KINETIC MODELLING OF ENHANCED BIOREMEDIATION OF HYDROCARBON POLLUTED SOIL

BY

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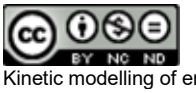

CERTIFICATION

This is to certify that this work entitled "Kinetic Modelling of Enhanced Bioremediation of Hydrocarbon Polluted Soil" was carried out by Musa, Akenn Sunday (20044448338) in partial fulfilment of the requirement for the award of PhD degree in Chemical Engineering.

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DEDICATION

This work is dedicated to Almighty God, the giver of life, knowledge and wisdom.

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NOMENCLATURE

Symbol	Description	Unit
C	Transportation rate of ground water	mg/l
k	First order kinetics rate constant	$week^{-1}(yr^{-1})$
PPM	Parts per million	nil
RCF	Root concentration factor	nil
S	Substrate concentration	mol%
T	Transportation rate of vegetation	l/day
t	time	week (yr)
TPH	Total petroleum hydrocarbon	mol%
TSCF	Transportation stream concentration factor	ml
U	uptake rate of contaminant	mg/day
X	Biomass concentration	cfu/g
Y	Yield	cfu/(g.mol%)
μ	Microbial growth rate	$week^{-1}$
γ	Logistic growth constant	nil

SUBSCRIPTS

o	initial value
∞	Final or ultimate value
G	used for growth
mG	used for growth and maintenance

ABSTRACT

Remediation of hydrocarbon polluted soil applying all existing techniques can be slow, expensive and requires close monitoring. Bioremediation and phytoremediation augmented bioremediation have been used to treat petroleum contaminated soil and even the difficult poly aromatic hydrocarbon polluted soil which takes longer time. What has not been studied was the effect of heavy metals present in petroleum which slows down the substrate degradation by microorganisms. After the primary and secondary recovery method for petroleum contaminated site, Phyto-remediation augmented bioremediation using locoweed was selected amongst twenty species as most suitable for hyper-accumulation of heavy metals in contaminated soil into the rhizosphere of the plant tissues. Application of the augmented technique was carried out after the microorganism can no longer degrade substrate contaminant any further during the stationary phase. The results obtained for the six different models based on biomass growth and enhanced remediation showed that the logistic model was the most fitting models. The results revealed that bioremediation and phytoremediation augmented bioremediation are based on the same mechanism. During both processes the biomass grows according to the logistic model (with inhibition as the amount of substrate is depleted) and the rate of production of biomass per unit substrate consumed can be considered constant. Bioremediation and phytoremediation are first order processes whose rates are directly proportional to the substrate concentration driving force. Phytoremediation enhances the rate of the bioremediation process by reducing the ultimate substrate concentration achievable through bioremediation alone, though the first order rate constant is reduced in the process. The method employed reduces the contaminant concentration by about 65%, while when augmented with Phytoremediation, the contaminant concentration was reduced by 69% and 88% for Sunflower and Locoweed respectively. With respect to time savings of achieving 60% contaminant removal from 1 (100%) mol to 0.4 (40%) mol concentrations, the results gave total required time of 9.7055 weeks, 7.5652 weeks and 8.1014 weeks for bioremediation, phytoremediation (locoweed) and phytoremediation (Sunflower) respectively. Also, in terms of cost savings, phytoremediation (Locoweed) showed the lowest average total cost savings of $6,597.32 for locoweed and $7,119.0 for Sunflower when compared to $13,344.09 for bioremediation or 49.44% and 45.86% respectively. Locoweed showed higher effectiveness in enhancing the remediation process in comparison to Sunflower. Phytoremediation augmented with bioremediation is therefore recommended as a viable means for remediation of polluted soil and should be backed by legislative and regulatory frameworks.

Keywords: polluted soil, bioremediation, model, phytoremediation, substrate concentration and total petroleum hydrocarbon.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Petroleum hydrocarbons represent a complex mixture of organic compounds mainly grouped into four fractions: alkenes, aromatics, resins and asphaltenes (Ruijuan et al., 2013). Hydrocarbon pollution of the environment has remained a major challenge for man over the years and has been escalating in proportions with increase in industrial activities. Such pollutions are usually occasioned by human error, equipment failure, vandalism, wars and natural disasters. Prominent among the deleterious effects of such pollutions on land is the destruction of natural flora and fauna thereby ultimately reducing the capacity of the ecosystem to support life. Several techniques have been developed over the years to combat this menace. These techniques are grouped broadly into two namely; In-situ methods (such as leaching or washing, isolation and containment, volatilization, bioremediation and passive bioremediation) and Ex-situ methods (such as incineration, solidification and stabilization, soil washing, and land farming) (Das & Mukherjee, 2007).

Bioremediation is a term that describes the deliberate use of organisms to remove or reduce man-made pollution. Bioremediation is the use of biological methods in restoring contaminated land, principally by the addition of bacteria and other micro-organisms that consume or neutralize contaminants in the soil (Gibson & Salyer, 1992). Microorganisms have been known to degrade hazardous compounds considered recalcitrant and resistant to biodegradation. Advantages of biological bioremediation compared to other treatment methods include destruction rather than transfer of contaminants to another medium, minimal exposure of workers to contaminants, long-time protection of public health and possible reduction in the duration of the remediation process

(Okoh & Trejo-Hernandez, 2006; Machin-Ramitez et al., 2008). The principle of bioremediation is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism. Microorganism such as bacteria (Protista) and fungi are very good at degrading complex molecules and incorporating the breakdown products into their metabolisms. The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms. Investigations into the use of bioremediation as a means of treating contaminated soil has been on since the late 1940's but gained widespread interest following the Exxon Valdez oil spill in 1989 (Margesih & Schinner, 1997; Jackson & Pardue, 1998). Petroleum can be degraded only by bacteria with the ability to produce enzymes that select petroleum as a substrate. These enzymes are substances that act as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without it being altered in the process.

The natural bioremediation process usually needs to be enhanced because hydrocarbon biodegradation in soil can be limited by many factors such as nutrients, pH, temperature, moisture, oxygen, soil properties and contaminant presence (Atagana, 2008). Most of these limiting factors can be controlled but metals concentration poses more difficulty as mobility of microbes' enzyme degradation is impaired. This limiting factor and moisture content can be minimized through the employment of phytoremediation technology. Phytoremediation is based upon the basic physiological mechanisms taking place in higher plants and associated microorganisms such as transpiration, photosynthesis, metabolism and mineral nutrition (Marmilori et al., 2006). Enhanced bioremediation can also be in the form of Bio-stimulation which involves the modification of the environment to stimulate existing bacteria capable of bioremediation and can be done by addition of various forms of limiting nutrients and electron acceptors such as Phosphorus, Nitrogen, Oxygen and Carbon (Elektorowicz, 1994; Piehler et

al.,1999; Rhykerd et al.,1999). It can also be in the form of Bio-augmentation which is the addition of oil-degrading microorganisms to supplement indigenous microbial populations which may not be capable of degrading the wide range of potential substrates present in complex mixtures such as petroleum (Leahy & Colwell, 1990).

Despite the progress made so far in contaminated soil remediation, extensive research is still on with a view to development of the most cost and technically effective method for remediation of contaminated soils.

1.2 PROBLEM STATEMENT

In-situ technologies such as bioremediation are very popular methods of soil remediation because they do not require complex and expensive excavation work which characterise ex-situ schemes. The effectiveness of bioremediation depends greatly on the presence of suitable microorganisms and nutrients in the subsurface. Bioremediation occurs naturally by the action of the indigenous microorganisms present in soil but the rate is usually so slow that it will require a very long time for appreciable impact to be observed. This is because bioremediation is affected by certain factors which limit its efficiency such as the presence of recalcitrant contaminants in soil, very high level of contamination and difficult geological conditions such as low permeable clay presence limiting water and air migration in soil. In order to make the process attractive, enhanced bioremediation techniques targeting reduction of total time for the complete remedial process and environmental compliance is required.

Several methods of bioremediation enhancement have been developed to increase technological efficiency. The most effective have proven to be chemical and physical methods of soil aeration, nutrient application with mostly nitrogen and phosphorus compounds, addition of surfactants,

addition of bacterial strains (bio-augmentation) and phytoremediation. The use of living green plants for reduction and/or removal of contaminants from polluted soil, water and air are called Phyto-remediation. This is an emerging biotechnology that has found application in many fields of remedial processes and includes genetically manipulated (GM) plants used to clean up polluted soil. A very promising approach to effective remediation of hydrocarbon contaminated soil is to combine bioremediation with phytoremediation in a hybrid scheme. Investigation into the effectiveness of this scheme and development of models to predict its operations is the major challenge addressed by this research.

1.3 AIMS AND OBJECTIVES

1.3.1 AIMS

The aim of this research is to reduce total time required for cost effective bioremediation of a given petroleum polluted site by deliberate enhancement and acceleration of degradation process of contaminant using suitable microbes in a fitting environment and the application of phytoremediation techniques.

1.3.2 OBJECTIVES

From the foregoing aim, the following are the objectives of research:

- To study the effects of remedial parameters in decontaminating petroleum polluted soils;
- To determine the physicochemical and microbial properties of the soil and crude oil samples;
- To establish the appropriate substrate kinetic models based on the order of reaction;

The word ‘phyto’ is derived from Greek term (puto) – ‘plants’ and the Latin word ‘remedium’ which means ‘remediation’ or ‘restore balance’.

- To find out the appropriate substrate kinetic model based on microbial growth;
- To verify the appropriate microbial growth and decay model;
- To select the appropriate product functional parameter models for N_2, O_2, etc.

1.4 SIGNIFICANCE OF STUDY

The increasing demand for petroleum or petroleum products and requirement for agricultural produce have exposed the soil to pollution, especially with crude oil, heavy metals and other hydrocarbon contaminants. Removal of such harmful compounds has become essential to ensure compliance with regulatory requirements, provide habitable environment, protection of the fragile ecosystem and sustainable corporate social responsibility.

1.5 SCOPE OF THE STUDY.

The scope of this work is limited to bioremediation and phytoremediation studies of hydrocarbon contaminated soils without reference to other treatment methods. Also the phytoremediation study is limited to the use of sunflower and locoweed. Determination of appropriate microbial growth models considered exponential growth models (with constant and varying yield), logistic growth models (with constant and varying yield) and Gaussian (or biomass death) growth models (with constant and varying yield). Determination of the appropriate product kinetic models was not considered in this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 POLLUTION

Pollution is the introduction of the contaminants into the natural environment that can have harmful effects on the ecosystem. Throughout the industrial world, petroleum is the primary source of fuel. Petroleum production can lead to contamination of soil and water by vandalisation of pipelines, accidental spill and ruptured of oil pipelines, oil well drilling production operations etc. (Rovina & McDougall, 1967; Hutchison et al., 2001).

2.2 TOTAL PETROLEUM HYDROCARBONS (TPH)

Total Petroleum Hydrocarbons (TPH) is made up of diverse mixture of hydrocarbons which can be found at pipelines vandalized area, waste disposal pits and refineries. TPHs are considered hazardous pollutants. They include compounds that can bio–concentrate and bio-accumulate in food chains. Also they are toxic in nature (Paul et al., 2000).

TPHs are divided into two categories namely:

- Gasoline range organics (GRO)
- Diesel range organics (DRO)

2.2.1 GASOLINE RANGE ORGANICS (GRO)

This corresponds to alkenes having between four carbon atoms to ten carbon atoms, with low boiling point of 60–170^{0}C such n-butane, n–pentane, 2-methylbutane, 2, 2–dimethylpropane,

etc.It also includes volatile aromatic compounds such as toluene, benzene, xylene etc. (Schwab & Bank, 1999). The structure of some GRO is given in the following:

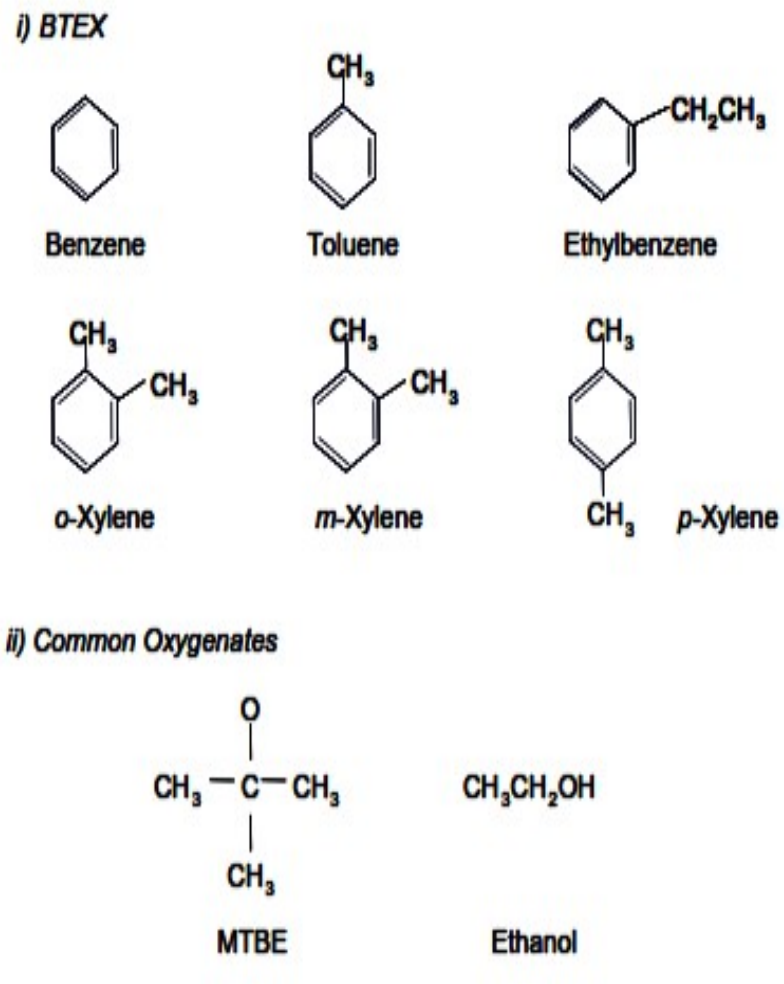

Figure 2.1: Structure of some gasoline range organics

2.2.2 DIESEL RANGE ORGANICS (DRO)

This includes long chain alkanes that is of carbon 10 to 40 and also hydrophobic chemical such as polycyclic aromatic hydrocarbons PAH (Pyrene, Phenanthrene, Benzo(a)pyreneetc).The structure of some DROs is given is figure 2.2 (Van Hamme et al., 2003).

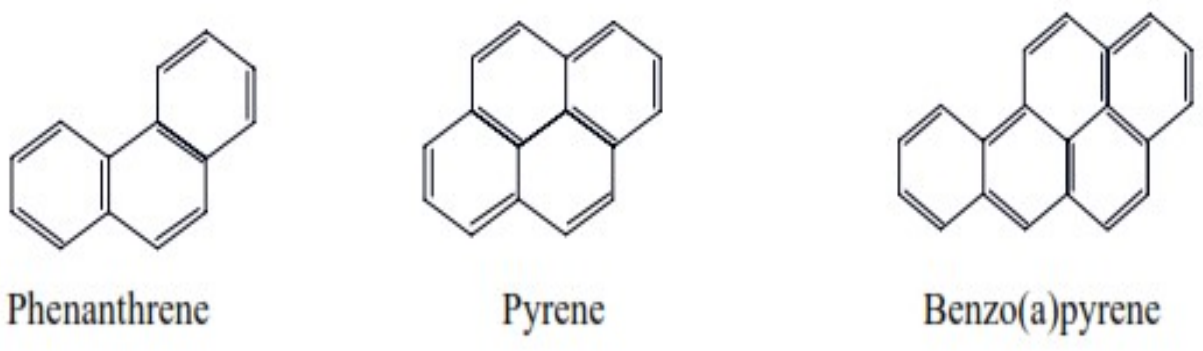

Fig. 2.2: Structure of Some Diesel Range Organics

Table 2.1: Properties of Nigerian Crude Oil and Other Petroleum Compounds

Source: Bonny Light Crude Oil Assay – Toboc.com.

Bonny Light Crude Oil Assay

WHOLE CRUDE	
Gravity, °API	32.9
Specific Gravity	0.86
Sulfur, wt %	0.16
Nitrogen, ppm	1170
Pour Point °F	6.1
Pour Point °C	-14.4
Acid Number, mg KOH/g	0.19
Back-Blended Acid, mg KOH/g	0.17
Viscosity @ 40 °C (104 °F), cSt	4.99
Viscosity @ 50 °C (122 °F), cSt	4.05
Asphaltenes, C7, %	0.0032
Nickel, ppm	4.16
Vanadium, ppm	0.42
Characterization Factor, K	11.68

TBP YIELDS, VOL %	
Butanes and Lighter	0.92
Light Gasoline (55-175 °F)	4.25
Light Naphtha (175-300 °F)	13.73
Heavy Naphtha (300-400 °F)	10.12
Kerosene (400-500 °F)	13.28
Atm. Gas Oil (500-650 °F)	22.69
Lt Vacuum Gas Oil (650-800 °F)	16.81
Hvy Vacuum Gas Oil (800-1050 °F)	13.26
Vacuum Residuum (1050 °F+)	4.96

LIGHT GASOLINE (55-175 °F)	
Gravity, °API	80.5
Specific Gravity	0.67
Mercaptan Sulfur, ppm	0.15
Octane Number, Research, Clear	75.7

LIGHT NAPHTHA (175-300 °F)	
Gravity, °API	54.9
Specific Gravity	0.76
Mercaptan Sulfur, ppm	0.41
Naphthenes, vol %	53.25
Aromatics, vol %	12.45
Octane Number, Research, Clear	68

HEAVY NAPHTHA (300-400 °F)	
Gravity, °API	45.2
Specific Gravity	0.80
Sulfur, wt %	0.014
Mercaptan Sulfur, ppm	0.381
Naphthenes, vol %	66.43
Aromatics, vol %	14.59
Smoke Point, mm (ASTM)	24

KEROSENE (400-500 °F)	
Gravity, °API	35.1
Specific Gravity	0.85
Sulfur, wt %	0.05
Mercaptan Sulfur, ppm	0.55
Naphthenes, vol %	62.1
Aromatics, vol %	20.18
Freezing Point, °F	-52.1
Freezing Point, °C	-46.7
Smoke Point, mm (ASTM)	18.6
Acid Number, mg KOH/g	0.04
Viscosity @ 50 °C (122 °F), cSt	1.66

ATM. GAS OIL (500-650 °F)	
Gravity, °API	30.9
Specific Gravity	0.87
Sulfur, wt %	0.13
Nitrogen, ppm	83.3
Acid Number, mg KOH/g	0.06
Pour Point °F	8.3
Pour Point °C	-13.2
Viscosity @ 50 °C (122 °F), cSt	3.7
Cetane Index	48.5
Characterization Factor, K	11.61

2.3 EFFECT OF THE TOTAL PETROLEUM HYDROCARBON AND POLYCYCLIC AROMATIC HYDROCARBON ON THE ECOSYSTEM.

2.3.1 Effect of the Total Petroleum Hydrocarbon on the Ecosystem

It is useful to measure the total amount of TPH at a site. Petroleum hydrocarbons Ranges are monitored at various levels depending on the state and testing sites. TPH is the sum of VPH and EPH. VPH refers to Volatile Petroleum Hydrocarbon, also known as Petrol Range Organics, PRO and includes hydrocarbons from $C_2 – C_5$. EPH refers to Extractable Petroleum Hydrocarbons, also known as Diesel Range Organics, DRO and includes hydrocarbons from $C_6 – C_{40}$. (US-CDC Agency).

1. **Effect on soil nutrients:** There is a direct relationship between the quantity of oil spillage and accumulation of manganese and ferrous ions in the soil. Plant growth in TPH contaminated soil is adversely affected due to changes in the nutrient status of the soil and disruption of microbial activities (Schneekloth et al., 2002).
2. **Effect on germination and growth crop:** The ability of crops to germinate or grow on crude oil pollution soil is dependent on the level of crude oil spillage on soil, that is, the high level of crude oil pollution of soil impairs germination of seedlings.
3. **Effect on human health:** Toxic components in oil may exert their effects on man through inhibition of protein synthesis, nerve synapse function, and disruption in membrane transport system and damage to plasma membrane (Njoku et al., 2009).

2.3.2 Effect of the Polycyclic Aromatic Hydrocarbon on the Ecosystem

To understand the effect and nature of PAH, the chemistry explains that the simplest of its compounds are the naphthalene and anthracene which both contain two or three fused aromatic rings. Small molecules, such as benzene are not PAHs. The compounds may contain four, five six or seven member rings, but those with five rings are most common. PAHs composed of only six – membered called 'Alternant' PAHs (NASA, 2005). Certain alternant PAHs are called 'Benzenoid' PAHs. The name originates from benzene, an aromatic compound with six carbon chain or six-membered ring. A set of alternant PAHs is closely related to a set of mathematical entities called polyhexes, which are planar, figures consisting of conjoining regular hexagons of identical size.

However, PAHs containing up to six fused aromatic rings are often known as 'Small' PAHs and those containing more than six aromatic compounds are called 'Large' PAHs (ATSDR, 1996). Due to availability of samples of the various small PAHs, the bulk of research on PAHs has been of those of up to six rings (Ukiwe, L. N, 2012). The biological activity and occurrence of the large PAHs does appear to be a continuation of the small PAHs. They are found as combustion products, but at lower levels than the small PAHs due to the kinetic limitation of their production through addition of successive rings. In addition, with many more isomers possible for large PAHs, the occurrence of specific structures is much smaller. PAHs possess very characteristics ultraviolet (UV) absorbance spectra. These often possess many absorbance bands and are unique for each ring structure. For this reason, for a set of isomers, each isomer has a different uv absorbance spectrum than the others, this is particularly useful in the identification of PAHs. Most PAHs are also

fluorescent, emitting characteristics wavelengths of light when they are excited or when the molecules absorb light. The extended pi-electron electron structures of PAHs lead to these spectra, as well as to certain large PAHs also exhibiting semi-conducting and other behaviours.

In general, PAHs are hydrophobic, with aqueous solubility decreasing almost linearly with increase molecular mass (Parish, et al., 2004). The United States Environmental Protection Agency (EPA) has designated sixteen PAHs compounds as priority pollutants. These include; naphthalene, acenapthythene, fluorine, phenanthrene, anthracene, fluorathene, purene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene (EPA, 2007). High molecular weight PAHs (four – membered rings and above) have high resonance energies due to their dense clouds of pi-electrons surroundings the aromatic rings, making them persistent in the environment and recalcitrant to microbial degradation (Johnson et al., 2005). Low aqueous solubility and high soil sorption also account for their persistence and recalcitrance nature (Parrish et al., 2004). When PAHs enter a subsurface environment, various physical, chemical and biological processes impact their fate and transport. Because PAHs constitute a variety of compounds with a broad range of properties, the individual compounds are impacted in different ways. Depending on the nature of the particular subsurface environment, the individual compounds may remain as a non-aqueous phase liquid, desorbs into the aqueous phase, be metabolized by microorganisms, taken up by plants, volatilize into the interstitial void spaces or sorbs onto soil or organic matter. Desorption from the solid phase is a critical factor in the degradability of PAHs. The tendency of a substance to sorbs to soil can be quantified using

the soil-water partition coefficient which is useful in determining the probable subsurface fate of PAHs. The High Molecular Weight (HMW) PAHs (those with four or more rings) are prone to irreversible sorption, while Low Molecular Weight (LMW) PAHs (those with two or three rings) are more likely to be directly taken up by roots. LMW PAHs are relatively water soluble, but PAHs containing four or more rings are quite hydrophobic and insoluble. Hence, they usually remain bound solid particles, sediments, or organic matter and are not transported with groundwater or surface water (Cerniglia & Heitkamp, 1989). Generally, the higher the molecular weight of a PAH, the more likely it is to sorb the soil organic matter. This tendency to strongly sorbs on particulate matter renders the HMW PAHs less available and thus less susceptible to bioremediation. When HMW PAHs are permanently sorbed to particles, the potential for off-site migration and adverse environmental effects is greatly reduced.

PAHs in soil are also degraded through biotic processes. Oxidation reactions are the most important in this content, although photochemical reactions may contribute significantly to the degradation on the surface of soils (Kochany & Maguire, 1994). In addition, most of the oxidants that commonly initiate the oxidation reactions in the environments, such as organic peroxides, hydrogen peroxides, ozone and radicals such as alkoxy radicals, and hydroxyl radicals are directly or indirectly generated through photochemical processes. However, some can also be produced from inorganic salts and oxides, especially those of iron and manganese (Kochany & Maguire, 1994). Chemical oxidation reactions involving hydroxyl radicals, generated from hydrogen peroxide, and ozone are strong radicals that react with aromatic compounds such as PAHs at near diffusion – controlled rates by abstracting hydrogen atoms or by addition to double bonds (Haag & Yao, 1992). The ozone molecule may attack double bonds directly, it can also form reactive hydroxyl radicals by

decomposing water (Legrini et al.,1993, Gurol & Singer 1982, Yao et al., 1998a & Yao et al., 1998b). The reaction pathways that follow are very complex and numerous intermediates are formed. The final reaction products include for both oxidants a mixture of ketones, quinones, aldehydes, phenols and carboxylic acids (Lee et al., 2001, Rivas et al., 2000, Reisen & Arey, 2002, Koeber et al., 1997). Photochemical degradation of PAHs often involves the same oxidative species that are produced during the pure chemical oxidation of PAHs, i.e. oxygen, hydroxyl radicals and other radicals. Consequently, the reaction products include similar complex mixtures of ketones, quinones, aldehydes, phenols and carboxylic acids (Barbas et al., 1996, David, Boule, 1993 & Mallakin et al., 2000).

During chemical reactions, PAHs are largely transformed into other polyaromatic carbons i.e they do not lose their aromatic character. The aromaticity is conserved since the non-aromatic hydrocarbons are much richer in energy. Thus, considerable amounts of energy are required to change an aromatic compound into a non-aromatic compound. The molecules that are most reactive have positions within the PAH structure.

Low levels of oil and hence polycyclic aromatic hydrocarbons (PAHs) are naturally present in the marine environment, although levels have increased significantly following human extraction and use of oil and gas. Other major anthropogenic sources of PAHs include smelters, the use of fossil fuels in general, and various methods of waste disposal, especially incineration. There are two major sources for PAHs to marine ecosystems in Norway: the inshore smelter industry, and offshore oil and gas production activities. A distinction is generally made between petrogenic (oil-derived) and pyrogenic (combustion-derived) PAHs. Although petrogenic PAHs appear to be bioavailable to a large extent, pyrogenic PAHs are often associated with soot particles and less available for uptake into organisms. There is extensive evidence linking sediment-associated PAHs to induction of

phase-I enzymes, development of DNA adducts, and eventually neoplastic lesions in fish. Most studies have focused on high-molecular-weight, carcinogenic PAHs such as benzo[a]pyrene. It is less clear how two- and three-ring PAHs affect fish, and there is even experimental evidence to indicate that these chemicals may inhibit some components of the phase I system rather than produce induction. There is a need for increased research efforts to clarify biological effects of two- and three-ring PAHs, PAH mixtures, and adaptation processes in marine ecosystems (Hylland, K., 2006).

2.4 Bioremediation Process

Bioremediation is a biotechnological approach of rehabilitating areas degraded by pollutant. It is the ability of the organisms to degrade or detoxify organics contaminated area by transforming the harmful substance into non–toxic compounds. The basis of bioremediation of organic pollutants is the detoxification or mineralization of the contaminated species to CO_2 and H_2O. Therefore, this makes it environmental friendly and relatively cost effective alternative to conventional physicochemical techniques, which rely mainly on incineration, volatilization or immobilization, attacks alkanes terminally whereas some perform sub–terminal oxidation. Primary attachment on intact hydrocarbons always requires the action of oxygen. In the case of alkenes, monoxygenase attack result in the production of alcohol. The alcohol product is oxidized finally into an aldehyde and finally, to a fatty acid. The latter is degraded further by beta–oxidation. An extensive methyl branching interface with the beta–oxidation process and necessitates di–terminal attack or other by pass mechanisms. Therefore, normal alkenes (n-alkenes) are degraded more readily than iso–alkanes (Schneekloth et al., 2002; Chinenye et al., 2013; Ajoku & Oduola, 2013).

2.5 FACTORS AFFECTING DEGRADATION OF CRUDE OIL

The factors are:

i. Soil Type and Physical Properties
ii. Moisture Content
iii. Oxygen Content
iv. pH Level
v. Nitrogen and phosphorus.

2.5.1 SOIL TYPE AND PHYSICAL PROPERTIES

Biodegradation will occur in all soil types such as sandy soil, loamy soil, and clay soil even though some of the soil need special treatment before the soil could be degraded. In the case of clay soil, it needed to be amended with bulking agents, in order to improve oxygen transport. Sandy soil needs to be amended with organic matters to improve the soil water holding capacity. From the research work performed at various research institutes, it was observed that biodegradation rates are lower for sandy soil than for clay and loam soil. The reasons for this may be low water holding capacity, low total organic carbon content and low surface area available for microbial growth in sandy soils. Biodegradation rates for the clay soils were similar or better than those for the loamy soils. However, in both studies moisture content was maintained at optimum levels to improve soil tilt (Emon, 2008). The rate of soil degradation is dependent on texture, nature and characteristics.

2.5.1.1 Soil Texture

Soil texture is the size distribution of primary inorganic particles in soils. The particle sizes are either sand (2.0 – 0.05mm), silt (0.05 – 0.002mm) or clay (<0.002mm). The surface area of a clay is 100 to 10,000 times greater than a sand. A loam is a combination of the three particle sizes which exhibits the properties of sand, silt and clay. In terms of soil texture, soil types usually refers to the different sizes of mineral particles in a particular sample. Soil is made up in part of finely ground rock particles, grouped according to size as sand and silt in addition to clay, organic material such as decomposed plant matter. Each component and their size, play an important role. For example, the largest particles, sand, determine aeration and drainage characteristics, while the tiniest, sub-microscopic clay particles are chemically active, binding with water and plant nutrients. The ratio of these sizes determines soil type: clay, loam, clay-loam, silt-loam, and so on. In addition to the mineral composition of soil, humus (organic material) also plays an important role in soil characteristics and fertility for plant life. Soil may be mixed with larger aggregate, such as pebbles or gravel. Not all types of soil are permeable, such as pure clay. (Henry, D.F., 1990).

Soil is generally composed of particles of broken rock that have been altered by chemical and environmental processes that include weathering and erosion. Soil differs from its parent rock in their morphological, physical, chemical, and mineralogical characteristics due to interactions between the lithosphere, hydrosphere, atmosphere, and the biosphere. (Lambe, T. W. 1969)

Soil particles pack loosely, forming a soil structure filled with pore spaces. These pores contain soil solution (liquid) and air (gas). Accordingly, soils are often treated as a three state-system. Most soils have a density between 1 and 2 g/cm^3. It supports a complex ecosystem,

which supports the plants on the surface and creates new soil by breaking down rocks and sand. This microscopic ecosystem has co-evolved with the plants to collect and store water and nutrients in a form usable by plants. There are many recognized soil classifications, both international and national.

