure 4.14 also shows the effect of earthworm concentration on the mean weight diameter of the soil. The mean weight diameter decreased from 1.406mm in the sample without worm to 1.191mm in the sample with the highest concentration of worms (40 worms/kg of soil) in the six weeks study. The presence of earthworms clearly has an effect on the particle size distribution, with a *P* value of 0.029 at a confidence interval of 0.050.The decrease in soil mean weight diameter evened out at earthworm concentration of 20 earthworms/400g of soil, and may be considered as optimal should the effect on particle size be the singular most important factor in earthworm assisted bioremediation of used engine oil contaminated soil.**Barois *et al.*, (1993)** hypothesized that in this reduction in particle size might exist potential for earthworm assisted release of soil associated organic contaminants, which will probably result in an increase in bioremediation rate.

4.4.2.5 Uptake of Metals by Earthworm in Simulated Used Engine Oil Contaminated Soil

Table 4.23 compares the concentration of heavy metals in the earthworms to the concentration of metals in the various concentrations of used engine oil contaminated soil. The table shows that for most of the metals detected in the worms (apart from V, Mn and Ti), the dry body burden of the worm (concentration in the dried carcasses of the earthworms) were higher than those in all contaminated soil concentrations. Only a marginal difference existed between the concentration of Fe and Ba in the earthworm and the used engine oil contaminated soil at t = 0. Based on these and should the metals in the soil be 100% bioavailable, it is expected that elimination should take place for metals with higher concentration in the worm and accumulation for the metals with lower concentrations in the worms. Fe and Ba should remain relatively constant in the worms

with time. The table also shows that the concentration of the metals for the various UEO concentrations did not vary much.

Table 4.23: Initial elemental composition of earthworm and simulated used engine oil contaminated soil.

	Worms	Concentration of used engine oil in contaminated soil (g UEO/kg soil)				
		12.5	25	50	75	100
Al	0.280	0.148	0.078	0.041	0.022	0.012
Si	0.000	32.977	31.483	31.856	30.782	30.689
K	0.000	3.369	4.216	4.307	4.482	4.008
Ca	16.214	0.446	0.478	0.448	0.479	0.602
Ti	0.527	0.857	1.121	1.067	1.198	1.180
V	0.028	0.039	0.062	0.053	0.051	0.052
Cr	0.178	0.030	0.055	0.047	0.075	0.041
Mn	0.000	0.060	0.060	0.0550	0.057	0.074
Fe	9.434	5.202	7.638	7.212	8.456	7.876
Ni	0.204	0.010	0.033	0.031	0.050	0.023
Cu	0.232	0.011	0.042	0.041	0.063	0.029
Zn	0.739	0.003	0.030	0.034	0.043	0.034

The one-compartment model of **Van Straalen *et al.* (2005**) was used to analyze the development of earthwormbody concentrations with time, and to calculate uptake and elimination rateconstants. Equation (4.4)was used for all elements.

$$C_{iworm}(t) = C_i(0)e^{-k_2 t} + \frac{a}{k_2}\left(1 - e^{-k_2 t}\right) \quad (4.4)$$

In addition,

$$k_1 = \frac{a}{C_e} \quad (4.5)$$

Where: $C_{iworm(t)}$ = metal concentration in the earthworm (µg g^{-1} dry weight) at time t (days), a = flux of metals into the earthworm (mg_{metal} kg^{-1} animal day^{-1}),k_2 = elimination rate constant (day^{-1}). $C_i(0)$ is the initial body concentration (µg g^{-1}dry weight), which is measured in the animals before exposure.

Other parameters of interest include:

R^2: coefficientof correlation

C_e: external metal concentration (concentration in the soil, µg g^{-1} dry weight)

k_1: uptake rate constant (from bulk concentrations $k_1$soil, gsoil ganimal^{-1} day^{-1};

Mathematically, k_1 is defined as;

C_{SS}: calculatedsteady-state metal concentration, mg kg^{-1}

t_{SS}: calculated time taken to reach steady state, days

BAF: bioaccumulation factors (from bulk concentrations)

Figures 4.15 and 4.16 are typical examples of the kinetic model against the experimental data. The kinetic parameters generated for Zn, Cu, Ni, Cr, Ti and Mn were tabulated in Tables 4.24 – 4.26. Tables 11 – 16 in**Appendix C**shows the raw experimental kinetic data for elimination of the elements Zn, Cu, Ni and Cr from the worms as expected (as the earthworm body concentration of these were higher than the contaminated soil concentration) and accumulation of Ti and Mn were accumulated also as expected. The R^2 values obtained varied widely ranging from 0.01 to 0.99 but were very similar to those reported by **Nahmani*et al.* (2009)** and higher than those reported by **Gimbert *et al.*(2006)**.

For V_2O_5, the concentration in the worms remained relatively unchanged irrespective of UEO concentration throughout the period of the study. The value of t_{SS} for all elements considered (Tables 4.24 – 4.26) ranged from 2 to 18 days for the elements that attained steady state concentration in the worms on exposure. The 14 days study period using in the earthworm survival assessment of **Section 4.4.1** is therefore generally reasonable, but future works on earthworm survival in similar environment may consider a longer period, especially to consider the effects of those metals that did not attain steady state tissue concentration.

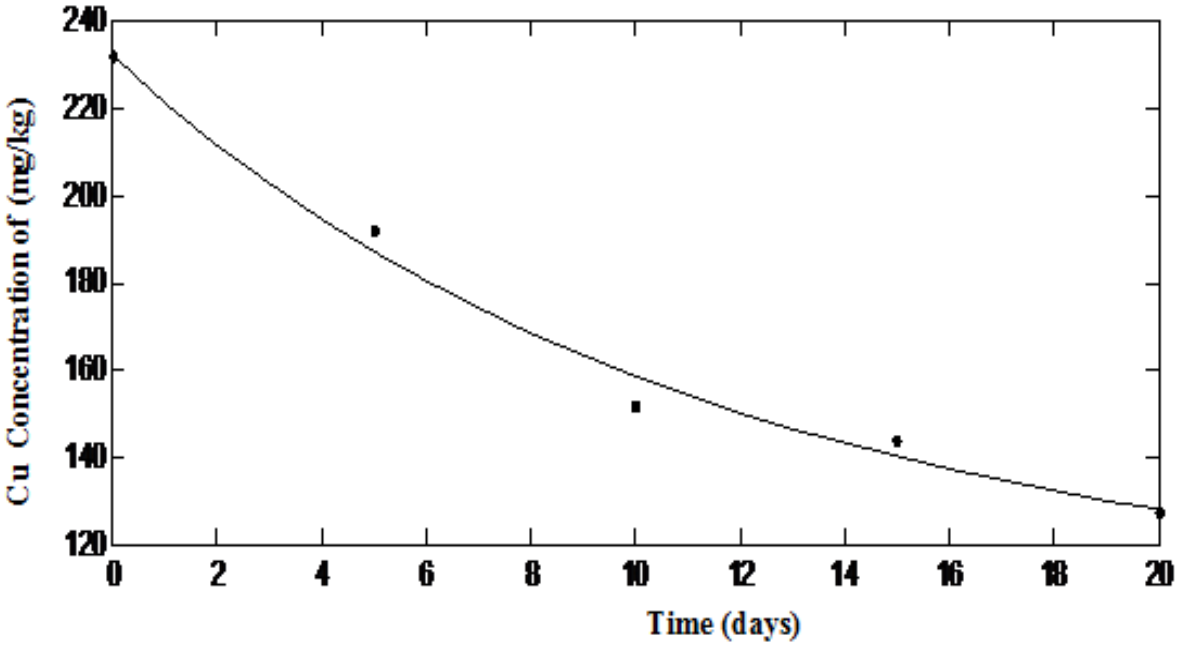


Figure 4.15: Modeling of Cu elimination kinetics for soil contaminated with 75g/kg of UEO.

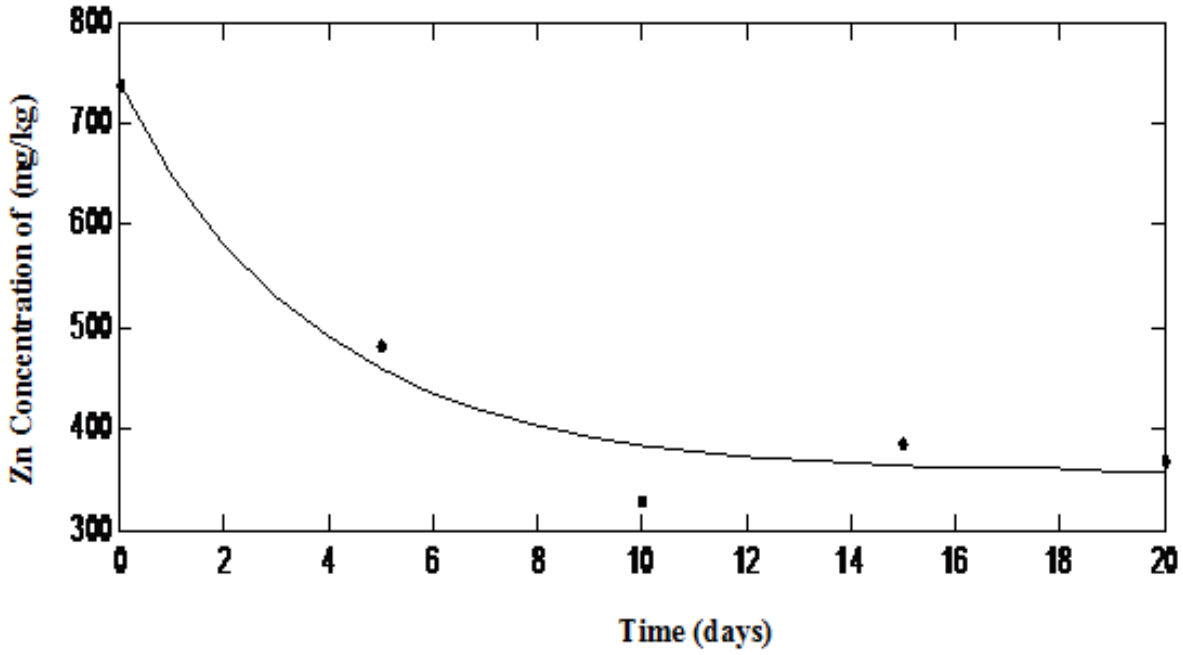


Figure 4.16: Modeling of Zn elimination kinetics for soil contaminated with 100g/kg of UEO.

Table 4.24: Elimination kinetics of Zn and Cu by the earthworm (*E. eugeniae*) exposed to simulated UEO contaminated soil.

	Zn					Cu				
	UEO concentration, g/kg soil					UEO concentration, g/kg soil				
	12.5	**25**	**50**	**75**	**100**	**12.5**	**25**	**50**	**75**	**100**
a	1268.70	1495.00	1424.30	108.45	93.82	674.77	6.50	589.91	9.46	42.93
k_2	4.40	4.40	4.20	0.28	0.26	4.12	0.08	4.22	0.09	-0.18
R^2	0.82	0.99	0.87	0.93	0.96	0.11	0.65	0.72	0.99	0.88
C_{ss}	280.00	337.50	330.00	380.00	362.50	170.00	NSS	140.00	NSS	NSS
C_e	3.21	29.72	33.73	43.37	33.73	11.18	42.33	40.73	63.10	28.75
k_1	394.87	50.31	42.22	2.50	2.78	60.34	0.15	14.48	0.15	1.49
BAF	89.74	11.43	10.05	8.85	10.55	14.64	1.99	3.43	1.70	-8.14
t_{SS} **(Days)**	2.00	2.00	2.00	18.00	18.00	2	NSS	2	NSS	NSS

NSS = steady state not attained

Table 4.25: Elimination kinetics of Ni and Cr by the earthworm (*E. eugeniae*) exposed to simulated UEO contaminated soils.

	Ni					Cr				
	UEO concentration, g/kg soil					UEO concentration, g/kg soil				
	12.50	**25.00**	**50.00**	**75.00**	**100.00**	**12.50**	**25.00**	**50.00**	**75.00**	**100.00**
a	759.62	582.38	618.20	9.36	-97.61	646.48	533.34	561.63	561.63	-22.69
k_2	4.16	4.17	4.19	0.08	-0.48	4.11	4.05	4.38	4.06	-0.13
R^2	0.01	0.49	0.37	0.82	0.60	0.01	0.27	0.20	0.68	0.44
C_{ss}	185.00	140.00	148.00	NSS	NSS	160.00	132.00	130.00	139.00	NSS
C_e	10.22	33.02	30.66	50.32	22.80	30.10	54.72	47.20	75.24	41.04
k_1	74.32	17.64	20.16	0.19	-4.28	21.48	9.75	11.90	7.47	-0.55
BAF	17.89	4.23	4.81	2.36	8.97	5.23	2.41	2.72	1.84	4.40
$t_{SS\ (Days)}$	2.00	2.00	2.00	NSS	NSS	1.00	2.00	2.00	2.00	NSS

NSS = steady state not attained

Table 4.26: Uptake kinetics of Ti and Mn by the earthworm (*E. eugeniae*) exposed to simulated UEO contaminated soils.

	Ti					Mn				
	UEO concentration, g/kg soil					UEO concentration, g/kg soil				
	12.50	**25.00**	**50.00**	**75.00**	**100.00**	**12.50**	**25.00**	**50.00**	**75.00**	**100.00**
a	90.17	248.82	-11.15	264.61	62.84	7.25	25.02	12.83	298.80	41.07
k_2	0.10	0.26	-0.04	0.36	0.08	0.04	0.30	0.28	4.45	0.53
R^2	0.82	0.64	0.66	0.51	0.79	0.97	0.78	0.99	0.91	0.84
C_{ss}	NSS	975.00	NSS	745.00	NSS	NSS	83.00	46.00	76.90	78.00
C_e	856.86	1120.50	1066.58	1198.40	1180.42	59.60	59.60	54.95	56.50	74.30
k_1	0.11	0.22	-0.01	0.22	0.05	0.12	0.42	0.23	5.29	0.55
BAF	1.15	0.87	0.27	0.62	0.64	2.97	1.42	0.84	1.19	1.05
$t_{SS\ (Days)}$	NSS	18.00	NSS	16.00	NSS	NSS	16.00	18.00	2.00	16.00

NSS = steady state not attained

4.6 EFFECT OF EARTHWORM INOCULATION ON TOTAL PETROLEUM HYDROCARBON OF SIMULATED USED ENGINE OIL CONTAMINATED SOIL

4.5.1 The Effect of Variable Initial Used Engine Oil Concentration on Bioremediation by Vermicomposting

The effect of variable concentration of UEO in soil is presented in Figure 4.17. It was in the soil with the highest initial concentration of UEO (that is TPH) that the degradation was highest a result also reported by **Schaefer (2001)**. The % drop in TPH after 42 days increased from 16.91% to 36.28% as the initial UEO concentration was increased from 5 g/kg to 20 g/kg. This may be as a result of increased metabolic activities by the microbes as more carbon is present, facilitated by earthworm activities. The seeming slight fluctuations (increase and decrease) may be attributed to the heterogeneity of the sample as earthworm activity may not be uniform throughout the sample.

Inthe heavy metals bioaccumulation study **Section 4.4.2.5**, equal number of worms were introduced into samples with variable initial UEO concentration. The drop in TPH of the samples at the end of 20 daysis shown in Figure 4.18. The figure show that increasing initial concentration of used engine oil contamination results in a higher drop in TPH (a higher rate of biodegradation): in complete agreement with the result shown in Figure 4.17. These suggest that vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil may be more effective at high initial used engine oil concentrations which fortunately is the case with most mechanic workshop soil (Table 4.9). However, this trend cannot continue indefinitely as earthworm survival reduces with increasing UEO concentration.

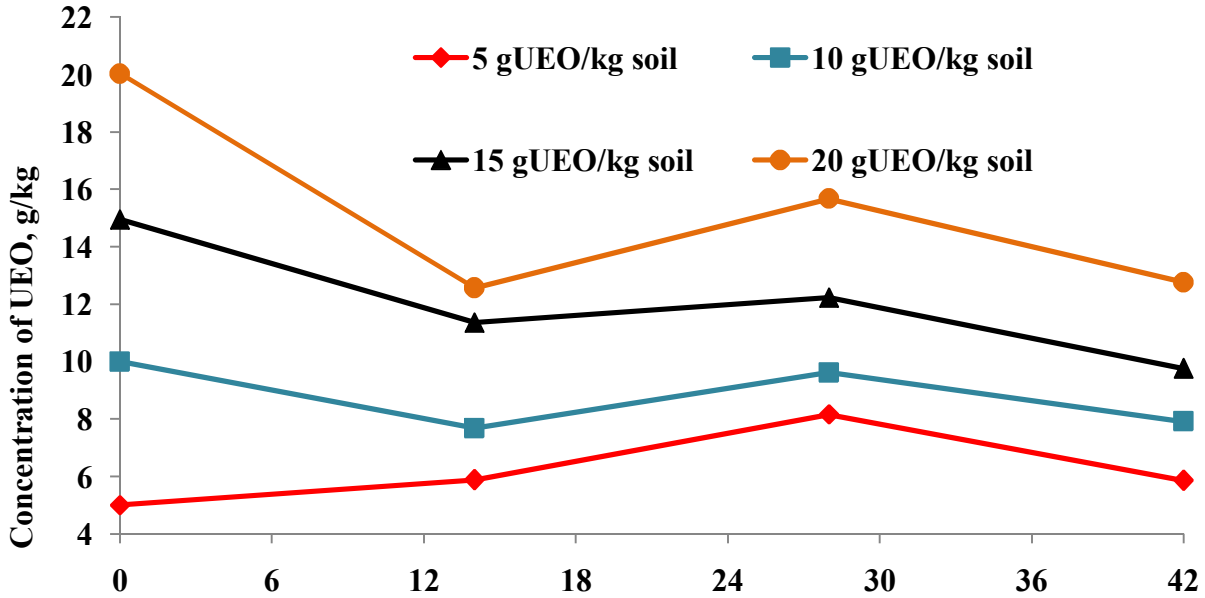


Figure 4.17: Effect of initial used engine concentration on bioremediation by vermicomposting

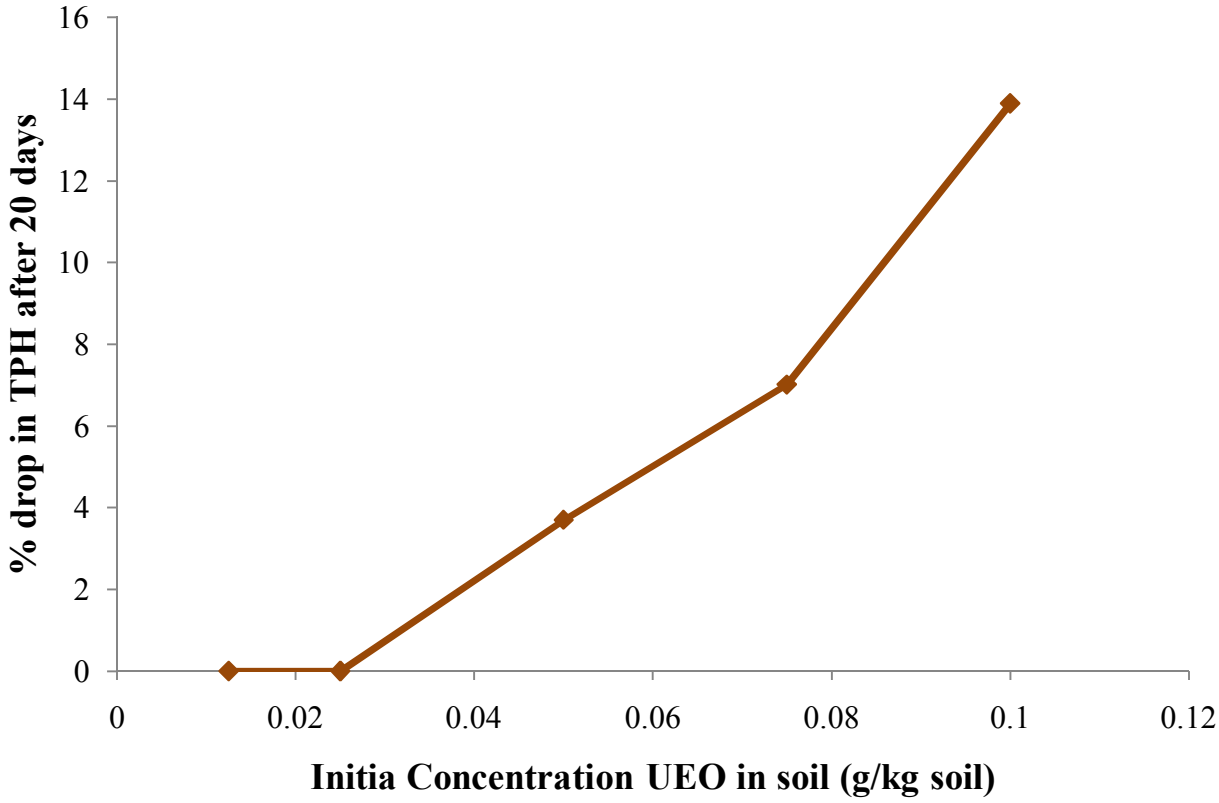


Figure 4.18: Effect of initialUEO concentration on vermi-assisted bioremediation (TPH analysis of metal uptake samples)

4.5.2 Effect of Variable Earthworm Concentration

Low initial UEO concentration

The effect of varying concentration of earthworm on the rate of bioremediation is shown in Figure 4.19. As shown in Figure 4.19, there was an initial increase in TPH for some of the samples probably as a result of the extraction of biogenic organic compounds by toluene from the organic matter **(Chaîneau *et al.*, 1997)**. Samples with 0, 15 and 20 worms showed the highest drop in TPH with time. The samples with 5 and 10 earthworms had the least drop in TPH over the 42 day period considered. The percentage drop in TPH for the samples with 0, 5, 10, 15 and 20 earthworms on day 42 was computed as 31.95, 11.75, 8.00, 25.93 and 28.98% respectively. Under the condition of the study, the earthworms will eventually starve to death as the nutrient (manure) is not replenished. This implies that without addition of nutrients (in this case manure) the earthworms will eventually begin to starve to death starting with the samples with the highest concentration of worms. It is however possible that lower worm concentrationscan translate to more available food (as competition will be less) and the worms may ultimately effect the same level of bioremediation as the samples higher worm concentration (that is for systems where there is no replenishing of food) as they may survive longer. This remains to be proven.

Generally the sample without worms performed better than all the samples with worms. It can be seen that with regards to TPH drop alone (and within the range of earthworm and used engine oil concentrationsand considered), there is no significant advantage in introducing earthworms for bioremediation of used engine oil contaminated soil. It has been reported that although the action of earthworm generally increase the mineralization of soil carbon, earthworms can also decrease

mineralization of carbon by contributing to the formation of stable soil aggregates in which carbon is protected from further breakdown – this may be a possible reason for the result.

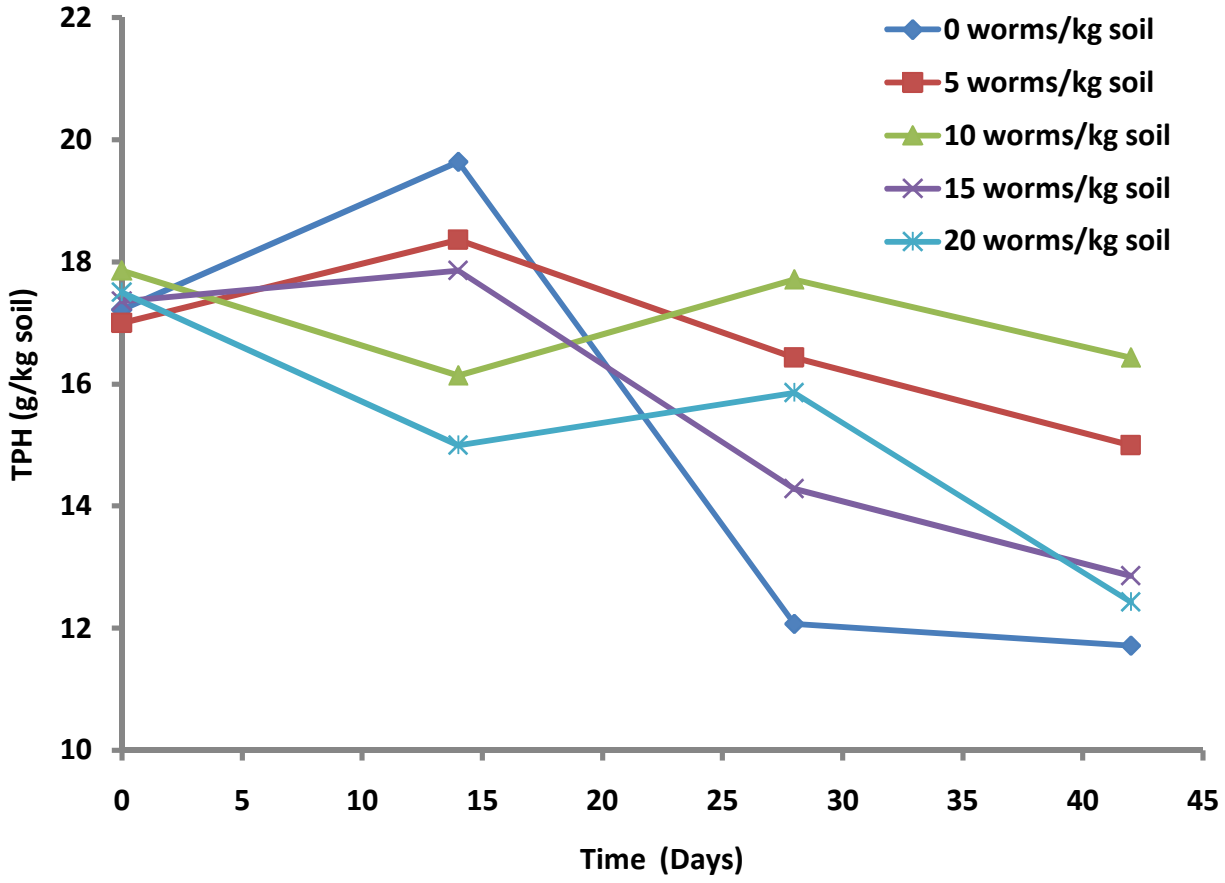


Figure 4.19: Effect of earthworm concentration on bioremediation by vermicomposting

The result of this investigation differs widely from the finding of **Maike and Filser, 2007:**TPH concentration decreased by 30–42% in samples with *E. eugeniae*, after 28 days of incubation. Also the TPH concentration of the samples without earthworms was not significantly decreased after 28 days (9–17%) when earthworm was used in the bioremediation of crude oil contaminated soil. They also concluded that addition of earthworms improved bioremediation rate more than manual tilling (in the absence of earthworms).However, **Callaham et al. (2002)**observed no difference in TPH concentrations associated with *E. fetida* or wheat

treatments when applied to petroleum contaminated soils,whilst **Stom *et al.* (2003)**, who investigated the effect of earthworm (*E. fetida*) application (n=5) to crude oil contaminated soils (25 g kg^{-1}) for 40 days with the co-application of microorganisms also observed minimal losses (~15 g kg^{-1} loss for the microbial inoculation and earthworm co-application; ~9 g kg^{-1} loss for the microbial inoculum alone, and only ~3 g kg^{-1} loss for sole earthworm treatments).Generally for the samples inoculated with worms, increase in earthworm concentration resulted in an increased rate of bioremediation. This result suggests that the kinetics of bioremediation may be dependent on the initial concentration of used engine in soil.The result of Figure 4.19 indicates that increase in earthworm number could result in an increase in the rate of bioremediation. Comparing this with the result of Figure 4.18 it may be deduced that earthworm assisted bioremediation of UEO contaminated soil is favored by both high UEO concentration and high earthworm concentration. However, a limit would naturally exist for both concentrations.

Figures 4.20 – 4.22 presents the GC analysis for effect of earthworm concentration on bioremediation. The corresponding MS resultis shown in Table 4.27. At initial time (t = 0), C30 and above fractionshad the highest proportion in the oil, being 56.78%. By 42 day, the C30 and above fraction had drop to 0% for the earthworm free samples and the earthworm treated sample (20 worms). However, the earthworm treated sample had a higher % composition of C20 -C29 when compared with the earthworm free sample. This is in line with Figure 4.19 which presents the sample with 20 worms as having a slightly higher TPH at 42 days than the earthworm free sample.

High initial UEO concentration

Analysis of the TPH of the samples of **Section 4.4.2.3** (in which earthworm concentration was varied at a fixed initial UEO concentration)showed a similar trend in TPH drop with earthworm

concentration. After 6 weeks of earthworm treatment, the sample with the highest concentration of worm recorded the highest drop in total petroleum hydrocarbon content as shown in Figure 4.23. The sample with 0, 10, 20, 30 and 40 earthworms/kg of soil recorded 28.08, 28.48, 29.19, 32.46 and 34.35% reduction in TPH respectively. This behavior is what would be expected of a catalyst: the more the active sites, the faster the reaction. Thus bioremediation using earthworms should be favored by higher earthworm concentration. The major difference between Figure 4.19 and 4.23 is that whereas for Figure 4.19 where the sample without worms recorded the highest drop in TPH after 42 days, Figure 4.23 indicates that the sample with the highest worm concentration recorded the highest drop in TPH after 35 days. This indicates that the effectiveness of earthworm assisted bioremediation of used engine oil contaminated soil is UEO concentration dependent.

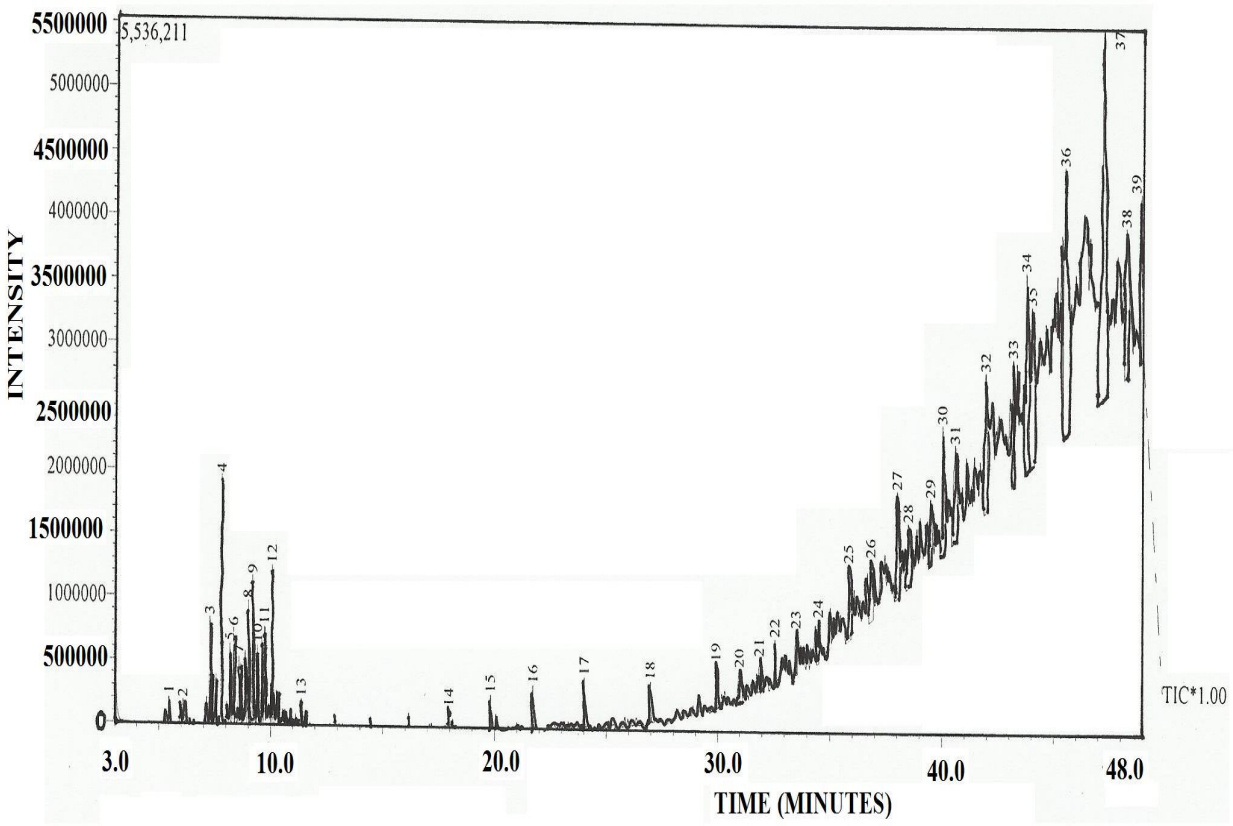


Figure 4.20: GC of extract from soil at t= 0 day

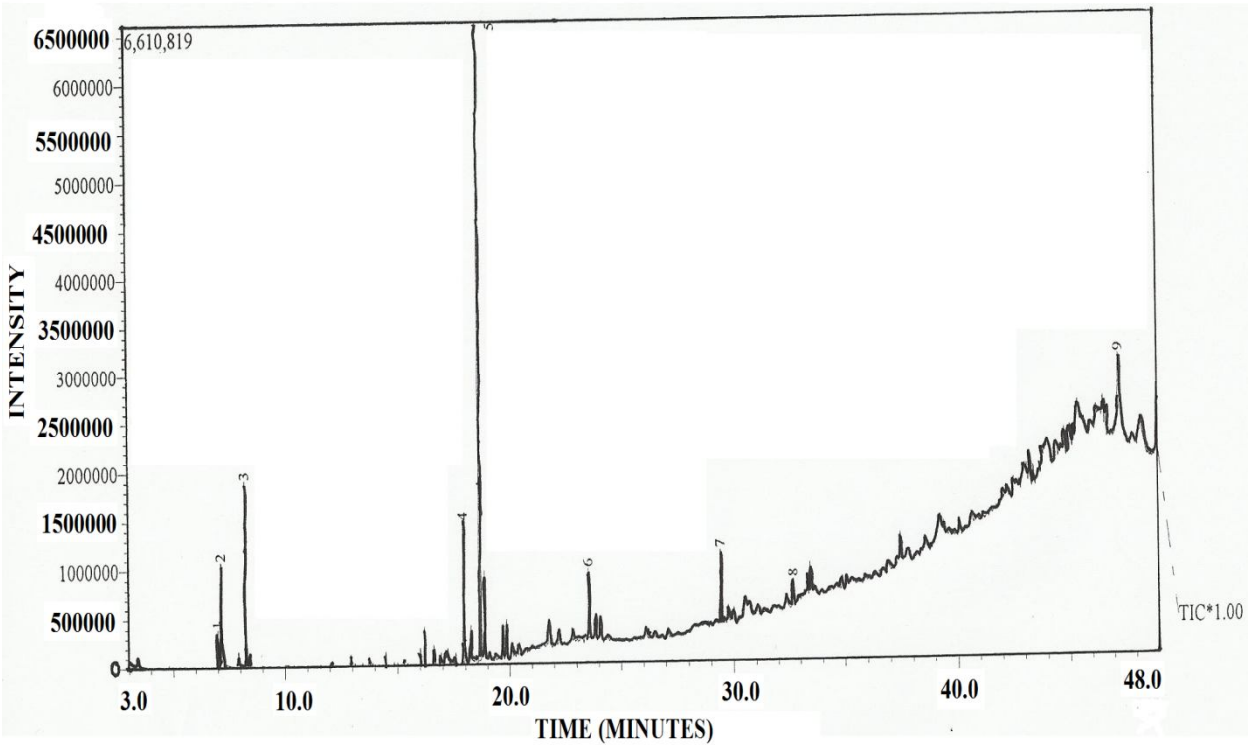


Figure 4.21: GC of extract from soil sample with 20 worms at t = 42 days

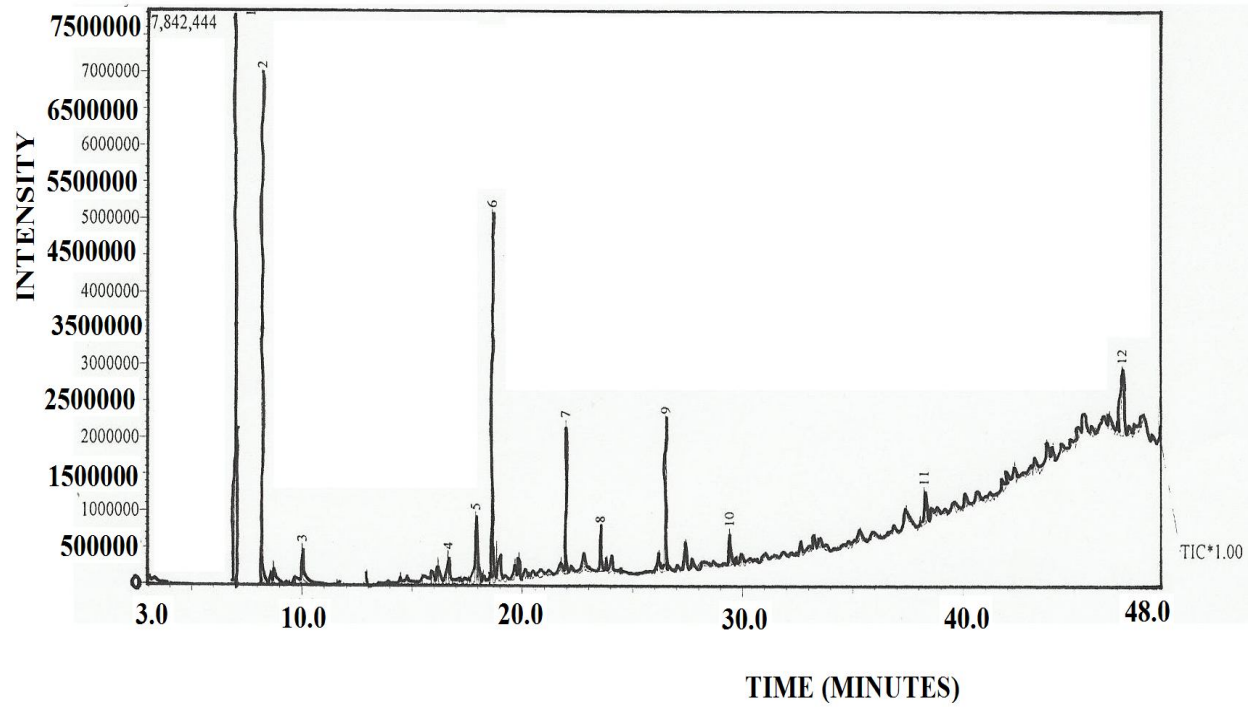


Figure 4.22: GC of extract from soil sample with no worms at t = 42 days

Table 4.27: MS analysis on the effect of earthworm concentration on the composition of UEO in bioremediation

	Composition of UEO		
	Day 0	**No worm t = 42 day**	**20 worms, t = 42 days**
C9 - C19	15.8	97.29	92.08
C20 - C29	26.42	2.71	7.91
C30 and Above	56.78	0	0

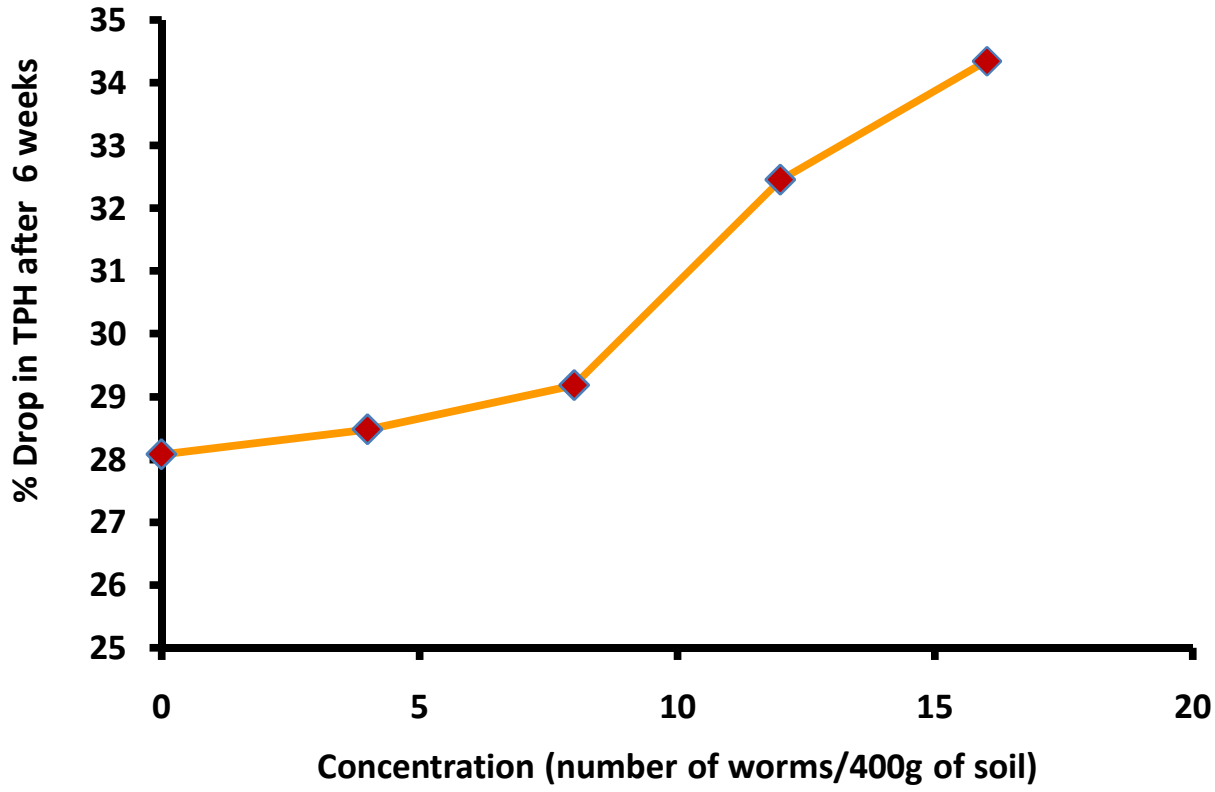


Figure 4.23: Effect of earthworm concentration on bioremediation by vermicomposting (initial TPH = 100g/kg soil – high UEO concentration)

4.5.2.1 *Effect of worm concentration on bioremediation and microbial count.*

The effect of the presence of earthworm on the microbial community is as shown in Figure 4.24. The microbial count was found to decrease and increase indiscriminately. According to **Granatstein and Bezdicek (2003)** there needs to be at least a 10-fold difference in microbial number to be meaningful because of variability in measuring organisms. Figure 4.24therefore shows that presence of earthworms had no significant effect on microbial count that all samples (untreated/earthworm treated) had similar number of bacteria at the various times and would perform the same if all else was equal. There however appears to be a general decrease in microbial count with time. The result of this investigation suggests that the effect earthworm inoculation has on bioremediation kinetics is not a direct follow up of its effect on microbial count, since earthworm treated and untreated sample recorded similar microbial counts.

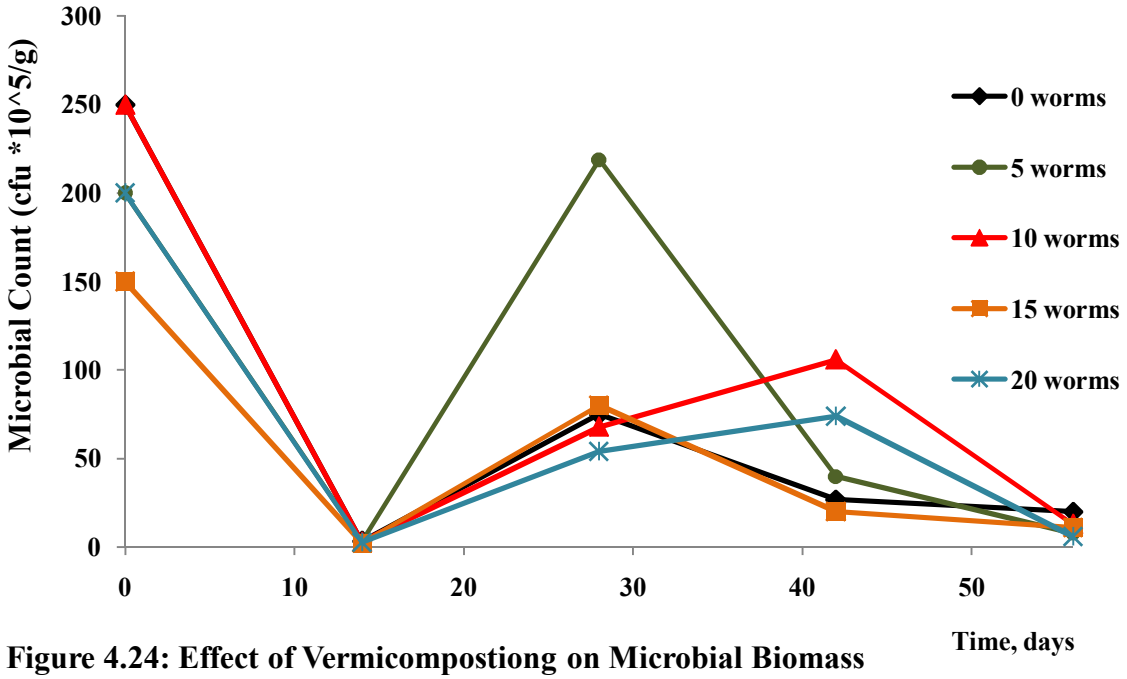


Figure 4.24: Effect of Vermicompostiong on Microbial Biomass

4.5.3 The Effect of Tilling (Mixing) and Earthworm on Bioremediation

As shown in Figure 4.25,positive drop in TPH for most of the samples began after the fifth week. At t = 7 weeks the samples with 30 earthworms/kg soil had the highest drop in TPH, which was comparable to the untilled sample (24.16% and 25% respectively**),** thereafter the TPH of this sample (with 30 earthworms/kg soil) increased probably as a result of starvation and death of the worms. The tilled (mixed) sample and the sample with 20 earthworms/kg soil indicated a slower initial drop in TPH but the mixed sample picked up after the t = 10 weeks to give the highest drop in TPH at t = 14 weeks of 36.67%. This result showed that the drop in TPH is a function of earthworm concentration with higher concentrations favoring higher rates, similar to the discussion in **Section 4.5.2**. It also shows that when earthworms are considered for use in bioremediation it is important to reintroduce nutrient (poultry manure in this case) to sustain the worms (since they cannot feed on used engine oil) and to maintain the pace of the bioremediation process. Comparison of the with-earthworm and the without-earthworms samples of both Figures 4.19 and 4.25 suggests that in term of TPH removal, the use of earthworms (*E. eugeniae*) did not offer a significant advantage (that for the UEO concentrations considered).The fluctuations in the TPH values with time may have been as a result of the heterogeneity of the samples as TPH values were not determined using the whole sample but homogenized small samples collected from different parts of the larger sample. It is important that other organic amendments as well UEO concentrations in soil (especially higher concentrations) be considered in future works as the bioremediation process is a function of a myriad of factors.