2.5.1.1.1 Particle and Bulk Density

Particle density is density of the soil particles. Bulk density is a measure of the weight per unit volume (g/cm^3) of soil or waste. Sandy soils have greater bulk density than well-aggregated finer textured soil percent pore space is the space available in the soil for air or water. For example, the bulk density for textural class of sands or sandy loam is within the range 1.2 – 1.8 g/cm^3, while that for silt loam, clay loam, clay are within the density range of 1.0 – 1.6 g/cm^3. Typically, the relationship between soil texture, bulk density and porosity can be presented thus for sand, sandy loam, fine sandy loam, loam, silt loam, clay loam, clay and aggregate clay have bulk density (g/cm^3) & porosity (%) as 1.55 & 42, 1.40 & 48, 1.30 & 51, 1.2 & 55, 1.15 & 56, 1.10 & 59, 1.05 & 60 and 1.05 & 62 respectively. Generally, the greater the bulk density of a soil, the lower % of porosity it has. Macropores are larger pores allowing the ready movement of air and percolation of water. Micropores are small pores in which air movement is impeded and water moves slowly due to capillary rise. Sandy soils have lower porosity than clay, but air and water movement are rapid because of the presence of more macropores. On the other hand, in clays, water and air movement is slow even though porosity is greater than that of sands. Water infiltration is faster in loamy sand than a clay loam.

Soil aeration is important to keep soil conditions under aerobic processes. Aeration is affected by texture, structure, total porosity and macropores versus micropores.

Bulk density of soil is the mass of dry soil per unit of bulk volume. The bulk volume is determined before drying at 105^{O} C to constant weight.

$$Porosity, \% = \left[100 - \left(\frac{Bulk\ Density}{Particle\ Density}\right) \times 100\right] \quad 2.1$$

$$Particle\ Density = \frac{Dw\ (Ws - Wa)}{(Ws - Wa) - (Wsw - Ww)} \quad 2.2$$

Where,

Dw = Density of water at observed temperature

Ws = Weight of flask plus soil

Wa = Weight of flask filled withair

Wsw = Weight of flask filled with soil and water

Ww = Weight of flask filled with water at observed temperature

2.5.1.2 Soil Nature and Characteristics

Soil is composed of particles of broken rock that have been altered by chemical and environmental processes that include weathering and erosion. Soil differs from its parent rock in their morphological, physical, chemical, and mineralogical characteristics due to interactions between the lithosphere, hydrosphere, atmosphere, and the biosphere. Soil particles pack

loosely, forming a soil structure filled with pore spaces as shown in figure 2.1. These pores contain soil solution (liquid) and air (gas). Accordingly, soils are often treated as a three state-system. Most soils have a density between 1 and 2 g/cm^3. It supports a complex ecosystem, which supports the plants on the surface and creates new soil by breaking down rocks and sand. This microscopic ecosystem has co-evolved with the plants to collect and store water and nutrients in a form usable by plants (Henry, D.F., 1990).

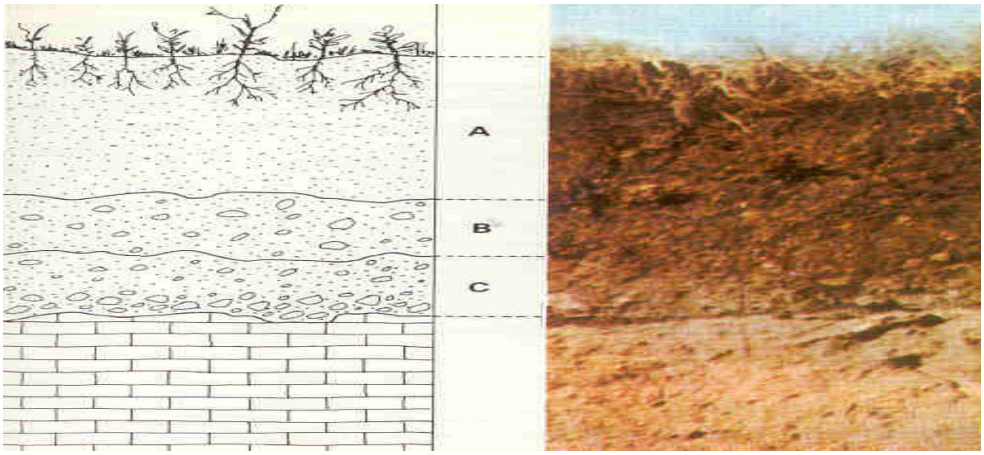

Figure 2.1: Soil Profile; A represents Top Soil; B represents Laterite, a Regolith; C represents Saprolite, a less-weathered Regolith; D represents Bedrock.

Source: Field Book for Describing and Sampling Soils – National Soil Survey Center Natural Resources Conservation Service – U.S. Department of Agriculture. Version 3.0, 2012

2.5.1.3 Soil forming factors

Pedogenesis or soil evolution (formation) is the process by which soil is created. It is the main topic of the science of pedology. Soil formation, or pedogenesis, is the combined effect of physical, chemical, biological, and anthropogenic processes on soil parent material. Soil genesis involves processes that develop layers or horizons in the soil profile. These processes involve additions, losses, transformations and translocations of material that compose the soil. Minerals

derived from weathered rocks undergo changes that cause the formation of secondary minerals and other compounds that are variably soluble in water, these constituents are moved (translocated) from one area of the soil to other areas by water and animal activity (Henry, D.F., 1990). The alteration and movement of materials within soil causes the formation of distinctive soil horizons. The weathering of bedrock produces the parent material from which soils form. An example of soil development from bare rock occurs on recent lava flows in warm regions under heavy and very frequent rainfall. In such climates, plants become established very quickly on basaltic lava, even though there is very little organic material. The plants are supported by the porous rock as it is filled with nutrient-bearing water which carries, for example, dissolved minerals and guano. The developing plant roots are themselves or associated with mycorrhizal fungi, gradually break up the porous lava and organic matter soon accumulates. But even before it does, the predominantly porous broken lava in which the plant roots grow can be considered a soil. How the soil "life" cycle proceeds is influenced by at least five classic soil forming factors that are dynamically intertwined in shaping the way soil is developed, they include: parent material, regional climate, topography, biotic potential and the passage of time (Ezekiel, A. A., 2009).

2.5.1.4 Parental Material Factor

The material from which soils form is called parent material. It includes: weathered primary bedrock; secondary material transported from other locations, e.g. colluvium and alluvium; deposits that are already present but mixed or altered in other ways - old soil formations, organic material including peat or alpine humus; and anthropogenic materials, like landfill or mine waste. Few soils form directly from the breakdown of the underlying rocks they

develop on. These soils are often called “residual soils”, and have the same general chemistry as their parent rocks. Most soils derive from materials that have been transported from other locations by wind, water and gravity. Some of these materials may have moved many miles or only a few feet. Windblown material called loess is common in the Midwest of North America and in Central Asia and other locations. Glacial till is a component of many soils in the northern and southern latitudes and those formed near large mountains; till is the product of glacial ice moving over the ground. The ice can break rock and larger stones into smaller pieces; it also can sort material into different sizes. As glacial ice melts, the melt water also moves and sorts material, and deposits it varying distances from its origin. The deeper sections of the soil profile may have materials that are relatively unchanged from when they were deposited by water, ice or wind. Weathering is the first stage in the transforming of parent material into soil material. In soils forming from bedrock, a thick layer of weathered material called saprolite may form. Saprolite is the result of weathering processes that include: hydrolysis (the replacement of a mineral’s cations with hydrogen ions), chelation from organic compounds, hydration (the absorption of water by minerals), solution of minerals by water, and physical processes that include freezing and thawing or wetting and drying. The mineralogical and chemical composition of the primary bedrock material, plus physical features, including grain size and degree of consolidation, plus the rate and type of weathering, transforms it into different soil materials.

2.5.1.4 Climatic Factors

Soil formation greatly depends on the climate, and soils from different climatic zones show distinctive characteristics. Temperature and moisture affect weathering and leaching. Wind

moves sand and other particles, especially in arid regions where there is little plant cover. The type and amount of precipitation influence soil formation by affecting the movement of ions and particles through the soil, aiding in the development of different soil profiles. Seasonal and daily temperature fluctuations affect the effectiveness of water in weathering parent rock material and affect soil dynamics. The cycle of freezing and thawing is an effective mechanism to break up rocks and other consolidated materials. Temperature and precipitation rates affect biological activity, rates of chemical reactions and types of vegetation cover (Henry, D.F., 1990).

2.5.1.5 Biological Factors

Plants, animals, fungi, bacteria and humans affect soil formation. Animals and micro-organisms mix soils to form burrows and pores allowing moisture and gases to seep into deeper layers. In the same way, plant roots open channels in the soils, especially plants with deep taproots which can penetrate many meters through the different soil layers to bring up nutrients from deeper in the soil. Plants with fibrous roots that spread out near the soil surface; have roots that are easily decomposed, adding organic matter. Micro-organisms, including fungi and bacteria, affect chemical exchanges between roots and soil and act as a reserve of nutrients. Humans can impact soil formation by removing vegetation cover; this removal promotes erosion. They can also mix the different soil layers, restarting the soil formation process as less-weathered material is mixed with and diluting the more developed upper layers. Some soils may contain up to one million species of microbes per gram, most of those species being unknown, making soil the most abundant ecosystem on Earth.

Vegetation impacts soils in numerous ways. It can prevent erosion from rain or surface runoff. It shades soils, keeping them cooler and slowing evaporation of soil moisture, or it can

cause soils to dry out by transpiration. Plants can form new chemicals that break down or build up soil particles. Vegetation depends on climate, land form topography and biological factors. Soil factors such as soil density, depth, chemistry, pH, temperature and moisture greatly affect the type of plants that can grow in a given location. Dead plants, dropped leaves and stems of plants fall to the surface of the soil and decompose. There, organisms feed on them and mix the organic material with the upper soil layers; these organic compounds become part of the soil formation process, ultimately shaping the type of soil formed.

2.5.1.6 Time Factor

Time is a factor in the interactions of all the above factors as they develop soil. Over time, soils evolve features dependent on the other forming factors, and soil formation is a time-responsive process dependent on how the other factors interplay with each other. Soil is always changing. For example, recently-deposited material from a flood exhibits no soil development because there has not been enough time for soil-forming activities. The soil surface is buried, and the formation process begins again for this soil. The long periods over which change occurs and its multiple influences mean that simple soils are rare, resulting in the formation of soil horizons. While soil can achieve relative stability in properties for extended periods, the soil life cycle ultimately ends in soil conditions that leave it vulnerable to erosion. Despite the inevitability of soil retrogression and degradation, most soil cycles are long and productive.

Soil-forming factors continue to affect soils during their existence, even on “stable” landscapes that are long-enduring, some for millions of years. Materials are deposited on top and materials are blown or washed away from the surface. With additions, removals and alterations, soils are

always subject to new conditions. Whether these are slow or rapid changes depend on climate, landscape position and biological activity.

2.5.1.7 Soil Characteristics

Soil degradability is affected by its characteristics namely; colour, structure, texture, electrical resistivity, organic matter content, humus content, and contaminant types.

2.5.1.7.1 Soil Colour:

Soil color is primarily influenced by soil mineralogy. Many soil colors are due to the extensive and various iron minerals. The development and distribution of color in a soil profile result from chemical and biological weathering, especially redox reactions. As the primary minerals in soil parent material weather, the elements combine into new and colorful compounds. Iron forms secondary minerals with a yellow or red color, organic matter decomposes into black and brown compounds, and manganese, sulfur and nitrogen can form black mineral deposits. These pigments produce various color patterns due to effects by the environment during soil formation. Aerobic conditions produce uniform or gradual color changes, while reducing environments result in disrupted color flow with complex, mottled patterns and points of color concentration.

2.5.1.7.2 Soil structure

Soil structure is the arrangement of soil particles into aggregates. These may have various shapes, sizes and degrees of development or expression. Soil structure affects aeration, water movement, resistance to erosion and plant root growth. Structure often gives clues to texture, organic matter content, biological activity, past soil evolution, human use, and chemical and mineralogical conditions under which the soil formed.

2.5.1.7.3 Soil Texture

Soil texture is defined by size distribution or mass fractions of soil primary particles both individual grains and particles. Particle size distribution can be determined by any of these methods; sieving, sedimentation, particle counting or laser/light diffraction. www.engr.uconn.edu Soil texture refers to sand, silt and clay composition as shown in figure 2.2. Soil content affects soil behavior, including the retention capacity for nutrients and water. Sand and silt are the products of physical weathering, while clay is the product of chemical weathering. Clay content has retention capacity for nutrients and water. Clay soils resist wind and water erosion better than silty and sandy soils, because the particles are more tightly joined to each other. In medium-textured soils, clay is often translocated downward through the soil profile and accumulates in the subsoil.

2.5.1.7.4 Soil Electrical Resistivity

The electrical resistivity of soil can affect the rate of galvanic corrosion of metallic structures in contact with the soil. Higher moisture content or increased electrolyte concentration can lower the resistivity and thereby increase the rate of corrosion. Soil resistivity values typically range from about 2 to 1000 Ω·m, but more extreme values are not unusual.

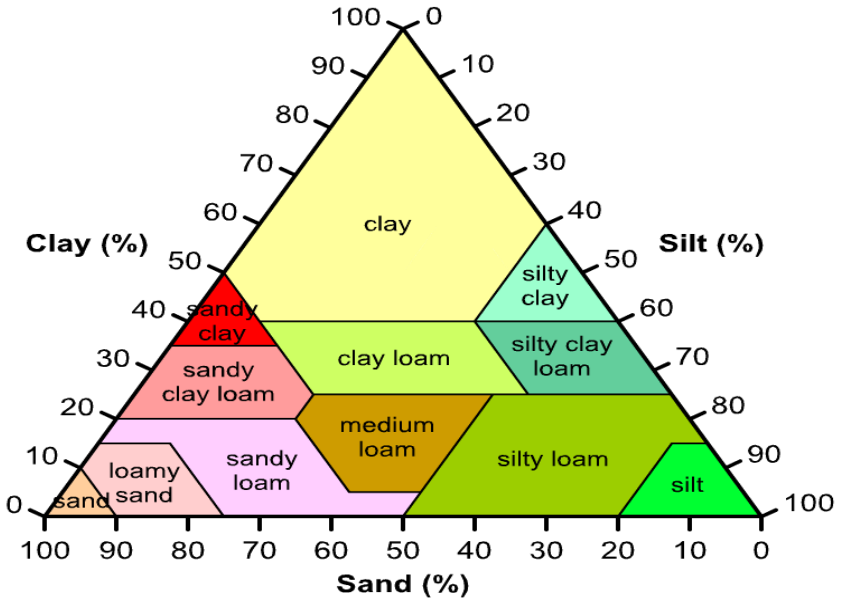

FIGURE 2.2: Soil Types by Clay, Silt and Sand

Source: USDA Soil Texture Triangle – CMG Garden Notes #213, Managing Soil Tilth.

2.5.1.7.5 Soil Organic matter

Most living things in soils, including plants, insects, bacteria, fungi and are dependent on organic matter for nutrients and energy. Soils often have varying degrees of organic compounds in different states of decomposition. Many soils, including desert and rocky-gravel soils, have no or little organic matter. Soils that are all organic matter, such as peat (histosols), are infertile.

2.5.1.7.6 Humus

Humus refers to organic matter that has decomposed to a point where it is resistant to further breakdown or alteration. Humic acids and fulvic acids are important constituents of humus and typically form from plant residues like foliage, stems and roots. After death, these plant residues begin to decay, starting the formation of humus. Humus formation involves

changes within the soil and plant residue, there is a reduction of water soluble constituents including cellulose and hemicellulose; as the residues are deposited and break down, humin, lignin and lignin complexes accumulate within the soil; as microorganisms live and feed on the decaying plant matter, an increase in proteins occurs. Lignin is resistant to breakdown and accumulates within the soil; it also chemically reacts with amino acids which add to its resistance to decomposition, including enzymatic decomposition by microbes. Fats and waxes from plant matter have some resistance to decomposition and persist in soils for a while. Clay soils often have higher organic contents that persist longer than soils without clay. Proteins normally decompose readily, but when bound to clay particles they become more resistant to decomposition. Clay particles also absorb enzymes that would break down proteins. The addition of organic matter to clay soils, can render the organic matter and any added nutrients inaccessible to plants and microbes for many years, since they can bind strongly to the clay. High soil tannin (polyphenol) content from plants can cause nitrogen to be sequestered by proteins or cause nitrogen immobilization, also making nitrogen unavailable to plants. Humus formation is a process dependent on the amount of plant material added each year and the type of base soil; both are affected by climate and the type of organisms present. Soils with humus can vary in nitrogen content but have 3 to 6 percent nitrogen typically; humus, as a reserve of nitrogen and phosphorus, is a vital component affecting soil fertility. Humus also absorbs water, acting as a moisture reserve that plants can utilize; it also expands and shrinks between dry and wet states, providing pore spaces. Humus is less stable than other soil constituents, because it is affected by microbial decomposition, and over time its concentration decreases without the addition of new organic matter. However, some forms of humus are highly stable and may persist over centuries if not millennia: they are issued from the slow oxidation of charcoal, also called black carbon,

like in Amazonian Terra preta or Black Earths, or from the sequestration of humic compounds within mineral horizons, like in podzols.

2.5.1.7.7 Climate and organics

The production and accumulation or degradation of organic matter and humus is greatly dependent on climate conditions. Temperature and soil moisture are the major factors in the formation or degradation of organic matter, they along with topography, determine the formation of organic soils. Soils high in organic matter tend to form under wet or cold conditions where decomposer activity is impeded by low temperature or excess moisture.

2.5.1.8 Soil Solution and Degradation

2.5.1.8.1 Soil Solutions

Soils retain water that can dissolve a range of molecules and ions. These solutions exchange gases with the soil atmosphere, contain dissolved sugars, fulvic acids and other organic acids, plant nutrients such as nitrate, ammonium, potassium, phosphate, sulfate and calcium, and micronutrients such as zinc, iron and copper. Some arid soils have sodium solutions that greatly impact plant growth. Soil pH can affect the type and amount of anions and cations that soil solutions contain and that exchange with the soil atmosphere and biological organisms.

2.5.1.8.2 Soil Degradation

Land degradation is a human-induced or natural process which impairs the capacity of land to function. Soils are the critical component in land degradation when it involves acidification, contamination, desertification, erosion or salination. While, soil acidification of alkaline soils is beneficial, it degrades land when soil acidity lowers crop productivity and increases soil

vulnerability to contamination and erosion. Soils are often initially acid because their parent materials were acid and initially low in the basic cations (calcium, magnesium, potassium and sodium). Acidification occurs when these elements are removed from the soil profile by normal rainfall, or the harvesting of forest or agricultural crops. Soil acidification is accelerated by the use of acid-forming nitrogenous fertilizers and by the effects of acid precipitation.

Vertical infiltration of light crude oil spilled on native soil spreads in the direction of the surface drainage with pooling in depressions. The infiltration can be controlled by:

- Surface condition
- Soil porosity
- Pore size distribution
- Soil moisture
- Soil structure
- Viscosity and gravity of the oil

Factors affecting vertical infiltration of crude oil are permeability or porosity of soil, surface soil condition and soil moisture content. Though, there is not much that may be done on the varying temperatures for the dry and wet seasons but the constant monitoring of the moisture will affect the rate degradation and/or remediation. Seasonal weather changes around the location of sample indicate temperature variations between 24 to 35 degrees Centigrade. Dry weather conditions are registered between the months of October to March, while that for raining season begins from April to September every year with little variations of $\pm$2 weeks of delayed reappearance of climatic conditions. The amount of oil percolating into a soil profile is controlled by the

permeability of the least pervious horizon or layer of soil in the vertical cross section. The stained soil defines the surface boundaries of oil spill or polluted land area. Unless specific information dictates otherwise, samples collected in vertical profile define concentrations with depth and profiles are sampled initially in 0-6, 6-12, 12-24, 24-36 inches in depth intervals. Profiles should be taken at various points along a transection which defines surface drainage of the site. Also, the sample of the site should be in grid fashion where drainage patterns and internal percolation are not well defined. Typically, bioremediation is not effective below 6 – 10 inches in depth and accordingly, contaminated soil below that depth must be excavated and treated on the surface.

Similarly, microbial degradation of petroleum hydrocarbon does not occur when soil temperatures remain below $5^{o}C$ and accordingly we must consider composting operations when soil temperatures remain below $10^{o}C$.

2.5.2 Effect of Moisture Content

A soil that contains little or no moisture content may not be friendly to survival and growth of microorganisms. Thus, highly contaminated soil would not permit the retention of moisture. Only lightly polluted soil would have capillarity for the movement of water with the soil and hence provide moisture for the purpose of degrading the crude oil by the microorganism, large quantities of metal salt may also become toxic to the biotic components of the contaminated environment and become recalcitrant inhibiting biodegradation of the crude oil contaminated soil.

It may also affect the survivability of the microorganisms in the soil. In such a situation additional treatment may be necessary. Combination of phytoremediation and bioremediation may be effective in the treatment of metal salt and crude oil combined of soil (Odokuma and Dickson, 2003). Irrigation of affected areas with regular introduction of water about 1 in/week in dry climates (arid and semiarid) can control moisture content, while humid regions with spurious rainfall distribution patterns may require seasonal irrigations to overcome drought conditions.

2.5.3 Oxygen Content / Aeration

Biodegradation is effective in an area where there is adequate oxygen. Only in some exceptional cases can biodegradation take place in anoxic area (i.e. area where is sufficient oxygen). In an open marine ecosystem, there is a highly oxygenated surface. This makes it to be favourable for biodegradation processes. But in stagnant water areas, there is decrease in the amount of oxygen; this contributes to the low rate of biodegradation (Schneekloth et al., 2002). Tilling of soil infuses oxygen and the application of (N), the tillage operations increases fertilizer efficiency and creation of an oxygen deficit by nutrient enrichment.

2.5.4 Nitrogen and Phosphorous

These two elements are limiting factors for biodegradation, and their availability to bacteria can affect their ability to consume oil products. The addition of nitrogen and phosphorous increases the proliferation of biodegrading bacteria, resulting in an increase in degradation rates (Okoh, 2003). Typically, bioremediation that shows long lag phase results from limited microbial populations and the additions of commercial microbes reduces the lag phase. Therefore, fertility adjustments in the form of fertilizer amendments, aeration and moisture are necessary for the remediation program. Fertilizer amendments include nitrogen (N), phosphorous (P) and

potassium (K). Ammonium nitrate (NH_4NO_3), ammonium sulphate ($(NH_4)_2SO_4$ and urea are good nitrogen sources. These sources of nitrogen should be applied to increase efficiency; because nitrogen is often lost to the atmosphere with time. Superphosphate and muriate of potash are suitable sources of Phosphorous and potassium. Moisture content should be monitored constantly and maintained preferably at 50 – 80% of field capacity to effect degradation rates.

2.5.5 pH

The soil pH is a measure of acidity or alkalinity of water. The pH of the environment can significantly affect microbial activity, and therefore the bioremediation rate. Most microorganisms thrive within a neutral pH range. Bioremediation studies in the laboratory and field have demonstrated that pH ranging from 6.5 to 7.5 is sufficient for optimal bacteria growth of hydrocarbon-degrading microorganisms. However, many acidic or alkaline soils support a viable microbial population capable of degrading the crude-oil contaminants. Higher acid or alkaline conditions generally inhibit microbial activity, and most bacteria favour neutral conditions. Nevertheless, bacteria are well adapted to acidic or basic conditions have been reported. For example, the sulphur–oxidizing bacteria, an obligate aerobic chemo-autotrophic genus that produce sulphurous acid though oxidation of hydrogen sulphide (H_2S) has been found to function well at pH value of 1. According to the pH ranges in which they function best, bacteria have been classified as neutrophiles, acidophiles or alkaliphiles respectively. Neutrophiles grow in the range of pH 5-8 with optimally growth near neutral pH of 7. Acidophiles grow optimally at a pH above 8.5 (Njoku et al., 2009).

Excess acidity is managed with lime (CaO) or using agricultural limestone ($CaCO_3$). Use of aluminium sulphate ($Al_2(SO_4)_3$) or elemental sulphur control and adjust alkalinity.

The soil pH also affects the solubility of phosphorus, an important nutrient for microbes, and the transport of hazardous metals in soil. Phosphorus solubility is maximized at a pH level of 6.5 and metal transport is minimized at a pH level greater than 6. Furthermore, the pH has a profound effect on biotic contaminants reaction within these soils (Inoni et al., 2006).

2.6 Phytoremediation of Crude Oil Contaminated Soil

Phytoremediation is the use of plants and their associated microorganisms in conjunction with agronomic techniques to remove or degrade environmental contaminants. Therefore, plant selection and soil amendments are important for successful phytoremediation. Several research works have been carried out on degradation of contaminated soil both in various universities and research institutes.

Phytoremediation is one of the many methods for cleaning up polluted soil or water that is considered new or innovative (USEPA, 2001).

It consists of de-polluting contaminated soils, water or air with plants able to contain, degrade or eliminate metals, pesticides, solvents, explosives, leachates, crude oil and its derivatives and other various contaminants from the mediums that contain them. In addition, the plants also help prevent wind rain and ground water from carrying pollution away from sites to other areas (USEPA, 2008). It is clean, efficient, inexpensive and non-environmentally disruptive, as opposed to processes that require excavation of soil.

Phytoremediation works best at sites with low to medium amount of pollution. It takes advantage of a plants natural ability to absorb, accumulate or metabolise contaminants from polluted soil, streams and ground water in which it grows, through its roots.

Plants can clean up chemicals as deep as their roots can grow; trees are, therefore, used to reach pollution deeper in the ground since their roots grow deeper.

Once inside the plants, chemicals can be stored in the roots, stems or leaves, changed into less harmful chemicals within the plant of changed into gases that are released into the air as the plant transpires.

Phytoremediation can occur even if the chemicals are not taken into the plants by the roots. Chemical can stick or sorbs to plant roots or through interaction between the plants and microorganisms that live in the soil, by which harmful chemicals are changed into less harmful ones by bugs and microbes.

The potentials of phytoremediation has been investigated, using plants such as sunflower, ragweed, cabbage and germanium as well as other less well known species. Some plants called hyper-accumulators are known for their ability to absorb more metal than other plants, including metals that do not appear necessary for plant function (USEPA, 2001; Bioportal, 2008).

2.6.1 Types of Phytoremediation

There are six basic types of phytoremediation categorised by the type of material they remediate – metals or organic chemicals.

2.6.1.1 Impact of Metal Contaminants

2.6.1.1.1 Phytoextraction (phytoaccumulation)

Heavy metals adversely impact humans and vegetation directly or indirectly. Soluble chlorides salts and excess exchangeable sodium are the major ions that migrate in soil. These ions cause harmful effects on the soil and plant growth (Mosley, 1993; Miller & Honarvar, 1975).

High soluble salt levels increase the osmotic potential in the soil lowering the amount of water available to plants from the soil. Also, the increased osmotic potential interferes with the plant uptake of required nutrients (Murphy & Kehew, 1984).

Soils having high organic content and moderate percentages of clay appear less sensitive to high sodium and soluble salt concentrations than coarser textured soils or loamy or clayey soils in a dry environment. The availability or mobility of metals in soil is dependent upon pH, reduction / oxidation potential and total metals concentration (Moseley, 1983; Duel, 1991 & Miller, 1975).

This involves uptake of metal contaminants from soil sediments and water through the roots of plants and their concentration into the plant biomass (stem and leaves). Phytoextraction has been growing widely in popularity world-wide for the last twenty years or so, especially in the extraction of heavy metals. Hyper-accumulators are often used in this process for their enhanced capacity to extract metal contaminants from the environment.

The plants are finally harvested and disposed appropriately, and typically have contaminants concentrated in much smaller volumes of the plant than in the initially contaminated soil or sediment (USEPA, Bioportal, 2008). Mining with plants or phytomining is also being experimented. There are two versions of phytoextraction;

(i) Natural Hyper-accumulation: In this, plants naturally take up the contaminants in soil unassisted.

(ii) Induced or Assisted Hyper-accumulation: In this a conditioning fluid containing a chelator or another agent is added to soil to increase metal solubility or mobilisation so that the plant can absorb them more easily.

Examples of phytoextraction from soils include:

- Arsenic using the sun flower (Helianthus annuus) or Chinese brake fern (Pteris spp.) – a hyper-accumulator that stores arsenic in its leaves.
- Cadmium and Zinc using alpine pennycress (Thlaspi caerulescens)
- Lead using Indian mustard (Brassica juncea), Ragweed (Ambrosa arteniisii folia), Hemp Dogbane (Apocynum cannabinum) or poplar trees.
- Extraction of Sodium chloride using salt tolerant (moderately halophytic) barley and/or sugar beets, to reclaim fields previously flooded by sea water.
- Uranium, using sun flowers
- Mercury, selenium and organic pollutants such as polychlorinated biphenyls (PCBS) using transgenic plants containing genes for bacterial enzymes (Meagher, 2000).

2.6.1.1.2 Phytostabilization

This involves using plants to reduce the mobility of soil and water contaminants in the environment. It focuses on long term stabilisation and containment of the pollutant. The plant's presence can reduce wind erosion, or the plant's roots can prevent water erosion, immobilize the pollutants by adsorption or accumulation (absorption) and provide zone around the roots where the pollutants can precipitate and stabilise. Unlike phytoextraction, phyto-stabilisation mainly focuses on sequestering pollutants in soil near the roots but not in plant tissues. Pollutants become less bio-available and livestock, wildlife and human exposure is reduced. This has been applied in use of vegetative cap to stabilise and contain mine tailings (Bioportal, 2008; Mendez & Maier, 2008).

2.6.1.1.3 Rhizofiltration

This method applies specifically to surface and ground water remediation. In this process, contaminants are extracted either directly or absorption through the roots or indirectly through root adsorption, meaning that contaminants are attracted to and held by the roots. Plants that have been grown in clean water are transplanted to the contaminated water site. When the roots become saturated with the contaminants, they are harvested and new ones are planted. This has been applied in the use of constructed wetlands to treat wastewater and landfill leachate (Bioportal, 2008).

2.6.1.2 Organic Chemical Contamination

Phytotransformation

This involves chemical modification of organic chemical in contaminated soil and water through plants metabolic activities, often resulting in inactivation, degradation (phytodegradation) or immobilisation (phytostabilisation). In some cases plants such as Cannas render organic pollutants (pesticides, explosives, industrial chemicals and other xenobiotic substances) non-toxic by their metabolism, while in other cases microorganisms living in association with plant roots metabolise these substances in soil and water. The complex and recalcitrant compounds that cannot be broken to water and carbon dioxide are chemically transformed to less toxic forms (Boyd and Martens, 1998).

There are three phases in phytotransformation:

Phase I metabolism is the stage after uptake of the xenobiotic substance when the plant enzymes (for example, nitroreductases) increase the polarity of the xenobiotic by adding functional groups such as hydroxyl groups (-OH).

In phase II metabolism, plant bio-molecules such as glucose and amino acids are added to the polarised xenobiotic to further increase the polarity,

Phase I and phase II reactions serve to increase the polarity and reduce the toxicity of the compounds and allow for easy transport of xenobiotics along aqueous channels,

In phase III metabolism a sequestration of the xenobiotic occurs within the plant. The xenobiotics polymerize in a lignin-like manner and get a complex structure which is sequestered in the plant to ensure safe storage of xenobiotic in plant without affecting the functioning of the plant. Plants involved in phyto-transformation may need to be maintained in closed enclosure as studies have shown that they can be toxic to small animals, like snails.

Trinitrotoluene phytotransformation has been studied and a transformation pathway proposed (Subramanian, Oliver & shanks, 2006).

Phytostimulation (Rhizosphere degradation or Rhizodegradation)

This plant assisted bioremediation involves plant stimulation of the growth of microorganisms in the area around their roots (called rhizosphere) through the release of natural substances. These microorganisms such as yeast, fungi and bacteria, degrade contaminants through their metabolic processes (Bioportal, 2008).

Phytovolatilisation: This involves the removal of toxic substances from soil and water with release into the air, sometimes as a result of phytotransformation to more volatile and/or less polluting substances.