The GC chromatogram for the samples at t = 0, for the mixed sample at t = 14 weeks and for the sample with 15 worms at t = 14 weeks are presented as Figures 4.26, 4.27 and 4.28 respectively.

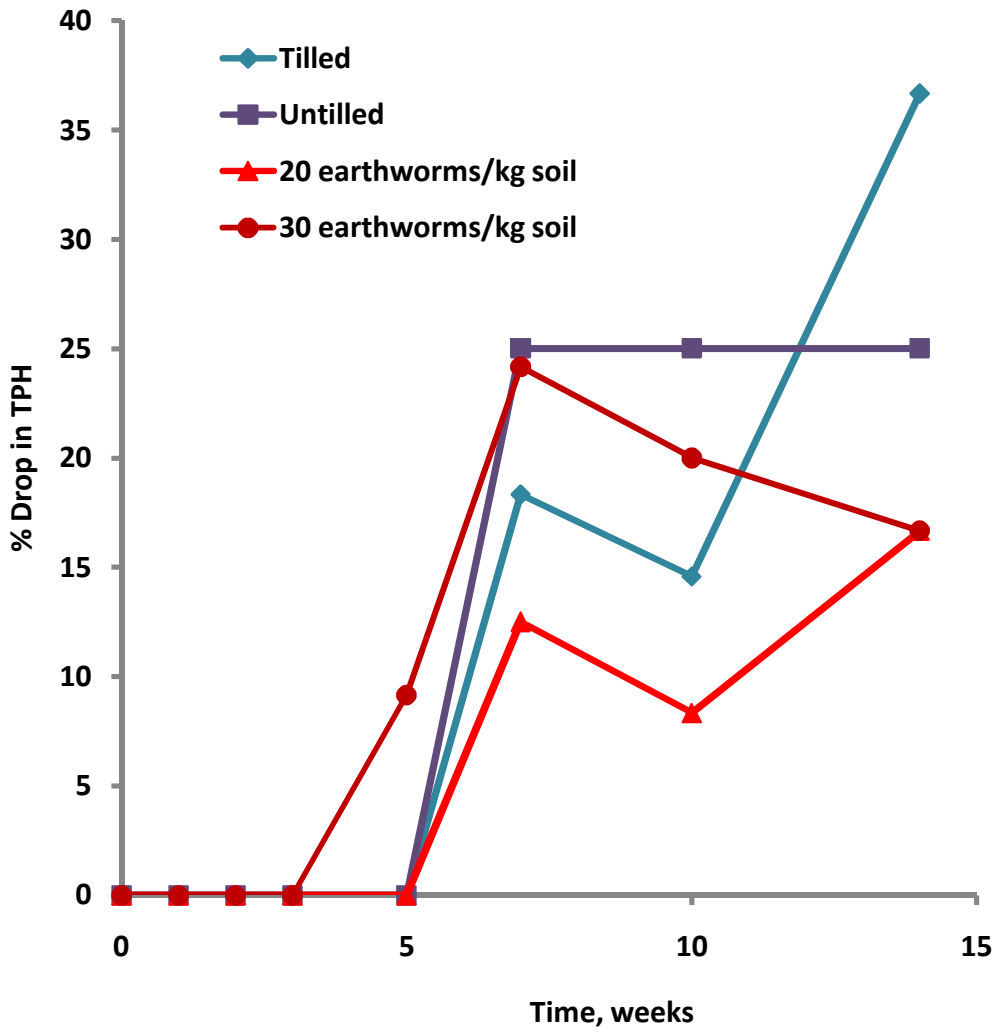


Figure 4.25: Comparison of the effect of manual mixing with the activity of earthworm on bioremediation

The MS analysis for these samples is presented in Table 4.28. Comparison of Figures 4.26, 4.27 and 4.28 show that at time t = 0 the GC of the contaminated recorded more peaks than those of 14 weeks of bioremediation for both earthworm treated and untreated (mixed) samples. Table 4.28shows that after 14 weeks of treatment all the C30 and above had been eliminated from the contaminant. The table also indicated that the earthworm treated sample recorded 100% composition of C9 – C19 whereas the mixed sample had 88.83% C9 – C19 and 11.18% C20 – C29.The TPH analysis of the samples on day 14 however indicated that the mixed sample had a

higher TPH drop (36.67%) as against the 16.67% recorded by the sample treated with 20 earthworms. Thus, higher molecular weight hydrocarbons were broken down faster in the earthworm inoculated sample.

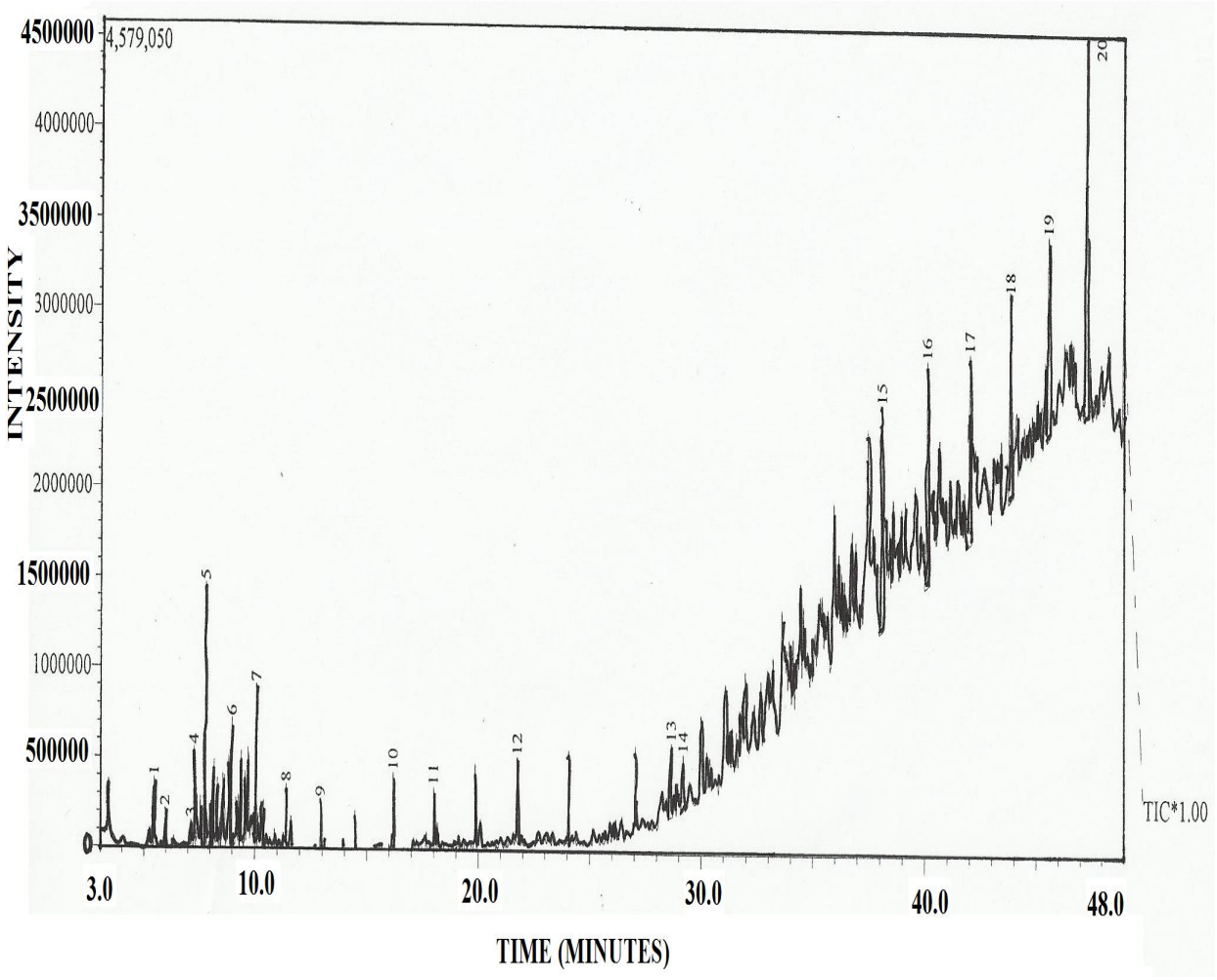


Figure 4.26: GC of extract from soil at t = 0 weeks

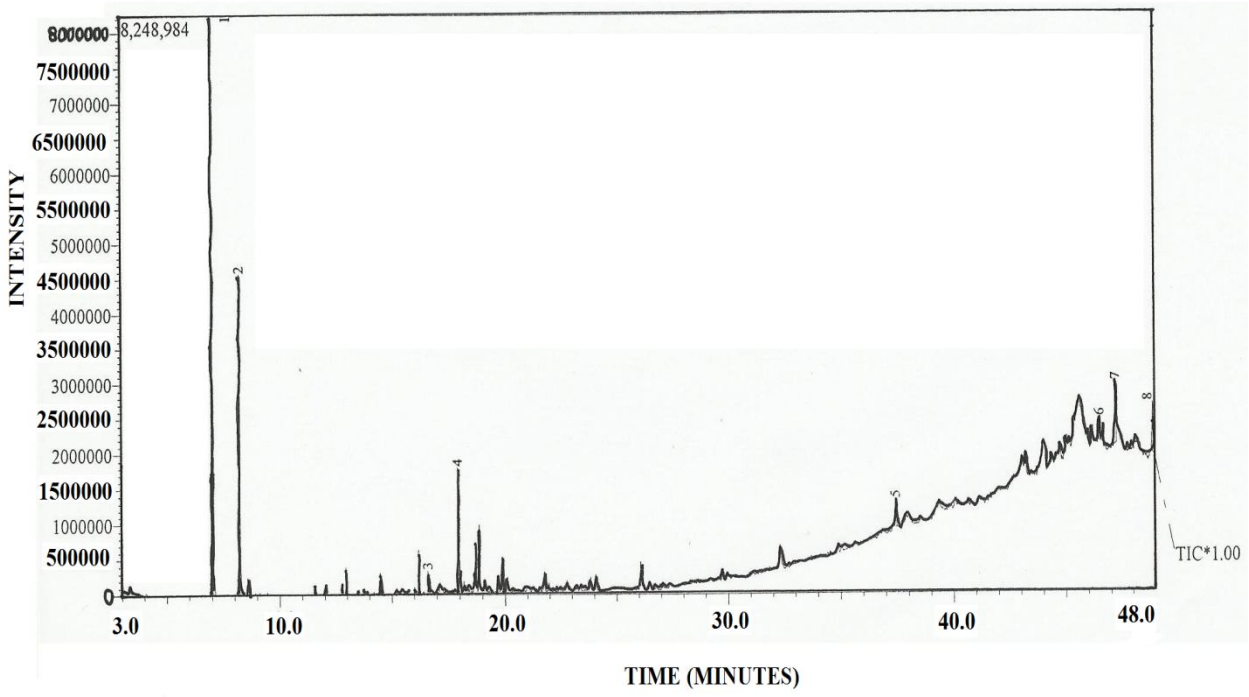


Figure 4.27: GC of extract from mixed soil sample at t = 14 weeks

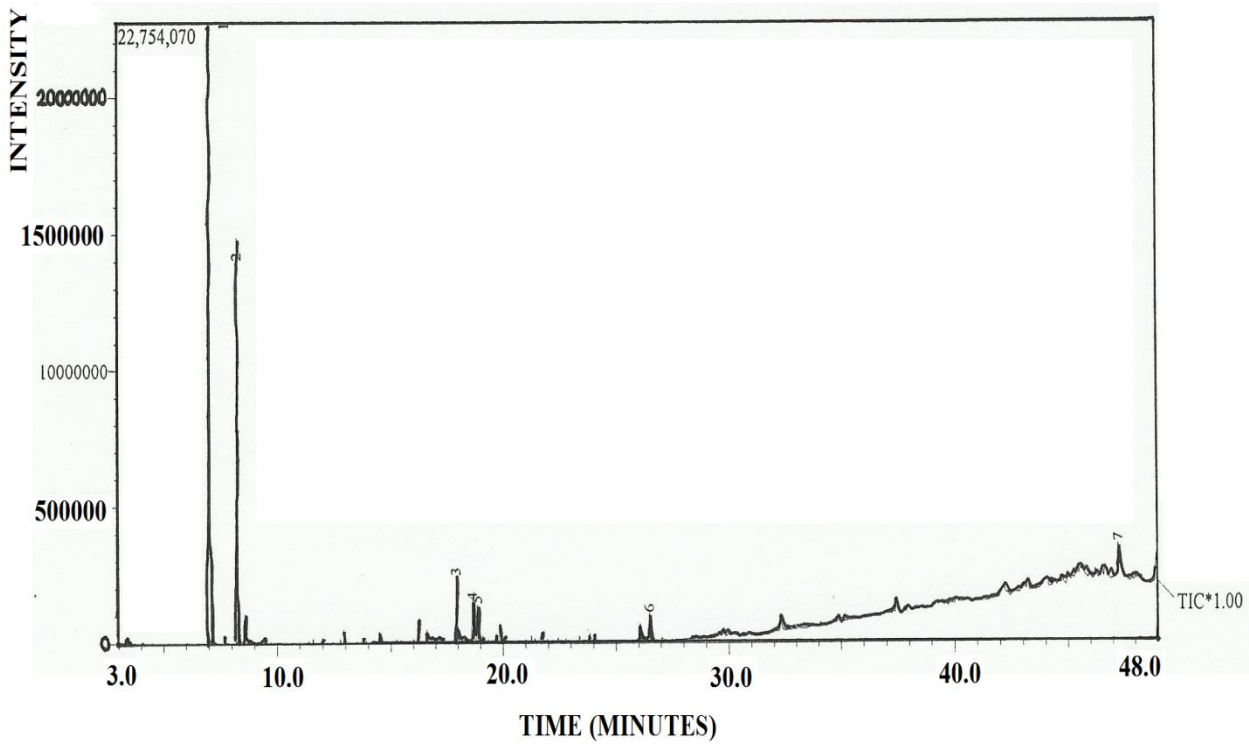


Figure 4.28: GC of extract from soil sample with 15 worms at t = 14 weeks

Table 4.28: MS analysis of the effect of earthworm to manual mixing on bioremediation of UEO contaminated soil

	Composition of UEO		
	t = 0	**15 worms, t = 14 weeks**	**Mixed, t = 14 weeks**
C9 - C19	54.58	100	88.83
C20 - 29	10.37	0	11.18
C30 and Above	35.05	0	0

4.5.3.1 Effect on microbial count

Again,as with Figure 4.24, Figure 4.29shows that there is no significant difference in microbial count between the earthworms treated and untreated (tilled and untilled) samples. All the samples showed a drop in microbial count after day 0. This drop was probably a result of the toxic effect used engine oil had on microbial community. However, the tilled sample on and after day 35 had the highest microbial count up to day 70. For the corresponding length of time (day 35 to 70) the tilled sample as shown in Figure 4.29, did not record the highest TPH drop. Therefore, the effect that the organic amendment and the presence of earthworm have on TPH reduction in bioremediation,may not be as a result of the effect they have on the microbial biomass alone.

4.5.3.2 Microbial Identification

Table 4.29 presents the result of the isolation and identification of the earthworm inoculated sample after 14 weeks of treatment. Similar to the earthworm free cases of **Section 4.3.1.4** the organism isolated was *Bacillus cereus.*

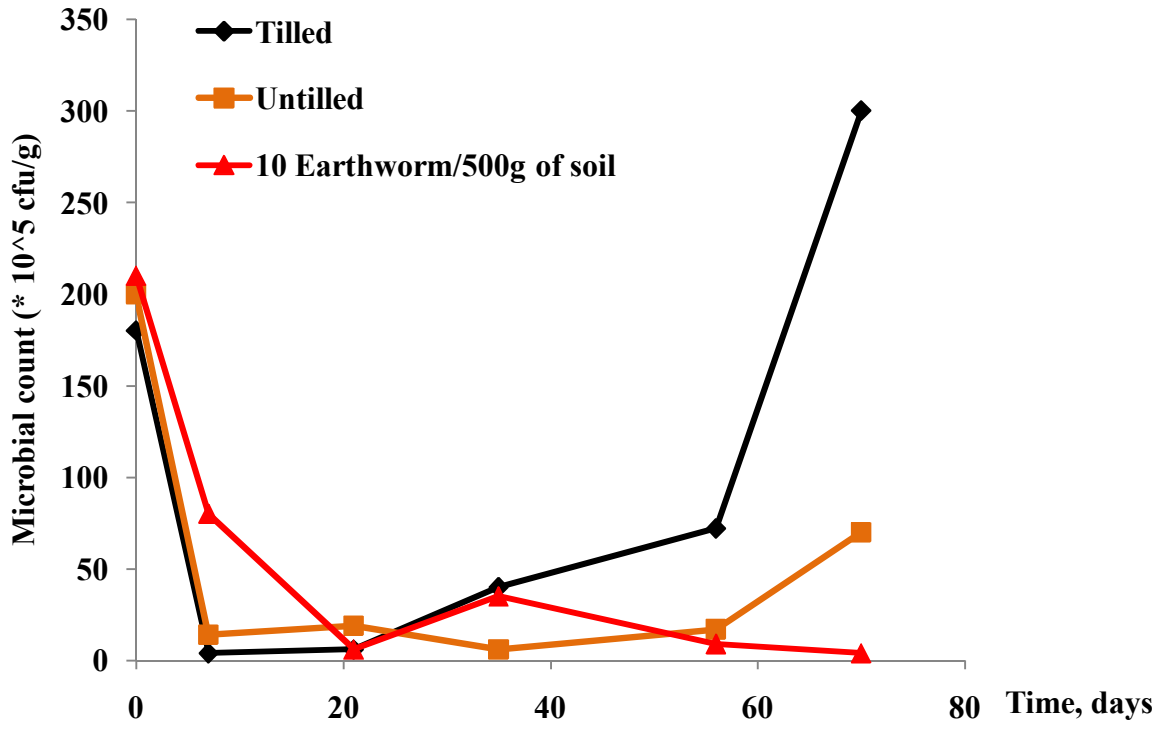


Figure 4.29: The efffect of tilling on microbial biomass

Table 4.29: Identification of bacteria for used engine oil contaminated soil biostimulated with 5g poultry manure and treated with 30 earthworms/kg soil

Gram reaction	Shape	Indole	MR	VP	Citrate	Catalase	Coagulase	Organism
+	Rod	-	-	-	-	+	-	*Bacillus cereus*

4.5.4 The Effect of Initial Organic Amendment Concentration on Bioremediation by Vermicomposting

The effect of manure concentration on bioremediation is presented in Figure 4.30.As shown in the figure, apart from the sample with 0gmanure/kg soil, all other sample indicated a positive drop in TPH at the end of 15 weeks. The sample with 10, 20, 30 and 40 g manure/kg soil recorded 15, 14.3, 23.1 and 15.4% drop in TPH respectively. These results suggest an optimum

food concentration in earthworm inoculated samples for bioremediation. That is, it seems an optimum nutrient concentration is required by earthworms in assisting bioremediation of used engine oil contaminated soil. This is in line with the report of **Schaefer *et al.* (2005)** who noted that residual TPH in the with-food system was greater than the without-food system. The TPH of the sample with zero worms remained relatively unchanged up to the week 5 after which there was a sharp increase in TPH as determined by the toluene cold extraction method. It was also observed that the worms started dying after week 5. It is therefore important when considering earthworms for bioremediation to intermittently replenish nourishment for the worms as they cannot feed on engine oil.

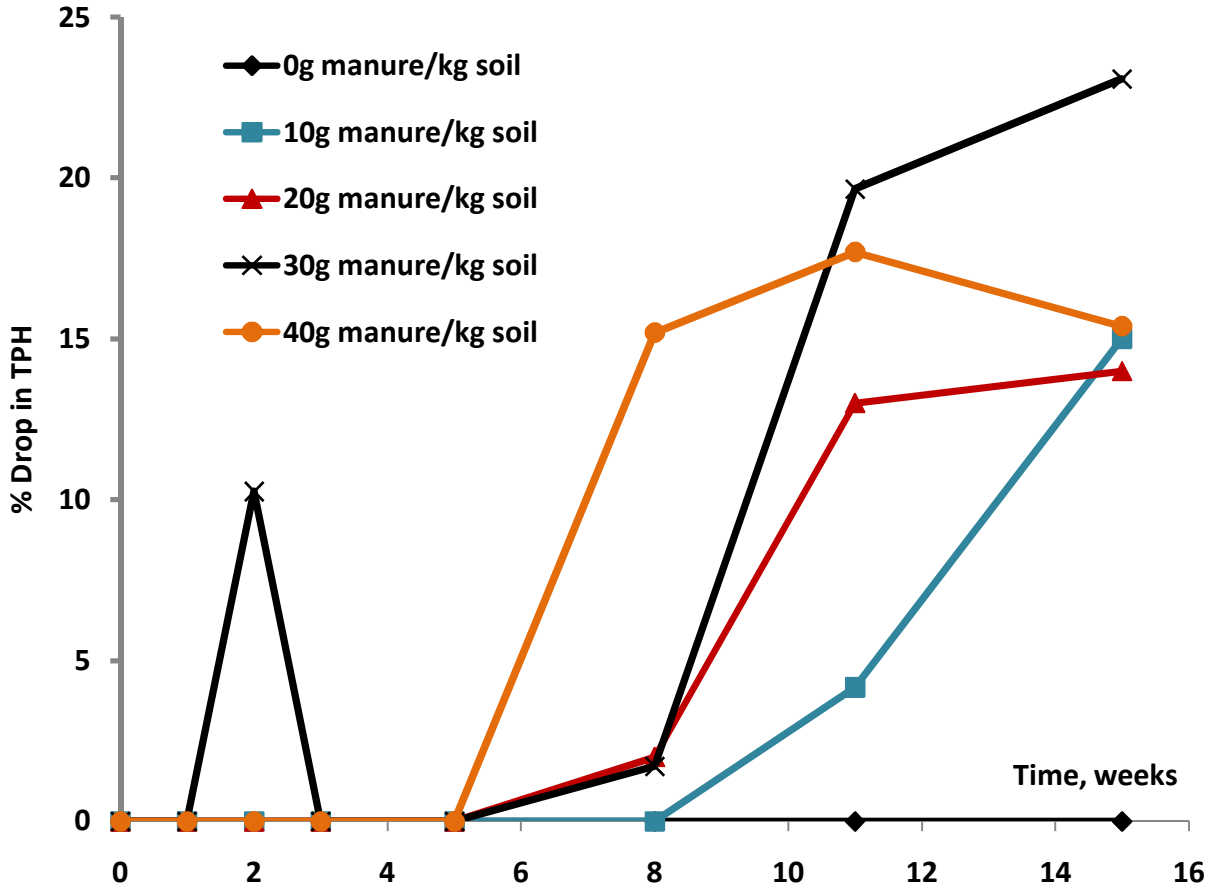


Figure 4.30 :Effect of initial manure concentration on earthworm assisted bioremediation

4.6 THE EFFECT OF EARTHWORM ACTIVITY ON THE BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOIL FROM MECHANIC WORKSHOP

Water holding capacity (WHC) and total petroleum hydrocarbon content of the auto mechanic workshop and motorcycle mechanic workshop soils are presented in Table 4.30. Table 4.31 presents the result of 35 days vermi-assisted bioremediation on the TPH and survival of the worms. For soil from both types of mechanic workshop considered, the use of earthworm inoculation proved to have an effect on the rate of degradation of petroleum hydrocarbon as shown in Table 4.31. All the samples treated with worms had higher drops in TPH than the corresponding control samples (without worms). The least effect was observed for the **MCU** samples (undiluted soil from motorcycle mechanic workshop) in which earthworm survival dropped to 0% (all the worms died) in less than 24. There was only a marginal difference in the drop in TPH recorded between the **MCU** and the control (24.27 and 24.57 respectively).

Table 4.30: Physicochemical property of soil from mechanic workshop

Sample	WHC (g water/kg soil)	TPH (g oil/kg soil)
MM	46.90	99.73
MC	50.00	150.20
DMM	42.27	42.29
DMC	49.79	76.43

MM: motorcar mechanic workshop soil, MC: motorcycle mechanic workshop soil, DMM: diluted motorcar mechanic workshop soil, DMC: diluted motorcycle mechanic workshop soil.

Table 4.31: Effect of earthworm inoculation on the bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshops

	Initial TPH, g/kg	% TPH Decrease after 35 days	% earthworm survival after 35 days
Soil from motorcar mechanic workshop			
MMUNW	75.45	34.97	
MMUW	75.45	36.35	30
MMDNW	31.61	29.50	
MMDW	31.61	39.10	80
Soil from motorcycle mechanic workshop			
MCUNW	136.36	24.57	
MCUW	136.36	24.27	0
MCDNW	69.27	28.84	
MCDW	69.27	44.18	70

MMUW: undiluted motorcar mechanic workshop soil treated with worms, MMUNW: undiluted motorcar mechanic workshop soil without worms, MMDW: diluted motorcar mechanic workshop soil treated with worms, MMDNW: diluted motorcar mechanic workshop soil treated without worms, MCUW: undiluted motorcycle mechanic workshop soil treated with worms, MCUNW: undiluted motorcycle mechanic workshop soil without worms, MCDW: diluted motorcycle mechanic workshop soil treated with worms, MCDNW: diluted motorcycle mechanic workshop soil treated without worms

4.6.1 Effect of Vermicomposting on the Total Petroleum Content of Mechanic Workshop Soil

It may also be observed that the highest drops in TPH for both mechanic workshop soil samples were recorded in the earthworm-treated dilute samples (44.18% and 39.10% for MCDW and MMDW respectively). Incidentally, these samples recorded an appreciable survival of earthworm over the duration of the study: on day 35, **MCDW** recorded 70% earthworm survival whereas **MMDW** recorded 80% survival of the earthworms.

For the undiluted soil samples (UD), it was observed in the course of the experiment that**MCUW** recorded 100% death of the worms in less than 24 hours. It was thus expected to have very similar bioremediation rate with the control (**MCUNW**) since all the worms died within 24 hours after introduction. Table 4.31 confirmed this as % drop in TPH for **MCUW** and **MCUNW** was found to be 24.26461 and 24.56509 respectively. The table also shows that **MMUW** recorded an earthworm survival rate of 30% at the end of the study (35 days) and also recorded a slightly higher TPH drop than the control (36.35 and 34.97 respectively). The death of the worms may be as a result of any or a combination of the following reasons: intolerable levels of petroleum hydrocarbon contamination, the pH of the soil, intolerable levels of heavy metal and carbon black (soot).

Figure 4.31 and 4.32presents the result of the fit of the bioremediation data obtained from the mechanic workshop soil samples (motorcar mechanic workshop soil and motorcycle mechanic workshop soil respectively) to the 1st order bioremediation kinetics. The results indicate a poor fit to the first order bioremediation kinetics (R^2 generally less than 0.9). However considering the duration of the study (only 35 days) it may be possible to obtain higher R^2 value for longer study periods.

Based on the result of this investigation the use of earthworm for the bioremediation of petroleum hydrocarbon contaminated soil from mechanic workshop would be recommended. Apparently, the presence of earthworms effected a net positive acceleration of the bioremediation process. Since bioremediation is mass transfer limited, the presence of the worms must have resulted in the production of biosurfactant(s), capable of facilitating the availability of oil in the aqueous phase.

More research work needed be undertaken to understand the mechanism. Possible pathways include: stimulation of biosurfactant production in the microorganisms, selective increase in the population of surfactant producing microorganisms, release of aged organic contaminants from soil as a result of size reduction, interplay between earthworm gut content, microorganism and contaminated soil as earthworms takes up and excrete soil etc.

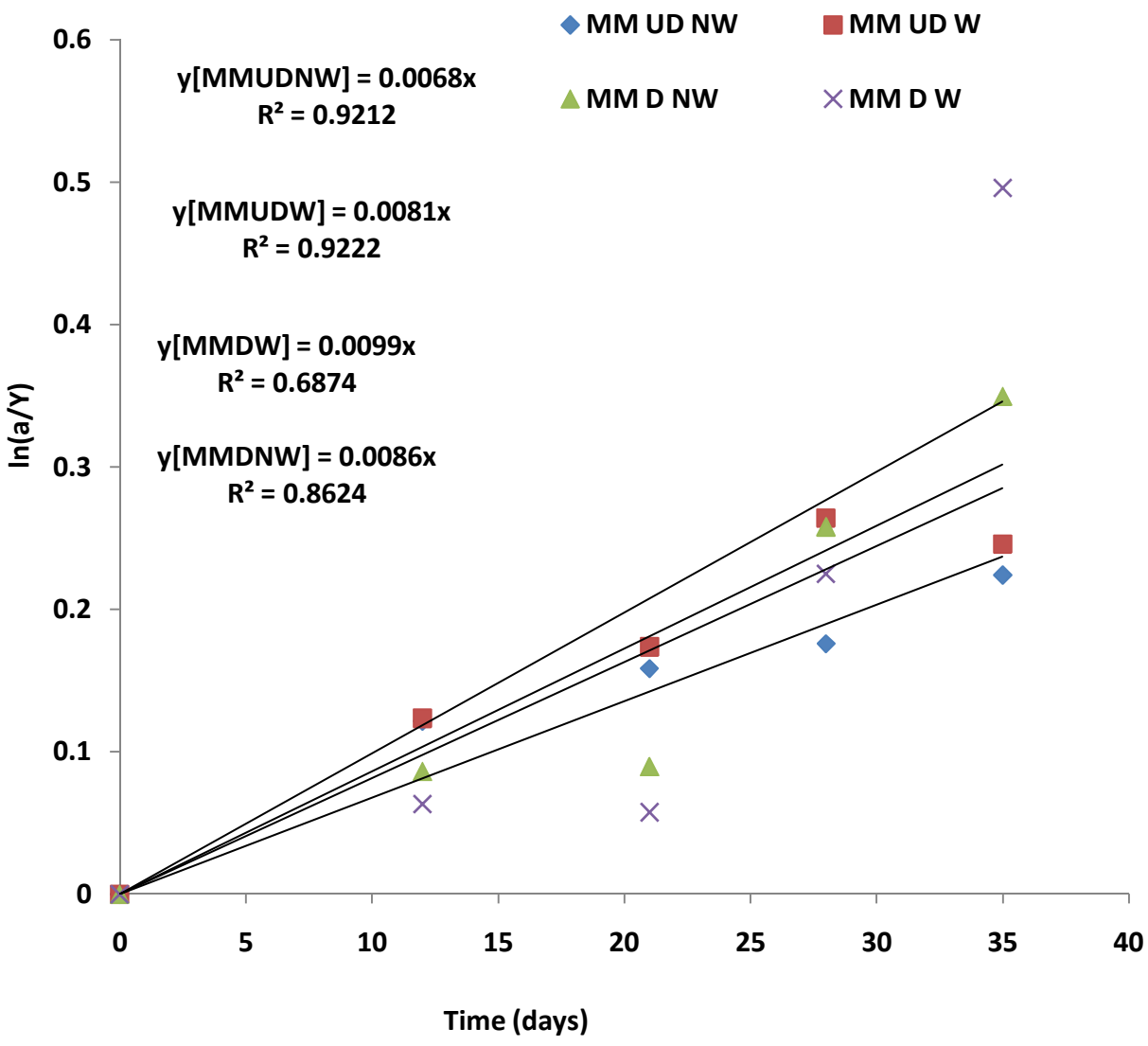


Figure 4.31: Effect of earthworm innoculation on the bioremediation kinetics of petroleum hydrocarbon contaminated soil from motorcar mechanic workshop

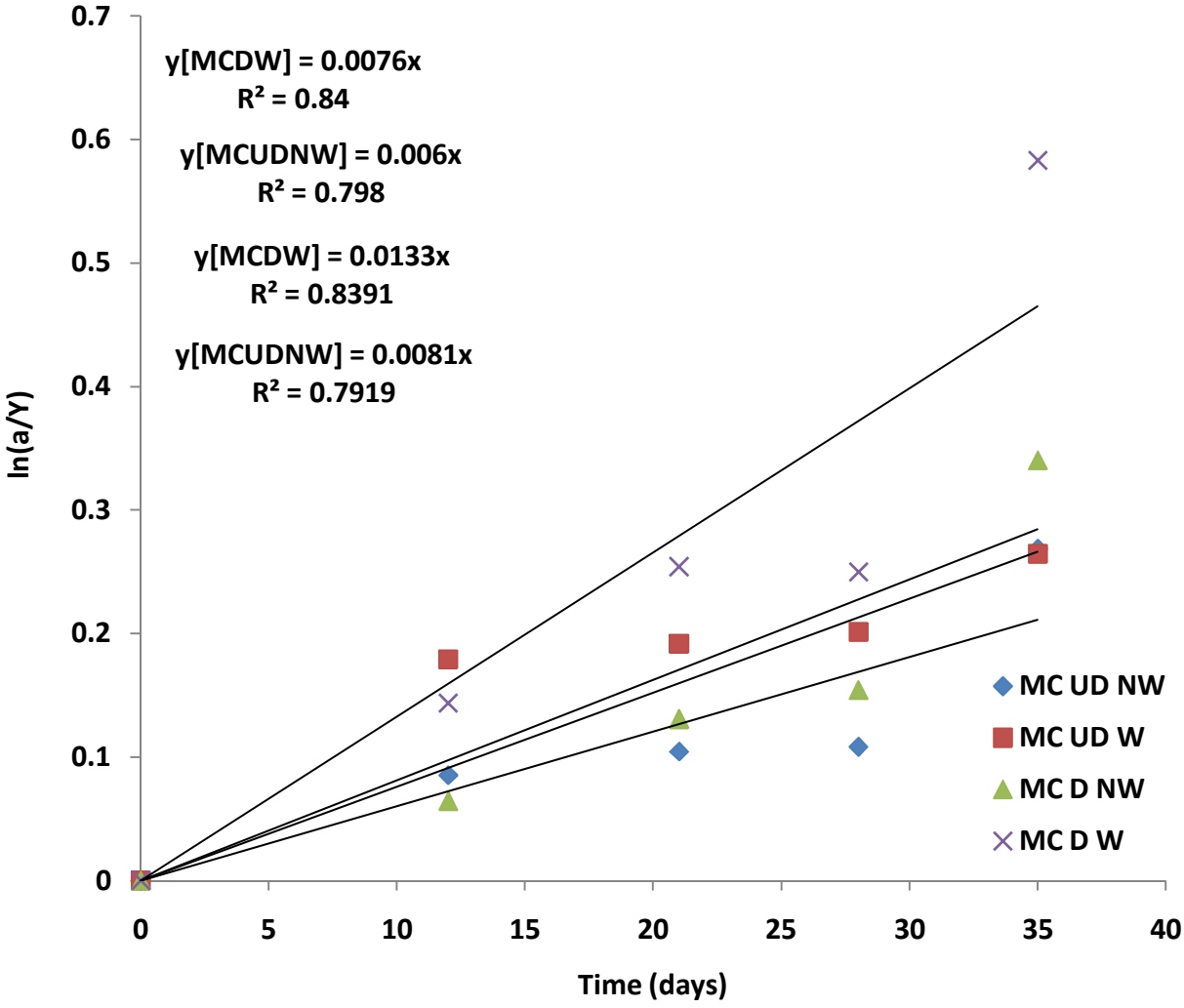


Figure 4.32: Effect of earthworm innoculation on the bioremediation kinetics of petroleum hydrocarbon contaminated soil from motorcycle mechanic workshop

Figures 4.33 and 4.34 present the result of the GC analysis of the diluted motorcycle mechanic workshop soil which recorded the highest drop in TPH while Table 4.32 presents the MS analysis for the same samples. The MS analysis of Table 4.32indicated that the C11 – C20 fraction of the contaminating petroleum hydrocarbon was most affected by the bioremediation

process, dropping from 95.47% to 92.31%. The C1 – C10 and the C20 –C30 fractions appeared to have increased. For the C1 – C10, this may be as a result of the breakdown of C11 – C20 resulting in the formation of more C1 – C10, whereas for the C20 –C30 the apparent increase in composition is most likely a result of the elimination of C11 – C20 fraction. In Table 4.31, MCDW recorded a TPH drop of 44.2% indicating that the microbial degradation of the C11 – C20 resulted in the formation of volatile organic materials which left the system or CO_2 and H_2O.

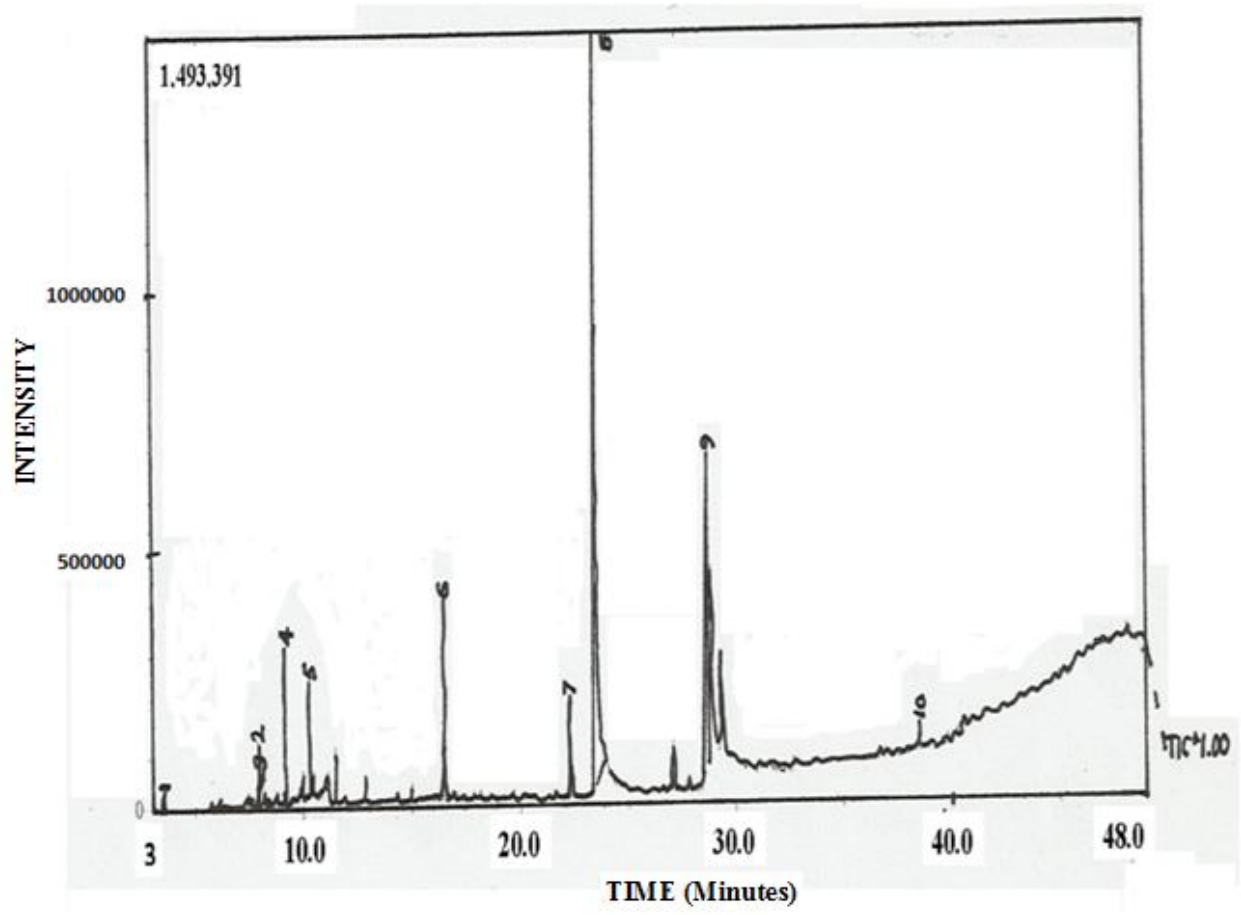


Figure 4.33: The GC chromatogram of the petroleum hydrocarbon contaminated of soil from motorcycle workshop prior to bioremediation (MCO).

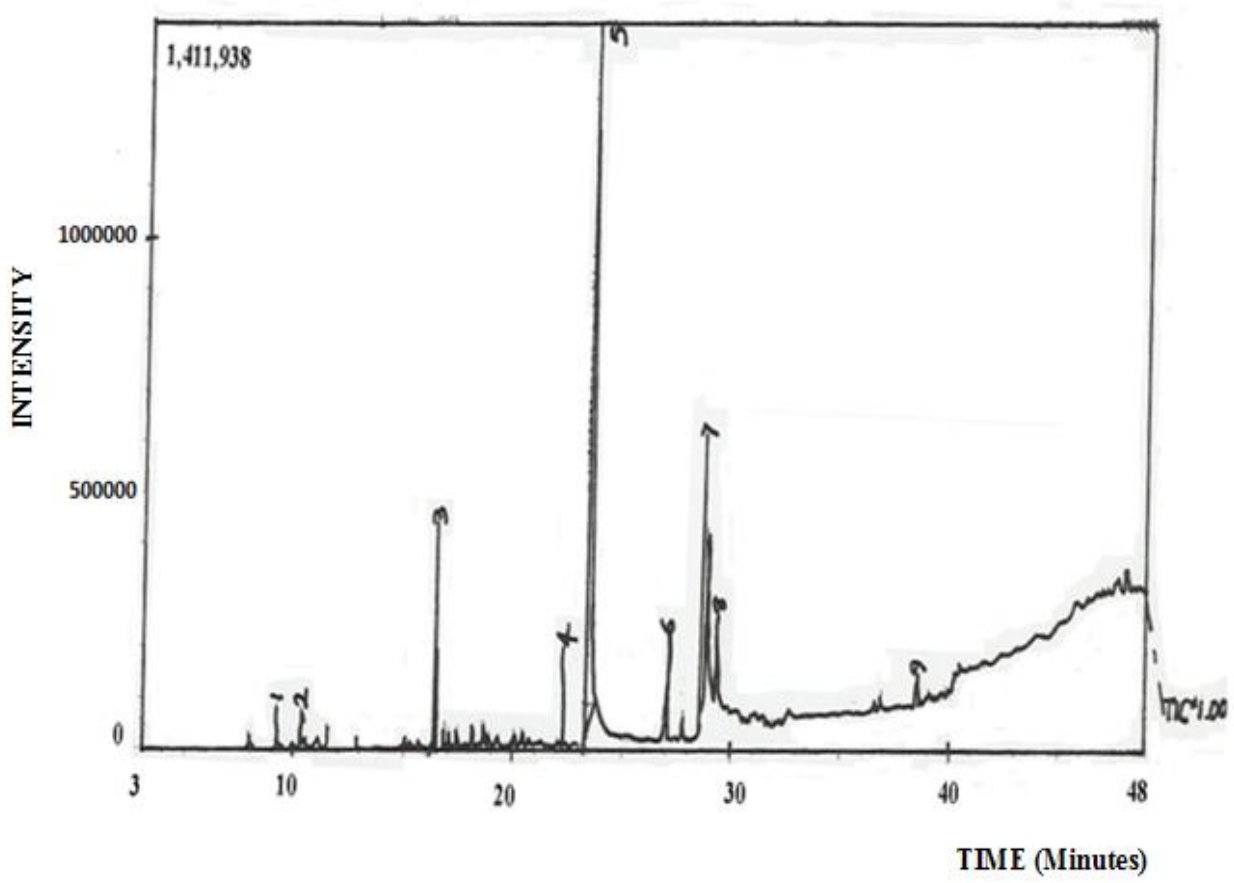


Figure 4.34: The GC chromatogram of the petroleum hydrocarbon contaminated of soil from motorcycle workshop after 35 days of vermin-assisted bioremediation (MC35).

Table 4.32: Effect of Vermin-Assisted Bioremediation on the Composition of Petroleum Hydrocarbon Content of Motorcycle Mechanic Workshop Soil.

	MCO	**MC35**
	% Composition	
C1 - C10	3.70	6.19
C11 - C20	95.47	92.31
C20 - C30	0.83	1.50

4.6.2 Effect of Vermi-Assisted Bioremediation on Microbial Community of Mechanic Workshop Soil

Table 4.33 presents the result of microbial count and identification performed on the various samples of mechanic workshop soil prior to bioremediation and after 35 days of bioremdiation.

From the table it can be seen that the microbial count of the raw motorcar and motorcycle mechanic workshop soil (MM and MC respectively) was very low. For instance at a dilution of 10^5 no microbe was enumerated in both samples. This shows the negative effect petroleum hydrocarbon contamination has on soil microbial community. After amendment with fermented cassava peels with earthworm inoculation (in some samples) microbial activity picked up as shown at the end of the 35 day study. The microbial count increased by a factor of at least 17, which is significant. In fact for MC at dilution of 10^4, the count increased by more than 300 times. This increase in microbial count however may not directly be the reason for the reduction in TPH: the samples without worms both indicate slightly higher microbial counts than the corresponding samples with worms though the TPH analysis indicated that the samples with worms recorded lower values of TPH compared to the corresponding samples without worms. Thus increased microbial count may have contributed but other factors may have also influenced the TPH of the samples. The microorganisms isolated in all samples were similar: *Bacillus sp, pseudomonas sp* and *staphylococcus sp. Staphylococcus sp.*which was not identified in the raw mechanic workshop soils now appeared in bioremediated samples. This may be from the cassava peels. All these microorganisms are known to produce biosurfactants of interest for application in petroleum hydrocarbon contaminated sites.

Table 4.33: Effect of Vermi-assisted Bioremediation on the Microbial Count and Identification of Mechanic Workshop Soil Contaminated with Petroleum Hydrocarbon.

	10^3	10^4	10^5	Organisms Isolated
MCDW	TNTC	3.18*10^5	8.15*10^5	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MCDNW	TNTC	3.34*10^5	8.7*10^6	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MMDW	TNTC	4.08*10^5	1.21*10^6	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MMDNW	TNTC	3.39*10^5	9.4*10^6	*Bacillus sp, Pseudomonas sp and Staphylococcus sp*
MC (Raw)	5.0*10^3	1.0*10^3	NIL	*Pseudomonas sp*
MM (Raw)	1.19*10^4	2.0*10^4	NIL	*Bacillus sp and Pseudomonas sp*

TNTC = too numerous to count

MM (Raw): soil obtained from motorcar mechanic workshop (without organic amendment/earhworms), MC (Raw): soil obtained from motorcycle mechanic workshop (without organic amendment/earhworms) MMDW: diluted motorcar mechanic workshop soil treated with worms, MMDNW: diluted motorcar mechanic workshop soil treated without worms, MCUW: undiluted motorcycle mechanic workshop soil treated with worms, MCDW: diluted motorcycle mechanic workshop soil treated with worms, MCDNW: diluted motorcycle mechanic workshop soil treated without worms

4.6.3 Effect of Earthworm innoculation on the Carbon and Nitrogen Contents of Bioremediated Mechanic Workshop Soil

Table 4.34 presents the result of carbon and nitrogen content of the various samples of mechanic workshop after 35 days of bioremediation.Comparing the results from the diluted samples (**MMDW/MMDNW**, and **MCDW/MCDNW**) which recorded high earthworm survival in the 35 day study, the trend in the carbon/nitrogen contents followed the same pattern.