2.6.2 Advantages and Limitations of Phytoremediation

Advantages

- It is environmentally friendly, cost-effective (in situ or ex situ) and aesthetically pleasing.
- The plants can be easily monitored.
- Metals absorbed can be recovered and re-used (for example, phytomining).
- It can be used to clean up a large variety of contaminants.
- It may reduce the entry of contaminants into the environment by preventing their leakage into the ground water system.

Limitations

- Phytoremediation is limited to surface and depth occupied by the roots. Typically three to six feet underground for herbaceous plants and ten to fifteen feet for trees.
- It relies on natural cycle of plants and therefore requires long-term commitment due to slow growth.
- Possible bioaccumulation of contaminants which then pass into the food chain, from primary level consumers upwards.
- Survival of the plants is affected by the toxicity of the contaminated land and the general condition of the soil.
- It is not possible to completely prevent leaching of contaminants into the ground water (USEPA, 2001; Bioportal, 2008). .

2.6.3 Plants Harvest and Disposal

Plants that have absorbed and accumulated contaminants are harvested and discarded. If the organic chemical contaminants are degraded into molecules like water and carbon dioxide, the plants may not require any special method of disposal.

The most common method used to dispose plants that have absorbed large amounts of contaminants is controlled incineration, to produce ashes which can be discarded in an appropriate waste site. For plants that have absorbed metals, the ash produced has a high metal content.

Sun drying, composting and leaching are still being considered as disposal and metal recovery methods (Bioportal, 2008).

2.6.4 Genetics in Phytoremediation

Breeding programs and genetic engineering are powerful methods for enhancing natural phytoremediation capabilities, or for introducing new capabilities into plants.

The genes control the solubility of metals into root cells and up into the plant's shoots. Genes for phytoremediation may originate from a microorganism or may be transferred from one plant to another variety better adapted to the environmental conditions at the clean-up site.

Genes encoding a nitroreductase from a bacterium were inserted into tobacco and showed faster removal of trinitrotoluene (TNT) and enhanced resistance to the toxic effects of TNT (Hannink et al, 2001).

2.6.5 Types of Sites and Contaminants Treated by Phytoremediation

There is potential to use phytoremediation beneficially under a wide variety of site conditions. Types of sites at which phytoremediation has been applied or evaluated include: pipelines; industrial and municipal landfills; agricultural fields; wood treating sites; military bases; fuel storage tank farms; gas stations; army ammunition plants; sewage treatment plants; and mining sites. Phytoremediation is being tested and evaluated for its effectiveness in containing and treating a wide array of contaminants found at brownfields sites. While much more testing is

needed, current results indicate that plants have the potential to enhance remediation of the following types of contaminants:

- Petroleum hydrocarbons
- Benzene, toluene, ethylbenzene, and xylene (BTEX)
- Polycyclic aromatic hydrocarbons (PAH)
- Polychlorinated biphenyls (PCB)
- Trichloroethene (TCE) and other chlorinated solvents
- Ammunition wastes and explosives
- Heavy metals
- Pesticide waste
- Radionuclides
- Nutrient wastes (such as phosphates and nitrates)

One of the more optimal applications of phytoremediation is as a containment technology. Since many brownfields sites are characterized by wide-spread contamination at low concentrations that are close to target clean-up levels, phytoremediation is a good containment alternative if geology and rainfall amounts are favourable.

Table below lists types of sites at which phytoremediation has been employed with some level of success in cleaning up the sites. The table provides only a representative sample of sites and contaminants.

Table 2.2: Selected Phytoremediation Projects

Source: USEPA, Bioportal, 2008.

Contaminant(s)/ Purpose of Project	Media/ Mechanism	Plant Species	Location (Scale*)	Point of Contact
Chlorinated solvents/ Control groundwater migration at an urban brownfields site and remove TCE and derivatives from groundwater	Groundwater/ Phytoextraction, phytovolatilization, rhizodegradation	Hybrid poplar and willow	Findlay, OH (Full scale)	Steve Synder, Ohio Environmental Protection Agency (OEPA) (419) 352-8461 Ed Gatliff, Applied Natural Sciences, Inc. (ANS) (513) 942-6061
Chlorinated solvents/ Biologically (pump and treat) contaminated groundwater	Soil/ Rhizodegradation, phytovolatilization	Hybrid poplar, white willow, native species	Solvents Recovery Systems of New England, Southington, CT (Full scale)	Steve Rock, U.S. EPA (513) 569-7149 Ari Ferro, Phytokinetics (801) 750-0950
Chlorinated solvents/ Control groundwater migration and remove solvents from groundwater	Groundwater/ Phytovolatilization, rhizosphere biodegradation, phytodegradation	Eastern cottonwood	Carswell AFB, TX (Pilot)	Steve Hitt, U.S. EPA (214) 665-6736 Greg Harvey, USAF (937) 255-7716
Heavy metals/ Reduce lead concentration in soil	Phytoextraction	Indian mustard	Trenton, NJ Brownfields Site (Pilot)	Larry D'Andrea, U.S. EPA (212) 637-4314 Dr. Michael Blaylock, Edenspace (703) 961-8700
BTEX compounds/ Treat petroleum and organic contaminants; prevent contaminated groundwater from migrating	Soil and groundwater/ Hydraulic control, phytoextraction, phytovolatilization, rhizodegradation	Hybrid poplar	Ashland Chemical Co, Milwaukee, WI (Full scale)	Scott Ferguson, Wisconsin Department of Natural Resources (WDNR) (414) 263-8685 Dr. Louis Licht, Ecolotree (319) 358-9753
PAH's/Control groundwater and surface water migration, stabilize soil, and degrade contaminants	Soil and groundwater/ Hydraulic control, rhizodegradation	Grasses, hybrid poplar	Oneida, TN (Full scale)	Dr. John Novak, VA Tech (540) 231-6132 Dr. Louis Licht, Ecolotree (319) 358-9753
Explosives and fertilizers/ Contain and treat toxic solvents	Soil and groundwater/ Phytodegradation, phytovolatilization	Hybrid poplar	Aberdeen Proving Ground, MD (Pilot)	Harry Compton, U.S. EPA (732) 321-6751 Steve Hirsh, U.S. EPA (215) 814-3352
Wood preservatives/ Treat PAHs and DNAPLs	Soil and groundwater/ Rhizodegradation, hydraulic control	Herbaceous species and hybrid poplar	Laramie, WY (Full scale)	Marisa Latady, Wyoming Department of Environmental Quality, (307) 777-7752 Jennifer Uhland, CH_2M Hill (303) 771-0900

2.6.6 Plants Species Used for Phytoremediation

Plants species are selected for use according to their ability to treat the contaminants of concern and achieve the remedial objectives for redevelopment (for example, time frame and risk management), and for their adaptability to other site-specific factors such as adaptation to local climates, depth of the plant's root structure, and the ability of the species to flourish in the type of soil present. Often the preferred vegetation characteristics include: an ability to extract or

degrade the contaminants of concern to nontoxic or less toxic products, fast growth rate, adaptability to local conditions, ease of planting and maintenance, and the uptake of large quantities of water by evapotranspiration. The selection and use of plant species must be conducted with care to prevent the introduction of non-native species into areas where those species are not already present. Plant species that are benign under most circumstances may become a problem when introduced into a new area. For example, water hyacinth is considered a noxious aquatic weed that should be used only in isolated bodies of water from which there are no risks of unintentional transport (for example, by flood).

Maintenance requirements should be considered when selecting plant species for use at brownfields sites; those requirements may include the frequency with which the plant must be mowed; the need for fertilizer; and the need for replanting, pruning, harvesting, and monitoring programs.

Several types of plants and sample species frequently used for phytoremediation are listed below:

- Hybrid poplars, willow, and cottonwood trees
- Grasses (rye, Bermuda grass, sorghum, and fescue)
- Legumes (clover, alfalfa, and cowpeas)
- Aquatic and wetland plants (water hyacinth, reed, bullrush, and parrot feather)
- Hyperaccumulators for metals (such as alpine pennycress for zinc or alyssum for nickel)

Herbaceous species, such as mustard, alfalfa, and grasses, can be used in the remediation of contaminants in surface soil.

Hybrid poplars, willows, cottonwood, and other woody species that have rapid growth rates, deep roots, and high transpiration rates (resulting in uptake of abundant quantities of water), can

be used in the remediation of contaminants in groundwater or can be used to provide hydraulic control.

Constructed wetlands also are being used to remediate contaminated sites. There are two broad categories of wetland plants -- emergent and submerged species. Emergent plants, those rooted in shallow water with most of the plant exposed above the water's surface, transpire water and can be easier to harvest, if necessary. Submerged species, which lie entirely beneath the water's surface, do not transpire water but provide more biomass (increased vegetative growth and density) for the uptake and sorption of contaminants. Plant species that have a relatively high biomass generally improve the overall effectiveness of phytoremediation.

Table 2.3: Types of Plant, Contaminant and Media
Source: USEPA, 2008

Type of Contaminant	Medium	Type of Plant													
		Alfalfa	Alyssum	Bald cypress	Black locust	Cottonwood	Grasses	Hybrid poplars	Indian mustard	Pennycress	Red Mulberry	Stonewort	Sunflower	Water hyacinth	Willow
Organic	Soil			▲ PD RD			▲ RD	▲ PD RD			▲ RD	▲ PD			▲ PD RD
	Sediment			▲ PD RD			▲ RD	▲ PD RD			▲ RD	▲ PD			▲ PD RD
	Groundwater			▲ PD		▲ HC		▲ HC PD				▲ PD			▲ HC PD
Inorganic	Soil	▲ PV	▲ PE		▲ PV		▲ PS	▲ PE PS PV	▲ PE PS PV	▲ PE			▲ PE		
	Sediment	▲ PV	▲ PE		▲ PV		▲ PS	▲ PE PS PV	▲ PE PS PV	▲ PE			▲ PE		
	Groundwater					▲ HC		▲ HC	▲ RF				▲ RF	▲ RF	▲ HC

▲ Plant is effective for the type of contamination and medium shown.
HC Hydraulic control
PD Phytodegradation
PE Phytoextraction
PS Phytostabilization
PV Phytovolatilization
RD Rhizodegradation
RF Rhizofiltration

Table 2.4: Phyto-extraction Potential of Different Species in a Field Experiment in Belgium

Source: USEPA, Biportal, 2008.

Species	Cd ($mg kg^{-1}$ DW)	BCF	Biomass ($t ha^{-1}$)	Cd removal ($kg ha^{-1} year^{-1}$)	Cleanup time (year)
Maize	3	0.6	20	0.06	188
Rapeseed	6	1.2	8	0.05	234
Sunflower	12	2.4	10	0.1	117
Tobacco	24	4.8	8	0.19	58
Poplar – twigs	11	2.2	8	0.09	255
Poplar – leaves	28	5.6	2.4	0.07	
Poplar twigs + leaves				0.16	144
Willow – twigs	24	4.8	8	0.19	117
Willow – leaves	60	12	2.4	0.14	
Willow twigs + leaves				0.34	67

Given are Cd contents in aerial part ($mg kg^{-1}$ DW), bioconcentration factor (BCF), biomass production ($t ha^{-1}$), Cd removal ($kg ha^{-1} year^{-1}$) and predicted cleanup time supposing a linear extraction

[a] Calculation based on 25 cm soil depth for agricultural crops; 50 cm for willow and poplar and linear extrapolation

2.6.7 Practical Considerations and Limitations

As is true of any clean-up alternative, phytoremediation offers a number of advantages, as described in the preceding section. However, it also has technical limitations related to the types and levels of contaminants present, soil properties, acceptable exposure risks, and other site specific considerations. Discussed in this section are a number of factors that decision makers may find necessary to consider when evaluating phytoremediation as a clean-up option for their site.

The total length of time required to clean up a site through phytoremediation may be too long to be acceptable for some redevelopment objectives. Phytoremediation is limited by the natural growth rate of plants and the length of the growing season.

Several growing seasons may be required before phytoremediation systems become effective, while traditional methods may require a few weeks to a few months. Therefore, low removal rates may prohibit the use of phytoremediation in cases in which the time period available for clean-up is limited and is a key criterion in selecting a technology.

The growth rate of a plant species will have a direct effect on the potential for use at a particular site. For example, fast-growing grasses will begin treating soil contamination more quickly than a tree, which must establish deeper roots to treat target contaminants. As plants, particularly trees used in phytoremediation, mature their root structures deepen and their capacity to treat deeper levels of contamination improves. Phytoremediation can provide a number of benefits during the course of vegetation maturation. Plantings during initial stages can provide a cover that minimizes water infiltration. As the tree roots mature, phytodegradation, rhizodegradation, and/or phytovolatilization processes can take place to treat contaminants at increasing depths below the surface. In fully mature stages, phytoremediation cover can develop a hydraulic control, hydrostatic barrier function.

Figure 2.3, below illustrates the progressive development stages for phytoremediation to support capping to reduce infiltration, degradation, and then hydraulic control.

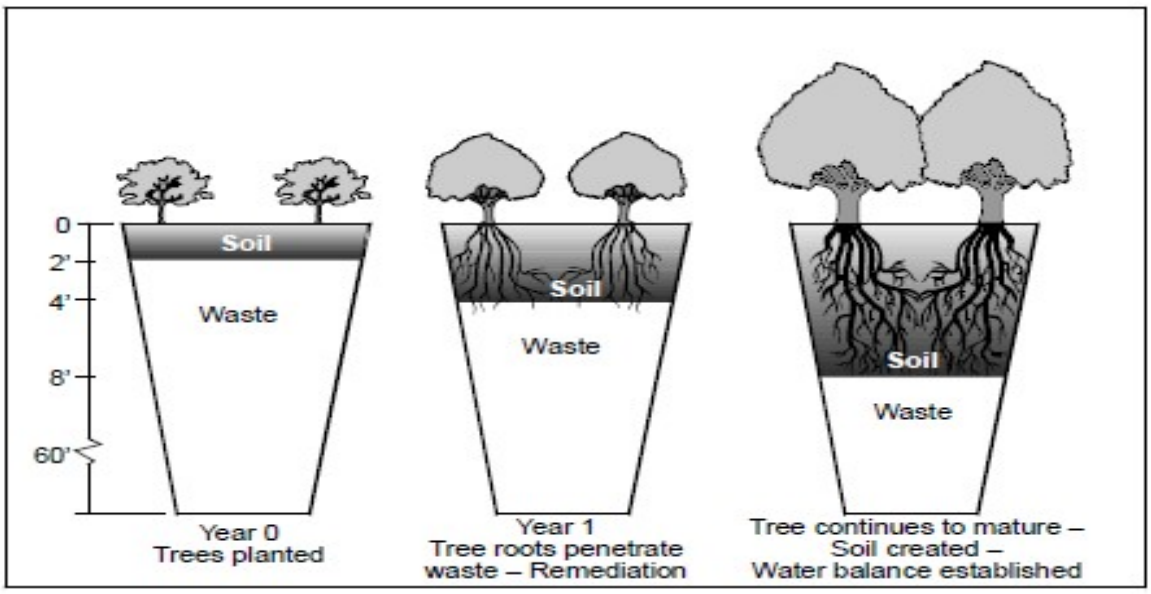

Figure 2.3: Phytoremediation Developmental Stages

Source: EPA 2000 Introduction to phytoremediation – National Risk Management Research Laboratories; Brownfields technology primer selecting and using phytoremediation for site

While this developmental process can be beneficial, consideration also must be given to whether phytoremediation is a safe and protective remedy during the time it takes for the plants to establish themselves to a point at which they are treating the contaminants effectively.

It must be determined whether phytoremediation can be effective for the site-specific conditions and contaminants.

For example, phytoremediation works better in shallow soils and groundwater, unless deep-rooted plants are suitable for the site. In addition, phytoremediation works best on certain types of contaminants or mixed waste and may be less effective when used on other combinations of waste. For example, phytoremediation may not be the most effective treatment option if levels of contamination are so high that concentrations of contaminants are toxic to plants (phytotoxic).

In some cases, phytoremediation might not provide adequate protection, from an eco-receptor perspective. For example, contamination that is below ground can be transferred into the leaves and stems of plants that are a food source. Further, in some cases, contaminants are not destroyed in the phytoremediation process; instead, they are transferred from the soil onto the plants and then are transpired in to the air. Phytoremediation could also increase the rates of bio-accummulation of contaminants than might otherwise occur. Potential costs associated with monitoring and maintaining the phytoremediation process at the site also must be factored into the selection process. Maintenance costs often are lower with phytoremediation than with conventional treatment technologies.

On the other hand, monitoring costs could be higher, especially if the clean-up rates are slower and monitoring of the site continues longer than monitoring for conventional treatment technologies. An activity that will increase the cost of long-term maintenance is the harvesting and proper disposal of plant materials that contain contaminants.

The state of phytoremediation technology is emerging, and more information from treatability studies and long-term applications are needed to support its consideration as a viable technology. Until that information is available, the diversity of opinions about the conditions and contaminants for which phytoremediation may be a well-suited clean-up technology will continue.

Consulting with technical experts to determine the applicability of phytoremediation on a site-by-site basis is advised. Further, in many cases, it will be important to identify a contingency plan for cleaning up the site in the event that phytoremediation will not meet clean-up objectives in an effective and timely manner.

2.6.8 Selection and Design of a Phytoremediation System

The design of a phytoremediation system varies according to the contaminants, the conditions at the site, the level of clean-up required, and the plants used. As previously noted, contaminants and site conditions are perhaps the most important factors in the design and success of a phytoremediation system. Other factors that influence the selection and design of a phytoremediation system are discussed below.

2.6.8.1 Technical Factors

Because phytoremediation is an agronomic process, it is highly dependent on climate and site-specific characteristics. Soil properties determine the ability of a plant species not only to become established in the soil, but also to maximize biomass and, therefore, removal of contaminants. Soil parameters typically analysed to determine whether phytoremediation is applicable include texture; pH; moisture content; organic matter content; lime content; cation exchange capacity; and content of nutrients, such as calcium, magnesium, potassium, phosphate, and sulphate. As with most treatment technologies, innovative or not, a treatability study should be conducted before a final remediation technology can be selected for use at a site to demonstrate that the technology will work at that specific site. Information to assess the effectiveness of phytoremediation also may be available in existing literature. For example, research may reveal phytotoxicity levels or regional agronomic practices for the simple application of phytoremediation, given adequate site characterization and monitoring.

Where treatability studies are necessary, site characterization and bench-scale tests may be used to determine system performance in the field and evaluate whether the design will meet the desired level of clean-up in the specified time period. For phytoremediation, it may be necessary to conduct treatability studies under laboratory conditions (for example, in an artificial hydroponic system) to simulate site conditions and obtain an initial result that proves the effectiveness of the design.

Acceleration of the process can be expedited by typical approaches, including artificial light, water, and temperature conditions. The advantage of such laboratory studies is that the process can be accelerated to provide early results and reduce implementation time. Local climatic conditions, particularly the length of the growing season, govern the type and number of crops that can be planted each year and therefore the annual rate of removal of contaminants. Climatic conditions, such as rainfall and temperatures, also influence irrigation strategies and the selection of plant species. Plant species that grow well in the Pacific Northwest may not survive in the arid Southwest.

Hydrologic models allow the calculation of the flow of water and how that flow might be affected by the application of phytoremediation. Irrigation flows can have an impact on groundwater conditions and ultimately on the movement of the contaminants to be treated. Although irrigation of plants may be necessary to ensure a robust start for a phytoremediation system, even in drought conditions, careful modelling may be necessary to predict with any certainty the effects of phytoremediation at a site.

Agronomic techniques include the addition of nutrients necessary for vigorous growth in vegetation. To maximize the efficiency of the phytoremediation treatment system, the soil type first must be determined. Analysis will help determine the need for amendments, such as nitrogen, potassium, phosphorous, manure, sewage sludge compost, straw, or mulch, which are added as required to improve the performance of the plant. For example, maintenance of the phytoremediation system may require the addition of chemicals to stabilize metals in the soil or the addition of chelates to ensure that plants take up the contaminants. A close working relationship with regulators is especially important in such a situation to quickly determine any rules, regulations, or prohibitions related to the addition of amendments to the subsurface. Any changes made in the soil through the application of soil amendments, however, should be evaluated and monitored for their effects on the site conditions.

Biomass is the amount of living or organic matter produced by plants. Increased biomass results in higher levels of treatment and containment because more materials available to the plant (including contaminants) are used to support growth. Phytoremediation designs commonly involve higher planting densities than standard agronomic rates for various species to overcome decreased germination because of contaminated soils and to maximize overall production of biomass for the area. Consulting with an experienced agronomist is essential to designing a healthy and productive phytoremediation system.

Because various plant species have different root structures, careful consideration must be given to selecting the most appropriate species to address contaminants at individual sites.

Figure 2.4 below illustrates typical root depths of four plants commonly used in phytoremediation and demonstrates the depth to which each of the species may be most effective. The figure also shows the potential limitation of phytoremediation to shallow soils.

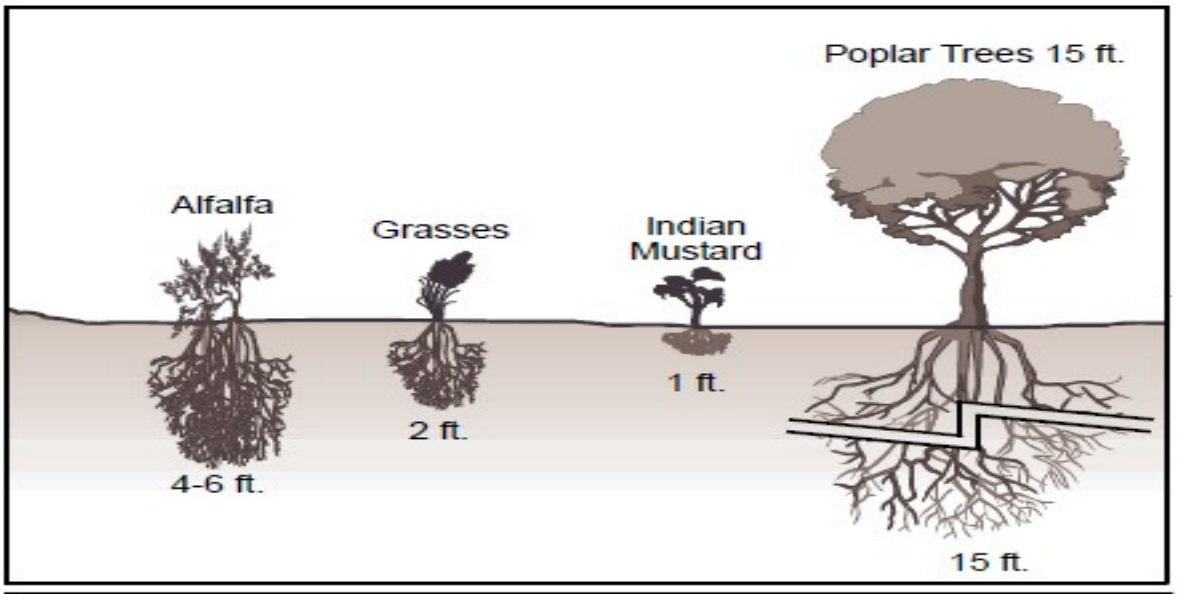

Figure 2.4: Plant Species and Root Depth Illustrations

Source: EPA, 2000; Brownfield technology primer selecting and using phytoremediation for site – National Risk Management Research Laboratory

2.6.9 Strategies for Contaminant Control

Phytoremediation can support a variety of clean-up strategies. One such strategy is to plant the contaminated area with a specific species known to extract the targeted contaminant, subsequently harvest the resulting biomass, and then reduce the harvested material by composting or burning.

The resulting pile then becomes a concentration of the extracted chemical that can be treated as hazardous waste or, if the contaminant is a metal, recycled. Another strategy, which focuses on containment, is to surround an underground plume of contaminants with a selected species of plants to prevent further movement of the plume through the establishment of a hydrostatic barrier of tree roots, that is, the groundwater is taken up by the tree roots and therefore does not migrate beyond the roots.

Hybrid poplars have achieved successes in such approaches. A common interim approach for brownfields sites has been capping or paving over a site to minimize infiltration of water. Several experiments have been conducted to create “phytocaps” as improvements of asphalt coverings. A phytocap is a combination of trees and other vegetation capable of absorbing and transpiring most of the infiltration water, thereby reducing the risk that contaminants will spread. A phytocap must be planted densely so that the rate at which the evaporative processes of the plants take place matches the rate of infiltration of water. The approach thereby eliminates the need to construct an impermeable surface.

Treatment or capture of contaminated groundwater under a site may require a certain minimum surface area and configuration of trees, depending on groundwater flow rates and considerations

related to the contaminant. Surface water buffers and corridors, groundwater interceptor strips, and vegetative covers are examples of applications of phytoremediation that can be integrated into redevelopment landscaping plans on both large and small sites.

2.6.10 Innovative Technology Treatment Trains

Phytoremediation can be an effective component of treatment train approaches that combine innovative technologies with traditional remediation technologies. The purpose of combining technologies can be to reduce the volume of material that requires further treatment, to prevent emission of volatile contaminants during excavation and mixing, or to treat several contaminants in a single medium.

An example might be to use phytoremediation as part of a treatment train involving soil vapor extraction and/or air sparging. If the volatized compounds are passed through a properly designed plant rhizoshere zone before being extracted or discharged to the atmosphere, there can be enhanced degradation of hazardous compounds.

Hybrid poplars or other deep-rooted species with high groundwater uptake rates could serve in a treatment wall capacity when installed in a way that intercepts migrating contaminated groundwater plumes. The groundwater that flows through the plant treatment wall would in many cases become adequately treated such that organisational (Michelin North America, NMA) requirements could be implemented as the final stage of the train. In shallow aquifer situations phytoremediation could replace more costly and intensive technologies such as pumping and treating.

Anaerobic, reducing conditions are required for effective degradation of chlorinated solvents and other organic compounds. A process whereby chemicals secreted from tree roots lead to

anaerobic degradation of chlorinated solvents currently is receiving research attention. This research has examined the process in naturally occurring trees, therefore in the context of MNA.

2.6.11 Design Team

It is important that the development and evaluation of a particular phytoremediation design and long-term performance strategy at a brownfields site be performed by an experienced multidisciplinary team. The design team can help the decision makers weigh the advantages and limitations of phytoremediation and select and design a system that best addresses the factors discussed in this section. The team might include experts in the following disciplines as shown in figure 2.5 below.

Figure 2.5: Design Team Disciplines for Remediation Project

Source: USEPA – Brownfield technology primer - phytoremediation

2.6.12 Phytoremediation Operation, Maintenance and Disposal

A phytoremediation treatment system must be monitored and evaluated periodically to measure the effectiveness of operations and progress toward attainment of the remedial objectives for brownfields redevelopment.

Monitoring can help determine the most effective course for continued operation and maintenance. This section describes responsibilities for operation and maintenance at a phytoremediation site.

2.6.13 Operation and Maintenance

Maintenance is required to obtain a healthy stand (or growth of plants). Weed control and irrigation probably are the two most important practices. Because of the proliferation of specific weeds, predators, and diseases that can cause significant reductions in yields, it may be necessary to rotate crops to maintain increased biomass production. Weeds also can be controlled by employing mechanical (cultivation) or chemical (herbicides) methods. Irrigation water should compensate for normal losses to evaporation and transpiration. The method of irrigation also must be considered carefully. Drip irrigation tends to minimize evaporation of water, improve efficiency, and reduce costs. The long-term maintenance needs of wetland systems typically are minimal and may consist of monitoring the distribution and level of water, removing vegetation and contaminants, and other predominantly land-management activities, such as control of access and maintenance of berms.

2.6.14 Disposal

In phytoextraction systems, plant material must be harvested and disposed of. Plants that accumulate contaminants may pose a risk of spreading contamination into the food chain if they are consumed by insects or other animals.

Consideration should be given to addressing the need to avoid consumption of contaminated plants by wildlife or livestock before plants are harvested. At brownfields sites, the end uses under a redevelopment plan can be a determining factor in the potential risk to human and

environmental receptors that accumulated contaminants may pose. The brownfields site redevelopment plan therefore can affect the need for disposal.

It is important to monitor the system and test whether the plants contain any hazardous substances. If there are no hazardous substances present, the material could be composted or worked into the soil on site. If that is not possible, off-site disposal will be required. The harvest of contaminated biomass and possible disposal of the material as hazardous waste would be subject to applicable regulations, such as those established under the Resource Conservation and Recovery Act (RCRA).

One option is disposal of contaminated material in a regulated landfill. Disposal under RCRA can add costs to a phytoremediation project. However, the removal and disposal of plant material used in phytoremediation generally involves the transporting and handling of materials that are of far less volume and that probably are less hazardous than materials generated by operations that involve soil excavation or other innovative or traditional remediation technologies. Therefore, phytoremediation can be a strategy for decreasing the costs of handling, processing, and possibly landfilling the materials.

2.6.15 Performance Evaluation and Monitoring

To evaluate the short-term performance and effectiveness of phytoremediation, the concentrations of contaminants and degradation products should be measured. Monitoring should be conducted for soil, groundwater, plant root and mass, and evapotranspiration vapour. Rigorous performance evaluation will help demonstrate the system's ability to meet clean-up goals and objectives. Because phytoremediation is an emerging technology, standard performance criteria for phytoremediation systems have not yet been established, and performance must be determined on a site-by- site basis.

Long-term monitoring typically is necessary for phytoremediation systems that require long time horizons to demonstrate their continued effectiveness. Monitoring may be continued after short-term clean-up goals have been met to determine the impact of the phytoremediation system on the ecosystem.

A monitoring plan should be developed to guide both short- and long-term monitoring. The plan should discuss the following elements: constituents or other parameters to be monitored; the frequency and duration of monitoring; monitoring and sampling methods; analytical methods; monitoring locations; and quality assurance and quality control (QA/QC) requirements.

2.6.16 Cost of Phytoremediation

Phytoremediation is an emerging technology; standard cost information still is being developed on the basis of experiences in implementing phytoremediation projects.

This section provides information that compares costs associated with the use of phytoremediation to costs associated with the use of conventional treatment technologies based on actual cost estimates for three sites, as well as other sample costs based on laboratory and pilot scale work and field information.

Many of those costs associated with phytoremediation are not unique to phytoremediation, but are common to remediation technologies.

The major cost components for the implementation of phytoremediation include the costs of:

* Site characterization

* Treatability studies

* Full-scale design (costs will vary according to the contaminants, the site characteristics, and the variety and amount of vegetation needed)

* Construction costs (includes direct capital costs for site preparation, plant material, and irrigation and monitoring equipment and indirect costs, such as those for permitting during construction, contingency design, and start-up)

* Operation and maintenance and monitoring costs (includes the cost of labour, materials, chemicals, utilities, laboratory analysis, disposal, and monitoring)

As discussed in other sections of this primer, start-up and maintenance costs often are less with phytoremediation than with conventional treatment technologies because:

(1) Phytoremediation is a natural process using solar energy;

(2) Phytoremediation is in situ and requires no digging or hauling of contaminated soil; and

(3) Little or no mechanical equipment is required to operate the phytoremediation process.

On the other hand, monitoring costs could be higher than with conventional treatment technologies because monitoring typically is required for a longer period of time at sites where phytoremediation is used.

In comparing the potential costs to use phytoremediation with the potential cost to use conventional treatment technologies at a site, care must be taken to compare the costs of the entire system for the entire life cycle.

Under phyto-extraction, the cost of processing and ultimate disposal of biomass generated is likely to account for a major percentage of overall costs.