Table 4.34: Effect of Vermi-assisted bioremediation on the Carbon and Nitrogen contents of Mechanic Workshop Soil Contaminated with Petroleum Hydrocarbon.

Sample	% Nitrogen	%Carbon
MMUW	0.49	11.88
MMUNW	0.53	9.77
MMDW	0.39	4.88
MMDNW	0.53	6.19
MCUW	0.53	11.72
MCUNW	0.49	11.72
MCDW	0.42	8.14
MCDNW	0.49	9.12

MMUW: undiluted motorcar mechanic workshop soil treated with worms, MMUNW: undiluted motorcar mechanic workshop soil without worms, MMDW: diluted motorcar mechanic workshop soil treated with worms, MMDNW: diluted motorcar mechanic workshop soil treated without worms, MCUW: undiluted motorcycle mechanic workshop soil treated with worms, MCUNW: undiluted motorcycle mechanic workshop soil without worms, MCDW: diluted motorcycle mechanic workshop soil treated with worms, MCDNW: diluted motorcycle mechanic workshop soil treated without worms

The carbon and nitrogen contents were lower in the earthworm inoculated samples when compared with the earthworm free samples. Nitrogen is lost mainly through ammonia volatilization or denitrification, The process is performed primarily by heterotrophicbacteria (such as Paracoccusdenitrificans and various pseudomonads). Carbon is lost in the form of CO_2

during respiration of earthworms/microbes. The results indicate heightened loss of nitrogen and carbon in the earthworm inoculated samples. The observed trend is differs from the results of **Section 4.4.2** and the findings **Li *et al.* (2002)** that earthworms had no significant influences on nitrogen content. It is possible that the organic amendment (fermented cassava peels) as well as the nature of the soil (contaminated with petroleum hydrocarbon over a long period of time which consequently affected the microbial community) used in the investigation offered a different condition that consequently affected the rate of mineralization of nitrogen.

4.6.4 Effect of Bioremediation on Earthworm Metal Uptake and Elimination

Table 4.35presents the result of metal uptake and elimination by the worms in the 35 day study. Comparison of the metal contents of Table 4.35 with the report of **Uba *et al.,* (2009)**in which heavy metal contents of worms around dumpsites in Zaria metropolis where analyzed, indicated that Zn, Cu and Mn were present in both results. However, whereas Pb and Cd were reported by **Uba *et al.,* (2009)**, they were not detected in Table 4.35. Furthermore, the Zn, Cu and Mncontents shown in the table worms were all higher than their findings.They also reported Cd, 0.55 - 8.13mg/kg; Zn, 105.82 - 380.95mg/kg; Cu, 0.66 - 10.25mg/kg; Pb, 5.01 - 265.40mg/kg and Mn, 1.26 - 10.23mg/kg. It should however be noted that whereas they used AAS method of analysis, this work used XRF analysis based on the body weight of dried earthworm carcass.

Elemental analysis of the soil (petroleum hydrocarbon free) from which the worms were obtained indicated that its iron (Fe) content was higher than that of all mechanic workshop soil considered.The composition of all other metals was close to or lower than the values recorded in the mechanic workshop soils. The earthworms prior to introductions into the mechanic workshop

soil (REW) showed a corresponding high Fe content. As was discussed in **Section *4.4.2.*5**, the metals of interest were Zn, Cu, Cr, Ni, Ti and Mn.

Table 4.35: Earthworm metal uptake and elimination in the vermin-assisted bioremediation of mechanic workshop soil

Element	Earthworm Sample				Soil Sample				
	REW	MMUW	MMDW	MCDW	UCWS	MMUW	MMDW	MCDW	MCUW
Al	6.878	0.000	6.878	5.820	6.243	2.540	6.878	5.450	4.127
Si	10.930	4.036	12.425	16.676	25.410	22.468	33.197	31.669	29.708
S	5.240	8.680	4.320	4.720	0.376	0.560	0.000	0.744	2.580
K	8.058	11.453	7.627	6.108	3.718	4.067	2.465	3.494	2.896
Ca	8.864	17.441	8.435	6.004	4.796	8.077	1.501	1.573	3.031
Ti	0.689	0.276	0.893	0.743	1.031	0.803	0.566	0.893	0.605
V	0.030	0.0336	0.032	0.027	0.050	0.051	0.0308	0.0437	0.026
Cr	0.044	0.151	0.052	0.032	0.054	0.045	0.0226	0.047	0.099
Mn	0.085	0.070	0.147	0.132	0.093	0.077	0.058	0.108	0.148
Fe	11.106	7.518	10.576	9.712	15.666	10.873	7.276	9.52	9.569
Ni	0.035	0.110	0.039	0.030	0.039	0.028	0.008	0.033	0.035
Cu	0.066	0.104	0.058	0.036	0.061	0.064	0.013	0.053	0.058
Zn	0.321	0.2892	0.249	0.193	0.225	0.201	0.023	0.145	0.276
Ba	0.251	0.269	0.215	0.134	0.305	0.430	0.000	0.260	0.134

(REW: Earthworm at t = 0, before introduction into mechanic workshop soil, MMUW: Undiluted motorcar mechanic workshop soil, MMDW: Diluted motorcar mechanic workshop soil, MCDW: Diluted motorcycle mechanic workshop soil, MCUW: Undiluted motorcycle mechanic workshop soil, UCS: petroleum hydrocarbon free soil used in diluting the mechanic workshop soil)

Just as with the simulated used engine oil contaminated soil, there was an appreciable uptake of Ti and Mn in MMDW and MCDW that recorded high survival rate of earthworm. MMUW which recorded a 70% death rate of the worms however recorded elimination of both Ti and Mn. This may be because the unfavorable environment affected the feeding behavior of the worms leading to the elimination of these metals.

For the metals Zn, Cu, Ni and Cr which recorded elimination in the study using simulated used engine oil contaminated soil: the earthworm body concentration of Zn for all samples decreased.

Theconcentration of Zn in the soil from which the worms were obtained was also found to be higher than all mechanic workshop soils considered. This suggests that a relationship exists between the earthworm body concentration of the metal and the soil. The concentration of Cu was found to be slightly higher in MMUW when compared to the UCWS but lower than MMDW and MCDW. MMDW and MCDW after the 35 day study recorded a drop in earthworm body concentration of Cu, as was recorded in the simulation study. The anomalous behavior with MMUW may again be attributed to unfavorable environment which may have resulted in the preferential excretion of other materials by the worms, resulting in an increased Cu Concentration. A definite relationship between earthworm body concentration and the concentration in the soil cannot be seen from the concentration in Ni and Cr. Heavy metals uptake/elimination becomes important when earthworms are considered for use in selective removal of metals from contaminated soil or when the possibility of transferring harmful metals down the food chain exist. With this in mind, it is of note that the concentration of the following metals in worms introduced into the following samples increased sharply: Cr, Cu and Ni (MMUW), Mn(MMDW and MCDW).

4.7 KINETIC MODELING OF BIOREMEDIATION

Microbes are primarily responsible for the degradation of petroleum hydrocarbon in the course of bioremediation. For bioremediation to take place, oxygen (O_2) and the petroleum hydrocarbon contaminant have to dissolve in water, after which they are absorbed by the microorganism present and via metabolism break down the petroleum hydrocarbons (PH's).

Hydrocarbons differ in their susceptibility to microbial attack and generally degrade in the following order of decreasing susceptibility: n-alkanes >branched alkanes >low-molecular

weight aromatics >cyclic alkanes **(Perry, 1984)**. In breaking down the hydrocarbons, the contaminants do not just directly get converted from PH's to harmless CO_2 and O_2: the most common pathway of alkane degradation is oxidation at the terminal methyl group; alkane → alcohol → fatty acid → ketone → CO_2 and H_2O. Formation of intermediate products take place, the presence of which may or may not affect the rate of bioremediation.When environmental conditions (pH, toxicity, etc.) are corrected and microbes are acclimated, the bioremediation rate depends on the biodegradation rate of the oil (within the microbes), the supply of oxygen and nutrients, and the mass transfer of contaminants to the right environment **(Li et al., 1995; Koren *et al.,* 2003)**. This mass transfer rate of oil is in general smaller than either the oxygen transfer rate or the biodegradation rate and therefore is the controlling step of bioremediation of oil-contaminated soil due to the strong hydrophobicity and low water solubility of these compounds **(Li et al., 1995)**. Another rate-limiting step for bioremediation of polyaromatics hydrocarbon (PAH) -contaminated soil is the strong sorption between the contaminant and soil. For contamination levels in which the soil is saturated with oil, rate limitation by strong sorption between contaminants and soil would be negligible and mass transfer limitations will dominate.

From the growth rate curve of Figure 2.2, the lag phase is preceded by the phase of exponential growth in which microbial count increase sharply followed by a stationery phase then the death phase. If an assumption is made that the biodegradation starts at the stationery phase then proceeds through the death phase, then bioremediation may be likened to a deactivating catalyst process. The microbial count may in the analogy be taken to be synonymous with active sites: reduction in microbial count to deactivation of catalysts active sites.With this in mind and drawing from the types of catalyst deactivation **(Levenspiel, 1999)**, independent deactivation

would be the process under consideration. For a constant density batch-solid reactor **(Levenspiel, 1999):**

$$-r_A' = k' C_A{}^n \quad \ldots\ldots (4.6a)$$

$$-\frac{da}{dt} = k_d a^m \quad \ldots\ldots (4.6b)$$

Where

a = activity of the catalyst

k' = reaction rate constant

C_A = concentration of reactant A

k_d = deactivation co

$-r_A$'= rate of reaction

n = order of reaction

m = order of deactivation

This represents n^{th} -order reaction and m^{th}-order deactivation which is also concentration independent. For bioremediation of petroleum hydrocarbon (PH's), the reaction may be summarized as:

$$\textbf{PH's} + \textbf{O}_2 \quad \rightarrow \quad \textbf{H}_2\textbf{O} + \textbf{CO}_2$$

According to **(Li *et al.*, 1995)** only when the oil transfer rate is enhanced by at least 10 times does the oxygen transfer process become the rate-controlling step. Thus it may be assumed that without enhancement, the reaction takes place in the presence of excess O_2. The reaction may therefore be considered 1^{st} order with respect to PH's as the solubility and hence availability of PH's in water is very low.

In developing an expression relating the changing fluid (in this case oil) concentration with time and using time as the one independent variable throughout the run the kinetic expressions of Equation 4.6 become

iv. For a nth order reaction and unit initial activity which remains constant (i.e no deactivation) the expression becomes:

$$-\frac{dC_A}{dt} = \frac{W}{V}\left(-\frac{1}{W}\frac{dN_A}{dt}\right) = \frac{W}{V}(-r'_A) = \frac{W}{V}kC_A^n = k'C_A^n \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.7)$$

Plugging in the 1st order kinetics:

$$(-r'_A) = \frac{dC_A}{dt} = k'C_A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.8)$$

$$ln\left(\frac{C_{Ao}}{C_A}\right) = k't \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.9)$$

This gives the simple first order kinetics also reported by Yeung *et al.* (1997)

v. For first order kinetics and zero order deactivation

$$-\frac{dC_A}{dt} = (-r'_A) = k'C_A\mathbf{a} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.10)$$

$$-\frac{da}{dt} = k_d a^o = k_d \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.11)$$

Integrating,

$$a = 1 - k_d t \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.12)$$

Substituting in Equation (4.9)

$$-\frac{dC_A}{dt} = k'C_A(1 - k_d t) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.13)$$

$$ln\left(\frac{C_{Ao}}{C_A}\right) = k'\left(t - \frac{k_d t^2}{2}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.14)$$

vi. For first order kinetics and first order deactivation:

$$ln\frac{C_{Ao}}{C_A} = \frac{k'}{k_d}\left(1 - e^{-k_d t}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.15)$$

Other kinetic models that may be considered include the shift-order kinetic model which usually characterizes enzyme-substrate reactions:

$$-r_A = \frac{k_1 C_A}{k_2 + C_A} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.16)$$

4.7.1 Testing of kinetic models to experimental bioremediation data

Table 4.37 presents the result of the simulation of experimental bioremediation data of **Section 4.3.1.1** with the model developed **(Equations 4.9, 4.14, 4.15 and 4.16)**. Whereas Table 4.37presents the result of the simulation of experimental bioremediation data of **Section 4.6.1.**For all the models considered as shown in Table 4.36 and 4.37, the shift order model of Equation gave the worst fit as depicted in the R^2 values which were very low (≤0.831). Next to shift order model on the worst model list is the first order kinetic model with unit catalyst activity (Equation 4.9) which was also proposed by **Yeung *et al.* (1997)**. Followed by the first order kinetics and zero order deactivation model (Equation 4.14) and finally the model with the best fit was the first order kinetics and first order deactivation model (Equation 4.16).

Table 4.36: Modeling of bioremediation kinetics

Sample	Equation (4.16)	Equation (4.9)	Equation (4.15)	Equation (4.14)
a_E = 0.514	0.4028	0.9404	nc	nc
a_E = 0.572	0.831	0.912	nc	nc
a_E = 0.335	0.1581	0.9809	0.9829	nc
a_E = 0.432	0.0293	0.9671	0.9791	nc

Table 4.37: Modeling of bioremediation kinetics

Sample	Equation (4.16)	Equation (4.9)	Equation (4.15)	Equation (4.14)
MCUDNW	nc	0.798	0.8288	0.8007
MCDW	nc	0.8391	0.8757	0.8464
MCDNW	nc	0.84	0.9026	0.8576
MMUDNW	nc	0.9212	0.9737	0.9763
MMDNW	nc	0.8624	0.9302	0.879

nc = not checked

However, though most of the R^2 for Equation (4.14) were lower that the corresponding ones obtained for Equation (4.16), it was slightly higher for MMUDNW. However, Table 4.37 shows that some of the R^2 for Equation (4.16) were less than 0.9 (for example MCDW), in which case the fits were not very good though better than the order models considered in this work. In conclusion, the 1st order reaction kinetics and 1st order deactivation kinetics (Equation 4.16) is proposed as the kinetic model for biostimulated and vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil.

Table 4.38 presents the values of the kinetics parameter k^1 and k_d for the samples shown in Table 4.37. k^1 is the 1st order chemical reaction rate constant and should have unit of per time. k^1 would be expected to vary with varying bioremediation conditions especially when mass transfer is enhanced. The deactivation constant k_d (also with per time unit) is expected to be dependent on nature and number of microbes, the concentration and nature of contaminant, the quantity and quality of organic amend and any other factor that can affect the microbial community. These may be responsible for the variation in k_d and k^1 values between similar samples (for example samples $a_E = 0.335$ and $a_E = 0.432$ on one hand and MCDW AND MCDNW on the other). It may also be observed in Table 4.39 that the k'/k_d values for all MC's are fairly constant.

Table 4.38: The empirical constants *k'* and k_d for the 1st order reaction and 1st order deactivation bioremediation model

	k_d	k'	k'/k_d
a_E = 0.335	0.05368	0.02823	0.52589
a_E = 0.432	0.03355	0.02093	0.62384
MCUDNW	0.00118	0.028256	23.946
MCDW	0.00261	0.062499	23.946
MC D NW	0.00156	0.035975	23.0611
MM UD NW	0.33163	0.074521	0.22471
MMDNW	0.00193	0.040785	21.1319

CHAPTER 5

CONCLUSIONS

This section gives the conclusions at the completion of the investigation.

The following conclusions were drawn from the results of this work.

11. Thesoil from mechanic workshops contains high levels of petroleum hydrocarbon and heavy metal worth considering for bioremediation.
12. Earthworm survival tests in used engine oil contaminated soil showed that earthworms (*E. eugeniae*) can survive in as high a contamination level of 150g UEO/kg of soil for up to 30 days. Concentration of organic amendment can affect earthworm survival in used engine oil contaminated soil.
13. Initial used engine oil concentration level affected bioremediation by vermicomposting with higher used engine oil concentrations favoring high bioremediation rates. Theuse of earthworms (*E. eugeniae*) had a significant advantage at high concentrations of used engine oil in soil.
14. Earthworm concentration affects the rate of bioremediation with higher rates favoured by higher earthworm concentrations.
15. The presence of earthworms also did not significantly affect soil microbial count.
16. Metals eliminated from earthworm when introduced into used engine oil contaminated soil of concentrations 12.5 – 100g/kg soil included Zn, Cu, Ni and Cr whereas those accumulated included Ti and Mn.
17. The most predominant bacteria in the sample treated with poultry manure and poultry manure/earthworm was *bacillus cereus*.

18. Earthworm inoculation was found to accelerate the bioremediation of mechanic workshop soil and the survival of the worms established to be an important parameter.
19. Use of earthworms (*E. eugenia*) for bioremediation of petroleum hydrocarbon potentially offers a means of accelerating the limiting step (the mass transfer resistancein bioremediation) and should be exploited for maximum benefit.
20. The kinetic model based on the 1^{st} order reaction kinetics and 1^{st} order deactivation kinetics is proposed biostimulated and vermi-assisted bioremediation of petroleum hydrocarbon contaminated soil.

CHAPTER 6

RECOMMENDATIONS

This section gives the recommendationsat the completion of the investigation.

9. Earthworm survival in mechanic workshops soil needs to be studied as the soil composition may be different from that used in this work. Mechanicworkshop soilstend to be contaminated with other material than the used engine oil considered in this work.
10. Other sources of organic amendments, such as cattle dung,should be used for biostimulation of earthworm inoculated used engine oil contaminated soil.
11. Owing to the benefit the environment stands to gain from success in this field, a GCMS machine and spectrophotometer should be made available for timely analysis of samples.
12. Earthworm assisted bioremediation needed be studied on other sources of petroleum hydrocarbon contamination e.g. diesel.
13. Bioremediation using some other earthworm species needed be considered: this work consider only*E. eugeniae*.
14. Proper legislation should be enacted to forestall the indiscriminate dumping of used engine oil in mechanic workshops. Other remediation strategies needed be studied to address the problems of pollution already in place at mechanic workshops
15. Innovative research should be encouraged in this field: who knows, one may come up with a means to change waste to wealth.
16. Metal uptake by earthworm from soil obtained from mechanic workshops needs to be studied as the soil may contain elevated levels of metals than that considered in this work.

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APPENDICES

APPENDIX A

This section gives the details of the standard experimental procedures used in characterizing soil and manure.

K. Soil pH determination

4. 50-ml plastic beaker
5. Glass rods
6. pH meter

Procedure: weight 20gm of air-dry soil (passed over 2-mm sieve) into 50ml beaker and add 50ml of distilled water. Stir the suspension several times during the next 30 minutes with a glass rod. Let the soil suspension stand for about 30 minutes to allow most of the suspended clay to settle out from the suspension. Insert the electrodes of the pH meter into the partly settled suspension and measure the pH **(Iwuafor *et al.* 1990)**.

L. Walkley-Black Method of Soil organic carbon determination(Iwuafor *et al.* 1990)

Apparatus

5. Burettes; 50ml or 25ml
6. Conical flasks, 250ml or 500ml
7. Measuring cylinder
8. pipette

Reagents:

7. Potassium dichromate ($K_2Cr_2O_7$): 1N – dissolve 49.04g of $K_2Cr_2O_7$ in distilled water and dilute to 1 – liter.
8. Concentrated sulphuric acid (H_2SO_4) if chloride is present in the soil add Ag_2SO_4 to the acid at the rate of 15g per liter.
9. O – phosphoric acid (H_3PO_4), concentrated.
10. O – phenanthroline – ferrous complex 0.025M (ferrous). When the indicator is not available it can be prepared by dissolving 14.85g of O – phenanthroline monohydrate and 6.95g of $FeSO_4.7H_2O$ in water and diluting to 1 liter.
11. Barium diphenylamine sulphonate (0.16%). This can be used in place of O – phenanthroline – ferrous complex (0.25M) dissolve 7.425g of O – phenanthroline and 3.475g of ferrous – sulphate in distilled water and dilute to 500ml.
12. Ferrous sulphate (0.5N) – dissolve 140g of $FeSO_4.7H_2O$ in water; add 15ml conc. H_2SO_4, cool and dilute to 1 liter. Standardize this reagent daily or each time before determining organic C by titrating against 10ml 1N K_2CrO_7.

Procedure:

11. Take a representative sample and grind to pass through 0.5mm sieve.
12. Weigh out 1.00g soil and place in the 250ml flask. Use 2.00g soil if organic C is suspected to less than 1%.
13. Pipette 10ml of 1N $K_2Cr_2O_7$ solution accurately into each flask and swirl gently to disperse the soil.

14. Rapidly add 20ml conc H_2SO_4 from a measuring cylinder. Immediately swirl the flask gently until soil and reagents are mixed, then swirl more vigorously for one minute. Rotate flash again and allow to stand on a sheet of asbestos for about 30 minutes. End point is easier to observe if suspension is cold.
15. Add 100ml of distilled water after standing for 30 minutes and let cool again. Filter suspension if cloudy.
16. Add 5ml of O-phosphoric acid to sharpen the color change at the end point (optional).
17. Add 3 – 4 drops of indicator and titrate with 0.5N ferrous sulphate solution on a white background. Note: as the endpoint is approached, the solution takes on a greenish; cast and then changes to darkgreen. At this point, add the ferrous sulphate drop until the color changes sharply from blue to red (maroon color) in reflected light against a white background
18. Make the blank determination in the same way but without soil to standardize the dichromate.
19. Calculate the results according to the following formulae:

$$\%\ organicCinsoil(air-drybasis) = \frac{\left((meqK_2Cr_2O_7 - meqFeSO_4) * 0.003\ * 100 * f\right)}{gofair-drysoil}$$

Where f = correction factor = 1.33

meq = Normality of solution * ml of solution used OR

$$\%\ organicCinsoil(air-drybasis) = \frac{\left((Blanchtitre - actualtitre) * 0.3\ * m * f\right)}{gofair-drysoil}$$

Where f = correction factor = 1.33

m = concentration of FeSO4

% organic matter (OM) may be calculated thus:

$$\% OM = \%OC * 1.729$$

20. % organic matter C may also be expressed on oven-dry basis after correction for moisture content in air-dry soil.

M. Bray No. 1 Method of Soil available phosphorus determination(Iwuafor *et al.* 1990)

Apparatus

6. Centrifuge
7. Mechanical shaker
8. Centrifuge tubes
9. Spectrophotometer
10. Volumetric flask

Reagents

4. Ammonium fluoride (NH4F), 1.0N (dissolve 37g of NH4F in distilled water and make upto 1 liter. Store in polyethylene bottle.
5. Hydrochloric acid HCl, 0.5N – (dilute 20.2ml of conc HCl with distilled water and make upto 500mls.
6. Extracting solution – Add 15ml of 1.0N NH4F and 25 ml of 0.5N HCl to 460ml distilled water.

Procedure

6. Weigh 1g of air-dried soil into a 15 ml centrifuge tube.
7. Add 7ml of the extracting solution
8. Shake for 1 minute on a mechanical shaker and centrifuge the suspension at 2,000rpm for 15 minutes.
9. Decant into an acid-washed volumetric flask.
10. Determine phosphorus in the extract colorimetrically using either the ascorbic acid molybdate blue or vanadomolybdate (yellow) methods.

N. Determination of total N in soil

Regular Macro-Kjedahl Method(Iwuafor *et al.* 1990)

Apparatus

4. Macro-Kjeldahl digestion-distillation apparatus/Aluminium block digestor.
5. Macro- Kjedahl Flask, 500ml or tubes.
6. Volumetric Flasks, 100ml

Reagents

6. Concentrated sulphuric acid.
7. Catalyst – 500gm sodium sulphate (Na2SO4) or potassium sulphate (K2SO4). 50g of anhydrous cupper sulphate 0.5g of selenium powder (mix these different reagents and ground to fine powder).
8. Boric acid (2%) – dissolve 20g of boric acid in 1000cm3 volumetric flask.
9. 40% NaOH – weigh out 400g of NaOH and dissolve in 800cm3 distilled water leave to cool before making to mark in 1 litre volumetric flask.

10. 0.01M HCl – measure 0.86ml of conc. HCl and make it up in 1000cm3 volumetric flask.

Mix Indicators

Weigh out 0.2g of methyl blue into a 100cm3 volumetric flask also add 0.4g of methyl red into another 100ml volumetric flask. Dissolve both indicators with ethanol. Mix both flask in a 200cm3 volumetric flask and mix thoroughly.

Or

Methyl red – bromocresol green mixed indicator. Dissolve 0.5g of bromocresol green and 0.1g of methyl red in 100ml of 95% ethanol adjust pH to 4.5 with 0.1M of NaOH or HCl.

Procedure

14. Weigh out 2g soil into Kjedahl flask or digestion tube.
15. Add 20ml of distilled water, swirl the flask for a few minutes and allow it to stand for 30 minutes.
16. Add about 3g of catalyst and 20ml of conc H_2SO_4.
17. Heat contiously on a low heat. When the water has been removed and frothing has ceased, increase the heat until the digestion clears. Then boil the mixture for 5 hours. Regulate the heating during this boiling so that the H_2SO_4 condenses about half way up the neck of the flask or tube.
18. Allow the flask to cool and slowly with shaking add some distilled water.
19. Carefully transfer the digest into another clean flask. Retain all sand particles in the original digestion flask because sand can cause severe bumping during distillation. Wash

the sand residue with 50 ml of distilled water for about four times and transfer each portion into the same flask. Make to mark with distilled water.

20. Add 10ml H_3Bo_3 solution into a 500ml Erlenmeyer flask which is then placed under the condenser of the distillation apparatus. The end of the condenser should be at about 4 cm above the surface of the H_3Bo_3 solution.
21. Add 10ml of 40% NaOH slowly into 10ml of digest in the flask which has been attached to the distillation apparatus. Raise temperature until it boils.
22. Keep condenser cool (below 30^oC) by allowing sufficient water to flow through and regulate heat to minimize frothing and prevent suck back.
23. Collect about 50ml distillate and then stop distillation.
24. Titrate NH_3 liberated with standard HCl or H_2SO_4. Add 2 – 3 drops of indicator. The colour change at the end point is from green to pink.
25. Calculate the %N content in the soil thus
26. Run a blank similarly but without soil sample

$$\%\ N = \frac{(0.14 * VD * N * 100 * TV)}{wtofsoil * AD}$$

Where VD = vol. of digest

N = normality of acid

TV = titre value

AD = Aliquot of digest.

O. Determination of soil textural class(Iwuafor *et al.* 1990)

50g of 2mm sieved soil was weighed and put into a plastic beaker and 100ml of calgon was added to it and stirred with glass rod. 100ml of distilled water was then added and again stirred. The mixture was left to stand for about 30 minutes with occasional stirring though. The mixture was transferred into a 250ml plastic container and shaken on an end to end shaker for ten minutes. After this, it was transferred into $1000cm^3$ measuring cylinder with the contents completely washed into the cylinder and made up to $1000cm^3$ with water. After again shaking the mixture in the cylinder with a long paddle plunger, a hydrometer was used to take readings. Readings were taken for 40 seconds, 4 minutes, 2 hours, 8 hours, and 24 hours. A blank cylinder was also prepared by making 100ml of 50% calgon solution up to the 1 litre mark with water. The blank hydrometer readings were taken side by side the suspension readings. Temperatures of the suspension and the blank were taken.

After the last suspension reading was taken, the suspension was poured through a 2mm sieve and the sand grains retained on the sieve washed with water. The sand was then transferred into a beaker and dried in an oven at 105^0C. After drying the sand was weighed.

Calculations:

The corrected hydrometer readings C(g/L) are obtained by subtracting the blank reading R_L(g/L) from the hydrometer readings in the soil suspensions R(g/L) and adding 0.36g/L for every degree above 20^0C.

$$C = R - R_L + (0.36T)$$

Where T = room temperature minus20^0C.

The percentages by weight of the SILT + CLAY + SAND fractions are given by:

$$\%Clay = \frac{corrected\ 8\ hours reading - Blank}{wtofsoiltaken} * 100$$

$$\%Silt = \frac{(Corrected\ 40\ seconds reading - Blank)}{wtofsoiltaken} - \%Clay$$

%Sand = 100 − %Clay − %Silt

The sample can then be classified according to the USDA or International system of textural classification.

PARTICLE SIZE ANALYSIS ON SOIL (USAD) chart

CLASS	**% SAND**	**% SILT**	**% CLAY**
SAND	>90	< 10	< 10
LOAMY SAND	>80	< 25	< 15
SANDY LOAM	>65	< 30	< 25
SANDY CLAY LOAM	>55 – 80	< 15	< 20 – 35
SANDY CLAY	>40 – 68	< 10	< 35 – 55
LOAM	>45 – 68	< 15 – 30	< 8 – 25
CLAY LOAM	>35 - 60	< 8 – 35	< 25 – 40
SILTY CLAY LOAM	>40	< 25 – 75	< 25 - 40

APPENDIX B

Procedure for Analysis of samples using EDXRF

Mini Pal is a compact Energy dispersive X-ray spectrometer designed for the elemental analysis of a wide range of samples. The system is controlled by a PC running the dedicated Mini pal analytical software.

The Mini pal 4 version in use is PW 4030 X-ray Spectrometer, which is an energy dispersive microprocessor controlled analytical instrument designed for the detection and measurement of elements in a sample (solids, powders and liquids), from sodium to uranium.

The sample for analysis is weighted and grounded in an agate mortar and a binder (PVC dissolved in Toluene) is added to the sample, carefully mixed and pressed in a hydraulic press into a pellet.

The pellet is loaded in the sample chamber of the spectrometer and voltage (30kV maximum) and a current (1mA maximum) is applied to produce the X-ray to excite the sample for a preset time (10mins in this case). The spectrum from the sample is now analyzed to determine the concentration of the elements in the sample (**PanAnalytical user's guide, 2007**).

APPENDIX C

RAW RESULTS OF ANALYSIS

EFFECT OF EARTHWORM ON WATER HOLDING CAPACITY AND MEAN WEIGHT DIAMETER OF SOIL

Table 1: Effect of earthworm on water holding capacity of UEO contaminated soil

Earthworm concentration (number of worms/kg of soil)	WHC, g H_2O/g soil
0	0.483
10	0.487
20	0.488
30	0.505
40	0.494

Table 2: Effect of worms on aggregate size distribution

Earthworm concentration (number of worms/kg of soil)	Mean weight diameter
0	1.406
10	1.264
20	1.172
30	1.190
40	1.191

Table 3: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with poultry manure

	% survival of 10 earthworms (*E. eugeniae*)		
g UEO/kg soil	**Motorcycle UEO**	**Motorcar UEO**	**Truck/lorry UEO**
5	100.00	100.00	100.00
10	100.00	100.00	100.00
20	100.00	91.67	100.00
30	100.00	90.99	100.00
40	100.00	86.67	100.00
50	100.00	83.33	100.00
100	100.00	66.67	91.67

Table 4: Effect of UEO concentration (from motorcycle, motorcar and diesel engine vehicles) on the survival of earthworms in soil spiked with cattle dung

	% survival of 10 earthworms (*E. eugeniae*)		
g UEO/kg soil	**Motorcycle UEO**	**Motorcar UEO**	**Truck/lorry UEO**
5	100.00	100.00	100.00
10	100.00	100.00	100.00
20	100.00	93.33	100.00
30	100.00	90.99	100.00
40	100.00	86.67	100.00
50	100.00	83.33	100.00
100	100.00	70.00	100.00

INVESTIGATION INTO THE EFFECT OF INITIAL USED ENGINE OIL CONCENTRATION ON BIOREMEDIATION

Table 5: Initial pH of samples

Sample/manure	0g/kg	5g/kg	10g/kg	15g/kg	20g/kg
pH	6.7	6.8	6.89	6.81	6.85

Table 6: Experimental Data on the investigation into the effect of initial concentration of used engine oil in soil (Concentration versus time data)

Time, Days	5g/kg	10g/kg	15g/kg	20g/kg
0	5.01476	10.0049	14.9466	20.03364
14	5.886824	7.6794	11.36145	12.57265
28	8.16388	9.61732	12.23351	15.67332
42	5.8626	7.92164	9.762664	12.76644

INVESTIGATION INTO THE EFFECT OF EARTHWORM CONCENTRATION ON BIOREMEDIATION

Each sample contains: 1000g wet soil, 20g UEO, 6gm poultry manure.
Initial pH of samples = 6.9

Table 7: Effect of variable earthworm concentration on bioremediation

Time, Days	TPH (g UEO/kg soil)				
	0 worms	5 worms	10 worms	15 worms	20 worms
0	17.21429	17	17.85714	17.35714	17.5
14	19.64286	18.35714	16.14286	17.85714	15
28	12.07143	16.42857	17.71429	14.28571	15.85714
42	11.71429	15	16.42857	12.85714	12.42857

Table 8: Effect of worm concentration on microbial count

Time, Days	0 worms	5 worms	10 worms	15 worms	20 worms
	Microbial count * 10^(5) cfu/g				
0	250	200	250	150	200
14	3.2	3	3.1	2.3	2.8
28	75	219	68	80	54
42	27	40	106	20	74
56	20	8	13	11	6

VERMI-ASSISTED BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOIL FROM MECHANIC WORKSHOP

Table 9: Effect of vermin-assisted bioremediation on motorcar mechanic workshop soil

	0 day	12 days	21 days	28 days	35 days
MMUNW	75.45	66.81	64.39	63.28	60.31
MMUW	75.45	59.13	63.42	57.95	59.03
MMDNW	31.61	29.00	28.90	24.42	22.29
MMDW	31.61	27.33	29.84	25.24	19.25

Table 10: Effect of vermin-assisted bioremediation on motorcycle mechanic workshop soil

	0 day	12 days	21 days	28 days	35 days
MCUNW	136.36	125.22	122.86	122.37	104.24
MCUW	136.36	114.00	112.54	111.50	104.65
MCDNW	69.27	64.96	60.78	59.37	49.29
MCDW	69.27	60.00	53.73	45.71	38.67

Table 11: Earthworm heavy metal uptake and elimination from soil contaminated with 12.5g/kg of UEO

Time (days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1	1.42	1.6	1.4
V_2O_5	0.05	0.06	0.06	0.06	0.06
Cr_2O_3	0.26	0.17	0.13	0.45	0.17
NiO	0.26	0.16	0.11	0.5	0.16
CuO	0.29	0.18	0.1	0.39	0.15
ZnO	0.92	0.3	0.28	0.56	0.28
MnO	0	0.05	0.094	0.1	0.15

Table 12: Earthworm heavy metal uptake and elimination from soil contaminated with 25g/kg of UEO

Time(days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1.3	1.9	1.3	1.71
V_2O_5	0.05	0.07	0.04	0.04	0.07
Cr_2O_3	0.26	0.14	0.26	0.15	0.22
NiO	0.26	0.16	0.24	0.14	0.17
CuO	0.29	0.19	0.25	0.13	0.15
ZnO	0.92	0.41	0.47	0.4	0.41
MnO	0	0.078	0.16	0.09	0.12

Table 13: Earthworm heavy metal uptake and elimination from soil contaminated with 50g/kg of UEO

Time(days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1	1.2	1	1.4
V_2O_5	0.05	0.04	0.04	0.04	0.05
Cr_2O_3	0.26	0.15	0.16	0.14	0.3
NiO	0.26	0.17	0.15	0.17	0.26
CuO	0.29	0.16	0.15	0.16	0.23
ZnO	0.92	0.29	0.39	0.53	0.46
MnO	0	0.05	0.063	0.06	0.07

Table 14: Earthworm heavy metal uptake and elimination from soil contaminated with 75g/kg of UEO

Time (days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	1.1	1.5	1.2	1.1
V_2O_5	0.05	0.06	0.07	0.05	0.08
Cr_2O_3	0.26	0.18	0.19	0.22	0.22
NiO	0.26	0.22	0.19	0.21	0.16
CuO	0.29	0.24	0.19	0.18	0.16
ZnO	0.92	0.55	0.59	0.46	0.45
MnO	0	0.11	0.08	0.086	0.11

Table 15: Earthworm heavy metal uptake and elimination from soil contaminated with 100g/kg of UEO

Time (days)	0	5	10	15	20
Components	**Concentration (%)**				
TiO_2	0.88	0.94	1.2	1.1	1.2
V_2O_5	0.05		0.02	0.04	0.03
Cr_2O_3	0.26	0.24	0.28	0.22	0.22
NiO	0.26	0.25	0.33	0.24	0.18
CuO	0.29	0.25	0.28	0.25	0.17
ZnO	0.92	0.6	0.41	0.48	0.46
MnO	0	0.1	0.14	0.12	0.08

Table 16: Metal uptake and elimination by earthworm in mechanic workshop soil.

Oxide	REWEW	MUDWEW	MMDWEW	MCDWEW	UCSWS	MMUWS	MMDWS	MCDWS	MCUWS
					% Composition				
Al2O3	13		13	11	11.8	4.8	13	10.3	7.8
SiO2	23.4	8.64	26.6	35.7	54.4	48.1	71.07	67.8	63.6
P2O5	10.4	15	10.4	10.8	0.6	0.91	0.38	0.44	0.81
SO3	13.1	21.7	10.8	11.8	0.94	1.4	0	1.86	6.45
Cl	2.03	3.16	1.96	1.63		0.33	0.242	0.083	0.44
K2O	9.71	13.8	9.19	7.36	4.48	4.9	2.97	4.21	3.49
CaO	12.4	24.4	11.8	8.4	6.71	11.3	2.1	2.2	4.24
TiO2	1.15	0.46	1.49	1.24	1.72	1.34	0.944	1.49	1.01
V2O5	0.053	0.06	0.057	0.048	0.089	0.091	0.055	0.078	0.047
Cr2O3	0.064	0.22	0.076	0.047	0.079	0.066	0.033	0.068	0.144
MnO	0.11	0.091	0.19	0.17	0.12	0.1	0.075	0.14	0.191
Fe2O3	12.35	8.36	11.76	10.8	17.42	12.09	8.091	10.59	10.64
NiO	0.045	0.14	0.049	0.038	0.05	0.036	0.0098	0.042	0.044
CuO	0.083	0.13	0.073	0.045	0.076	0.08	0.016	0.066	0.073
ZnO	0.4	0.36	0.31	0.24	0.28	0.25	0.028	0.18	0.343
Ga2O3			0.02	0.03	0	0.02		0.026	
Br	0.62		0.51	0.43					0.22
ZrO2		2					0.2		
Rb20						14			
Rh207					0.65				
BaO	0.28	0.3	0.24	0.15	0.34	0.48		0.29	0.15
MoO3							0.57		
Re2O7	0.05	0.3		0.06	0.06	0.06	0.067	0.14	0.3
PtO2		0.3							
IrO2	0.4				0.21				
Au			0.45				0.09		

APPENDIC D

Pictorial representations of major equipment use in the course of this investigation



Figure A: Equipment for elemental analysis using XRF, MiniPal PW4025, Philips Analytical.



Figure B: MiniPal PW4025, Philips Analytical.



Figure C: GCMS equipment (GCMS-QP2010 Plus Shimadzu, Japan).



Figure D: Spectrophotometer (B. Bran Scientific and Instrument Company, 722 – 2000 20D spectrometer).



Figure E: Sieve shaker

Sample of Result of XRF Analysis of Motorcycle Mechanic Workshop Soil (MC5)

05-Nov-2010 14:38:51

Sample results

Sample Ident

SAMPLE MC 5			

Application	<Standardless>
Sequences	1 of 1
Measurement time	05-Nov-2010 14:34:30
Position	2

Channel	Al	Si	S	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu
Compound	Al2O3	SiO2	SO3	K2O	CaO	TiO2	V2O5	Cr2O3	MnO	Fe2O3	NiO	CuO
Conc Unit	12%	61.5%	3.5%	3.86%	1.53%	0.046%	0.068%	0.16%	12.24%	0.029%	0.029%	0.14%

Channel	Zn	Rb	Sr	Y	Zr	Ru	Ba	Eu	Pb
Compound	ZnO	Rb2O	SrO	Y2O3	ZrO2	RuO2	BaO	Eu2O3	PbO
Conc Unit	0.286%	0.042%	0.038%	0.049%	0.446%	0.75%	0.1%	0.08%	0.12%

Sample of Result of XRF Analysis of Motorcar Mechanic Workshop Soil (MM8)

05-Nov-2010 14:30:49

Sample results

Sample Ident			
SAMPLE MM8			

Application	<Standardless>
Sequences	1 of 1
Measurement time	05-Nov-2010 14:28:15
Position	6

Channel	Al	Si	S	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu
Compound	Al2O3	SiO2	SO3	K2O	CaO	TiO2	V2O5	Cr2O3	MnO	Fe2O3	NiO	CuO
Conc Unit	12%	53.4%	0.83%	3.37%	8.75%	1.54%	0.06%	0.071%	0.11%	17.60%	0.024%	0.13%

Channel	Zn	Rb	Sr	Y	Zr	Ru	Ba	Re	Pb	Th
Compound	ZnO	Rb2O	SrO	Y2O3	ZrO2	RuO2	BaO	Re2O7	PbO	ThO2
Conc Unit	0.26%	0.06%	0.053%	0.049%	0.429%	0.90%	0.1%	0.04%	0.23%	0.12%

Sample of Result of XRF Analysis of Earthworms (20g/kg UEO Contaminated Soil on Day 5), in Metal Uptake/Elimination Study

07-May-2011 16:25:38

Sample results

Sample Ident			
20C5			

Application	<Standardless>
Sequences	1 of 1
Measurement time	27-May-2011 16:19:3
Position	12

Channel Compound	Al2O3	SiO2	P2O5	SO3	Cl	K2O	CaO	TiO2	V2O5	Cr2O3	MnO	Fe2O3
Conc Unit	16%	14.4%	11.6%	16.1%	2.71%	9.85%	15.1%	1.0%	0.04%	0.15%	0.05%	10.4%

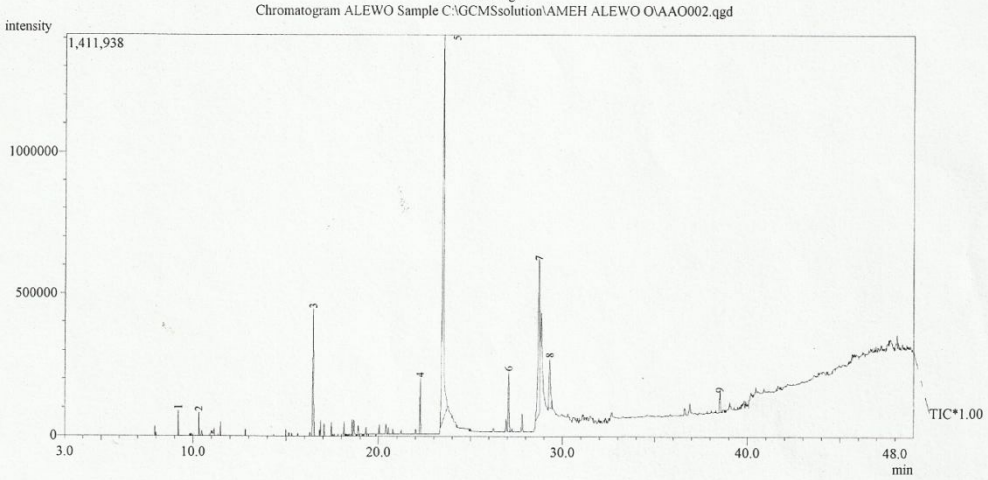
Channel Compound	NiO	CuO	ZnO	Br	BaO	Re2O7
Conc Unit	0.17%	0.16%	0.29%	1.1%	0.3%	0.71%

GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA
GCMS ANALYSIS

AMEH ALEWO O (SAMPLE-MC35W)

Chromatogram
Chromatogram ALEWO Sample C:\GCMSsolution\AMEH ALEWO O\AAO002.qgd



Method

[Comment]

===== Analytical Line 1 =====

[AOC-20i]

# of Rinses with Presolvent	:5
# of Rinses with Solvent(post)	:5
# of Rinses with Sample	:3
Plunger Speed(Suction)	:High
Viscosity Comp. Time	:0.2 sec
Plunger Speed(Injection)	:High
Syringe Insertion Speed	:High
Injection Mode	:Normal
Pumping Times	:5
Inj. Port Dwell Time	:0.3 sec
Terminal Air Gap	:No
Plunger Washing Speed	:High
Washing Volume	:8uL
Syringe Suction Position	:0.0 mm
Syringe Injection Position	:0.0 mm
Use 3 Solvent Vial	:1 vial

[GC-2010]

Column Oven Temp.	:60.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:100.2 kPa
Total Flow	:6.2 mL/min
Column Flow	:1.61 mL/min
Linear Velocity	:46.3 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:1.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	60.0	5.00
20.00	140.0	0.00
5.00	200.0	5.00

4.00 280.0 5.00

< Ready Check Heat Unit >
Column Oven : Yes
SPL2 : Yes
MS : Yes
< Ready Check Detector(FTD) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
SPL2 Carrier : Yes
SPL2 Purge : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait :No
Equilibrium Time :3.0 min

[GC Program]

[GCMS-QP2010 Plus]
IonSourceTemp :200.00 °C
Interface Temp. :250.00 °C
Solvent Cut Time :2.50 min
Detector Gain Mode :Relative
Detector Gain :0.00 kV
Threshold :8000

[MS Table]
--Group 1 - Event 1--
Start Time :3.00min
End Time :49.00min
ACQ Mode :Scan
Event Time :0.50sec
Scan Speed :1428
Start m/z :40.00
End m/z :700.00

Sample Inlet Unit :GC

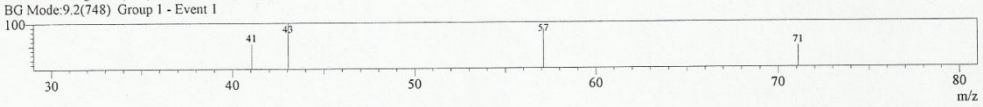
[MS Program]
Use MS Program :OFF

Peak Report TIC

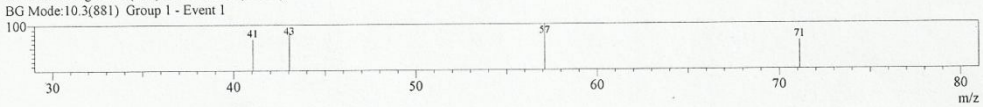
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	9.193	9.167	9.225	138777	0.86	88488	2.80	1.57		
2	10.304	10.275	10.342	142105	0.88	82651	2.61	1.72		
3	16.459	16.392	16.542	1449966	8.97	445279	14.08	3.26		
4	22.261	22.217	22.317	521713	3.23	195517	6.18	2.67		
5	23.504	23.333	23.742	8210420	50.79	1352224	42.75	6.07		
6	27.063	26.992	27.142	719721	4.45	207610	6.56	3.47		
7	28.709	28.517	28.775	3817164	23.61	546389	17.27	6.99		
8	29.301	29.217	29.408	923895	5.71	172626	5.46	5.35		
9	38.522	38.450	38.575	243249	1.50	72615	2.30	3.35		
				16167010	100.00	3163399	100.00			