2.6.17 Cost Savings Based on Actual Cost Estimates

Table 2.4 provides site-specific estimates that have been reported of the cost savings realized by using phytoremediation rather than conventional treatment technologies

Table 2.4: Sample Phytoremediation Costs

Source: Introduction to Phytoremediation. EPA/600/R-99/107. February 2000

Contaminant and Matrix	Phytoremediation		Conventional Treatment		Projected Savings
	Application	Estimated Cost	Application	Estimated Cost	
Lead in soil (1 acre)[a]	Extraction, harvest, and disposal	$150,000 -$250,000	Excavate and landfill	$500,000	50-65 percent
Solvents in groundwater (2.5 acres)[b]	Degradation and hydraulic control	$200,000 for installation and initial maintenance	Pump and treat	$700,000 annual operating cost	50 percent cost saving by third year
Total petroleum hydrocarbons in soil (1 acre)[c]	In-situ degradation	$50,000 - $100,000	Excavate and landfill or incinerate	$500,000	80 percent

The estimated 30-year costs (1998 dollars) for remediating a 12-acre lead site were $12,000,000 for excavation and disposal, $6,300,000 for soil washing, $600,000 for a soil cap, and $200,000 for phytoextraction.

The costs of clean-up of various heavy metals at the Twin Cities Army Ammunition Plant, Minneapolis-St. Paul, MN Project were reported in the Federal Remediation Technologies Roundtable to be $153 per cubic yard of soil over the life of the project.

The costs of removing radionuclides from water with sunflowers has been estimated to be $2 to $6 per thousand gallons of water. The costs of clean-up of explosives at the Milan Army

Ammunition Plant, Milan, TN were reported in the Federal Remediation Technologies Roundtable to be $1.78 per thousand gallons of water over the life of the project.

Estimated costs for hydraulic control of an unspecified contaminant in a 20-foot-deep aquifer at a 1-acre site were $660,000 for conventional pump-and-treat and $250,000 for phytoremediation.

Cost estimates indicate savings for an evapotranspiration cover compared to a traditional cover design to be 20-50%, depending on availability of suitable soil. Studies indicate that phytoremediation is competitive with other treatment alternatives, as costs are approximately 50 to 80 percent of the costs associated with physical, chemical, or thermal techniques at applicable sites. Figures 2.6, 2.7 and 2.8 show the phytoremediation decision tree for soil, sediments and groundwater.

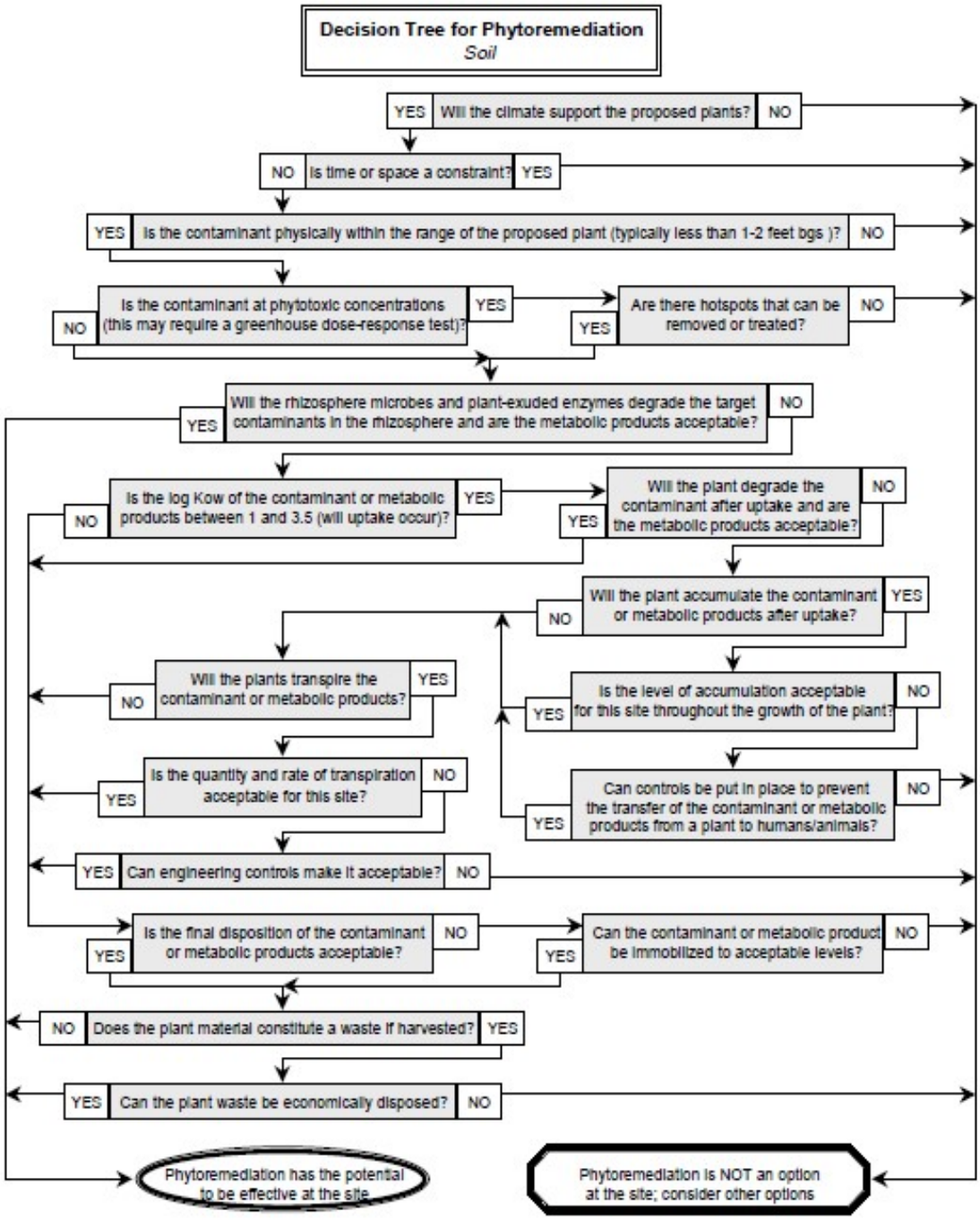

Figure 2.6: Decision Tree for Phytoremediation Soil

Source: Decision Tree Document – The Interstate Technology and Regulatory Cooperation

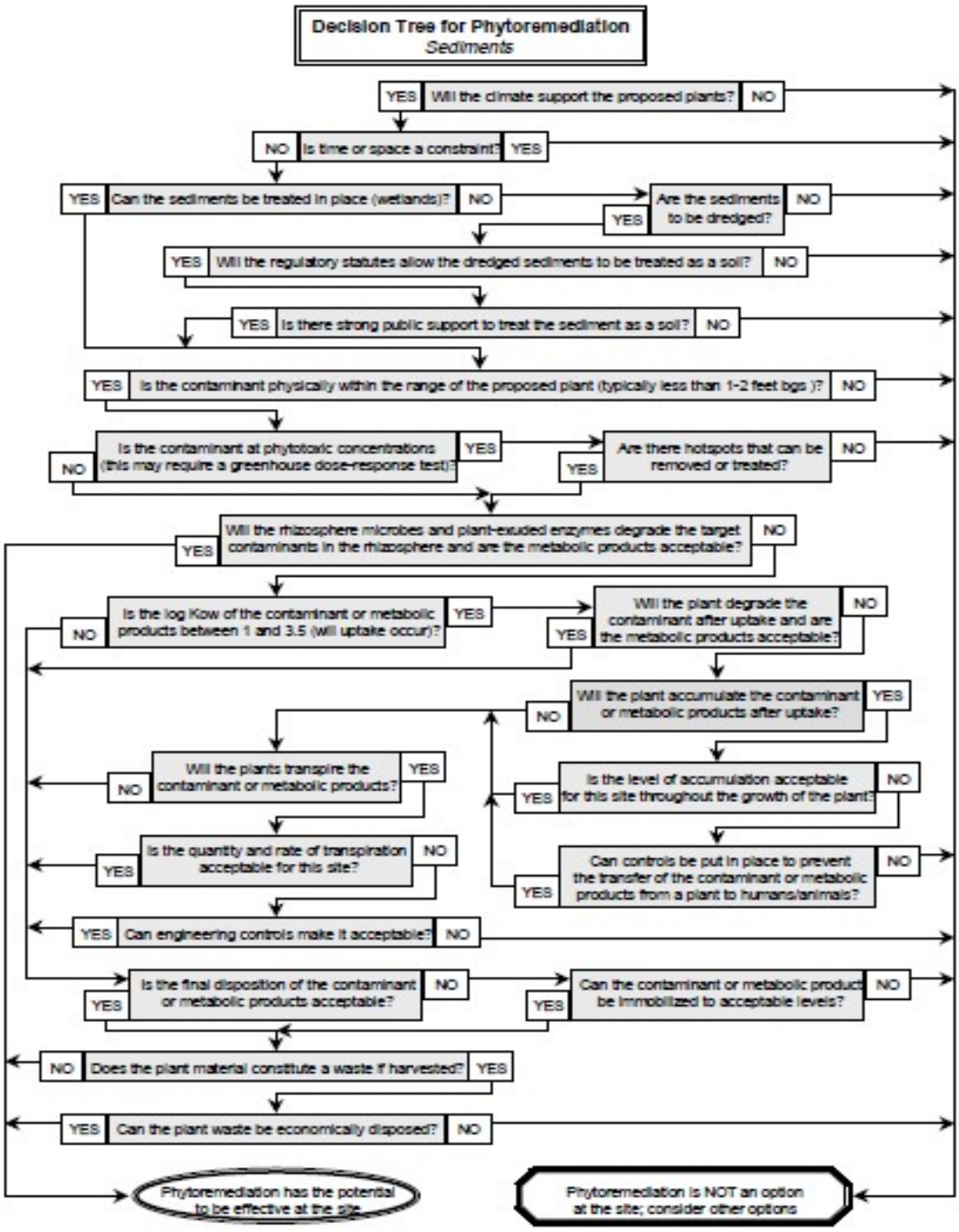

Figure 2.7: Decision Tree for Phytoremediation Sediments

Source: Decision Tree Document – The Interstate Technology and Regulatory Cooperation

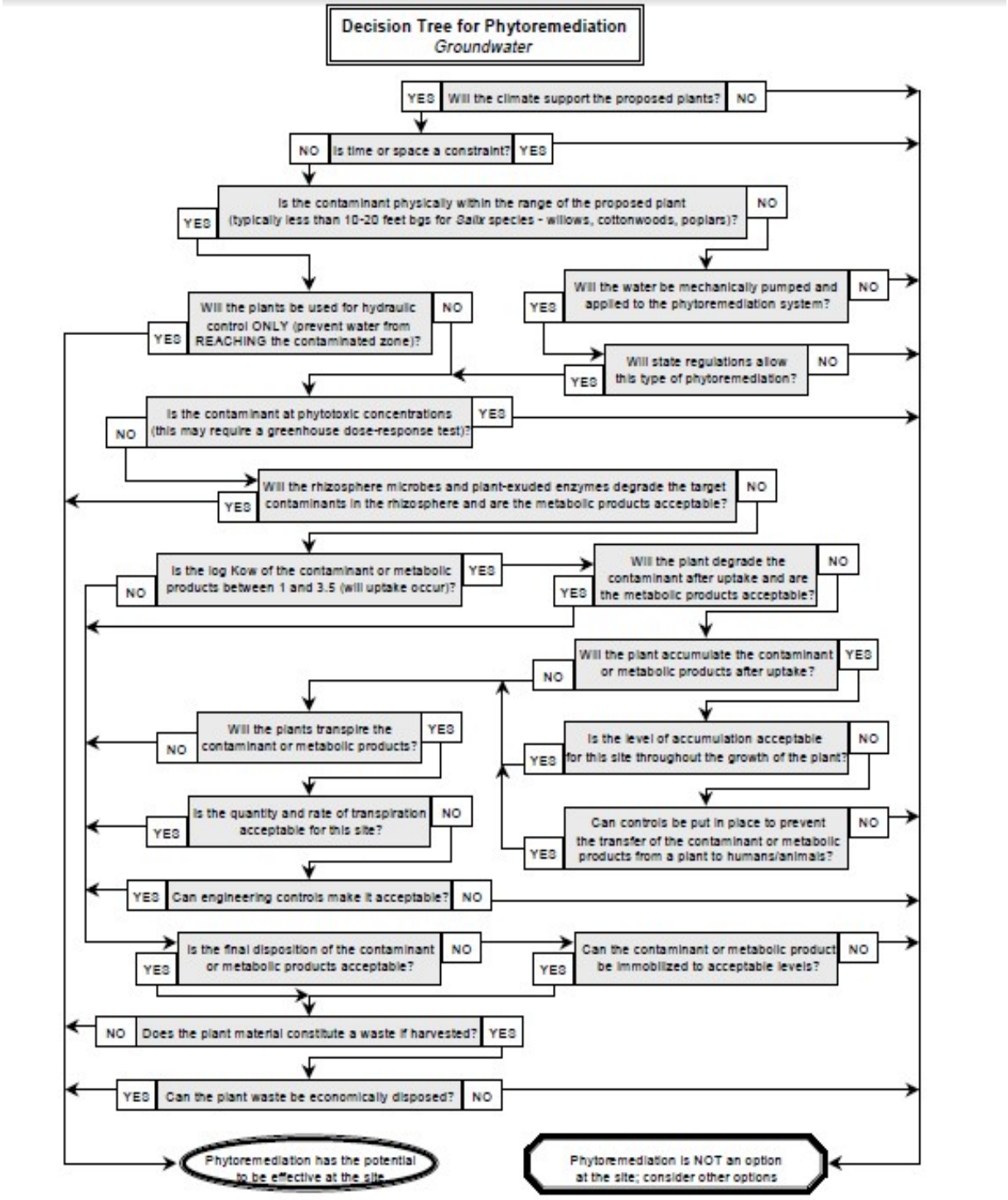

Figure 2.8: Decision Tree for Phytoremediation Groundwater

Source: Decision Tree Document – The Interstate Technology and Regulatory Cooperation

2.7 Review of Previous Works

Emon (2008) carried out research work on phytoremediation of oil contaminated desert soil using the rhizosphere effect. In his work, four plots of $2x2m^2$ each were delimited in an area with no history of pollution, 0 - 50cm depth was ploughed on each plot. Ploughed soils were mixed with wreathed crude oil so as to give initial concentration of 2.2-2.3 wt% soil. Each plot received the suitable nitrogen and phosphorus concentration. Plot 1 was seeded with 100 viable Faba seed at the beginning of January. Plot 2 was seeded with 100 viable grain Zea mays at the beginning of May. Plot 3 was seeded with 200 viable triticumaestivuml at the beginning of November while plot 4 was without seeding. After 60 days growth period of each plant, samples were taken from the rhizosphere and non-rhizosphere soil of each plant; also at the beginning of the experiment samples were collected. Residual oil and its fraction were determined by adding 10g of anhydrous sodium sulphates to 10g of air-dried soil samples. The hydrocarbons were extracted using a Soxhlet extractor and the extract was evaporated in a pre-weighed dish. The amount of the total petroleum hydrocarbons (TPHs) was determined and the loss (%) of TPH was calculated. Extracted residual oil was suspended in n-hexane and filtered through tared filter paper to remove and determine the insoluble fraction (asphaltenes).Hexane soluble fraction was fractionated by solid-liquid chromatography to have saturates, aromatic and resins. The result of the experiment is given below in Table 2.5, 2.6, and 2.7.

Table 2.5: Biodegradation of oil and its fraction in the rhizosphere of Vicafaba (RV) plant as compared to the non-rhizosphere soil (S) after 60 days growth period.

Source: Nature and Science Publication. 2010.

60 Days Growth Period					
	0 – time mg	S mg		RV mg	
Fractions	100g soil	100g soil	Loss %	100g soil	Loss %
saturates	800+ 8.0	634.+4.9	20.7	423.7+5.5	47.0
Aromatics	1080+ 20.0	1005.7+21.0	6.9	797.3+6.4	26.2
Resins	190.0+20.0	192.4+1.7	_	180.0+1.3	5.6
Asphaltenes	180.0+2.0	181.4+3.6	_	176.6+4.1	1.9
Total	2230.0+100	2013.6+19.3	10.3	1571.6+13.3	30.2

Table 2.6: Biodegradation of Oil and Its Fraction in the Rhizosphere of Zea Mays (RZ) as Compared to the Non-Rhizosphere Soil (S), After a 60 Days Growth Period.

Source: Nature and Science Publication. 2010.

60 Days Growth Period					
	0 – time mg	S mg		RV mg	
Fractions	100g soil	100g soil	Loss %	100g soil	Loss %
Saturates	840.0± 100	652.6±25	22.3	525.5±11.3	37.4
Aromatics	1010±40.0	995.9±3.4	6.9	982.0±2.6	8.2
Resins	180.0± 10.0	184.8± 2.7	_	181.3± 2.9	_
Asp halters	240.0±3.0	245.1± 4.3	_	250.7± 3.8	_
Total	2330.0	2078.3±16	10.8	1939.6± 15.2	16.8

From these results TPH was reduced by 30% in the rhizosphere soil of Viciafaba plant and by 16.8% and 13.7%. In Zea mays and triticum aestivuml respectively. TPH biodegration was enhanced in the rhizosphere soil of the legume plant (viciafaba) then other two monocotyledon plant.

Freshthe et al (2014) carried out experiment to investigate the quantitative uptake of phenanthrene's (as one of most important PAHs in crude oil) by salicomiaeurapea. In their research work salicomia plantlets were taken from Eshtehard plant which is located in Karaj, at Borz, Iran. They were transplanted and exposed to various concentration of crude oil (4.5-16-27.5-32.5 g/kg soil). Spectrophotometer and gas chromatography - mass spectroscopy methods were used to determine and identify, the phenanthrene uptake, in roots and stalks, also the plants

appearance were checked out. From their observation the highest uptake was at 8mg crude oil per 1g of soil.

Olujuyigbe & Aruwajoye (2014) carried out a rhizoremediation study on hydrocarbon contaminated soil with paspalumvaginatum, a stoloniferous, perennial grass of the family poaceae found mainly in the subtropics and tropical regions of the world. The contaminated soil analyses indicated a decrease in the level of hydrocarbons present after phytoremediation. There was equally, a significant reduction in growth parameters of the plant such as plant height, leaf number, tiller number and total dry weight, compared to the control. Anatomical studies of sections of the plants, stems did not reveal the presence of accumulated oil within the tissues but rather denatured internal parenchyma cells traction ways observed. Bacteria capable of degrading hydrocarbon were isolated from the rhizosphere of the grass. The isolates include *Arthrobactersp.,Bacilluspumilus, Bacillus sphaericus* and *Serratiamarcescens*. Growth in mineral salts medium supplemented with 0.5% crude oil for 21 days resulted in 95.9%, 95.6%, 98.3% and 96.7% degradation of oil for *Arthrobaiter sp., B. pumolus,S. Macescens* and *B.sphaericus* respectively. A soil microcosm set up with the consortium of the isolates resulted87.70% degradation of crude oil in 45 days.

Elena and John (2004) determined the effect of different levels of diesel oil contamination (25ml (T_1), 50ml (T_2), 75ml (T_3) and 100ml (T_4) of diesel oil per kg of soil) on seedling of khayasenegalensis and Terminalia superb. For 12 weeks, the growth performance (number of leaves, seedling collar diameter and height) and biomass accumulated by roots, stem and leaves of seedlings in each treatment were measured, fortnightly. At the end of their study, heavy metal analysis was done to determine the concentration of Lead (Pb) and Nickel (Ni) in the above and below ground parts of seedlings in each treatment. Data were analyzed using descriptive

statistics and anova at $p<0.05$. The diesel oil contamination had no significant effect on leaf production, collar diameter, height or biomass accumulated by both hardwood species. The K. senegalensis seedlings in the T1 (29.47 ± 13.69g) had the highest biomass while T3 (16.33 ± 0.14g) had highest for *T. superba* accumulated more heavy metals (Ni :5.62 – 7.52ppm; Pb :12.63 – 17.82ppm) than *K. senegalensis* (Ni :4.71 – 6.34ppm; Pb : 11.24 – 14.26ppm) with roots retaining more than 50% of each metal in most of the treatments. The tolerance of diesel oil contamination by the two hardwood species indicates their potential for phyto-extraction of heavy metals from hydrocarbon polluted area in the tropics. Thus, phytoremediation of diesel oil contaminated soils by tree species could be a cheap, effective and sustainable means of rehabilitating ecosystems in the tropics.

Ezonu (2013) studied the decomposition of used motor oil in soil as influenced by plant treatment. In their work soil was contaminated with used motor oil to a concentration of 1.5% w/w. The contaminated soil was seeded with soyabean (*Glycine max*) and green bean (*Phaseolas vulgaris*); sunflower (*Helianthus annus*) and Indian mustard (*Brassica juncea*); mixed grasses and maize (*zea mays*); and mixed clover (red clover, *Trifoliumpratense* and *ladino clover*, *Trifoliumrepens*) and incubated Soxhlet-extractable oil and grease remaining in the soil was monitored after 100 and 150 days. After 150days in the clover treatment, the added oil was no longer detected. A total of 67% of the oil was removed in sun-flower/mustard, and with addition of NPK fertilizer, the oil was completely removed. The grass/maize treatment resulted in a 38% oil reduction, which increased to 67% with fertilizer application. The control treatment reduced oil in the soil by 82% when fertilizer was added. At 150 days the sunflower/mustard and wheat/oats treatments produced the greatest biomass in the presence of used oil. Gas chromatograph/Mass spectroscopy (GC/MS) spectra of oil/ grease extracts revealed the presence

of new peaks associated with hydrocarbon decomposition. The presence of new hydrocarbons was corroborated by changes in Fourier- transformed infrared spectrometry (FTIR) spectra. Fertilizer to treatments resulted to negligible changes to FTIR bands. Based on oil/grease residues and biomass results, the clover and sunflower/mustard are considered superior to the other plant treatments in terms of overall phytodegradation of used oil hydrocarbons.

Njoku et al., (2009) examined the rate of biodegradation of crude oil under natural environment. In their experiment a 24kg of soil was divided into 3 groups of 8kg each, consisting of soil samples contaminated with petrol hydrocarbon (PHC) in the concentration of 5%, 10% and 20% w/w. After a week of soil preparation, 6 viable Zea mays seeds were planted on each plot. Nurseries of the Zea mays seeds were also raised and used for transplanting. After 8 weeks of growth, bio-accumulation of crude oil in leaves of the Zea mays as well as well as the quantity of crude oil remaining in the contaminated soil was determined.

Table 2.7: Crude Oil Bioaccumulation in mg/g of Maize Biomass after 8 Weeks of Growth

Source: Nature and Science Publication. 2010.

Test	Group A: Crude oil Bioaccumulation (mg/g of maize)	Group B:crude oil Bioaccumulation (mglg of maize)	Group C:Crude oil Bioaccumulation (mg/g of maize)	Mean ±S.D	Percentage of crude oil Bioaccumulation in maize
5% (PNC) Contaminated soil	0.0023	0.0022	0.0023	0.0023±0.00005	0.81
10% (PNC) Contaminated soil	0.0013	0.0013	0.0023	0.0016± 0.00005	0.34
20%(PNC) contaminated soil	0.0018	0.0025	0.0023	0.0022+±0.0003	0.33

The concentration of crude oil was extracted using 10ml of 1:1 ethanol/chloroform mixture to extract the crude oil from 1g of each contaminated soil as well as Zea mays biomass cultivated on each contaminated soil, which was extracted after 8 weeks of growth as shown below in table 2.8. The result from the finding indicated that 5%, 10% and 20% (PNC) contaminated soil had 0.0023± 0.00005mg/g, respectively of crude oil bioaccumulation in Zea mays cultivated in them.

Table 2.8: Bacterial Counts for Polluted and Treated Soil Samples

Source: Nature and Science Publication. 2010.

Time (Days)	Polluted Soil cfu/g x 10^4	NPK treated Soil cfu/g x 10^4	Poultry Manure treated Soil cfu/g x 10^4	Goat Dung treated Soil cfu/g x 10^4
0	8.8	4.4	2.0	9.3
28	9.0	4.6	2.3	9.5
56	9.2	4.7	2.7	9.6
84	9.9	4.9	2.4	9.8
112	7.6	5.2	3.0	9.9

Biodegradation of crude oil polluted soil by co-composting with agricultural wastes and inorganic fertilizer was carried out by White et al., (1995). In their study, soil experimentally polluted with Bonny Light crude oil (Rivers State, Nigeria) and supplementation with organic and inorganic nutrients (poultry manure, goat dung, saw dust and NPK fertilizer). The efficacy of the treatments was monitored for 112days by the measurement of total hydrocarbon utilizing bacteria load and some physico-chemical parameters. The mean microbial counts of the polluted soil (control) sample, sample treated with NPK fertilizer, sample treated with poultry manure and

sample treated with goat dung for days 0, 28, 56, 84, and 112 respectively are shown in table 2.8. These were differences in physico-chemical analyses from the diverse samples. After statistical analyses ($p \leq 0.05$) there was a significant difference between the different treated samples from the control. The result suggests that nutrient supplementation would be effective in the remediation of crude oil polluted soils.

Ajoy et al., (2012) evaluated the use of phytoremediation to clean up soils contaminated with weathered crude oil. A Greenhouse study was conducted to evaluate the effect of N rates on the growth of three warm–season grasses and a warm–season legume. Alkane's Total Petroleum Hydrocarbon (TPH) and polycyclic Aromatic Hydrocarbon (PAH) degrades level in the crude–oil contaminated soil was significantly higher in rhizosphere soil as compared to bulk soil. Results from their study showed that TPH levels at time 6months were significantly lower in vegetated fertilized plots than in non–vegetated, non–fertilized plots. Vegetation establishment and fertilized addition result in increased bacterial, fungal and PAM degrade levels. Their studies demonstrated the importance of plants, agromic techniques, and their effect on rhizospheres micro–organisms in the clean-up of crude oil–contaminated sites.

Arezon & Salmah, (2015) carried out work on large scale bioremediation of petroleum hydrocarbon contaminated waste at Indian refineries. In their research work an Indigenous microbial consortium was developed by assemble of far species of bacteria, isolated from various oil contaminated sites of Indian, which could biodegrade different fractions of total petroleum hydrocarbon (TPH) of the oily waste to environment friendly end products. The said consortium was applied on field scale at different oil refineries in India and successfully bio–remediated 48,914 tons of different types of oily waste. In 44 field case studies of different batch size of ex

site bio–remediation process, the initial TPH content varying from 83.50 to 53130m1kg of only waste, has been biodegraded to less than 10gm/kg of only wastes in major cases in 2-12 months . In one refinery due to coastal climate, the bioremediation time was greater them 20 months. The bio remediated soil was non-toxic and natural vegetation was found to be grown on the same.

Olusola & Ejiro (2011) studied biodegradation of waste crude oil contaminated soil amended by Bacillus 139SI to determine the rate of hydrocarbon remediation. Previously, bacillus 139SI was isolated from an agricultural soil in the Sender Agricultural Center, Mulasis. Within 60days, 14%oil loss was recorded in un-amended soil more rapidly than in the soil amendment with both strain and organic waste recorded above 89%. Utilizing bacteria counts were significantly higher in all amended treatment compared to control soil. Dehydrogenate activity in soil was markedly enhanced by the application of amendment. Waste crude oil composition monitored by GC/FID indicated complete degradation of n-C_9-C_{25}.

Chaudry et al., (1998) evaluated the growth of Amaranthus hydrides on a crude oil–polluted soil bio-remediated with *Pleurotuspulmonarius* (a white root fungus) and *Glomusmosseae* (a mycorrhizeal fungus). Nine different treatments were used for three replicated in randomized block design at the same age of the seedlings. Duncan's multiple range tests were used to compare means of plant height, number of leaves and leaf area per plant at the different crude oil concentrations. Growth was superior in crude oil–pollution soil inoculated with mycorrhiza (Glomusmosseas) followed by mycelium of the mushroom in both sterilized and or sterilized soil, respectively. It was observed that mushroom mycelium compost at high to concentrate of crude oil grew better than at low crude oil concentration.

They defined phytoremediation as an innovative technology employed to reclaim soils, sediments and water that have been polluted by industrial contaminant. The techniques are emerging as an attractive clean–up method compared to the more traditional physic–chemical technologic, due to its simplicity, relatively low cost and in situ approach. Further, phytoremediation offer selective removal of contaminant, while also facility improvement of soil structure in case of serves erosion. In the work, a particular attention is given to phytoaccumulation, which involves the cultivation and harvesting of know contaminant hyper accumulator plant. While various hyper accumulators have been reported from Europe and the USA, knowledge of suitable plants from countries in south East Asia is limited. Hence, the finding of major studies that have been completed for certain tress–metal (Cd, Cu, Pb, and Zn) contaminated sites of New South Wales, Australia, have been included.

2.7.1 Phytodegradation of Polycyclic Aromatic Hydrocarbons (PAH)

Phytodegradation also known as phytotransformation is a type of in-situ phytoremediation in which plants are used to remove contaminants from substrate (Newman & Reynolds, 2004). Tropical plants have been reported to indicate effective degradation tendency due to inherent properties such as deep fibrous root system and tolerance to high hydrocarbon and low nutrient availability (Dzantor et al., 2000). Recently published data revealed that the tall fescue grass (*Festuca arundinacea*) and switch grass (*Pannicum virgatum*) could degrade pyrene to about 38% in soil stem in 190 days (Chen et al., 2003). In a related study, Cambell et al., (2002) noted that, using industrial hemp (*Cannabis Sativa*), levels of benzo (a) pyrene were reduced in soil planted with the plant in pots. Recent advances in phytoremediation studies have come to light in the application of phytotoxity assays of selected plants to PAH contaminated

soils. Three selected plants; alfalfa, (*Medicago Sativa*) rape seed oil (*Brassica Napus*) and perennial ryegrass (*Lolium Perenne*) were tested for their ability to germinate and grow in soil contaminated with PAH. To the extent that the plant absorb PAHs into tissues, transported and distributed among plant cells via tissues and their partitioning in roots, stalks and leaves cells. Primary processes responsible for PAH transfer and distribution in plant tissues are;

- Transfer between plant tissues and cells driven by transpiration and the PAH concentration gradient across plant cell components.
- Accumulation of PAHs in plant tissues, with the extent related to plant lipid content (Gao & Collins, 2009), Paterson & Makay, (1994). PAHs and their degradation products have frequently been detected within plant tissue, hence Wild et al., (2006) suggested that *Zea Mays* could metabolize phenanthrene into polar products, while Hams (1996) indicated that anthracene and its metabolites are bound to several cell-wall components, such as pectin, lignin, hemicellulose and cellulose respectively. Similarly, Wild et al., (2005) have published data suggesting that the distribution of anthracene and its metabolites in *Zea mays* occurs predominantly in the cell wall.

Water hyacinth (*Eichhornia crassipes*) applications in accumulation organics has been investigated by Xia (2008). Two phyto-processes were found to be most important in the remediation of hydrocarbons in contaminated water. These include;

(i) Uptake, accumulation and phytodegradation of the organics by the plant accounting for 50% removal of the compound and

(ii) Microbial degradation associated with the rhizosphere, contributing about 7% removal of the substance. The study further concluded that water hyacinth is a good candidate for development as a phytoremediation system for hydrocarbon polluted water. The plant has been reported to accumulate high levels of five and above rings of PAHs as opposed to two and three rings PAHs compounds (Moutafa & Shara, 2009). As previously observed, plants system appear to adsorp hydrocarbons and further metabolize these compounds to other products.