Spectrum

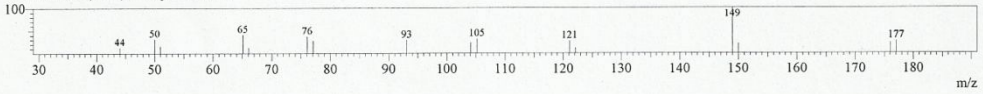
Line#:1 R.Time:9.2(Scan#:744)
MassPeaks:4
RawMode:Single 9.2(744) BasePeak:43(31085)
BG Mode:9.2(748) Group 1 - Event 1



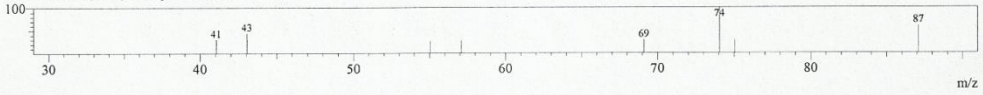
Line#:2 R.Time:10.3(Scan#:877)
MassPeaks:4
RawMode:Single 10.3(877) BasePeak:57(23526)
BG Mode:10.3(881) Group 1 - Event 1



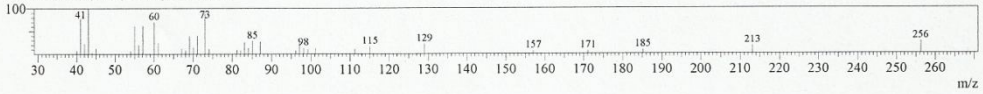
Line#:3 R.Time:16.5(Scan#:1616)
MassPeaks:16
RawMode:Single 16.5(1616) BasePeak:149(70201)
BG Mode:16.4(1612) Group 1 - Event 1



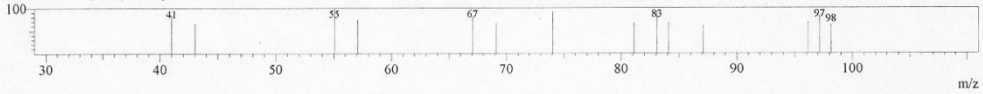
Line#:4 R.Time:22.3(Scan#:2312)
MassPeaks:9
RawMode:Single 22.3(2312) BasePeak:74(39686)
BG Mode:22.3(2316) Group 1 - Event 1



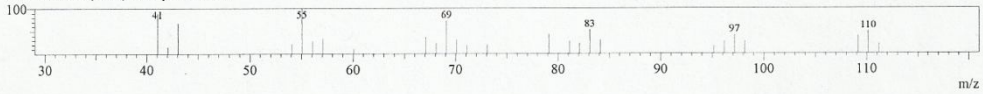
Line#:5 R.Time:23.5(Scan#:2461)
MassPeaks:38
RawMode:Single 23.5(2461) BasePeak:43(123093)
BG Mode:23.6(2468) Group 1 - Event 1



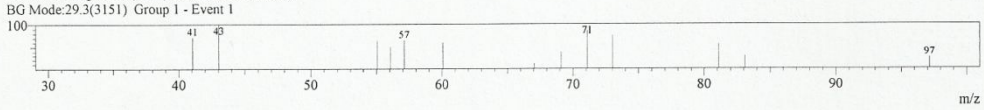
Line#:6 R.Time:27.1(Scan#:2889)
MassPeaks:15
RawMode:Single 27.1(2889) BasePeak:55(15231)
BG Mode:27.0(2884) Group 1 - Event 1



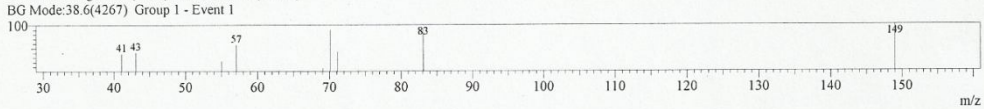
Line#:7 R.Time:28.7(Scan#:3087)
MassPeaks:27
RawMode:Single 28.7(3087) BasePeak:55(20756)
BG Mode:28.8(3091) Group 1 - Event 1



Line#:8 R.Time:29.3(Scan#:3157)
MassPeaks:14
RawMode:Single 29.3(3157) BasePeak:43(15928)
BG Mode:29.3(3151) Group 1 - Event 1



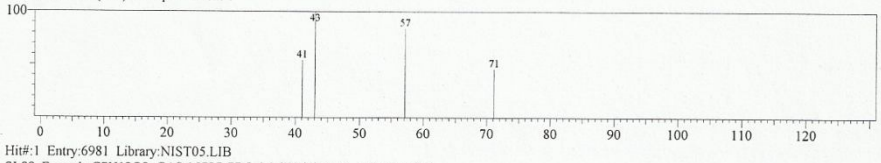
Line#:9 R.Time:38.5(Scan#:4263)
MassPeaks:10
RawMode:Single 38.5(4263) BasePeak:149(10017)
BG Mode:38.6(4267) Group 1 - Event 1



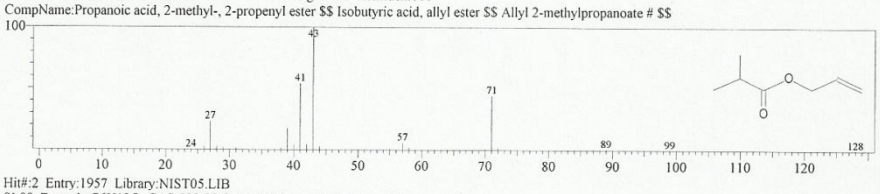
Spectrum Comparison

Library

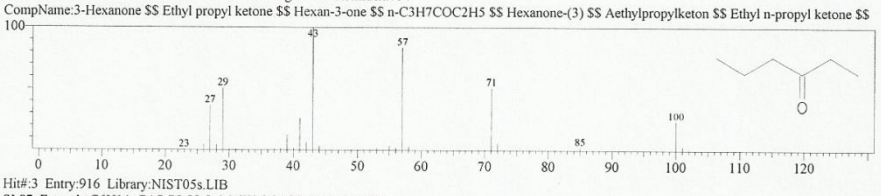
<< Target >>
Line#:1 R.Time:9.192(Scan#:744) MassPeaks:4
RawMode:Single 9.192(744) BasePeak:43.05(31085)
BG Mode:9.225(748) Group 1 - Event 1



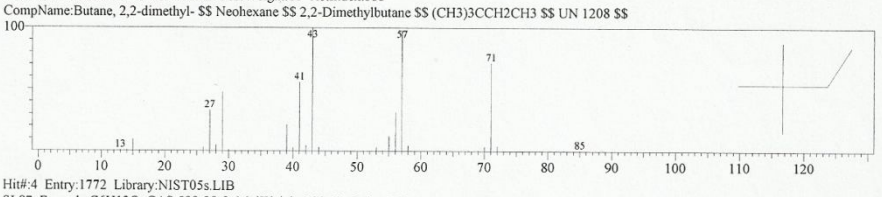
Hit#:1 Entry:6981 Library:NIST05.LIB
SI:89 Formula:C7H12O2 CAS:15727-77-2 MolWeight:128 RetIndex:810
CompName:Propanoic acid, 2-methyl-, 2-propenyl ester $$ Isobutyric acid, allyl ester $$ Allyl 2-methylpropanoate # $$



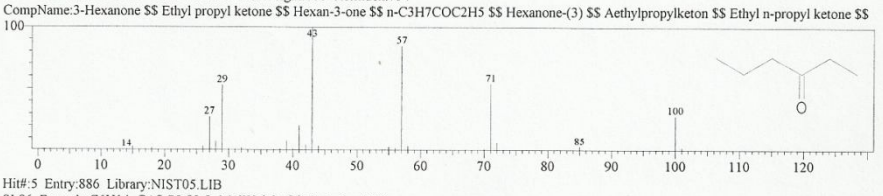
Hit#:2 Entry:1957 Library:NIST05.LIB
SI:88 Formula:C6H12O CAS:589-38-8 MolWeight:100 RetIndex:754
CompName:3-Hexanone $$ Ethyl propyl ketone $$ Hexan-3-one $$ n-C3H7COC2H5 $$ Hexanone-(3) $$ Aethylpropylketon $$ Ethyl n-propyl ketone $$



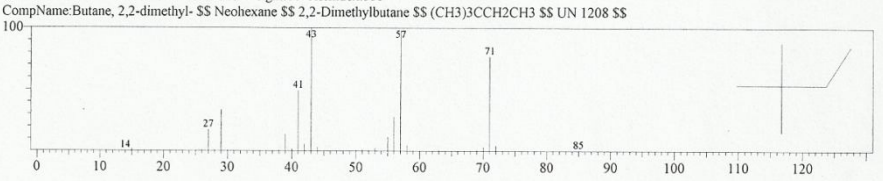
Hit#:3 Entry:916 Library:NIST05s.LIB
SI:87 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



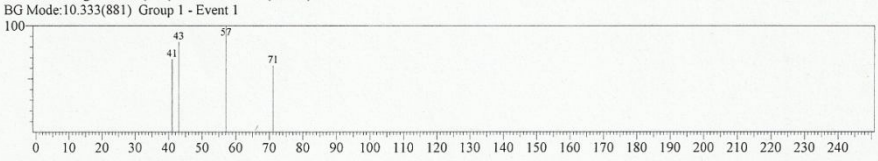
Hit#:4 Entry:1772 Library:NIST05s.LIB
SI:87 Formula:C6H12O CAS:589-38-8 MolWeight:100 RetIndex:754
CompName:3-Hexanone $$ Ethyl propyl ketone $$ Hexan-3-one $$ n-C3H7COC2H5 $$ Hexanone-(3) $$ Aethylpropylketon $$ Ethyl n-propyl ketone $$



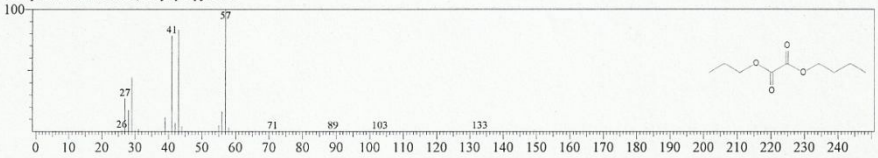
Hit#:5 Entry:886 Library:NIST05.LIB
SI:86 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



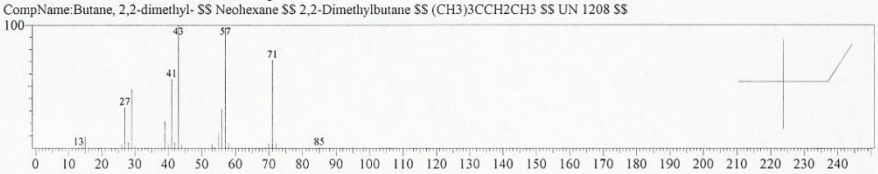
<< Target >>
Line#:2 R.Time:10.300(Scan#:877) MassPeaks:4
RawMode:Single 10.300(877) BasePeak:57.10(23526)
BG Mode:10.333(881) Group 1 - Event 1



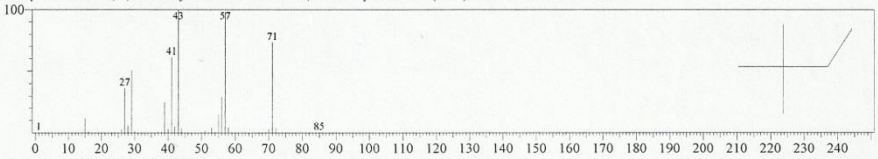
Hit#:1 Entry:34044 Library:NIST05.LIB
SI:88 Formula:C9H16O4 CAS:0-00-0 MolWeight:188 RetIndex:1250
CompName:Oxalic acid, butyl propyl ester



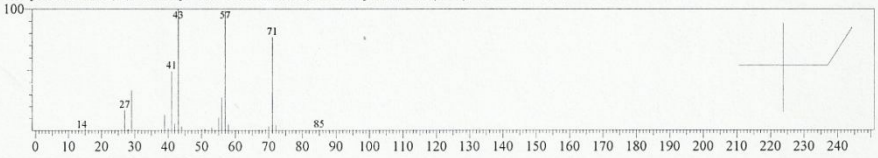
Hit#:2 Entry:916 Library:NIST05s.LIB
SI:88 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



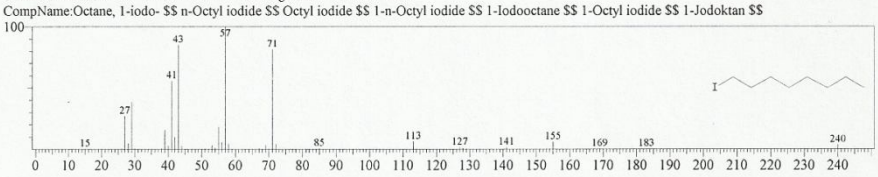
Hit#:3 Entry:915 Library:NIST05s.LIB
SI:87 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



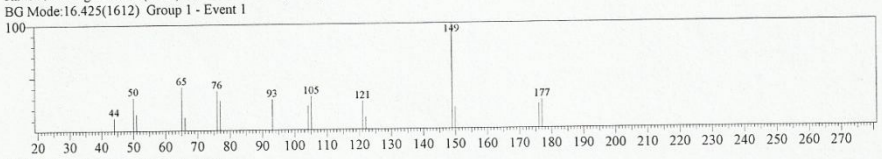
Hit#:4 Entry:886 Library:NIST05.LIB
SI:87 Formula:C6H14 CAS:75-83-2 MolWeight:86 RetIndex:533
CompName:Butane, 2,2-dimethyl- $$ Neohexane $$ 2,2-Dimethylbutane $$ (CH3)3CCH2CH3 $$ UN 1208 $$



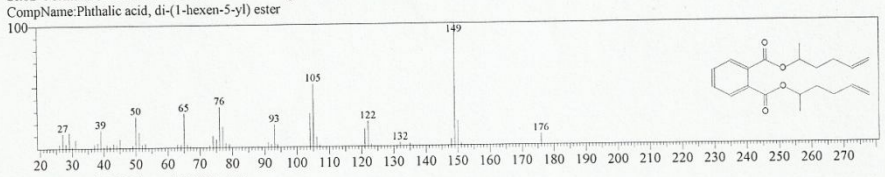
Hit#:5 Entry:20129 Library:NIST05s.LIB
SI:87 Formula:C8H17I CAS:629-27-6 MolWeight:240 RetIndex:1231
CompName:Octane, 1-iodo- $$ n-Octyl iodide $$ Octyl iodide $$ 1-n-Octyl iodide $$ 1-Iodooctane $$ 1-Octyl iodide $$ 1-Jodoktan $$



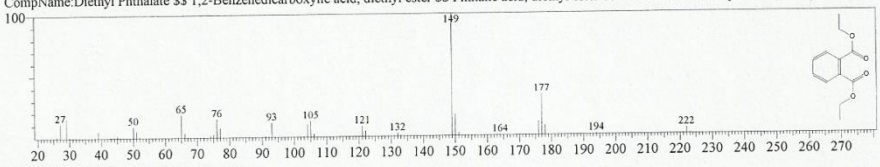
<< Target >>
Line#:3 R.Time:16.458(Scan#:1616) MassPeaks:16
RawMode:Single 16.458(1616) BasePeak:149.00(70201)
BG Mode:16.425(1612) Group 1 - Event 1



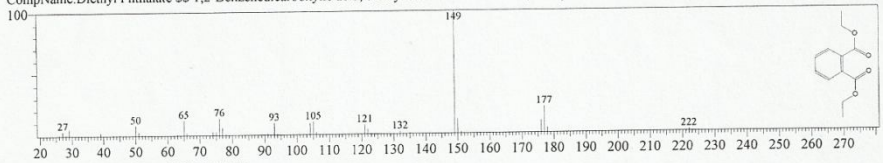
Hit#:1 Entry:117551 Library:NIST05.LIB
SI:82 Formula:C20H26O4 CAS:0-00-0 MolWeight:330 RetIndex:2286
CompName:Phthalic acid, di-(1-hexen-5-yl) ester



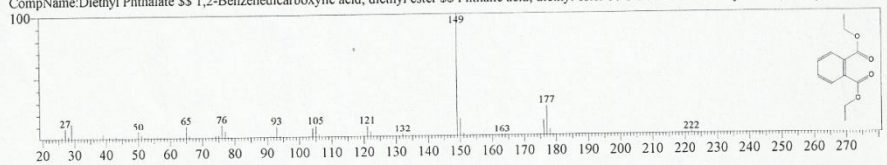
Hit#:2 Entry:18578 Library:NIST05s.LIB
SI:81 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:1639
CompName:Diethyl Phthalate $$ 1,2-Benzenedicarboxylic acid, diethyl ester $$ Phthalic acid, diethyl ester $$ o-Benzenedicarboxylic acid, diethyl ester $$



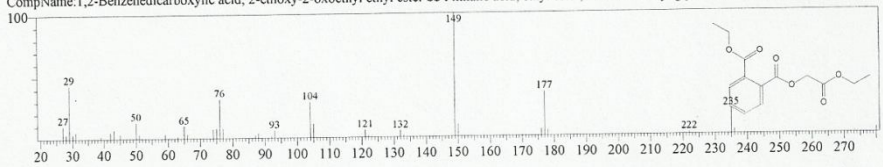
Hit#:3 Entry:18579 Library:NIST05s.LIB
SI:79 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:1639
CompName:Diethyl Phthalate $$ 1,2-Benzenedicarboxylic acid, diethyl ester $$ Phthalic acid, diethyl ester $$ o-Benzenedicarboxylic acid, diethyl ester $$



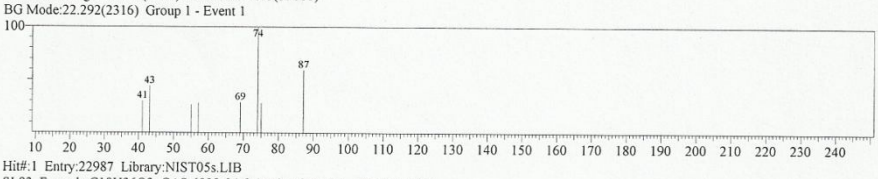
Hit#:4 Entry:18580 Library:NIST05s.LIB
SI:79 Formula:C12H14O4 CAS:84-66-2 MolWeight:222 RetIndex:1639
CompName:Diethyl Phthalate $$ 1,2-Benzenedicarboxylic acid, diethyl ester $$ Phthalic acid, diethyl ester $$ o-Benzenedicarboxylic acid, diethyl ester $$



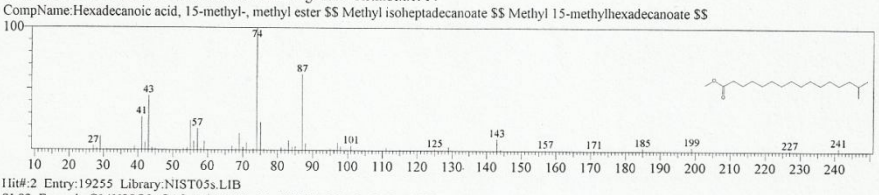
Hit#:5 Entry:22735 Library:NIST05s.LIB
SI:77 Formula:C14H16O6 CAS:84-72-0 MolWeight:280 RetIndex:2005
CompName:1,2-Benzenedicarboxylic acid, 2-ethoxy-2-oxoethyl ethyl ester $$ Phthalic acid, ethyl ester, ester with ethyl glycolate $$ Carbethoxymethyl ethyl



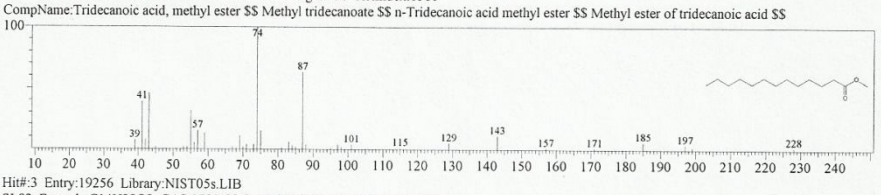
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Line#:4 R.Time:22.258(Scan#:2312) MassPeaks:9
RawMode:Single 22.258(2312) BasePeak:74.05(39686)
BG Mode:22.292(2316) Group 1 - Event 1



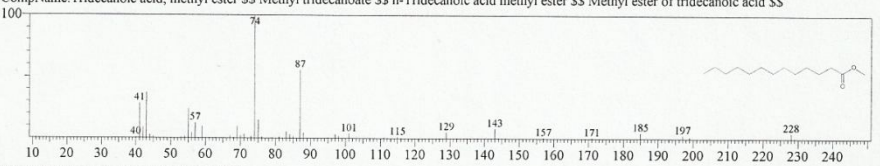
Hit#:1 Entry:22987 Library:NIST05s.LIB
SI:83 Formula:C18H36O2 CAS:6929-04-0 MolWeight:284 RetIndex:1914
CompName:Hexadecanoic acid, 15-methyl-, methyl ester $$ Methyl isoheptadecanoate $$ Methyl 15-methylhexadecanoate $$



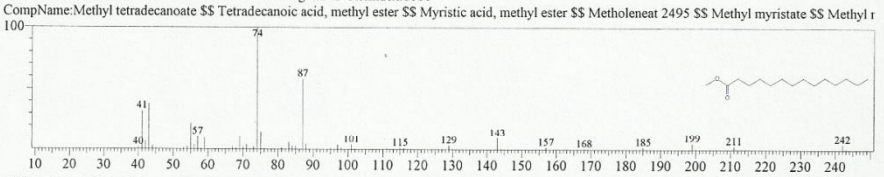
Hit#:2 Entry:19255 Library:NIST05s.LIB
SI:82 Formula:C14H28O2 CAS:1731-88-0 MolWeight:228 RetIndex:1580
CompName:Tridecanoic acid, methyl ester $$ Methyl tridecanoate $$ n-Tridecanoic acid methyl ester $$ Methyl ester of tridecanoic acid $$



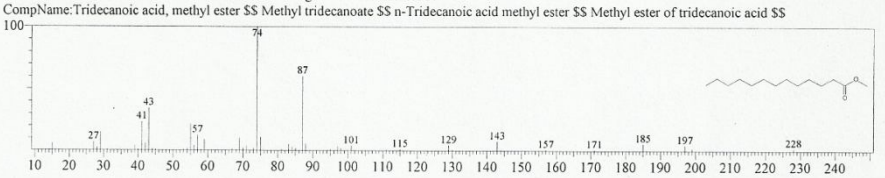
Hit#:3 Entry:19256 Library:NIST05s.LIB
SI:82 Formula:C14H28O2 CAS:1731-88-0 MolWeight:228 RetIndex:1580
CompName:Tridecanoic acid, methyl ester $$ Methyl tridecanoate $$ n-Tridecanoic acid methyl ester $$ Methyl ester of tridecanoic acid $$



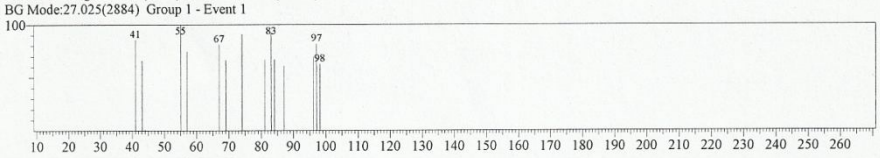
Hit#:4 Entry:20373 Library:NIST05s.LIB
SI:82 Formula:C15H30O2 CAS:124-10-7 MolWeight:242 RetIndex:1680
CompName:Methyl tetradecanoate $$ Tetradecanoic acid, methyl ester $$ Myristic acid, methyl ester $$ Metholeneat 2495 $$ Methyl myristate $$ Methyl r



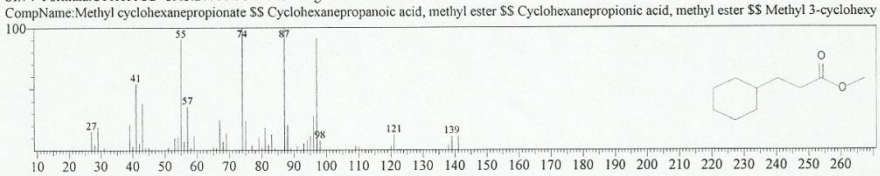
Hit#:5 Entry:19254 Library:NIST05s.LIB
SI:82 Formula:C14H28O2 CAS:1731-88-0 MolWeight:228 RetIndex:1580
CompName:Tridecanoic acid, methyl ester $$ Methyl tridecanoate $$ n-Tridecanoic acid methyl ester $$ Methyl ester of tridecanoic acid $$



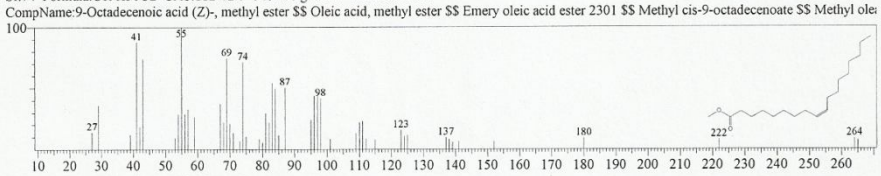
<< Target >>
Line#:6 R.Time:27.067(Scan#:2889) MassPeaks:15
RawMode:Single 27.067(2889) BasePeak:55.05(15231)
BG Mode:27.025(2884) Group 1 - Event 1



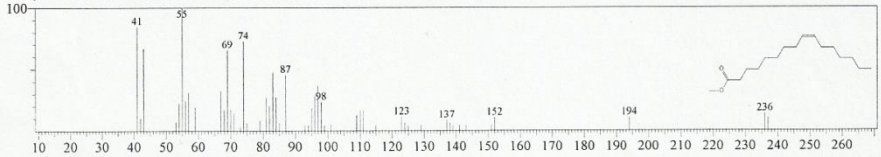
Hit#:1 Entry:24819 Library:NIST05.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



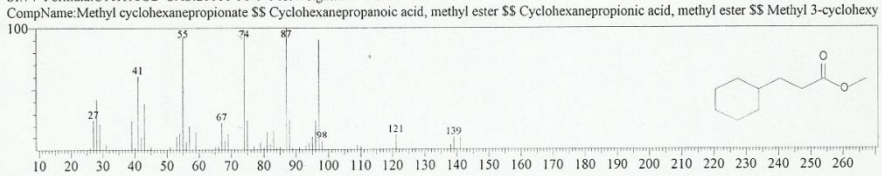
Hit#:2 Entry:98788 Library:NIST05.LIB
SI:74 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl ole



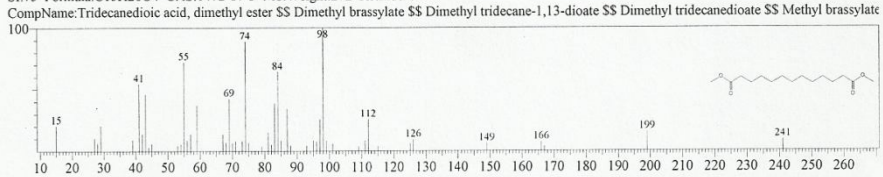
Hit#:3 Entry:22069 Library:NIST05s.LIB
SI:74 Formula:C17H32O2 CAS:1120-25-8 MolWeight:268 RetIndex:1886
CompName:9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate $$ Methyl palmitoleinate $$ Palmitoleic acid, methyl ester $$ Methyl (9Z)-9-he



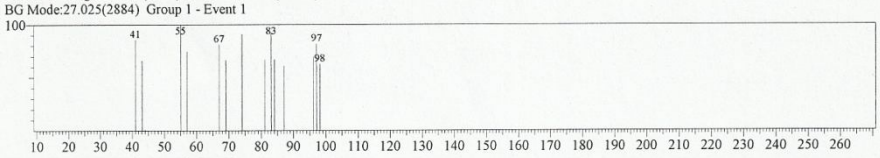
Hit#:4 Entry:11955 Library:NIST05s.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



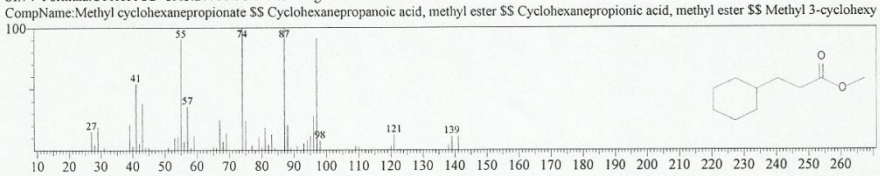
Hit#:5 Entry:22294 Library:NIST05s.LIB
SI:73 Formula:C15H28O4 CAS:1472-87-3 MolWeight:272 RetIndex:1847
CompName:Tridecanedioic acid, dimethyl ester $$ Dimethyl brassylate $$ Dimethyl tridecane-1,13-dioate $$ Dimethyl tridecanedioate $$ Methyl brassylate



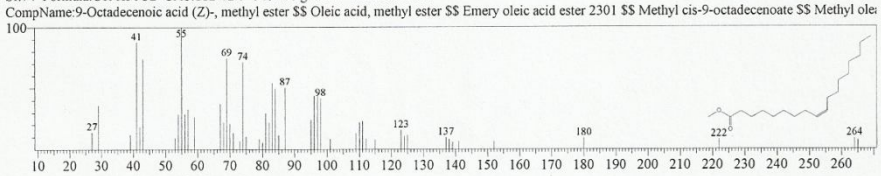
<< Target >>
Line#:6 R.Time:27.067(Scan#:2889) MassPeaks:15
RawMode:Single 27.067(2889) BasePeak:55.05(15231)
BG Mode:27.025(2884) Group 1 - Event 1



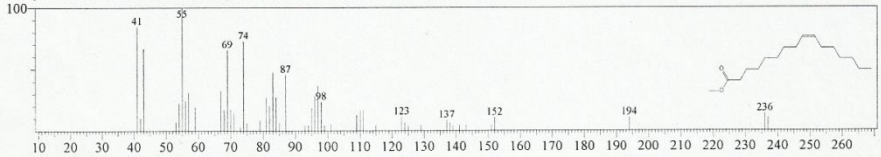
Hit#:1 Entry:24819 Library:NIST05.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



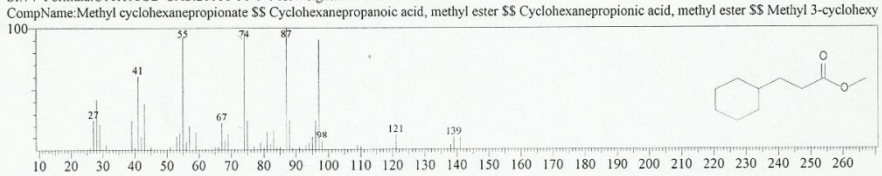
Hit#:2 Entry:98788 Library:NIST05.LIB
SI:74 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl ole



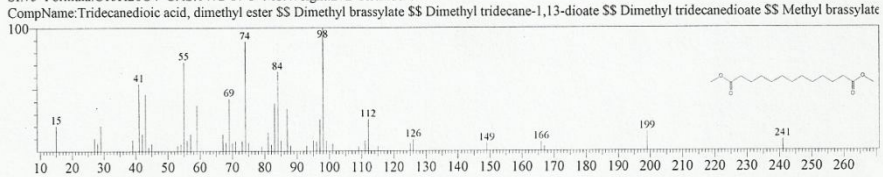
Hit#:3 Entry:22069 Library:NIST05s.LIB
SI:74 Formula:C17H32O2 CAS:1120-25-8 MolWeight:268 RetIndex:1886
CompName:9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate $$ Methyl palmitoleinate $$ Palmitoleic acid, methyl ester $$ Methyl (9Z)-9-he



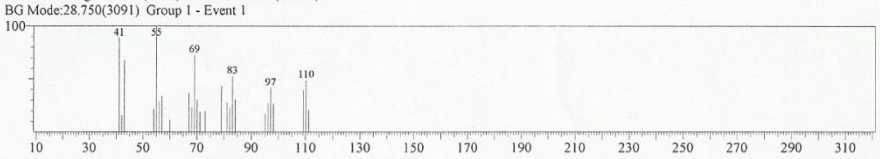
Hit#:4 Entry:11955 Library:NIST05s.LIB
SI:74 Formula:C10H18O2 CAS:20681-51-0 MolWeight:170 RetIndex:1246
CompName:Methyl cyclohexanepropionate $$ Cyclohexanepropanoic acid, methyl ester $$ Cyclohexanepropionic acid, methyl ester $$ Methyl 3-cyclohexy



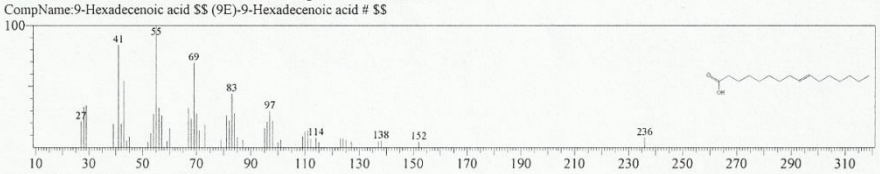
Hit#:5 Entry:22294 Library:NIST05s.LIB
SI:73 Formula:C15H28O4 CAS:1472-87-3 MolWeight:272 RetIndex:1847
CompName:Tridecanedioic acid, dimethyl ester $$ Dimethyl brassylate $$ Dimethyl tridecane-1,13-dioate $$ Dimethyl tridecanedioate $$ Methyl brassylate



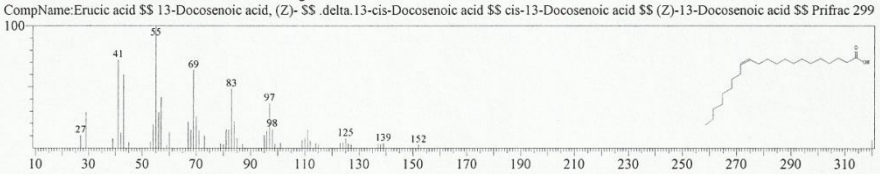
<< Target >>
Line#:7 R.Time:28.717(Scan#:3087) MassPeaks:27
RawMode:Single 28.717(3087) BasePeak:55.05(20756)
BG Mode:28.750(3091) Group 1 - Event 1



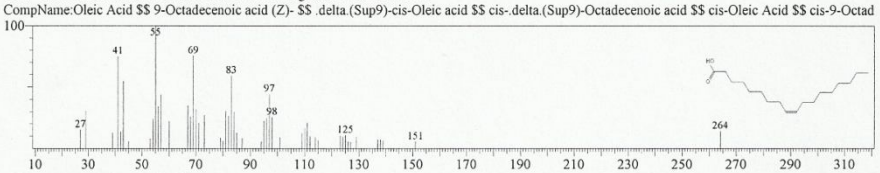
Hit#:1 Entry:73685 Library:NIST05.LIB
SI:88 Formula:C16H30O2 CAS:2091-29-4 MolWeight:254 RetIndex:1976
CompName:9-Hexadecenoic acid $$ (9E)-9-Hexadecenoic acid # $$



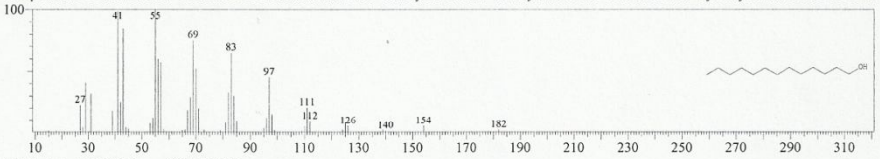
Hit#:2 Entry:121691 Library:NIST05.LIB
SI:87 Formula:C22H42O2 CAS:112-86-7 MolWeight:338 RetIndex:2572
CompName:Erucic acid $$ 13-Docosenoic acid, (Z)- $$ .delta.13-cis-Docosenoic acid $$ cis-13-Docosenoic acid $$ (Z)-13-Docosenoic acid $$ Prifrac 299



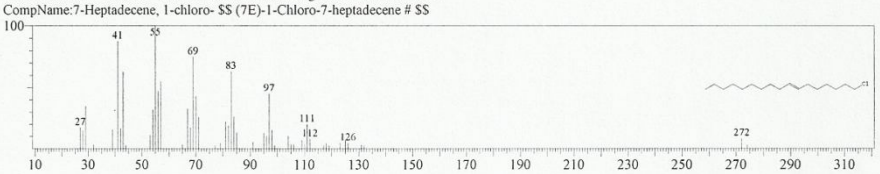
Hit#:3 Entry:90577 Library:NIST05.LIB
SI:87 Formula:C18H34O2 CAS:112-80-1 MolWeight:282 RetIndex:2175
CompName:Oleic Acid $$ 9-Octadecenoic acid (Z)- $$ .delta.(Sup9)-cis-Oleic acid $$ cis-.delta.(Sup9)-Octadecenoic acid $$ cis-Oleic Acid $$ cis-9-Octad



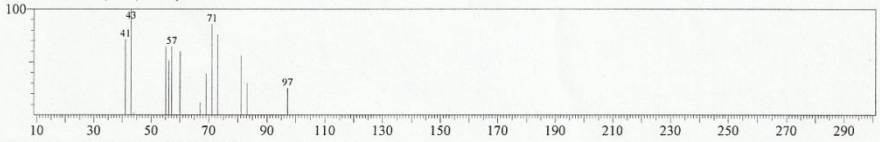
Hit#:4 Entry:41278 Library:NIST05.LIB
SI:86 Formula:C13H28O CAS:112-70-9 MolWeight:200 RetIndex:1556
CompName:1-Tridecanol $$ n-Tridecan-1-ol $$ n-Tridecanol $$ n-Tridecyl alcohol $$ Tridecyl alcohol $$ Tridecanol $$ 1-Hydroxytridecane $$ C13 Linea



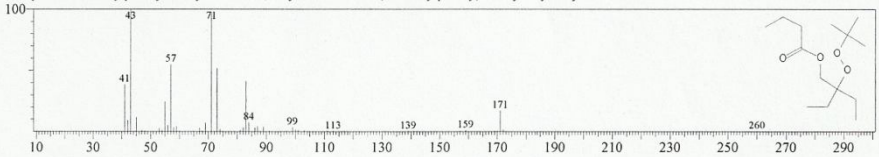
Hit#:5 Entry:84726 Library:NIST05.LIB
SI:86 Formula:C17H33Cl CAS:56554-78-0 MolWeight:272 RetIndex:1944
CompName:7-Heptadecene, 1-chloro- $$ (7E)-1-Chloro-7-heptadecene # $$



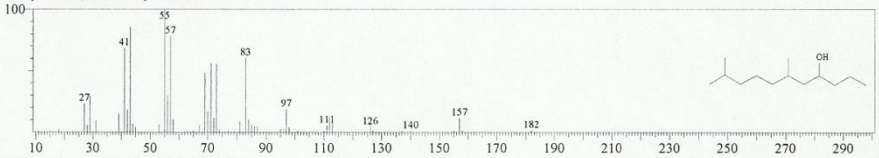
<< Target >>
Line#:8 R.Time:29.300(Scan#:3157) MassPeaks:14
RawMode:Single 29.300(3157) BasePeak:43.05(15928)
BG Mode:29.250(3151) Group 1 - Event 1



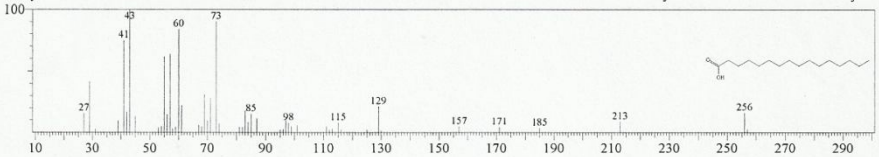
Hit#:1 Entry:77135 Library:NIST05.LIB
SI:78 Formula:C14H28O4 CAS:139727-33-6 MolWeight:260 RetIndex:1563
CompName:2-t-Butylperoxy-2-ethylbutan-1-ol, butyrate ester $$ 2-(tert-Butylperoxy)-2-ethylbutyl butyrate # $$



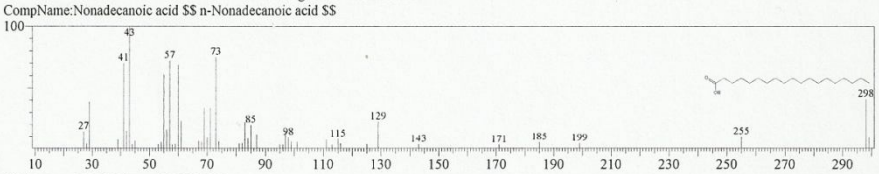
1Hit#:2 Entry:41279 Library:NIST05.LIB
SI:78 Formula:C13H28O CAS:92153-95-2 MolWeight:200 RetIndex:1348
CompName:6,10-Dimethyl-4-undecanol



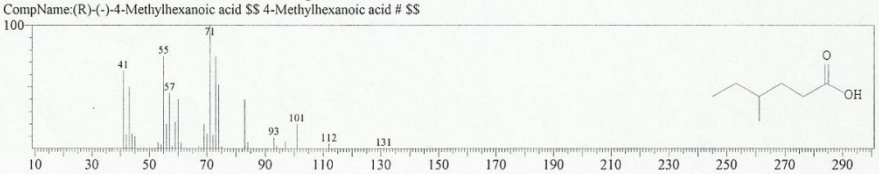
Hit#:3 Entry:74999 Library:NIST05.LIB
SI:77 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
CompName:n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecanecarboxylic



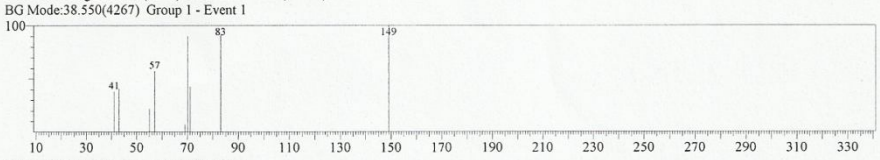
Hit#:4 Entry:100053 Library:NIST05.LIB
SI:77 Formula:C19H38O2 CAS:646-30-0 MolWeight:298 RetIndex:2266
CompName:Nonadecanoic acid $$ n-Nonadecanoic acid $$



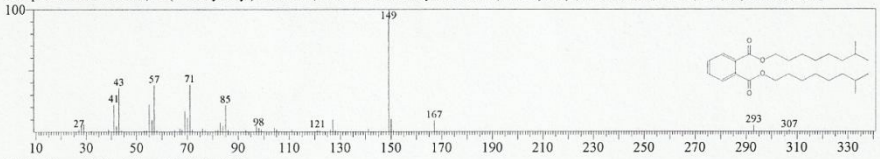
Hit#:5 Entry:7775 Library:NIST05.LIB
SI:76 Formula:C7H14O2 CAS:52745-93-4 MolWeight:130 RetIndex:1009
CompName:(R)-(-)-4-Methylhexanoic acid $$ 4-Methylhexanoic acid # $$



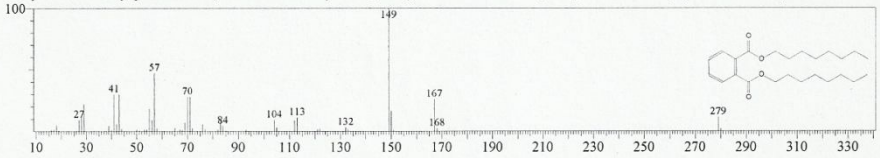
<< Target >>
Line#:9 R.Time:38.517(Scan#:4263) MassPeaks:10
RawMode:Single 38.517(4263) BasePeak:149.00(10017)
BG Mode:38.550(4267) Group 1 - Event 1



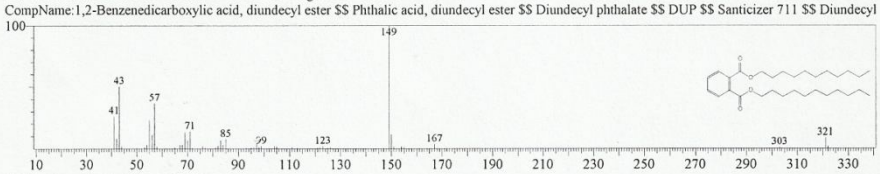
Hit#:1 Entry:26786 Library:NIST05s.LIB
SI:74 Formula:C26H42O4 CAS:20548-62-3 MolWeight:418 RetIndex:2902
CompName:Phthalic acid, bis(7-methyloctyl) ester $$ 1,2-Benzenedicarboxylic acid, bis(7-methyloctyl) ester $$ Bis(7-methyloctyl) phthalate # $$



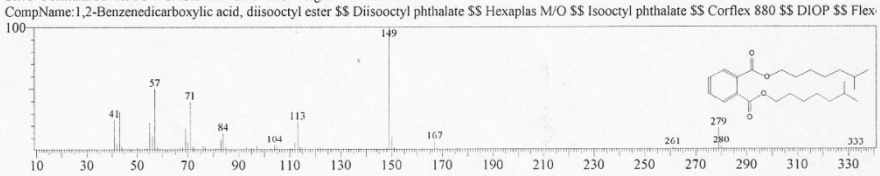
Hit#:2 Entry:26375 Library:NIST05s.LIB
SI:74 Formula:C24H38O4 CAS:117-84-0 MolWeight:390 RetIndex:2832
CompName:Di-n-octyl phthalate $$ 1,2-Benzenedicarboxylic acid, dioctyl ester $$ Phthalic acid, dioctyl ester $$ n-Octyl phthalate $$ Dinopol NOP $$ Dioc



Hit#:3 Entry:27198 Library:NIST05s.LIB
SI:74 Formula:C30H50O4 CAS:3648-20-2 MolWeight:474 RetIndex:3428
CompName:1,2-Benzenedicarboxylic acid, diundecyl ester $$ Phthalic acid, diundecyl ester $$ Diundecyl phthalate $$ DUP $$ Santicizer 711 $$ Diundecyl



Hit#:4 Entry:26376 Library:NIST05s.LIB
SI:73 Formula:C24H38O4 CAS:27554-26-3 MolWeight:390 RetIndex:2704
CompName:1,2-Benzenedicarboxylic acid, diisooctyl ester $$ Diisooctyl phthalate $$ Hexaplas M/O $$ Isooctyl phthalate $$ Corflex 880 $$ DIOP $$ Flex



Hit#:5 Entry:7895 Library:NIST05.LIB
SI:72 Formula:C8H18O CAS:6570-88-3 MolWeight:130 RetIndex:867
CompName:2,3,4-Trimethyl-1-pentanol