2.7.2 Degradation of PAH Using Solar Ultraviolet Radiation

Solar ultraviolet radiation has been shown to degrade and alter the quantity of natural organic matter as well as organic pollutants. Recent study by Bertilsson & Widerfalk (2002) had noted that photochemical degradation of anthracene and phenanthrene was possible under the influence of humic acid addition. However, the study did not observe appreciable photodegradation of naphthalene. Hence, photo-degradation was shown not to be a most likely removal mechanism for naphthalene. Different parameters such as temperature, soil particle size, soil depth, and humic acid concentration influence the rate of photo-degradation of PAHs (Zhang et al., 2010). However direct photolysis of PAHs has also been observed under simulated solar radiation, with pyrene degrading at a faster rate than anthracene and naphthalene (Javobs et al., 2008). Ultrasound frequencies of 24 and 80 KHz generate complete degradation of PAHs under operating conditions of temperature ($40^{o}C$), and applied voltage-ampere or electrical power of 150Watts (Psillakis et al., 2004). Wheat & Tumeo (1997) studied sonochemical degradation of phenanthrene and biphenyl in aerated aqueous solutions in the presence of Fe^{3+} ions, while in further studies, Wang et al., (2003) and

Manariotis et al., (2011) investigated the sonochemical degradation of PAHs of high frequency ultrasound. These authors published data revealing the fact that the presence of chlorinated solvent during sonication results in the formation of solvent radicals, which react with PAHs leading to the build-up of chloro-PAHs by-products. Godon & Cain (2003) extensively documented the use of titanium film annular photocatalytic reactor as a novel approach in degrading PAHs in dilute water streams. Nevertheless, in their contribution, Aishawabkeh & Sarahney (2005) examined the effect of current density on enhanced transformation of naphthalene and concluded that almost 88% of $13mgL^{-1}$ naphthalene was degraded after 8 hours treatment under 18.2 $mAL^{-1.}$ However, increasing the naphthalene concentration to 25mgL-1 produced similar degradation effects.

2.7.3 Conventional Clean – Up Methods for Petroleum Polluted Soil.

Several other technological methods are available for clean-up of hydrocarbon polluted soil. Much of these technologies are classified as either in situ or ex situ. Treatment methods for polluted soil includes, initial (primary & secondary) oil recovery process, landfill, incineration, solidification, thermal desorption, leaching, bio-augmentation, bio-filters, bioreactors, bio-stimulation, bio-venting, composting and land-farming, acid extraction, adsorption, etc.

Primary and Secondary Oil Response Method

One tool used occasionally in oil spill response as a secondary and alternative method is the category of chemical dispersants. Under strict approvals and a narrow set of conditions, dispersants can be sprayed aerially over oil spilled waters in Alaskan marine sites. Chemical dispersants break a slick into smaller droplets, promoting mixture of oil into the water column, and accelerating dilution and degradation.

Dispersants help prevent formation of water-oil emulsions, or "mousse," and they speed up biological breakdown of oil by natural marine organisms. They also reduce the adhesion of oil to sediments and other organisms in the water.

State law requires all companies which handle and ship large amounts of oil to write an oil spill prevention and contingency plan. In order for dispersants to be considered an option in spill response, those companies must make many preparations through their contingency plan, including the following:

1) Mechanisms to assess environmental consequences and provide continuous monitoring of its environmental effects;
2) An inventory of equipment and supplies, including their type and toxicity;
3) Identification of all permits, approvals, or authorizations and the timeline for obtaining them; and,
4) A plan for protecting environmentally sensitive areas, areas of public concern, and the public from any adverse effects of dispersant use.

For instance, Federal and state approval for dispersant application in Alaska is considered only when an effective conventional response is not feasible or not totally adequate in containing or controlling the spill. Even then, approval is given only when the impact of dispersants or dispersed oil is judged to be less harmful than that of non-dispersed oil.

The primary oil spill response method in Alaska and the United States is mechanical containment and recovery, which involves the use of containment booms, skimmers and other related equipment. The many hindrances to spill recovery, however, place a real advantage to having many "tools in the toolbox" – historically, no more than 10 percent of the oil has been recovered

from large marine spills. Current mechanical technology is not effective in waves greater than about 6 feet, winds greater than 20 knots, or currents greater than 1.0 knot. Colder air and water temperatures, and emulsification of the oil also limit recovery.

Conditions of use

- Dispersants are best used to protect shoreline: when the damage to the shore and nearby marine life would be worse than "dissolving" the oil into the off-shore water.
- They're best used on the leading edge of slicks that may get out of control and head ashore.
- Their use is avoided in near-shore areas especially near sensitive habitats.
- Dispersants must be applied soon after the oil is spilled and before the oil weathers or slicks are broken up. This means usually within a matter of several days.
- Conditions are best when the water is deep and when there is "mixing" action from waves, wind or currents. There must also be the right quantity of dispersants on hand to provide the proper oil-to-dispersant ratio, and they can be applied in the right place.
- Dispersants are used primarily on crude oil, but have been used on bunker oils too.

Land Fill Operations for Treatment of Polluted Soil

A landfill, also known as a dump is a site for the disposal of waste materials by burial and is the oldest form of waste treatment. Historically, landfills have been the most common methods of organized waste disposal and remain so in many places around the world. Landfills may include internal waste disposal sites (where a producer of waste carries out their own waste disposal at the place of production) as well as sites used by many producers. Many landfills are also used for other waste management purposes, such as the temporary storage, consolidation and transfer, or processing of waste material (sorting, treatment, or recycling).

A landfill also may refer to ground that has been filled in with soil and rocks instead of waste materials, so that it can be used for a specific purpose, such as for building houses. Unless they are stabilized, these areas may experience severe shaking or liquefaction of the ground in a large earthquake.

Incineration / Thermal Remediation of Polluted Soil

Fluidized bed combustion technology can be used for combustion or incineration of a wide range of materials, including heavy residues such as bitumen, tars, pitches, and other oily wastes. This technology has been tested for treatment of sludge from the Sydney Tar Ponds, whose remediation is an immediate priority for the government of Canada. The technology has also been evaluated, with the support of the government of France, for treating the oil-contaminated sand produced by the previously mentioned shipwreck of the Erika (Edward, J. A. & Jinsheng, W., 2004).

Soils contaminated with petroleum or different types of oils, caused normally by accidental spills or during normal maintenance of oil transportation pipes, are commonly found materials, specially in areas located inside or close to petrochemical or refinery plants. It is common to find soil contaminated with more than 25 % in weight of petroleum or oil. The spilled petroleum or oils may cause serious treat to the environment, endangering different vegetal and animal species, including human beings. In the same category, it can be included soils, sludges and similar materials contaminated with other organic compounds, such as pesticides and insecticides. In this case the amount of contamination could vary from tens of ppm (parts per million) to few percent. In all contamination cases mentioned above there is a considerable risk to the environment if the toxic or hazardous organic compounds are not removed from the soil or sludge and then properly treated (Szente, R. N. et al., 2005).

There are several possible methods to treat contaminated soils containing organic compounds. The most commonly used processes include incineration, the use of centrifuges and bioremediation. However, those processes present considerable drawbacks as shown below.

a) Incineration:

In the incineration process, the contaminated soil is normally fed into a rotating furnace that contains proper lining. Gas or oil burners are used to maintain the temperature inside the furnace in the range of 500 to 1,000 0C; air is allowed inside the furnace. The combination of the oxygen from the air and the temperature inside the furnace result in the partial or complete combustion of the contaminating organic compounds. The result of the process is material that in principle would be free of organic toxic or hazardous compounds. The process is conducted in dedicated incineration units or in cement kilns. The problems associated with the incineration process are the following:

i) Large volume of effluent gases that need to be properly treated (the effluent gases are the summation of entrained air for the process, the gases resulted from the combustion of the organic toxic compounds, the gases from the burners and water vapor), since the gases can contain toxic compounds; although dedicated incineration units present off gases cleaning systems, the same is not the case of cement kilns (normally only a removal of entrained particulate material is conducted), which increases the problem.

ii) Poor energy efficiency, since a great deal of the energy resulted from the oil/gas burners leave the furnace with the off gases, with typical energy efficiencies of less than 30 % for the overall process;

iii) No recovery of the initial organic materials is possible, since the organic compounds are burnt in the furnace; in the case of oil or petroleum contaminated soil, for instance, it could represent a significant loss of raw materials;

iv) Large costs and environmental risks associated with the transportation of the material to dedicated incineration plants (the contaminated soil needs to be properly placed inside drums or other vessels in order to be carried to the incineration site);

b) Centrifuges

The use of centrifuges to treat contaminated soil or sludges is also a common practice. In this process the contaminated material, containing variable amounts of organic toxic or hazardous compounds and eventually water, is fed into a centrifuge. In the centrifuge vessel, due to centrifuge forces resulted from the rotation at high speeds, the liquid organic compounds contaminating the soil or sludges and eventual water are separated from the solid materials, using different types of screens. The result of the process would be in principle material (soil or sludge) free of liquid organic contaminants and water, making it possible the recovery of such organic liquids in a separate vessel.

The main problems associated with that process are:

i) The impossibility of complete removal of the organic compounds; the soil or sludge after the treatment with centrifuges still contains typically more than 5 % in weight of organic contaminants (the legal limit for discarding hydrocarbon containing material is less than 1 %);

ii) Constant maintenance of the centrifuges required;

iii) Chemical agents need to be added to the charge regularly in order to improve the separation of the oil and the other components; this increase the costs of the process and the treatment of the recovered materials.

c) Steam extraction

The extraction of organic compounds from contaminated soil using steam consists in injecting saturated steam directly in the soil, resulting in a localized heating of the same, volatilizing, oxidizing and dissolving the organic compounds.

That process presents some problems:

i) Low removal efficiency, resulting in longer treatment periods;

ii) The off gases from the soil need, in principle, to be cleaned, since they may contain some organic compounds; the cleaning of the gases presents a difficult task.

iii) Some organic compounds will not be volatilized or oxidized by steam, remaining in the contaminated area.

d) Bioremediation: There are several variations of the bioremediation process, but all of them consist in the consumption of the organic compounds, present in the contaminated soil, by microorganisms or bacteria, resulting carbon dioxide and water vapor from the process.

Bioremediation presents some restrictions:

i) Can be used in cases where the contamination of the soil is not above 1 %;

ii) The soil cannot present harmful compounds to the microorganisms (such as heavy metals);

iii) Normally only few types of organic compounds can be consumed by the microorganisms; in the cases of several organic compounds exist, some of them will not be eliminated by the process.

As shown above, all the existing processes for treating contaminated soil present technical and/or economical and/or environmental restrictions. A new process for treating contaminated soil (soil contaminated with petroleum, oil, gasoline, diesel, benzene, toluene, organo-chlorinated compounds, mercury, etc) or petroleum sludges was developed; the process uses a plasma system in a clean and efficient manner as shown below. The process was developed in a joint collaboration between TSL Environmental Corporation and the Technological Research Institute of São Paulo.

The Plasma Process

A brief description of plasma is given below, followed by a description of the plasma process developed for treating contaminated soil and similar materials.

a) Thermal Plasmas

Thermal plasmas have been applied increasingly to industrial processes, in different areas. Thermal plasmas can be understood as an ionized gas, at high temperatures when compared to temperatures normally found in combustion or using electrical resistance heating. Thermal plasmas are produced using an electric arc that strikes between two (metallic) electrodes, inside an equipment called plasma torch. When a common gas, such as air, argon, nitrogen, steam and many others, are injected into the plasma torch, the molecules / atoms of the gases collided with the electrons present in the electric arc (the electrons are being produced in one electrode, accelerated and collected in the other electrode). The result of that process is the heating and ionization of the gas, producing a (thermal) plasma jet, that reaches very high temperatures as mentioned above. .

Thermal plasmas have been used for:

i) Environmental applications, such as:

(1) For the treatment of industrial residues, (2) treatment of hospital wastes, (3) vitrification of incineration ashes, (4) treatment of radioactive materials, (5) recovery of aluminum from drosses and others;

ii) Metallurgy applications, such as for tundish heating for continuous casting, ferroalloys production, blast furnaces and others;

iii) Materials applications, such as the production of titanium oxide, zirconia and several nitrites and carbides. A thermal plasma process was developed for the treatment of contaminated soil

and similar materials. The plasma process utilizes a plasma torch, operating at approximately 150^{o}C, for volatilizing the organic compounds present in the contaminated soil, in a controlled ambient; the volatilized organic compounds are recovered in the form of an oil, and can be readily reused. The process presents several advantages when compared to conventional technologies, as described below

b) Process Description

The developed plasma process can be understood as a controlled volatilization of the organic compounds initially present in the contaminated soil, in an ambient that does not contain oxygen. The energy for the process is provide by a plasma torch; a plasma gas, such as argon or nitrogen (not containing oxygen), is used in the torch. The organic vapors that leave the plasma reactor, with the plasma gas, passes through a series of condensers; the vapors after cooling, are recovered in the form of a light oil (diesel type) that is 100 % reusable. The plasma gas can be recycled in the process. The normal operating temperatures of the proposed plasma process is from 80 to 150^{o}C, in order to volatilize all the hydrocarbons contained in the treated material. The contaminated soil or sludge is continuously fed into the reactor from one end, while the oil free soil is removed from the other end of the reactor. The contaminated material, as it travel through the reactor, becomes increasingly free of organic contaminants; and it is clean once it has reached the outlet chute for removal from the reactor. The atmosphere inside the plasma reactor is maintained neutral or reducing in order to prevent the oxidation of the hydrocarbons and to permit their recovery after volatilizing them inside the reactor and condensing the hydrocarbons outside the plasma reactor. The developed process presents several interesting characteristics and advantages when compared to conventional technologies, such as:

i) Higher energy efficiency, since the plasma jet is at much higher temperature than, for instance, the flame produced by oil or gas burners employed in incineration (typically 1,500^{o}C for the plasma jet and 2,000^{o}C for the oil or gas flame), so that the heat transfer, dependent on the temperatures of the energy source and the heated substance, is significantly higher in the plasma process, increasing substantially the energy efficiency of the plasma process.

A typical energy efficiency above 80 %, is achieved with the plasma process as compared to around 20 % for the normal gas or oil burners);

ii) Possibility of recovering the organic compounds that were initially contaminating the soil, which can be a significant asset for the process especially when the soil or sludge contains more than 10 % in weight of hydrocarbons;

iii) Nil or almost nil off gases are produced in the process, since the plasma torch can operate with small amounts of gases (and the plasma gases can be recycled in the process) and the volatilized organic compounds are condensed and recovered.

iv) Soil after the plasma treatment contains less than 0.01 % in weight of hydrocarbons, even when treating soil containing more than 50 % organic compounds; which is less than 100 times the legal limit for discarding or reusing the soil.

v) Continuous operation process.

Traditional processes, such as incineration, cement kiln processing, centrifuging and others, used for treating soil contaminated with petroleum, oil, diesel, etc and or for treating oily sludges (generated from the exploration, production and refining of petroleum), present several environmental, technical and economical restrictions. A new process, based on the use of a plasma system, was developed for treating those materials, including petroleum contaminated soil and oily sludge. The process is based on the use of a plasma system, to transfer energy to the

material being treated. The contaminated material is heated in a controlled ambient, without the presence of oxygen, up to 120°C, using a plasma torch operating at temperatures around 1,500°C. The organic compounds volatilize under those conditions and leave the reactor; the vapors are cooled in condensers, recovering the organic compounds in the form of a light oil. The oil can be readily reused. The soil after the treatment presents less than 0.01% in weight of hydrocarbons (legal limit for discarding it is 1%); similar results were obtained when treating sludge containing up to 90 % hydrocarbons. Nil or almost nil off gases are generated in process. A demonstration unit was built (200 kg/h capacity) and industrial units are being designed and constructed for treating up to 5,000 kg/h of those materials.

Solidification Method for Remediation of Petroleum Contaminated Soil

Stabilization / Solidification (S/S) is a remediation technology that relies on the reaction between a reagent and the soil or waste in order to reduce the mobility of the envisaged contaminants. The notion "stabilisation", as most commonly used in the Anglo-Saxon countries, refers to the immobilization of the contaminants by producing more chemically stable constituents. "Solidification" on the other hand is the improvement of the soil or waste matrix in order to encapsulate the contaminants in a solidified matrix (Stany, et al., 2009).

In the EU often "immobilization" is used as a general term to describe the reduction of the contaminants' mobility, irrespective whether it is based on stabilization or on solidification. The S/S technology has initially been developed as a treatment concept for hazardous waste prior to landfilling. The last decades however it has also been applied as a remediation technology for contaminated soils, especially in the USA and the UK, and for a wide variety of contaminants such as organics and heavy metals. Most known cases are however based on solidification by means of a hydraulic binder such as cement. When contaminated soil is treated by a cement-

based formulation, the inevitable result is a tremendous solidification into almost concrete. Often this reduces the flexibility of the treated material for re-use, as it should be applied immediately after mixing and before curing. In addition, some chemical conditions, such as the high buffered pH, can be adverse to the groundwater quality. Finally, high dosages of cement are often required (10 % w/w or more) resulting in relatively high treatment costs and increase in mass and volume of the treated material.

The ideal immobilization technology for soil should be based on adding a small dosage of reagent and with only limited or at least necessary and controllable effects on the geotechnical properties as mentioned above. Treatment methods for soil or waste are mostly triggered by the presence of chemical pollutants, and in particular by the risk these pollutants cause. Whatever treatment method for contaminated soil or waste is envisaged, whether it is removal or immobilization of the pollutants, the recycling of the treated materials stays the main objective. So in any case of soil treatment optimal geotechnical properties are required such that reuse as engineered fill or other is possible.

Thermal Desorption Treatment Method for Oil Polluted Soil

Thermal desorption is a term applied to many different types of soil remediation technologies. All of these technologies consist fundamentally of a two-step process. In Step 1, heat is applied to a contaminated material, such as soil, sediment, sludge, or filter cake, to vaporize the contaminants into a gas stream that, in Step 2, is treated to meet regulatory requirements prior to discharge. A variety of gas treatment technologies are used to collect, condense, or destroy these volatized gases.

An in-situ method is disclosed for remediation and decontamination of surface and near-surface soils by evacuating the soil under a flexible sheet, which is impermeable to gases, and heating the soil surface with a relatively flat electric surface heater, which is permeable to gases.

The heater may be constructed, for example, from a mesh of electrically conductive metal wires. The surface heater is equipped with provision for electrical connections to supply electric power to the heater, and is placed in contact with the soil surface. Electrical power is supplied to the heater at power line frequencies of about 60 Hz. The surface heater is permeable to vapors that emanate from the soil when heated. Above the surface heater is placed a mat which is substantially more permeable to gases than the soil and which may also be a good thermal insulator. Above the permeable mat is placed a flexible impermeable sheet with at least one opening serving as a vacuum line connection. The impermeable sheet extends really beyond the surface heater and permeable mat, is impermeable to gases, and has the capability of sealing to the soil surface. A layer of thermal insulation may be placed above the impermeable sheet.

According to the teachings of this invention, a vacuum is applied through a manifold connected to the impermeable sheet. Creating a vacuum below the impermeable sheet will cause the sheet to be sucked tightly to the ground surface. The soil surface is sealed by the impermeable sheet as atmospheric pressure pushes the sheet against the soil surface. At the center of the pattern, the impermeable sheet will press the permeable mat and heater against the soil surface. In the peripheral region beyond the surface heater and permeable mat, the impermeable sheet will form a seal against the soil surface. Thus substantially only the air, soil moisture, and contaminants in the soil below the surface heater will be pulled toward the soil surface, minimizing the risk of spreading the surface contamination.

While applying a vacuum to the soil, the temperature of the soil is raised by applying heat to the surface of the soil with a surface heater. The surface heater can reach temperatures as high as 1000° C. or more, if necessary. A thermal front moves downward into the soil by thermal conduction, thereby vaporizing water and contaminants in the near-surface soil. For contaminants that are subject to thermal decomposition, at least a portion of the soil may be heated to a temperature sufficient to fragment contaminants into their decomposition products. Also, additional decomposition may occur as the vaporized contaminants pass through the very high temperatures at the surface heater. The vacuum is maintained throughout the period of heating and for a sufficient time after heating to avoid contaminant losses or dispersion.

The vacuum will cause vaporization or boiling to occur at a lower temperature than the normal boiling point at atmospheric pressure. At the same time, the high boiling point contaminants will be removed by steam distillation in the presence of water vapor within the soil at a temperature well below the normal boiling point of such contaminants. This will occur for all contaminants that are nearly immiscible in water, since the boiling point of the mixture of two immiscible fluids will always be less than the boiling point of either component. Contaminants with normal boiling points well above 300° C. can thus be removed by this process (Grant Publ., 1991).

Unlike other decontamination processes that require flow of electrical current within the soil, this process can be applied to soils which have low in-situ moisture content. Moreover, the decontamination process can be continued at temperatures well above the point at which moisture in the soil evaporates.

The water vapor and contaminants and/or decomposition products may be collected, for example, in a cold trap located between the opening in the impermeable sheet and a vacuum pump. Alternatively, the water and contaminant liquids can be separated on the basis of density

in a separator, while the gases can be reused, or incinerated, or otherwise disposed. Alternatively, the contaminants and/or their decomposition products can be trapped and concentrated, for example, on molecular sieve material, on activated carbon, or in a wet scrubber. Thereafter the concentrated contaminants and/or their decomposition products can be reused, or incinerated, or otherwise disposed. Alternatively, the contaminants and/or their decomposition products can be incinerated in line.

In one heating method, the surface heater is used to heat the soil continuously at a constant or varying temperature. In an alternative method, the heater is used to heat the soil surface to a high temperature, such as about 1000° C., then the heater is turned off, allowing a thermal front to propagate deeper into the soil. The heating process can be tailored to use the minimum amount of electrical energy to heat the soil to a predetermined minimum temperature at a minimum depth, required for volatilization and/or decomposition of the contaminants. When the initial moisture content is insufficient, or removed by heating, it may be advantageous to add water to enhance steam distillation. The process may consist of repetitive cycles of heating, adding moisture to the soil, heating, adding moisture, etc. to take advantage of steam distillation in reducing contaminant concentrations.

In an additional method of practicing this invention, once the near-surface soil has been evacuated and is at elevated temperatures, various gases and liquids can be supplied through an opening in the flexible sheet or through the surface at the periphery of the flexible sheet. Thus, for example, oxygen, hydrogen peroxide, or other reactants could be added to the soil to remove or decompose the contaminants further by chemical reaction at elevated temperature. This could be done in conjunction with additional heating to accelerate the reaction kinetics for decomposition of the contaminants.

Still another practice of this invention could embody other sequences of withdrawal of liquids prior to electrical heating. For example, liquid water could be removed by evacuation through the flexible sheet, thereby desaturating the soil and reducing the electrical power required for vaporization of the moisture.

Some of the contaminants that can be removed by this process include hydrocarbons, pesticides, chlorinated hydrocarbons such as PCBs, chemical warfare products, radioactive wastes such as tritium and titrated water, and heavy metal contaminants such as mercury, arsenic, etc (Grant Pub., 1991). The invention is in general applicable to any contaminant which has a vapor phase at elevated temperatures and reduced pressures, and/or may be decomposed at elevated temperatures and reduced pressures.

An in-situ method is disclosed for low pressure vaporization and recovery of contaminants from surface and near-surface soil by electrically heating the soil with a pattern of hollow electrodes and pulling a deep vacuum through the electrodes. The surface of the soil is sealed with an impermeable barrier supported by the soil. The contaminants are removed by vacuum distillation in the presence of water vapour at a temperature well below the normal boiling point of the contaminants (George, L. S., 1997).

Another in-situ method is disclosed for remediation and decontamination of surface and near-surface soils by electrically heating the soil through electrodes operated at power line frequencies of about 60 Hz. The electrodes are implanted substantially vertically in the soil in a line pattern which allows substantially uniform electrical heating in the region between rows of electrodes. The depth of electrode insertion is substantially equal to the depth of the contamination, but could be deeper or shallower. The process is particularly applicable to soils contaminated at depths of up to about 30 meters. The electrodes are hollow and perforated below the surface to

allow application of a vacuum to the soil through the electrodes. The electrodes are also equipped with provision for electrical connection and vacuum line connection, and also with the capability to be sealed to a barrier that is impermeable to gases, such as a flexible sheet, described herein below.

In one embodiment of this invention, a substantial vacuum is generated by, for example, a vacuum pump, and applied through a pumping manifold connecting to the array or pattern of hollow electrodes. The soil surface is covered by an impermeable flexible sheet which is sealed to the upper part of the electrodes and through which the electrodes protrude into the soil. Applying a vacuum through the electrodes will cause the flexible sheet to be sucked tightly to the ground surface and form a seal against air entering the pattern. The flexible sheet is supported solely by the soil, thereby avoiding bulky vapor containment structures. Substantially only the air, moisture, and contaminants in the pattern will be collected and removed from the hollow electrodes by a pump. Alternatively, the impermeable barrier may be a clay layer placed on the surface. As another alternative, an impermeable clay layer could be used to augment the flexible sheet.

Before applying, while applying and/or after applying electrical heat to raise the soil temperature, a substantial vacuum is created in the soil through the array of hollow electrodes. The vacuum will cause vaporization or boiling of water to occur at a lower temperature than the normal boiling point at atmospheric pressure. At the same time, the high boiling point contaminants will be removed by vacuum distillation in the presence of water vapor within the soil at a temperature well below the normal boiling point of such contaminants. This will occur for all contaminants that are nearly immiscible in water, since the boiling point of the mixture of two immiscible fluids will always be less than the boiling point of either component. Thus, in

many cases, it will not be necessary to raise the temperature of the soil above 100° C. This represents a substantial savings in electrical energy.

The hollow electrodes enable application of a much higher vacuum in the soil than that obtainable in a large vapor containment structure. Vacuums as low as 2 psi or below can typically be obtained. Contaminants with boiling points well above 200° C. can thus be removed by this process of vacuum distillation at temperatures of no more than about 100° C., and as low as about 50° C. Moreover, the amount of steam required per unit quantity of distillate will be diminished by lowering the total pressure. Thus the process can be applied in soils which have low in-situ moisture content.

The water vapor and contaminants may be collected, in a cold trap and/or condenser located between the pumping manifold and the vacuum pump. The water and contaminant liquids from the cold trap and/or condenser can be separated on the basis of density in a separator, while the gases can be reused, or incinerated, or otherwise disposed. Alternatively, the contaminants can be trapped and concentrated, for example, on molecular sieve material or on activated carbon, or in a wet scrubber. Thereafter the concentrated contaminants can be reused, or incinerated, or otherwise disposed.

By pulling moisture toward and into the hollow electrodes, and by concurrently providing only modest electrical heating, the soil near the electrodes will stay electrically conducting. Thus, inexpensive 60 Hz power from a power line transformer can be used for heating the soil, rather than radio frequency power with its attendant disadvantages. The applied vacuum assures that volatilized components are contained and not dispersed to surrounding soil.

In an additional method of practicing this invention, once the hollow electrodes and pumping manifold are in place, various gases and liquids can be introduced to the soil at the surface, or

injected through selected groups of hollow electrodes or injection wells and withdrawn from other groups of electrodes or other wells. Thus, for example, water, steam, oxygen, hydrogen peroxide, or other reactants, or combinations of these materials, could be injected to further remove or decompose the contaminants, as required. This could be done in conjunction with electrical heating to accelerate the reaction kinetics for the decomposition of the contaminants.

Other methods of practicing this invention include additional steps. For example, in very moist soil, liquid water could be removed by evacuation through the electrodes prior to electrical heating, thereby desaturating the soil and reducing the electrical power required for vaporization of the liquids.

Still another method of practicing this invention is applicable to deep contamination, well below the water table. Electrical heating is used to create a vapor zone in the contaminated region between the electrodes. In this case it is necessary to heat the contaminated region to higher temperatures, sufficient to vaporize the water at the downhole pressures which can be substantially above atmospheric pressure. At these depths, it may not be necessary to evacuate the hollow electrodes because the downhole pressures may be sufficient to drive the water vapor and contaminants to the surface.

Some of the contaminants that can be removed by this process include hydrocarbons, pesticides, and chlorinated hydrocarbons such as PCBs, chemical warfare products, and radioactive wastes such as tritium and titivated water. The invention is in general applicable to any contaminant which has a sufficient vapor phase at elevated temperatures and reduced pressures (George, L. S. et al., 1997).

Bio – augmentation Method for Treatment of Oil Contaminated Soil

The addition of archaea or bacterial cultures required to speed up the rate of degradation of a contaminant. In a place filled with contamination, microbial life usually finds it as a place to call home. The biological material that originated in this contaminated area is able to break down waste, but when the amount of waste overloads it needs help from a foreign form to increase performance in breaking down chemicals. Bio-augmentation develops the biological material in order to smoothly break down certain compounds. When a microbe is added to the contaminated area, they are able to improve the biological material's capability to behave in a manner as to break down contamination that was already broken up before (Yaohui, Xu, 2010).

This enhanced treatment can lead to the cure of contamination in wastewater and agricultural improving biological waste treatment systems. Usually the steps involve studying the indigenous varieties present in the location to determine if bio-stimulation is possible. If the indigenous variety do not have the metabolic capability to perform the remediation process, exogenous varieties with such sophisticated pathways are introduced.

Bio-augmentation is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of Microbial cultures, containing all necessary microorganisms (*B. licheniformis*, *B. thuringiensis*, *P. polymyxa*, *B. stearothermophilus*, *Penicillium s*., *Aspergillus sp*., *Flavobacterium, Arthrobacter, Pseudomona, Streptomyces, Saccharomyces, Triphoderma*, etc.). Whereas activated sludge systems are generally based on microorganisms like bacteria, protozoa, nematodes, rotifers and fungi capable to degrade bio degradable organic matter. There are many positive outcomes from

the use of bio-augmentation like the improvement in efficiency and speed of the process of breaking down substances and the reduction of toxic particles inhibiting an area.

Selective Enumeration of Aromatic and Aliphatic Hydrocarbon Degrading Bacteria by Most-Probable Number Procedure

A most-probable-number (MPN) procedure was developed to separately enumerate aliphatic and aromatic hydrocarbon degrading bacteria, because most of the currently available methods are unable to distinguish between these two groups. (Germida, H. G., 1996). Separate 96-well microtiter plates are used to estimate the sizes of these two populations. The alkane-degrader MPN method uses hexadecane as the selective growth substrate and positive wells are detected by reduction of iodonitrotetrazolium violet, which is added after incubation for 2 weeks at 20°C. Polycyclic aromatic hydrocarbon degraders are grown on a mixture of phenanthrene, anthracene, fluorene, and dibenzothiophene in a second plate. Positive wells turn yellow to greenishbrown from accumulation of the partial oxidation products of the aromatic substrates and they can be scored alter a 3-week incubation period. These MPN procedures are accurate and selective. For pure cultures, heterotrophic plate counts on a nonselective medium and the appropriate MPN procedure provide similar estimates of the population density. Bacteria that cannot grow on the selective substrates do not produce false positive responses even when the inoculum's density is very high. Thus, this method, which is simple enough for use in the field, provides reliable estimates for the density and composition of hydrocarbon-degrading microbial populations.

In general, the alkane fraction is the most biodegradable, whereas the polar fraction (i.e., the resins and asphaltenes) is resistant to biological degradation. The aromatic compounds,

especially the polycyclic aromatic hydrocarbons (PAHs), are of intermediate biodegradability, but these are of most concern owing to their toxicity and tendency to bioaccumulate.

Enumeration of oil-degrading bacteria usually involves growth on a medium that contains crude oil or a refined petroleum product as the selective substrate (Mulkins-Phillips & Stewart, 1974). Because these complex substrates contain both aliphatic and aromatic compounds, methods that use them cannot distinguish between alkane and PAH degraders.

2.8 MODEL DEVELOPMENT

2.8.1 Microbial Growth

The mathematical description of the rate of growth of a microbial culture frequently makes use of an exponential growth pattern. This is based on the premise that the growth rate is directly proportional to the existing population and the proportionality constant is a function of the organism type. Malthus' law gives exponential growth as:

$$\frac{dX}{dt} = \mu X \qquad (2.1)$$

On integration it gives:

$$X = X_0 e^{\mu t} \qquad (2.2)$$

This growth, however, cannot be sustained indefinitely and for one reason or another will lead to a stationary phase. Pearl and Reed (1920) modified the exponential growth equation by adding a further term to account for 'inhibition' at high biomass concentration:

$$\frac{dX}{dt} = \mu X - \mu\gamma X^2 \qquad (2.3)$$

On integration it gives:

$$X = \frac{X_0 e^{\mu t}}{1 - \gamma X_0 (1 - e^{\mu t})} \tag{2.4}$$

This is the 'logistic equation'.

2.8.2 Substrate degradation and yield coefficient

The growth of a microbial culture, consuming substrate for energy purposes, for incorporation into its own cellular material, or for synthesis of a product, gives rise to the concept of yield. Yield is ratio of mass of product obtained to that of reactant consumed and is expected to be constant for given reaction conditions. In more sensitive experiments the yield appears not to be a constant quantity, but a function of time as well as the physico-chemical environment. This is the result of the changing composition of the microbial cell and the phenomenon of adaptation. When the yield is considered constant we have:

$$Y = \frac{\Delta X}{-\Delta S} \tag{2.5}$$

A material balance for the consumption of substrate gives

$$\frac{dS}{dt} = \frac{1}{Y_G}\frac{dX}{dt} + mX \tag{2.6}$$

Substrate consumed for growth is usually much larger than that consumed for maintenance, such that equation (2.6) can be simplified thus:

$$\frac{dS}{dt} = \frac{1}{Y_G}\frac{dX}{dt} \tag{2.7}$$

2.8.3 Modification of Yield coefficient

Oyoh and Osoka (2007), in their study of NPK fertilizer enhanced bioremediation, proposed a new definition for the yield coefficient at times when it is not a constant quantity. In this definition averages of mass of product obtained (change in biomass concentration) and average of reactant consumed (change in substrate concentration) are used instead. They theorized that the use of averages will normalize the variation of yield with time, so we have:

$$Y_m = \frac{\Delta X / X}{-\Delta S / S} \tag{2.8}$$

Equation (2.7) will take the form:

$$\frac{1}{S}\frac{dS}{dt} = \frac{1}{Y_{mG}}\frac{1}{X}\frac{dX}{dt} \tag{2.9}$$

This may be written in the form:

$$\frac{d(\ln S)}{dt} = \frac{1}{Y_{mG}}\frac{d(\ln X)}{dt} \tag{2.10}$$

On Integration it gives:

$$S = S_o \left(\frac{x}{x_o}\right)^{\frac{-1}{Y_G}} \tag{2.11}$$

Equations (2.7) and (2.11) can be applied for either exponential growth model or logistic model.

If microbial growth rate is exponential in nature and yield is constant we have:

$$S = S_0 + \frac{X_0}{Y_G}\left[1 - e^{\mu t}\right] \tag{2.12}$$

If microbial growth rate is exponential in nature with the yield not being constant we have:

$$S = S_o \exp\ (\mu t)^{\frac{-1}{Y_G}} \tag{2.13}$$

If microbial growth rate is logistic in nature with the yield being constant we have:

$$S = S_0 + \frac{X_0}{Y_G}\left[1 - \frac{e^{\mu t}}{1 - \gamma X_0\left(1 - e^{\mu t}\right)}\right] \quad (2.14)$$

If microbial growth rate is logistic in nature with the yield not being constant we have:

$$S = S_o\left(\frac{\exp(\mu t)}{1 - \gamma x_o(1 - \exp(\mu t))}\right)^{\frac{-1}{Y_{mG}}} \quad (2.15)$$

The above equations can be used to fit experimental data in order to obtain the appropriate rate model for the degradation of the substrate through bioremediation.

2.9 KINETICS OF BIODEGRADATION

The kinetics of biodegradation is a set of empirically derived rate laws. Three suffice to describe most biological reactions:

$$\frac{dC_A}{dt} = -k_0 \quad \text{(Zero order)} \quad (2.16)$$

$$\frac{dC_B}{dt} = -k_1 C_A \quad \text{(First order)} \quad (2.17)$$

$$\frac{dC_B}{dt} = -k_2 C_A C_B \quad \text{(Second order)} \quad (2.18)$$

k_0, k_1, k_2 = rate constants mol/1-sec, 1/sec, 1/mol-sec, respectively

C_A, C_B = some reacting species (Simkins and Alexander, 1984)

Osoka and Onyelucheya (2010) presented a modified approach that will be adopted for zero order, first order and second order kinetics:

$$\frac{dS}{dt} = -k_0 \quad (2.19)$$

$$\frac{dS}{dt} = -k_1(S - S_\infty) \quad (2.20)$$

$$\frac{dS}{dt} = -k_2(S - S_\infty)^2 \quad (2.21)$$

Where S is substrate concentration, t is time, k is the reaction rate constant and S∞ is the ultimate substrate concentration.

2.9.1 ZERO ORDER

Integrating equation (2.19) subject to the condition that $S = S_o$ when $t = 0$, we have;

$$S = S_o - k\,t \quad (2.22)$$

Where S_o is the initial substrate concentration

2.9.2 FIRST ORDER

Integrating equation (29) subject to the condition that $S = S_o$ when $t = 0$, we have;

$$ln\left(\frac{S - S_\infty}{S_o - S_\infty}\right) = -k\,t \quad (2.23)$$

equation (2.23) gives;

$$\left(\frac{S - S_\infty}{S_o - S_\infty}\right) = \exp(-k\,t) \quad (2.24)$$

Re-arranging equation (2.24) gives;

$$S = S_\infty + (S_o - S_\infty)\exp(-k\,t) \quad (2.25)$$

If $S_\infty = 0$, equation (2.25) reduces to

$$S = S_o \exp(-k\,t) \quad (2.26)$$

Equation (2.26), which is the reduced form of equation (2.25), is one of the models presented by Simkins and Alexander (1984).

2.9.3 SECOND ORDER

Integrating equation (2.21) subject to the condition that $S = S_o$ when $t = 0$, we have;

$$\left(\frac{1}{S-S_\infty}\right)-\left(\frac{1}{S_0-S_\infty}\right)=k\,t \tag{2.27}$$

On simplification of the above equation we have;

$$\frac{S_0-S}{(S-S_\infty)\,(S_0-S_\infty)}=k\,t \tag{2.28}$$

Further re-arrangement of equation (2.28) gives;

$$S=\frac{S_0+S_\infty(S_0-S_\infty)k\,t}{1+(S_0-S_\infty)\,k\,t} \tag{2.29}$$

If $S_\infty = 0$, equation (2.29) reduces to

$$S=\frac{S_0}{1+S_0\,k\,t} \tag{2.30}$$

Table 2.9: Summary of the Kinetic Equations and Parameters for Different Reaction Orders

S/N	ORDER OF REACTION	KINETIC EQUATION	KINETIC PARAMETERS
1	ZERO ORDER	$S = S_o - k\,t$	K_0
2	FIRST ORDER	$S = S_o\,\exp(-k\,t)$	K_1
3	FIRST ORDER	$S = S_\infty + (S_o - S_\infty)\exp(-k\,t)$	K_1, $S\infty$

4	SECOND ORDER	$S = \frac{S_o}{1 + S_o\, k\, t}$	K_2
5	SECOND ORDER	$S = \frac{S_o + S_\infty (S_o - S_\infty) k\, t}{1 + (S_o - S_\infty)\, k\, t}$	K_2, $S\infty$

The above equations (table 2.5) will be used to fit the experimental data (substrate concentration and remediation time) to determine the model that best fit the phytoremediation enhanced bioremediation process, based on order of reaction.

2.10 Phytoremediation Mechanism: Uptake, Translocation and Transformation

The rate of contaminant removal is a function of uptake efficiency, which depends on plant species, age, health, properties of root zone among other things. An indirect measure of uptake efficiency of a plant with respect to a contaminant is the Transpiration Stream Concentration Factor (TSCF) (Kamath et al., 2004).

Briggs et al (1982) studied pesticide uptake by barley plant and developed a relationship for the Transpiration Stream Concentration Factor (TSCF) as:

$$TSCF = 0.784 \ \exp\left(-\frac{(\log K_{ow} - 1.78)^2}{2.44}\right) \qquad (2.31)$$

Burken and Schnoor (1998) studied uptake of a wide variety of organic compounds (including monoatomic hydrocarbons: benzene, toluene, ethylene and xylenes) by hybrid poplar trees using equation (2.31) and got relationship for the Transpiration Stream Concentration Factor (TSCF) as:

$$TSCF = 0.756 \ \exp\left(-\frac{(\log K_{ow} - 2.50)^2}{2.58}\right) \quad (2.32)$$

Where K_{ow} is the partitioning coefficient, which is referred to as a measure of contaminant affinity for root membranes.

The Root Concentration Factor (RCF) – which is ratio of contaminant in roots to the concentration dissolved in soil water - is used to study microbial degradation in the rhizosphere (around the root of the plant) which is the most significant mechanism for removal of diesel range organics in vegetated contaminated soils.

Briggs et al (1982) studied pesticide uptake by barley plant and represented the Root Concentration Factor (RCF) as:

$$\log (RCF - 0.82) = 0.77 \ \log K_{ow} - 1.52 \quad (2.33)$$

Burken and Schnoor (1998) studied uptake of a wide variety of organic compounds by hybrid poplar trees and got relationship for the Root Concentration Factor (RCF) as:

$$\log (RCF - 3) = 0.65 \ \log K_{ow} - 1.57 \quad (2.34)$$

2.10.1 Contaminant Uptake and Clean-up Time

The uptake rate of contaminants can be represented as:

$$U = (TSCF)(T)(C) \quad (2.35)$$

Where U is uptake rate of contaminant in mg/day, T is Transportation rate of vegetation in l/day and C is aqueous phase concentration in soil or ground water in mg/l.

First order kinetics can be assumed as an approximation for clean-up time (Kamath et al., 2004).

$$K = U/M_o \quad (2.36)$$

Where Mo is mass of contaminant initially in mg, K is first order rate constant for uptake in day^{-1} and U is contaminant uptake rate in mg/day.

$$M = M_o \exp(-kt) \quad (2.37)$$

Where M is mass remaining in kg and t is time in years.

$$t = -\ln\left(\frac{M}{M_o}\right)/K \quad (2.38)$$

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

The materials used were consistent with standard test methods, they included water pycnometer for specific gravity of soil solids (ASTM, D854). For soil sampling at depth, Augers and Thin-Wall Tube samplers, in line with the standard method was employed. (USEPA, ENV3.13; 1997). Sample collection, handling and storage procedures were carried out and consistent with standard methods prescribed for plastic containers, sampling cans or containers and storage under controlled temperatures. (ASMT, 4547-15). This guide describes sample collection and handling procedures designed to minimise losses of Volatile Organic Compounds (VOCs). The principal mechanisms for the loss of VOCs from materials during collection, handling and storage are volatilisation and biodegradation. Compounds with higher vapour pressure are more susceptible to volatilization than compounds with lower vapour pressures. Compounds that are

aerobically degradable are generally more susceptible to biodegradation than anaerobic compounds. Loss or gain of VOCs may lead to results that are inconsistent of field conditions. Soil moisture content was determined using drying oven and in desiccator at 60^{o}C. Moisture content of the soil samples were determined utilizing new or calibrated weighing scales which were employed for average weight before and after drying in line with standard method for laboratory determination of water (moisture) content of soil and rock by mass. (ASTM, D2216-10). For the purpose of soil pH determination, the test soil was sieved through a 2-mm (No.10) sieve. Test soil in a Calcium Chloride solution was also used to determine the pH levels. (ASTM, 4972-13).

Other materials used included, refrigerators for storage of samples broth microbes' culture, gas chromatograph for crude oil assay, calibrated thermometer with resolutions ±0.1^{o}C, rain boots, cutlass, shovel, hand gloves, hydrometer, first aid kits, weigh scales (Accuracy: ±0.2grams), scissors, label / tags, writing materials, camera, etc. for personnel entering the polluted site for sample taking, handling and storage.

3.2 METHODS

The soil samples were collected from Obite and Erema towns of Ogba-Egbema-Ndoni LGA of Rivers State, Nigeria. Experimental works were carried out in microbiology and Chemical/Petrochemical Engineering laboratories in Rivers State University of Science & Technology, Port Harcourt. Polluted sites where soil samples were collected was located at Erema village in Ogba-Egbema-Ndoni local government area of Rivers State, Nigeria. Soil samples were collected at three sites where pollution had occurred.

3.2.1 METHOD FOR SITE DETERMINATION

Two methods were used to obtain the geographical coordinates namely, by rough visual location in an enlarged map and more accurately by employing Google earth software. The

later method proves more accurate and the coordinates were obtained as: Latitude 5^{o} 13' and Longitude 6^{o} 42' at altitude of nearly 235 meters or 774 ft above sea level. Utilized the 2009 Europa GPS (3111) (Accuracy: ±1m), Ogba-Egbema-Ndoni LGA of Rivers State in Nigeria was determined to be geographically located at latitude 4^{o} 55' 55"N and longitude 6^{o} 32' 48" E.

It is important to note that the pollution of the Erema and Obite sites dates back from 1998 and 2003 respectively.

3.2.2 METHOD FOR SOIL SAMPLE COLLECTION

Procedure adopted for sample collection involves site investigation in line with ASTM, 4547 – 15; Standard Guide for Sampling Waste and Soil for Volatile Organic Compounds.

1. Prior to site visit for soil sample collection, hard rain boot, hand gloves, a first aid kit and suitable clothing was adorned personnel.
2. The field where polluted soil exist was divided into areas for sampling and a mixed composite sample was obtained from each area. Note, unusual areas for soil samples were avoided especially where human or animal wastes existed.
3. At site, shovels were used alongside cutlass to clear the selected spot were soil sample were taken.
4. Equipment called Auger was used as sampling device, and soil samples collected into plastic containers.
5. A depth of 30 inches of soil was collected from four (4) different location within the same site. Sample information (location name, date, soil depth, soil colour, age of pollution) was recorded on label attached to each sample. Another record of samples cans are jotted in note books.

6. Sand auger and mud auger were used to collect the undisturbed soil and muddy polluted soil.
7. Glass containers fitted with plastic or metal lids were used. Glass jars lined with Teflon were preferable for samples containing organic parameters such as benzene or TPH (Total Petroleum Hydrocarbons).
8. Samples were taken to the Rivers State Chemical Engineering laboratory for analysis for soil texture (particle size, grain / particle distribution, soil density, bulk density, moisture content, pH levels, organic matter content, etc.).
9. The samples were numbered and samples recorded.
10. Interpretation of samples analysed and results recorded.
11. Determination of amendments needed, fertilizer requirement, pH level adjustment for acidity or alkalinity, moisture content requirements, etc.

3.2.3 EXPERIMENTAL METHODOLOGY

Microbes so isolated were cultured and identified and report on results obtained submitted. One or more types of bacteria were present and therefore, the predominant type was counted and compared with the remaining microbial population. Predominant microbial type was identified as Pseudomonas.

Experimental procedure involved the inoculation of cultured bacteria (Pseudomonas) in controlled or uncontrolled samples with varying NPK and/with phosphorus as nutrients.

Plant types used were Sunflower and Locoweed and were planted in samples while one was used as control sample.

3.2.4 EXPERIMENTAL PROCEDURE

The equipment used was all tested for their functionality based on the following step by step procedures for all the experimental runs:

1. Mercury in glass thermometers (with precision / resolution ±0.1^{o}C) was used to record temperature changes, and hydrometers were calibrated within accuracy level of ±0.2 was used for testing specific gravity of crude oil samples.

2. The type of microorganism (Pseudomonas) and microbial population/density were determined.

3. Dormant samples were isolated and only active ones were used.

4. Active samples were divided into four equal parts and mixed – one control group and three experimental groups.

5. Each sample weight and microbial content was balanced so that they differ by an insignificant value and the values noted.

6. Nutrients were added to the three controlled samples and monitored at weekly intervals..

7. Records were as in item No.6 above were recorded with date and time and any observations or limitations noted.

8. All parameters were recorded as in item No.6 above for the uncontrolled samples.

9. Steps 9 to 12 were repeated for another one week for the controlled and uncontrolled samples and all changes recorded accordingly.

10. Step 13 was repeated after additional one week with indication for date and time and all observed changes noted.

3.2.5 Method for Enumeration and Isolation of Total Heterotrophic Bacteria

Tenfold serial dilution method of Ofunne (1999) was used to enumerate and isolate bacteria. In this method one gram (1g) of the soil sample was put into the test tube containing 10ml of normal saline (diluent), and thoroughly shaken to properly disperse the sample in the diluent to give 10^{-1} dilution.

From the 10^{-1} dilution, further serial dilutions were made up to 10^{-3} dilutions. Two drops (0.1ml aliquots) of 10^{-2} and 10^{-3} dilutions were inoculated onto the surface of sterile nutrient agar plates and spread with a sterile bent glass rod.

The inoculated plates were incubated in the microbiology laboratory using a bacteriological incubator at temperatures of $37^{o}C$ for 24 hours. Note that an incubator is a device used for growing and maintaining microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide and oxygen content of the atmosphere inside.

After incubation, the plates were examined and colonies that developed were counted and recorded; and taken as the total number of bacteria enumerated from the sample.

Also, the cultural characteristics of the colonies were observed and three bacterial types were isolated by sub-culturing the colonies onto sterile freshly prepared nutrient agar plates and incubated at $37^{o}C$ for 24 hours.

The colonies were characterized and identified and the results showed bacterial types identified include, Bacillus species, Staphylococcus species and Pseudomonas species.

3.2.6 Method for Preparation of Broth Cultures for Bioremediation

This was done by inoculating the three bacterial types mentioned above and isolated from the sample into 500ml of nutrient broth medium. The inoculated broth was incubated at 37^{o}C for 24 hours and used for study. Below was the ingredient agar for culturing of non-fastidious organisms.

The dominant microbial type seen was Pseudomonadaceae with other strains as oil bio-degraders.

Table 3.1: Nutrient Agar Composition and Quantity.

Source: Ofunne, 1999.

S/NO	**Composition**	**Grams/Litre**
1	Peptone	5.0
2	Beef extract	3.0
3	NaCl	8.0
4	$CaCl_2$	12.0
5	Agar	(pH – 7.3 ± 0.2)

A weighing scale (Accuracy: ±0.2grams), was used to measure 28 grams of powder of CaCl which was weighed and dispersed in 1 litre of deionised water.

It was allowed to soak for 10 minutes, swirled to mix, and then sterilized by autoclaving for 15 minutes at 121^{o}C. It was cooled to 47^{o}C, mixed properly and then poured into plates.

Table 3.2: Nutrient Broth ‘E’ and Compositions

Source: Ofunne, 1999.

S/NO	Formulation	Grams / Litre
1	Beef Extract	1.0
2	Yeast Extract	2.0
3	Peptone	5.0
4	Sodium Chloride	5.0 (pH – 7.4 ± 0.2)

The 13 grams of CaCl powder was weighed and dispersed in 1 litre of deionised water. It was heated to dissolve, and then dispensed into bottles. It was sterilized by autoclaving at 121^{o}C for 15 minutes.

Appearance: straw coloured, clear.

3.2.7 Method for Procedure for Determination of Moisture Content of a Soil Sample

The method described below was used to determine the percentage of water in a sample by drying the sample at a given temperature to constant weight. The water content is expressed as the percentage, by weight, of the dry sample.

Apparatus:

a) Drying equipment – An oven, hot plate, field stove or the like suitable for drying moisture samples at a uniform temperature not exceeding 239º F (115º C).

b) Balance– A weighing balance or scale sensitive to 0.1 percent of the minimum weight of the sample to be weighed and with a capacity equal to the maximum wet weight of the samples to be weighed.

SAMPLING:

i) A representative quantity of the moist sample based on the maximum particle size of the sample was selected.

ii) Quantities for approximate minimum weights are listed in the Table 3.3 below.

Table 3.3: Soil Particle Size and Minimum weights

Maximum Particle Size	**Minimum Weight of Sample, ounces (grams)**
No. 4 (4.75 mm)	4.3 (100)
¾ in. (19.0 mm)	17.1 (500)
2 in. (50 mm)	36.2 (1000)

Procedure:

i) Moist sample was weighed immediately and recorded as "wet weight of sample"

ii) The wet sample was dried to a constant weight, at a temperature not exceeding 115º C using the suitable drying equipment.

iii) The sample was allowed to cool.

iv) The cooled sample was weighed again, and recorded as the "dry weight of sample"

3.2.8 Method for Procedure on How to Change the Independent Variable(S)

1. One set of experimental trials was conducted before changing the independent variable to establish a set of data to compare the experiment to.

2. Sample size was increased and started with carbon/nitrogen ratio of 1/5, 2/5, 3/5, 4/5, 1 and 5/1, 5/2, 5/3, 5/4 for uncontrolled variables.

3. Each sample was left for one week intervals and analysed for changes in amount for biomass formation (BF) and HC concentrations.

4. The sample C/N ratio was increased by 2/5, and sample was left for one week and analysed for BF and HC concentrations.

3.2.9 Method for Maintaining Control Variables at Constant Value:

1. The nutrient size was increased and started with Carbon / Nitrogen ratio (C/N ratio) of 1/5, 2/5, 3/5, 4/5, 1 and 5/1, 5/2, 5/3, 5/4 or an equivalent of 20/100, 40/100, 60/100, etc.

2. All was left for the same period as the controlled variable for four (4) weeks unperturbed.

3. Ambient temperature, sample temperatures, oxygen level, BOD, COD, pH Level, Colour changes, density, viscosity, BF, Hydrocarbon (HC) Concentration, etc were recorded.

3.2.10 METHOD FOR NUMBERS OF RUNS ON REPEATABILITY OF RESULTS

1. At least five (5) experimental runs were done for each sample and also for each sample concentration.

2. For a given sample concentration,

Repeatability = {(Max. Conc.–Min. Conc.) / Min. Conc.} x 100% (3.4)

3. Standard Deviation, $\sigma = \sqrt{(\sum_{i=1}^{n} x^2)/n}$ (3.5)

4. Procedures as described above were used for duplication of experiment.

3.2.11 Method for General Precautions and Peculiarities for the Soil Sample Collection

The under listed precautionary measures were taken before, during and after the experiment in order to ensure better results.

1. That soil sample was collected from site in areas where only petroleum pollution had taken place by visual inspection.
2. Accurate measurement was taken and experimental results properly recorded.
3. All samples were labelled for easy identification.
4. All samples were kept away from rain or other experimental works to avoid dilution or mix-up.
5. Good records of results were maintained with name of laboratory, Attendant, date and time when readings were taken.
6. Other precautions were taken and noted as the experiment progressed.

3.2.12 METHOD FOR DETERMINATION OF SOIL SPECIFIC GRAVITY

This experiment was performed to determine the specific gravity of soil by using a pycnometer. Specific gravity is the ratio of the mass of unit volume of soil at a given temperature

to the mass of the same volume of gas –free distilled water at the same temperature. Similarly, specific gravity is the ratio of density of soil to the density of gas-free distilled water at the same temperature. Specific density is often used interchangeably as relative density.

Standard Reference:

ASTM D 854 – 00: Standard Test for Specific Gravity of Soil Solids by Water pycnometer.

Significance:

The specific gravity of a soil is used in the phase relationship of air, water and solids in a given volume of the soil.

Equipment:

Pycnometer, Balance, Vacuum Pump, Funnel, Spoon.

Test Procedure:

1) The weight of the empty clean and dry pycnometer were determined and recorded, W_p.
2) 10g of a dry soil sample was placed (passed through the sieve No.10) in the pycnometer. The weight of the pycnometer containing the dry soil was determined and recorded, W_{ps}.
3) Distilled water was added to fill about half to three-fourth of the pycnometer and the samples were soaked for 10 minutes.
4) A partial vacuum was applied to the contents for 10 minutes, to remove the entrapped air.
5) The vacuum was stopped and the vacuum line carefully removed from the pycnometer.
6) The pycnometer was filled with distilled (water to the mark) and the exterior surface of the pycnometer cleaned with a clean, dry cloth. The weight of the pycnometer and contents was determined, W_B.

7) The pycnometer was emptied and cleaned. It was then filled with distilled water only (to the mark). The exterior surface of the pycnometer was cleaned with a clean dry cloth. The weight of the pycnometer and distilled water was determined, W_A.

8) The pycnometer was emptied and cleaned.

3.2.13 Method for Plant (Sunflower / Locoweed) Seed Planting, Dressing, Nurturing, Transplanting, Harvesting and Disposals

1. Seeds of selected plant types e.g. sunflower and locoweed were planted in humus soil rich in organic nutrients under humid condition.

2. Weeds were eliminated when plants are young and a layer of mulch 4 to 8 inch were added to reduce water evaporation and soil erosion and enable growth.

3. The plants were continuously side-dressed with 10-10-10 aged manure or rich compost or a balanced liquid of fertilizer monthly.

4. When the seedlings were about 3 inches tall, the plants were thinned so that they were 10 inches apart to allow for aeration and oxygen flow and growth.

5. The plants were well watered throughout, 1 inch of water per week but sometimes applied more on hot conditions when ambient temperatures were generally high.

6. Plants were carefully removed with soil on the roots and root depth measured as initial recorded values.

7. Plants so removed were transplanted in sample soils and NPK added in measured quantity.

8. Plants and soil condition were constantly monitored for foliage coloration or infestation by pest or insects under adequate sunlight.

9. Weekly plant growth, soil conditions (pH level, contaminant concentration level, moisture content, etc.) were monitored and records kept.

10. Plants were harvested after 13 – 15 weeks when growth rate was observed insignificant and root depth or penetration height of rhizosphere into soil was measured and recorded. Deviations was recorded and averages of plant height and root depth were documented.

11. Plants roots, stem and leaves were taken and analysed for heavy metals concentrations and parameters recorded.

12. The ex – situ plant seedlings, growth and transplanting was repeated on soil samples and the above procedures 1 – 11 were repeated until contaminant concentration levels was negligible or sufficiently reduced to acceptable limits.

13. Plant types that were harvested (Sunflower and Locoweed) were disposed of by incineration method and ashes obtained disposed in suitable landfills or approved dump sites.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

The density, viscosity and surface tension of the crude oil sample and fresh water were measured and recorded. Results of the analysis showed surface tension of 0.040N/m, viscosity of 18.9cP, density of 0.834g/cm^3 for crude oil and 0.0727N/m, 1.1389cP and density of 1g/cm^3 for fresh water samples respectively.

Calculation of Soil Moisture Content:

The moisture content of the sample is calculated using the following equation:

$$\%W = \{(A - B) / B\} \times 100 \quad (4.1)$$

Where:

%W = Percentage of moisture in the sample,

A = Weight of wet sample (grams), and

B = Weight of dry sample (grams)

Volumetric water content, θ, is defined mathematically as:

$$\theta = \frac{V_w}{V_T} \tag{4.2}$$

Where V_w is the volume of water and $V_T = V_s + V_v = V_s + V_w + V_a$ is the total volume (that is soil volume + water volume + air space).

Gravimetric water content is expressed by mass (weight) as follows:

$$u = \frac{m_w}{m_b} \tag{4.3}$$

Where m_w is the mass of water and m_b is the bulk mass.

To convert gravimetric water content to volumetric water, multiply the gravimetric water content by the bulk specific gravity of the material.

Data Analysis:

Calculate the specific gravity of the soil solids using the following formula:

$$\text{Specific Gravity, } G_s = W_o/(W_o + (W_A - W_B)) \tag{4.4}$$

Where:

W_o = weight of sample of oven-dry soil, g = $W_{ps} - W_p$.

W_A = weight of pycnometer filled with water.

W_B = weight of pycnometer filled with water and soil.

SPECIFIC GRAVITY DETERMINATION - DATA SHEET

Date Tested: June 15, 2008

Tested By: UST Lab. Chemical Engineering Department

Research Topic Name: Kinetic Modelling of Enhanced Bioremediation of Hydrocarbon Polluted Soil.

Table 4.1: Sample Number: Erema 1 - Sample Description: Dark-Brown Clay Soil.

Specimen Number	1	2
Pycnometer Bottle Number	96	37
W_P = Mass of empty , clean pycnometer (grams)	37.40	54.51
W_{PS}= Mass of empty pycnometer + dry soil (grams)	63.49	74.07
W_B = Mass of pycnometer + dry soil + water (grams)	153.61	165.76
W_A = Mass of pycnometer + water (grams)	137.37	153.70
Specific Gravity G_S	2.65	2.61

Example Calculation: W_P = 37.40g, W_{PS} = 63.49g, W_B = 153.61g

W_A = 137.37g

W_O = 63.49 – 37.40 = 26.09g

$$\text{Specific Gravity} = \frac{26.09}{26.09 + (137.3 - 153.61)} = 2.65$$

Total microbial colonies were calculated as follows:

$$\text{Plate Count}\left(\frac{\text{cfu}}{\text{ml}}\right) = \frac{(\text{No. of Colonies on Plate}) \times (\text{Dilution Factor})}{\text{Volume Innoculated into Plates}} \qquad 3.7$$

Physico–chemistry (% TOC, pH, particle size, heavy metals) – about 1Kg soil in plastic bags. These samples were stored in a refrigerator at 4^{o}C.

Total Petroleum Hydrocarbon (TPH) – 100 ml glass bottle, the bottles were preheated with phosphate free detergent and rinsed with demineralised water. The samples were stored refrigerated at 4 -10^{O}C ($\pm$ 2).

In this research work, field investigation was used to assess the rate of degradation of crude oil in soil. A detailed review of bioremediation studies undertaken within the last thirty (30) years, when the public outcry against soil pollution arising from hydrocarbon spillages was at its peak constituted the initial step in this study. Also published kinetic models from such bioremediation investigations were collated, analysed and based on their relative advantages selection of the most appropriate model was made.

A specific model was developed on phyto-remediation in consideration of death of biomass (reduction in microbial count) and the final derived equation was substituted into a predominant parameter contained in the selected model equation. The model so developed and other existing models were tested with experimental data obtained from collected soil samples.

Table 4.2: Results for Crude Oil Degradation, Microbial Growth Rate and Product Concentration

Time (Weeks)	Crude Oil Conc. [S] mol%	Specific Rate (mol%/day) V_o	Microbial Conc. (cfu/ml)		1/[S]	$1/V_o$	Product Conc.
			B	F			
0	1.0	-	1000	1000	1.00	-	0.00
3	0.8	0.028	540	370	1.25	35.71	0.00
6	0.6	0.032	$6.4x10^4$	$1.5x10^4$	1.67	31.25	0.15
9	0.4	0.030	$9.7x10^4$	$8.1x10^4$	2.50	33.33	0.37
12	0.2	0.036	$1.3x10^5$	$1.0x10^5$	5.00	27.78	0.68
15	0.1	0.033	$4.1x10^3$	$3.7x10^3$	10.00	30.30	0.93

Where B = Bacteria and F = Fungi

Results presented in Table 4.2 illustrate the experimental and theoretical value obtained from the investigation such as the crude oil concentration per day, specific rate of degradation per day, microbial population of bacteria and fungi per day in (cfu/ml), the rate of product concentration

per day and the establishment of reciprocal microbial growth rate and specific rate of crude oil degradation.

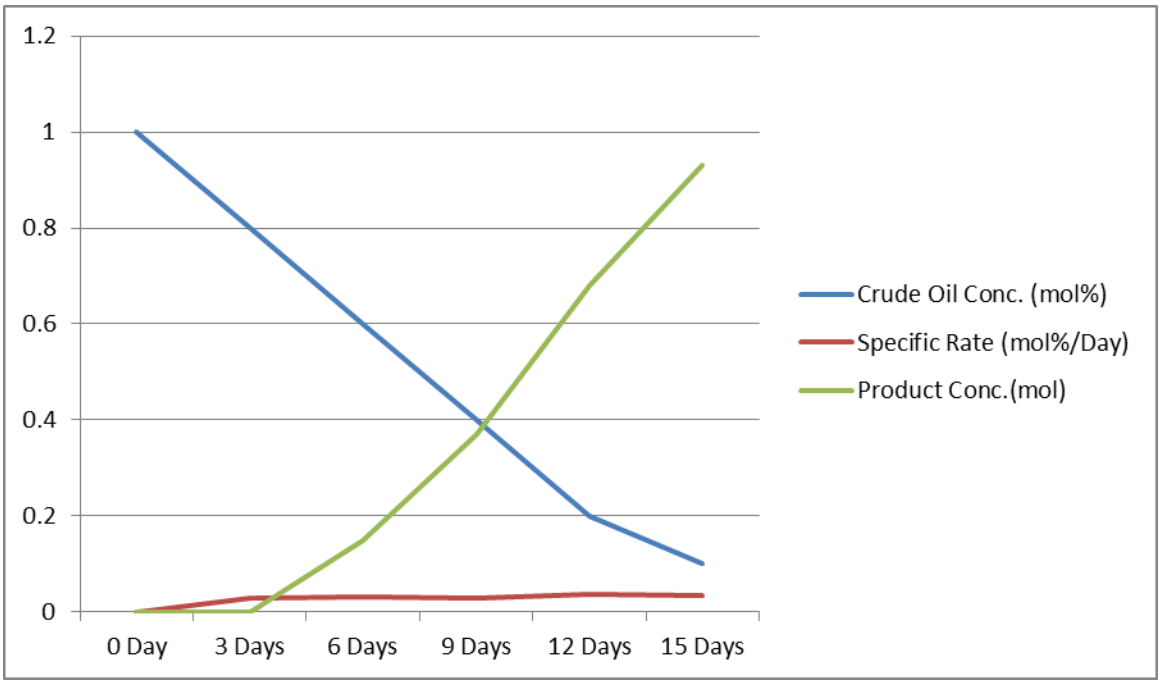

Figure 4.1: Crude Oil Degradation, Specific Rate and Product Concentration

Table 4.3: Crude Oil Degradation Rate in Bio-remedial Treatment and Phyto-remediation Application

Time (Weeks)	Crude Oil Conc. in Soil. (mol%)	Crude Oil Conc. mol% (Phyto-remediation Locoweed)	Crude Oil Conc. mol% (Sunflower)
0	1.00	1.00	1.00
2	0.80	0.80	0.80
4	0.60	0.60	0.60
6	0.40	0.40	0.40
8	0.40	0.35	0.35
10	0.38	0.34	0.35
12	0.38	0.32	0.34
14	0.38	0.28	0.34
16	0.38	0.24	0.34
18	0.37	0.15	0.33
20	0.37	0.10	0.33

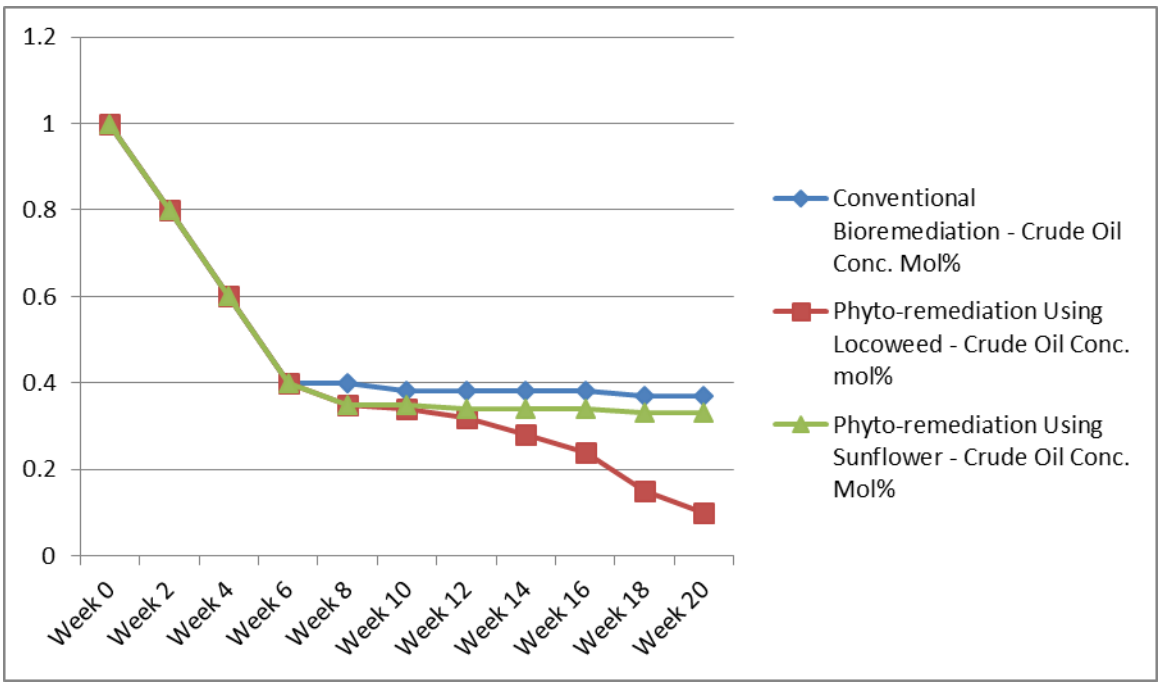

Figure 4.2: Crude Oil Degradation Rate in Polluted Soil Using Bioremediation and Phyto-remediation

The introduction of phyto-remediation method (Figure 4.2) was at the onset of the stationary phase (when there is no further substrate uptake or degradation by the microorganism due to inhibition and death especially because of heavy metals). The regulatory agencies do not permit continued bioremediation treatment of polluted site when further monitoring of the process reveals that there is constant substrate concentration over a period of two years. The main aim of introducing phyto-remediation is to minimize cost by reducing total time for remediation and decreasing substrate concentration below the stationary phase.

Typical periods of bioremediation are usually three to five years depending on substrate concentration and the elements. Additional two years after maximum substrate degradation where no further substrate uptake is significant is permitted before final approval. Below is a

typical performance curve in Figure 4.3 for substrate concentration (S-Conc.) uptake with respect to time. Substrate concentration is usually measured in Parts-Per-Million (PPM).

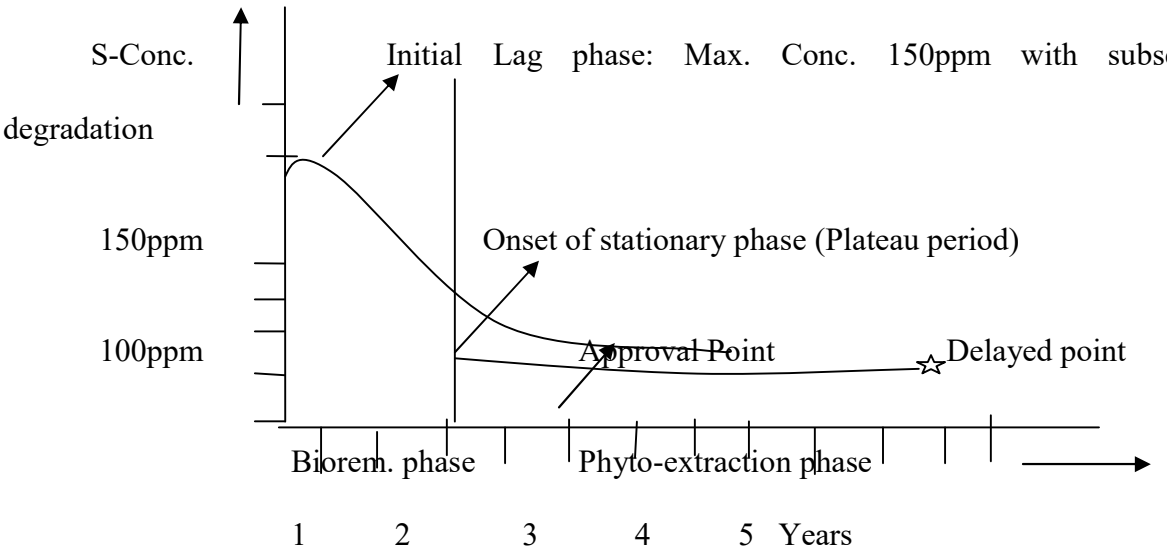

Figure 4.3: Performance Curve for Enhanced bioremediation with Phyto-Remediation

It is important to note that where there is high concentration of contaminant at initial stage, pre-treatment must be taken such as scooping, soaking and thermal desorption, until concentration level is below 60ppm in the soil before remedial action is put in place and subsequent bioremediation augmented with phyto-remediation technique.

Expected results from analytical methods for five to seven years in the first instance, is that the time savings in years can translate into cost savings in excess of fifteen (15) percent in comparison with conventional methods. The modest estimate is carried out considering variation factors, such as inflation, tax and other unforeseen cost.

Table 4.4: Physicochemical Properties of Nigerian Crude

SAMPLES	TEMP. ^0C	pH	Ca^{2+} Hardness (mg/l)	Mg^{2+} Hardness (mg/l)	SO_4^{2-} Dissolved Oxygen (mg/l)	NO_3^{2-} Dissolved Oxygen (mg/l)	Total Dissolbed Solids (g/l)	Density (g/cms)	viscosty	Surface tension
Bonny Light	18-40	7.22	188	44.2	35	35	5.5	0.880	166.6	.036
Bonny Medium	18-40	7.36	164	42.31	33	21.6	5.3	0.714	33.3	0.036

Microbial kinetics on degradation of Nigerian crude oil at different dilution rates of 0.2, 0.4 and 0.6 are shown in Table 4.4 above: The specific growth rate was determined.

Table 4.5: Microbial Growth Rate for Biodegradation of Petroleum In Obite Soil Sample

Time (Day)	MICROBIAL KINETICS (Kcal/h) at [S] Note: Initial Dilution Rate = 0.2									
	$[S]_1$	$[S_{plant}]$	$\square_p$	$\square_{plant}$	$1/[S]_1$	$1/[S]_{plant}$	$\square_p$	$\square_{plant}$	Q_1, Bonny light	Q_2
1	22	33	-	-	0.0455	0.0357	0.0244	0.0122	5.672	2.930
2	50	74	28	44	0.0200	0.0167	0.0110	0.0050	2.834	2.909
3	110	165	60	91	0.0091	0.0074	0.0049	0.0046	1.467	1.465
4	245	368	135	203	0.0041	0.0033	0.0022	0.0012	1.169	1.169
5	546	819	301	451	0.0018	0.0015	0.0010	0.0007	0.975	0.975
6	1215	1823	669	1604	0.008	-	-	-	0.973	0.973
AT DILUTION RATE OF 0.4										
1	18	27	-	-	0.0556	0.0303	-	-	-6.908	-4.30
2	33	49	15	22	0.0303	0.0204	0.0667	0.0455	2.138	2.129
3	60	91	27	42	0.0167	0.0110	0.037	0.024	1.425	1.49
4	110	165	50	74	0.0091	0.0061	00110	0.007	1.428	1.492
5	210	301	91	136	0.005	0.0033	0.011	0.007	0.879	0.87
6	366	549	165	248	0.0027	0.0018	0.006	0.004	0.730	0.73
AT DILUTION RATE OF 0.6										
1	15	22	-	-	0.0667	0.0455	-	-	-8.092	-5.67
2	22	33	7	11	0.0455	0.0303	0.143	0.091	1.325	1.41
3	33	50	11	17	0.0303	0.0200	0.059	0.951	0.988	0.99
4	40	74	16	24	0.0204	0.0135	0.063	0.042	0.708	0.71
5	74	111	25	37	0.0135	0.009	0.040	0.027	0.394	0.59
6	110	163	36	54	0.0091	0.0061	0.028	0.019	0.477	0.478

4.1.1 RESULTS FOR EREMA FIELD SOIL SAMPLES

Results for the Erema soil samples were recorded as treatment rates and the volume of oil applied to 12,550 cm^3 soil sizes with the nutrient supplement see below Table 4.5.

Table 4.6: Treatment Rates and Volume Applied to 12,550 Cm^3 Soil Sizes with Nutrient Supplement

S/NO	TREATMENT TYPES	Rate of nutrient application (g)	Volume of crude oil (ml)	% Pollution
Sample 1	Unpolluted soil	0	0	0
Sample 2	Polluted soil without nutrients	0	416	4
Sample 3	Polluted soil with nutrient (No aeration)	275	416	4
Sample 4	Polluted soil with nutrient and aeration	275	416	4

Results showed that the specific rate for substrate consumption slightly increases with the introduction of phyto-remedial process which is consistent with analysis. Investigative work also shows that because of the relatively high substrate present at the initial degradation process, the optimum point of phyto-remedial introduction is before the decline phase of microbial activities. Results also show that the rate which the micro-organisms consume the substrate (crude oil) is slower without nutrients or aeration and this is consistent with previous work done (Oyoh & Osoka, 2007; Osoka & Onyelucheya, 2010).

Therefore, the introduction of phyto-remedial process can be used to predict degradation time required for bioremediation.

4.1.2 NEW MODEL DEVELOPMENT FOR BIOMASS DEATH

Observation from Table 4.2 reveals a microbial concentration that has a profile which shows that biomass death has set in and which fits the Gaussian model as can be observed in the plot below:

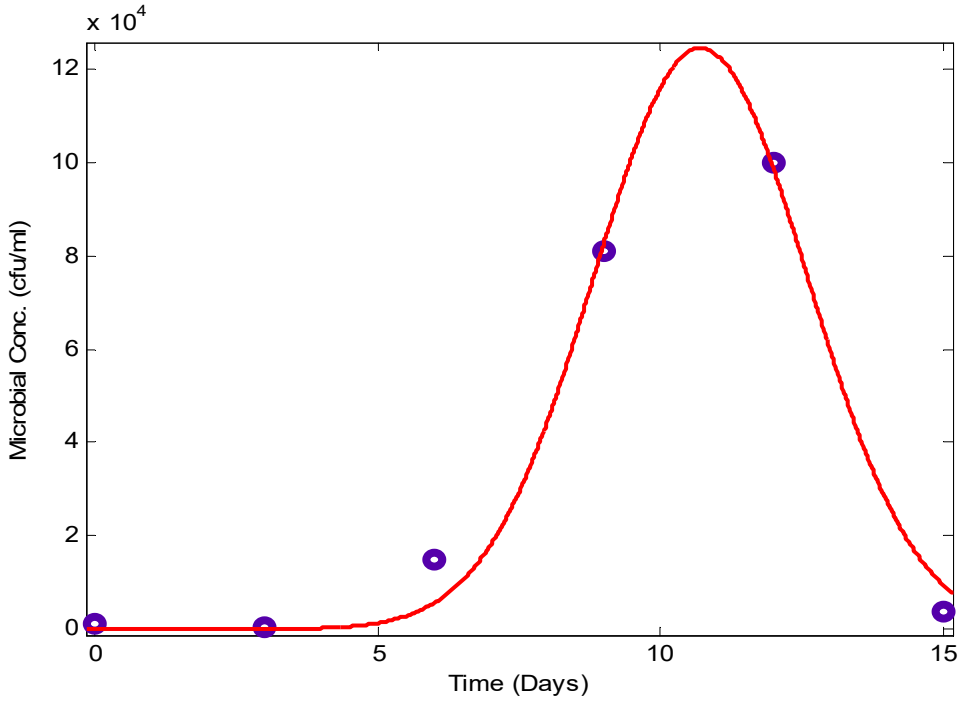

Fig. 4.4: Microbial growth profile with time

General model Gauss1:

$$f(x) = a_1 \exp(-((x - b_1)/c_1)^2) \quad (4.1)$$

Coefficients (with 95% confidence bounds):

a1 = 1.246 e+005

b1 = 10.7

c1 = 2.669

Goodness of fit:

R-square: 0.9873

Adjusted R-square: 0.9788

The data-driven model above can be represented as:

$$x = a\, exp(-(\frac{t-b}{c})^2) \quad (4.2)$$

Osoka and Olebunne (2010) applied a reverse-engineering technique for model development that will be used for this study. On differentiation of equation 4.2, we obtain the equation below

$$\frac{dx}{dt} = \frac{-2}{c^2}\, a\, exp(-\left(\frac{t-b}{c}\right)^2)(t-b) \quad (4.3)$$

Substituting equation (4.2) into (4.3), we obtain equation (4.4) to relate the rate of biomass growth ($\frac{dx}{dt}$) to the biomass concentration, x, at any time,

$$\frac{dx}{dt} = \frac{-2}{c^2}\, x\,(t-b) \quad (4.4)$$

Let the specific growth rate μ be substituted for the constant $\frac{2}{c^2}$ and $k = b$, thus

$$\frac{dx}{dt} = \mu\,(k-t)x \quad (4.5)$$

On re-arranging equation (4.5) we have;

$$\frac{dx}{x} = \mu\,(k-t)\,dt \quad (4.6)$$

Equation (4.6) can be integrated subject to the condition that $x = x_o$ when $t = 0$

$$\ln\frac{x}{x_o} = \mu\, t\,(k - t/2) \quad (4.7)$$

On re-arranging equation (4.7) we have;

$$x = x_o\, \exp\,(\mu\, t\,(k - t/2)) \quad (4.8)$$

Equation (2.7) which relates substrate concentration and biomass concentration at constant yield can be integrated to give;

$$S = S_o + \frac{1}{Y_G}(x_o - x) \quad (4.9)$$

Substituting equation (4.8) -which represents a situation when death of biomass has set in – into equation (4.9), we have;

$$S = S_o + \frac{X_o}{Y_G}(1 - \exp(\mu t(k - t/2))) \quad (4.10)$$

For situations where there is death of biomass and yield is not constant, we substitute equation (4.8) into equation (2.11) to obtain;

$$S = S_o \exp(\mu t(k - t/2))^{\frac{-1}{Y_{mG}}} \quad (4.11)$$

Table 4.7: Summary of the different rate equations based on the nature of biomass growth

S/no	Nature of Biomass Growth	Nature of Yield	Rate Equation	Parameters
1	Exponential Growth (No Inhibition)	Constant Yield	$S = S_0 + \frac{X_0}{Y_G}\left[1 - e^{\mu t}\right]$	$\frac{X_o}{Y_G}, \mu$
2	Exponential Growth (No Inhibition)	Varying Yield	$S = S_o \exp(\mu t)^{\frac{-1}{Y_G}}$	$\frac{1}{Y_G}, \mu$
3	Logistic Growth (with Inhibition)	Constant Yield	$S = S_0 + \frac{X_0}{Y_G}\left[1 - \frac{e^{\mu t}}{1 - \gamma X_0(1 - e^{\mu t})}\right]$	$\frac{X_o}{Y_G}, \mu, \gamma X_o$
4	Logistic Growth (with Inhibition)	Varying Yield	$S = S_o\left(\frac{\exp(\mu t)}{1 - \gamma x_o(1 - \exp(\mu t))}\right)^{\frac{-1}{Y_{mG}}}$	$\frac{1}{Y_G}, \mu, \gamma X_o$
5	Gaussian Growth (with Biomass Death)	Constant Yield	$S = S_o + \frac{X_o}{Y_G} - \exp(\mu t(k - t/2)))$	$\frac{X_o}{Y_G}, \mu, k$
6	Gaussian Growth (with Biomass Death)	Varying Yield	$S = S_o \exp(\mu t(k - t/2))^{\frac{-1}{Y_{mG}}}$	$\frac{1}{Y_{mG}}, \mu, k$

4.1.3: GRAPHICAL FIT RESULTS FOR BIO-REMEDIATION BASED ON THE NATURE OF BIOMASS GROWTH (Data: Table 4.2)

The following graphs show the graphical fits for the bioremediation based on the nature of biomass growth.

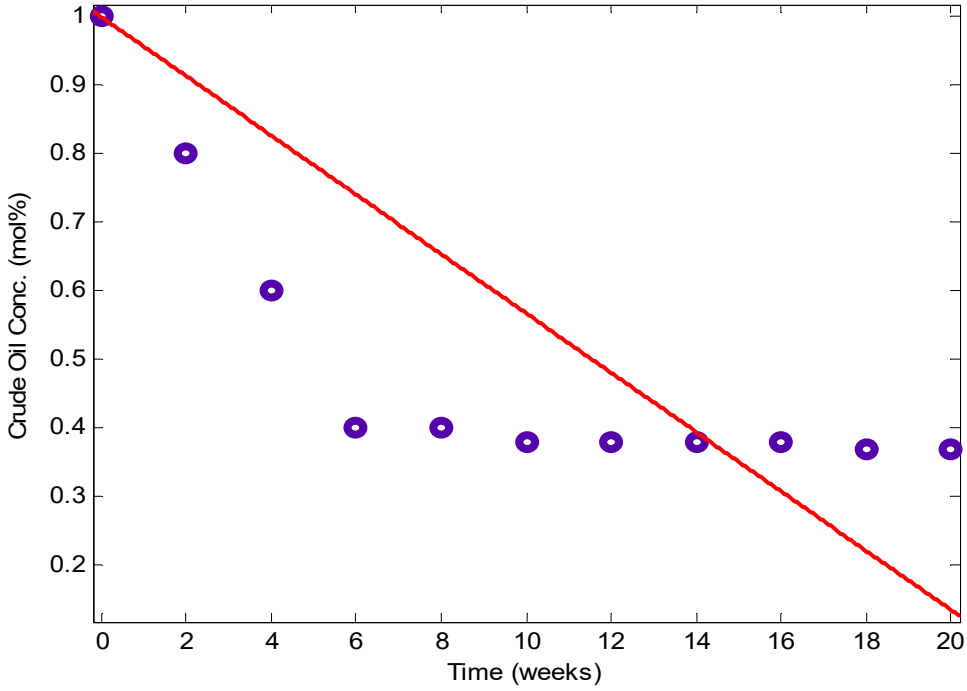

Fig. 4.5: Exponential Growth with Constant Yield fit to Bioremediation Data

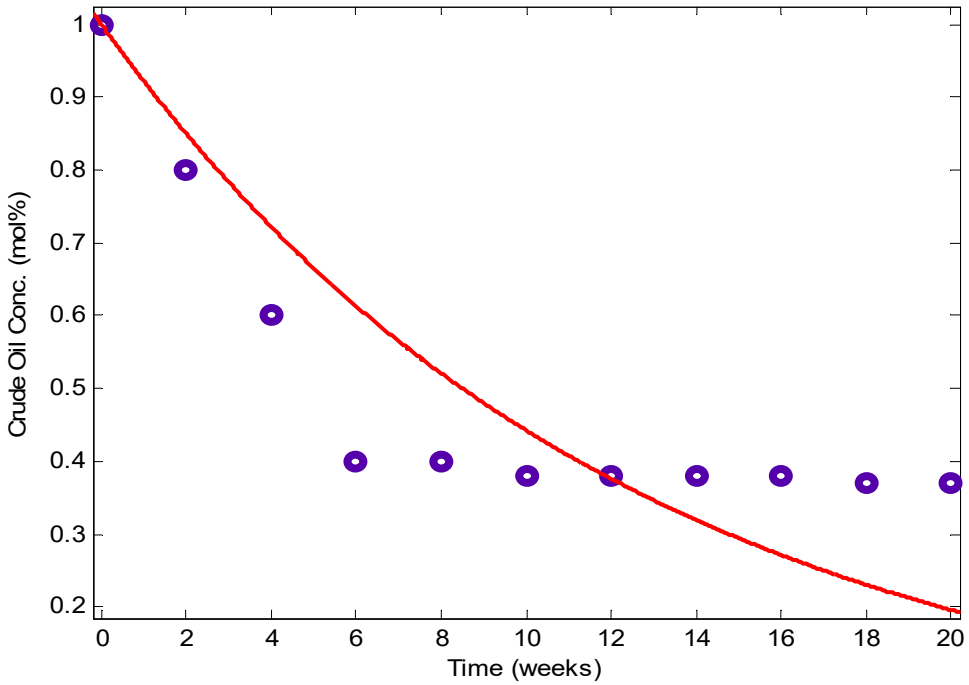

Fig.4.6: Exponential Growth with Varying Yield fit to Bioremediation Data

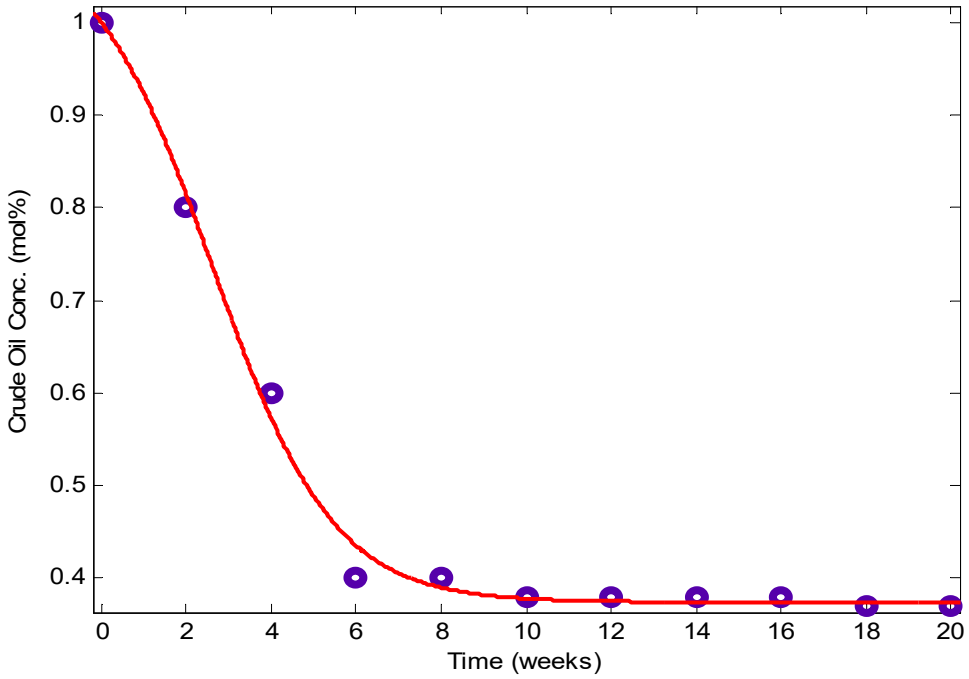

Fig. 4.7: Logistic Growth with Constant Yield fit to Bioremediation Data

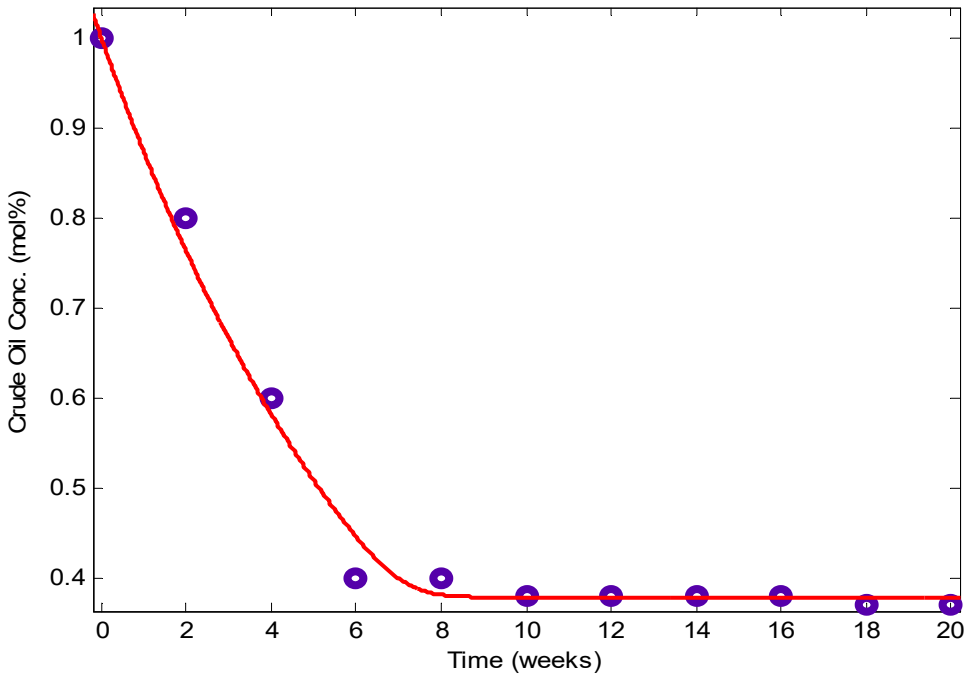

Fig. 4.8: Logistic Growth with Varying Yield fit to Bioremediation Data

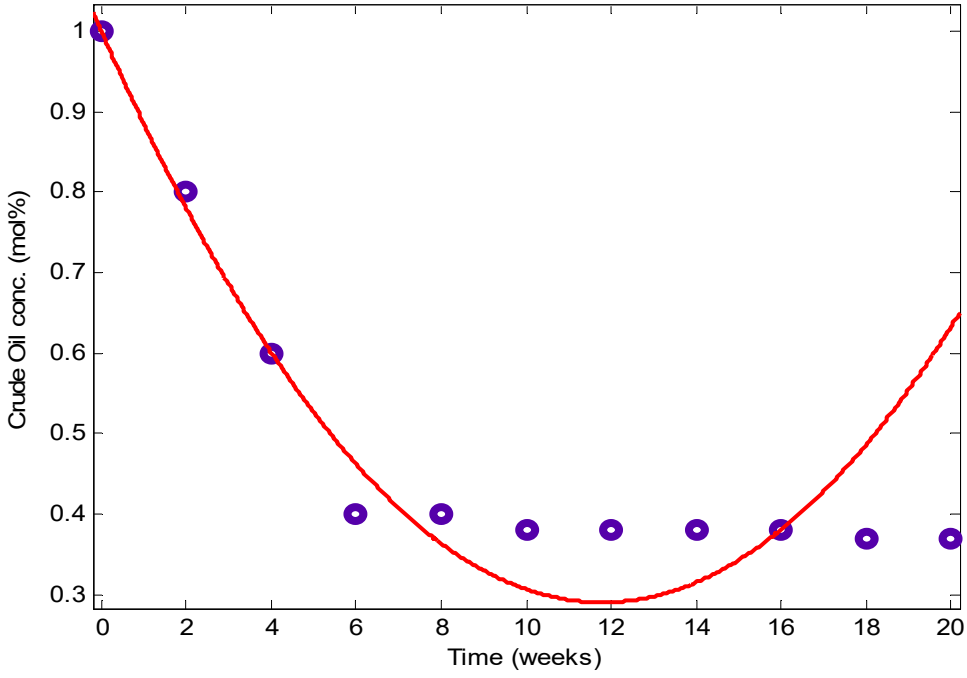

Fig. 4.9: Gaussian Growth with Constant Yield fit to Bioremediation Data

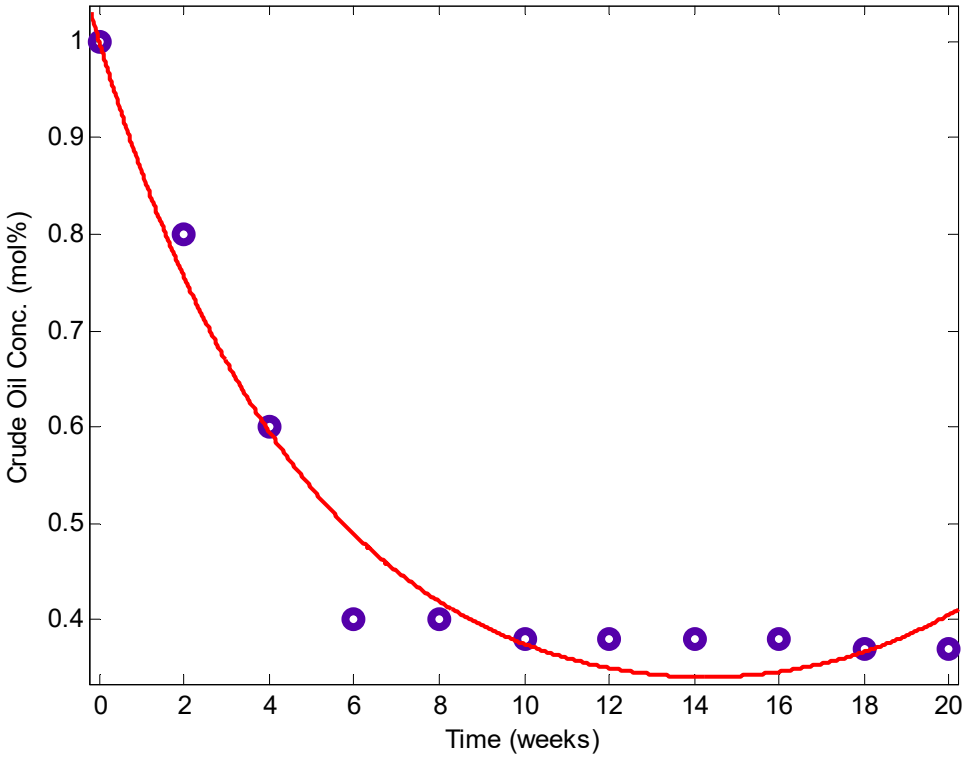

Fig. 4.10: Gaussian Growth with Varying Yield fit to Bioremediation Data

Table 4.8: Numerical Fit Results for Bio-remediation based on nature of Biomass growth.

Rate Model equation	$\frac{X_o}{Y_G}$ or Y_{mG}	μ	γX_o or k	R^2	Adj-R^2
$S = S_0 + \frac{X_0}{Y_G}\left[1 - e^{\mu t}\right]$	723.5	5.972e-5	-	0.1910	0.1012
$S = S_o \exp\ (\mu t)^{\frac{-1}{Y_G}}$	10.03	0.8162	-	0.6823	0.6471
$S = S_0 + \frac{X_0}{Y_G}\left[1 - \frac{e^{\mu t}}{1 - \gamma X_0\left(1 - e^{\mu t}\right)}\right]$	0.09935	0.7036	0.137	0.9948	0.9935
$S = S_o\left(\frac{\exp\ (\mu t)}{1 - \gamma x_o(1 - \exp\ (\mu t))}\right)^{\frac{-1}{Y_{mG}}}$	16.76	2.268	8.46e-8	0.9907	0.9897
$S = S_o + \frac{x_o}{Y_G}\ (-\exp\ (\mu t\,(-t/2)))$	332.7	3.061e-5	11.8	0.7708	0.7135
$S = S_o \exp\ (\mu t\,(k - t/2))^{\frac{-1}{Y_{mG}}}$	42.51	0.499	14.27	0.9670	0.9588

It can be observed from Fig. 4.5 to Fig. 4.10 that the model fit to experimental data is poor for Fig. 4.5, Fig. 4.6, Fig. 4.9 and Fig. 4.10 but good for Fig. 4.7 and Fig. 4.8. The numerical fit results based on the Adjusted-R^2 show that the bioremediation process fit most to the model for Logistic growth with constant yield. It thus suggests that the biomass during bioremediation grow according to the logistic model, with inhibition as the amount of substrate is depleted and the rate of production of biomass per unit substrate consumed is constant, which is in agreement with the constant yield idea.

4.1.4 GRAPHICAL FIT RESULTS FOR SUNFLOWER ASSISTED PHYTOREMEDIATION BASED ON THE NATURE OF BIOMASS GROWTH

The following graphs show the fit for the Sunflower assisted phytoremediation based on the nature of biomass growth.

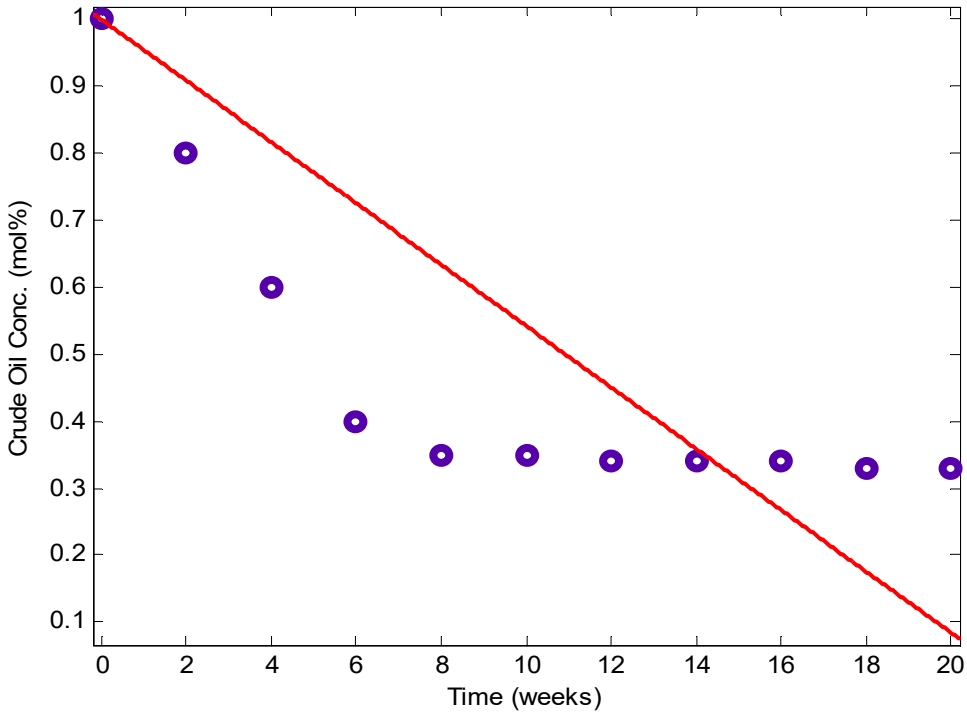

Fig. 4.11: Exponential Growth with Constant Yield Fit for Phytoremediation (Sunflower)

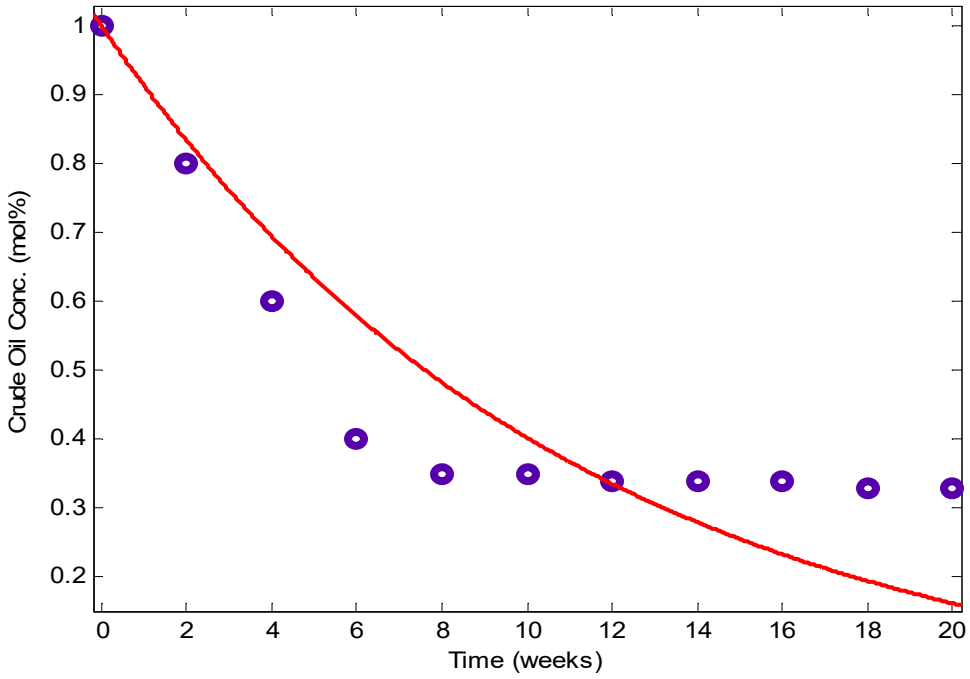

Fig. 4.12: Exponential Growth with Varying Yield Fit for Phytoremediation (Sunflower)

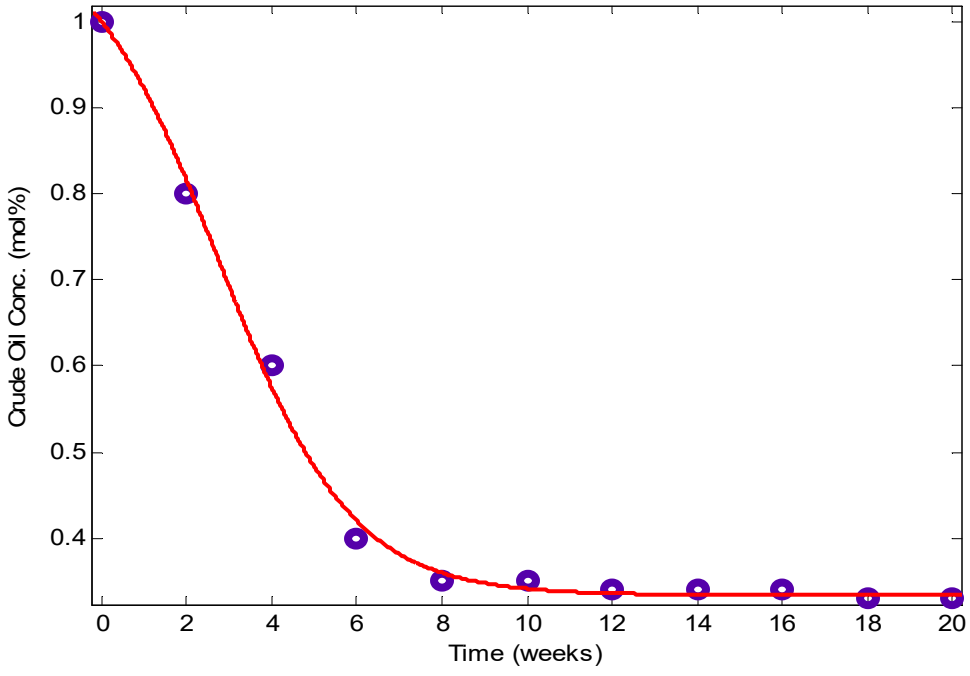

Fig. 4.13: Logistic Growth with Constant Yield Fit for Phytoremediation (Sunflower)

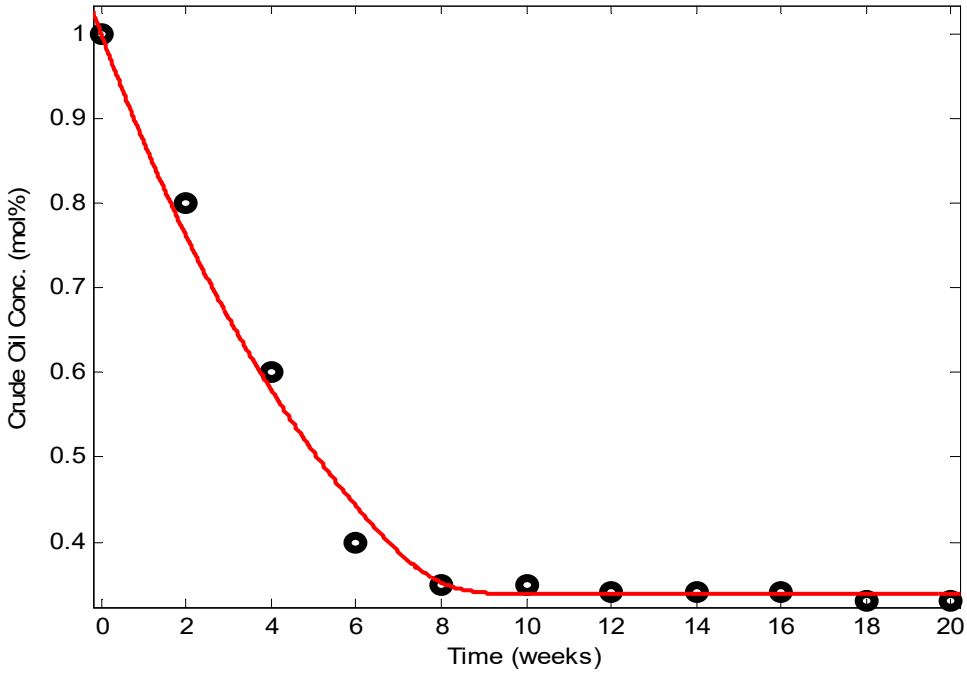

Fig. 4.14: Logistic Growth with Varying Yield Fit for Phytoremediation (Sunflower)

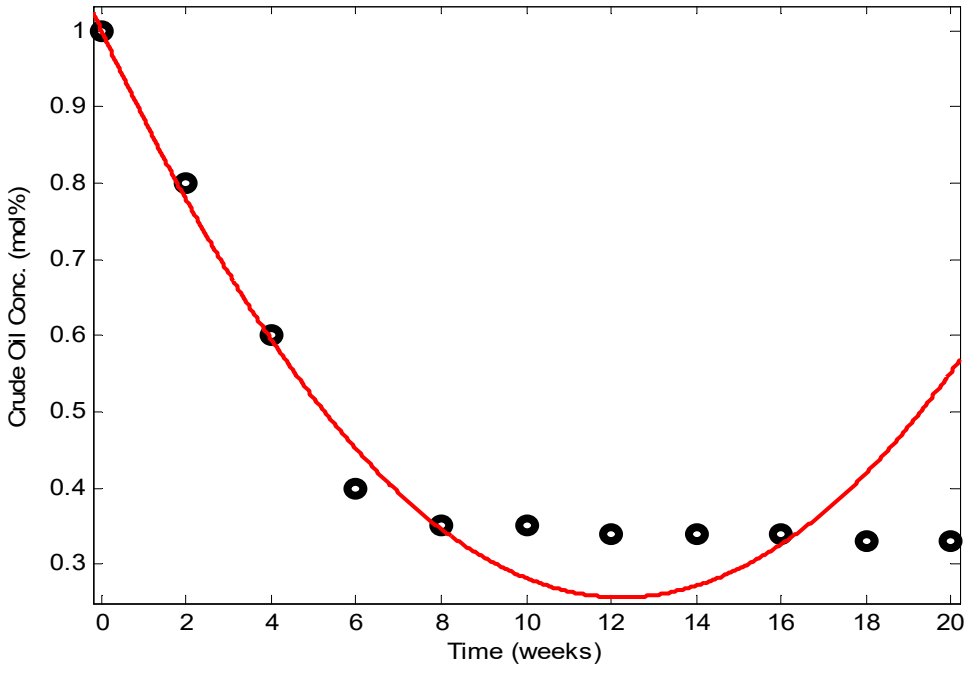

Fig. 4.15: Gaussian model with constant yield Fit for Phytoremediation (Sunflower)

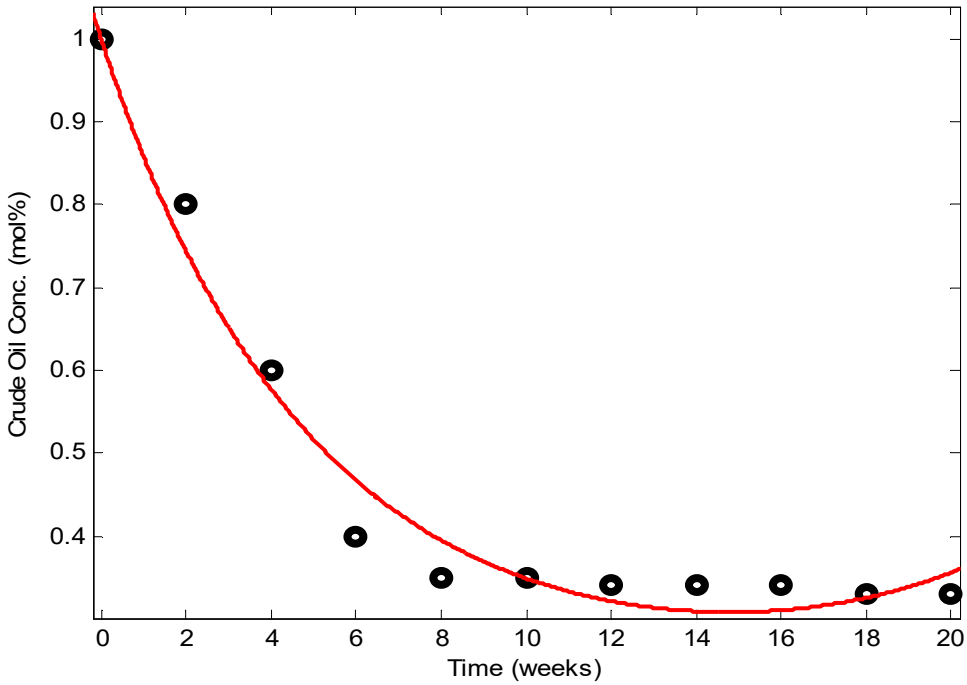

Fig. 4.16: Gaussian model with varying yield Fit for Phytoremediation (Sunflower)

4.1.5 NUMERICAL FIT RESULTS FOR THE SUNFLOWER ASSISTED PHYTOREMEDIATION BASED ON NATURE OF BIOMASS GROWTH.

The numerical fit result summary for the Phytoremediation with Sunflower based on the nature of biomass growth is given in table 4.8 below.

Table 4.9: Numerical fit result for phytoremediation with Sunflower

Rate Model equation	$\frac{X_o}{Y_G}$ or Y_{mG}	μ	γX_o or k	R^2	Adj-R^2
$S = S_0 + \frac{X_0}{Y_G}\left[1 - e^{\mu t}\right]$	1164	3.93e-5	-	0.2756	0.1951
$S = S_o \exp\,(\mu t)^{\frac{-1}{Y_G}}$	8.973	0.8162	-	0.7655	0.7394
$S = S_0 + \frac{X_0}{Y_G}\left[1 - \frac{e^{\mu t}}{1 - \gamma X_0\left(1 - e^{\mu t}\right)}\right]$	0.1160	0.6386	0.1483	0.9971	0.9963
$S = S_o\left(\frac{\exp\,(\mu t)}{1 - \gamma x_o(1 - \exp\,(\mu t))}\right)^{\frac{-1}{Y_{mG}}}$	15.57	2.121	4.58e-8	0.9926	0.9918
$S = S_o + \frac{X_o}{Y_G}\,(1 - \exp\,(\mu t\,(k - t/2)))$	250.9	3.93e-5	12.27	0.8552	0.8190
$S = S_o \exp\,(\mu t\,(k - t/2))^{\frac{-1}{Y_{mG}}}$	44.52	0.4770	14.82	0.9751	0.9689

It can be observed from Fig.4.11 to Fig. 4.16 that the model fit to experimental data is poor for Fig. 4.11, Fig. 4.12, Fig. 4.15 and Fig. 4.16 but good for Fig. 4.13 and Fig. 4.14. The numerical fit results, based on the Adjusted-R^2 show that the phytoremediation augmented bioremediation process using Sunflower fit most to the model for Logistic growth with constant yield. It thus suggests that the biomass during phytoremediation augmented bioremediation process grows according to the logistic model, with inhibition as the amount of substrate is depleted and the rate of production of biomass per unit substrate consumed is constant, which is in agreement with the constant yield idea.

4.1.6 GRAPHICAL FIT RESULTS FOR LOCOWEED ASSISTED PHYTOREMEDIATION BASED ON THE NATURE OF BIOMASS GROWTH

The graphical results for the locoweed assisted phyto-remediation based on the nature of the biomass growth are given in the following graphs.

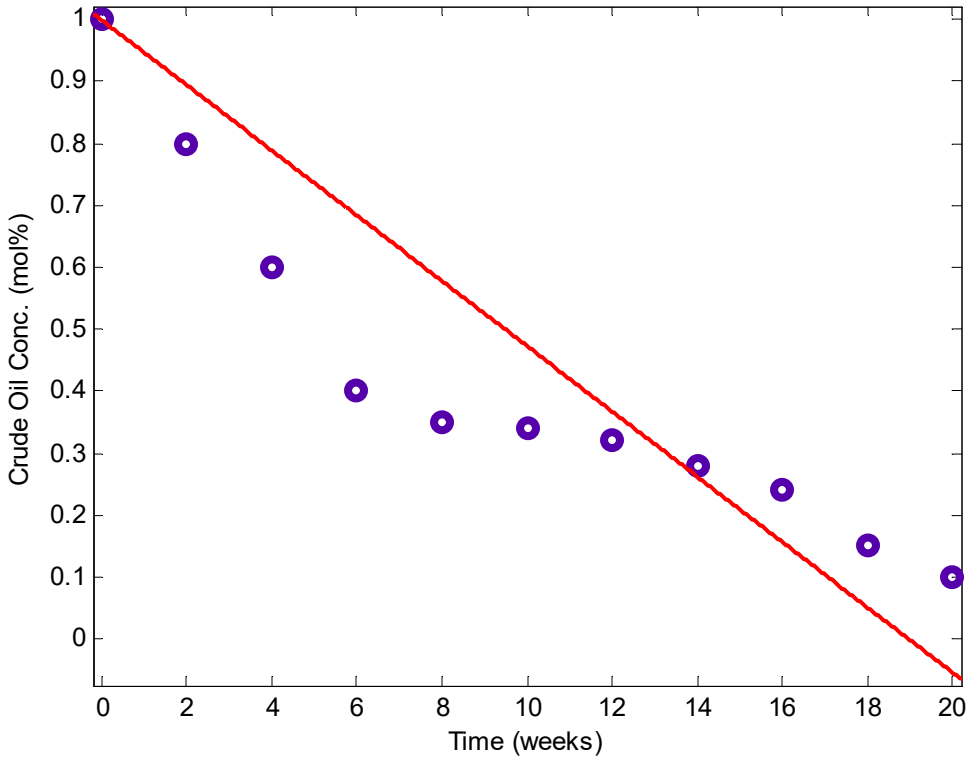

Fig. 4.17: Exponential Growth with Constant Yield Fit for Phytoremediation (Locoweed)

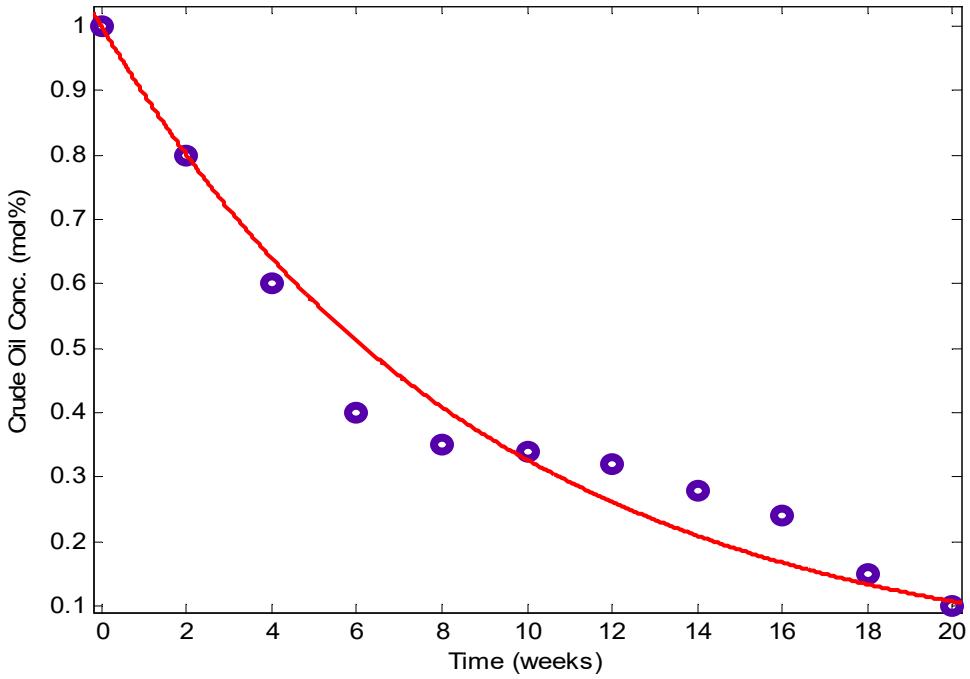

Fig. 4.18: Exponential Growth with Varying Yield Fit for Phytoremediation (Locoweed)

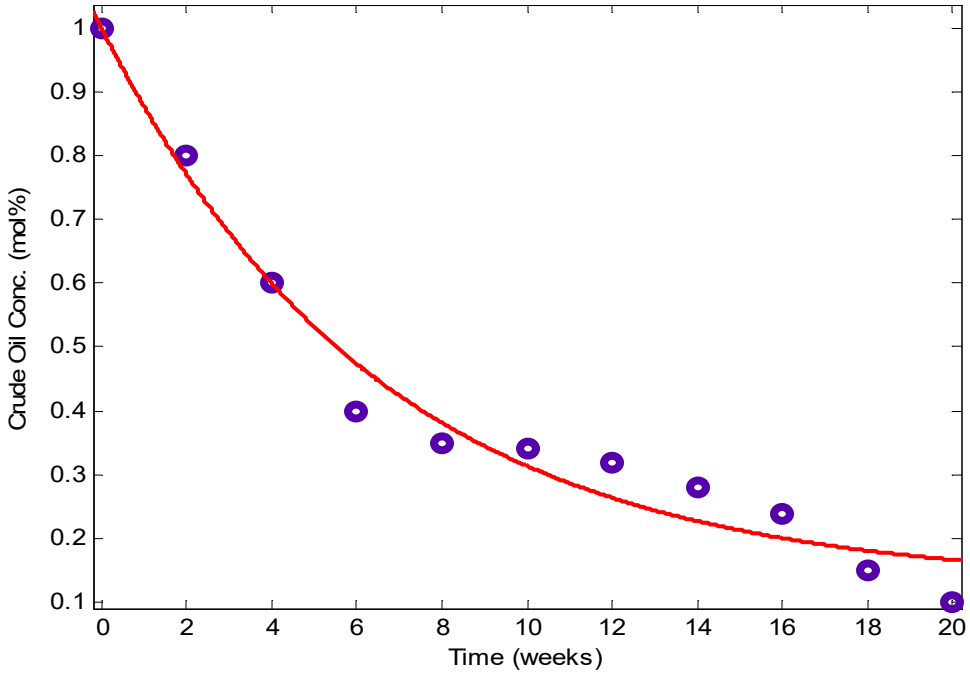

Fig. 4.19: Logistic Growth with Constant Yield Fit for Phytoremediation (Locoweed)

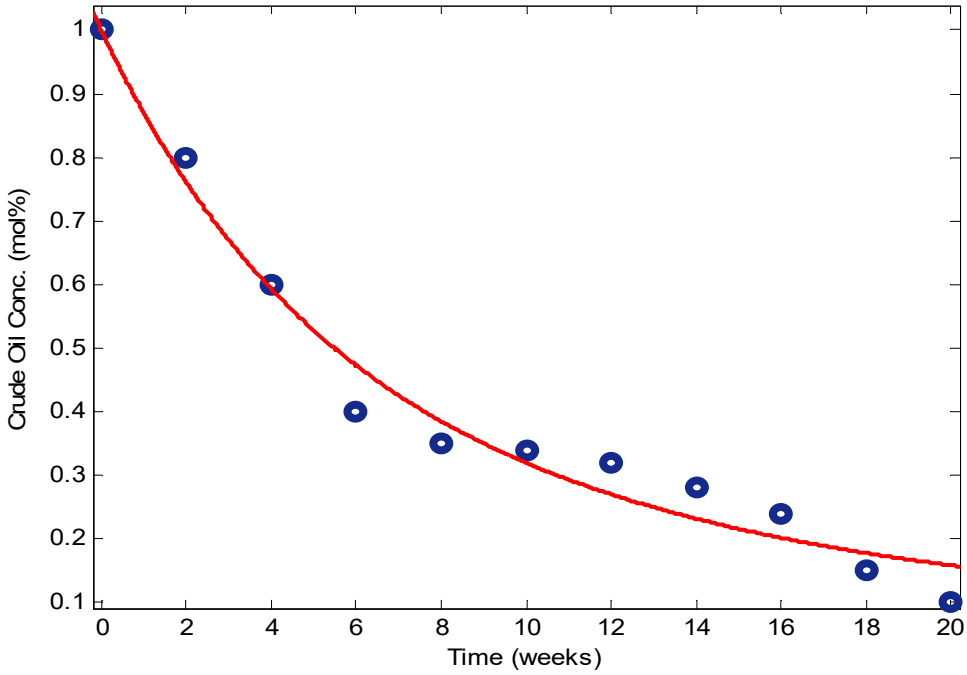

Fig. 4.20: Logistic Growth with Varying Yield Fit for Phytoremediation (Locoweed)

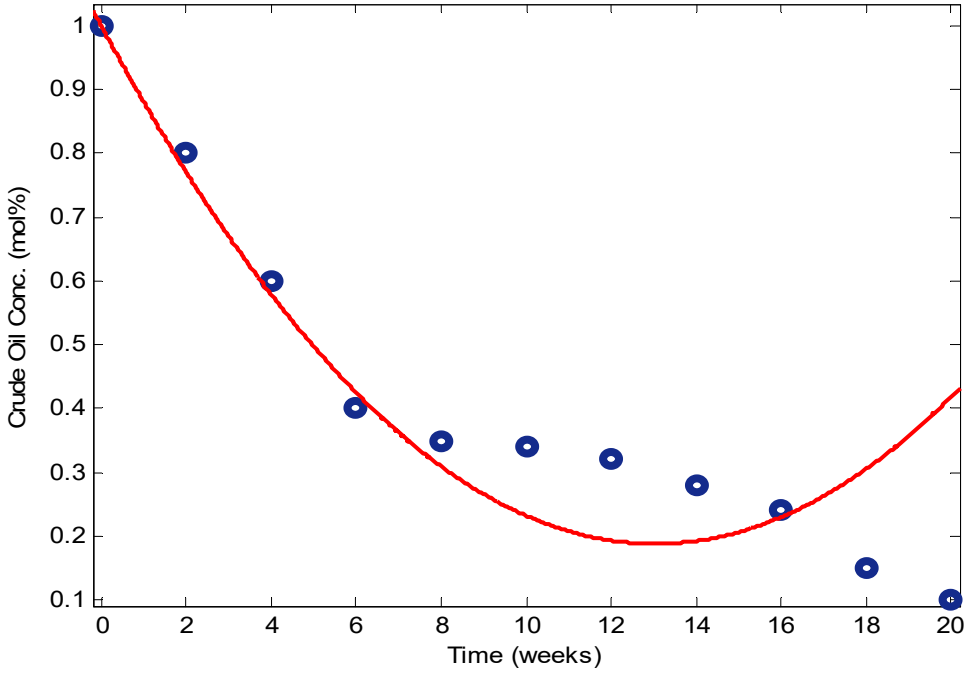

Fig. 4.21: Gaussian model with constant yield Fit for Phytoremediation (Locoweed)

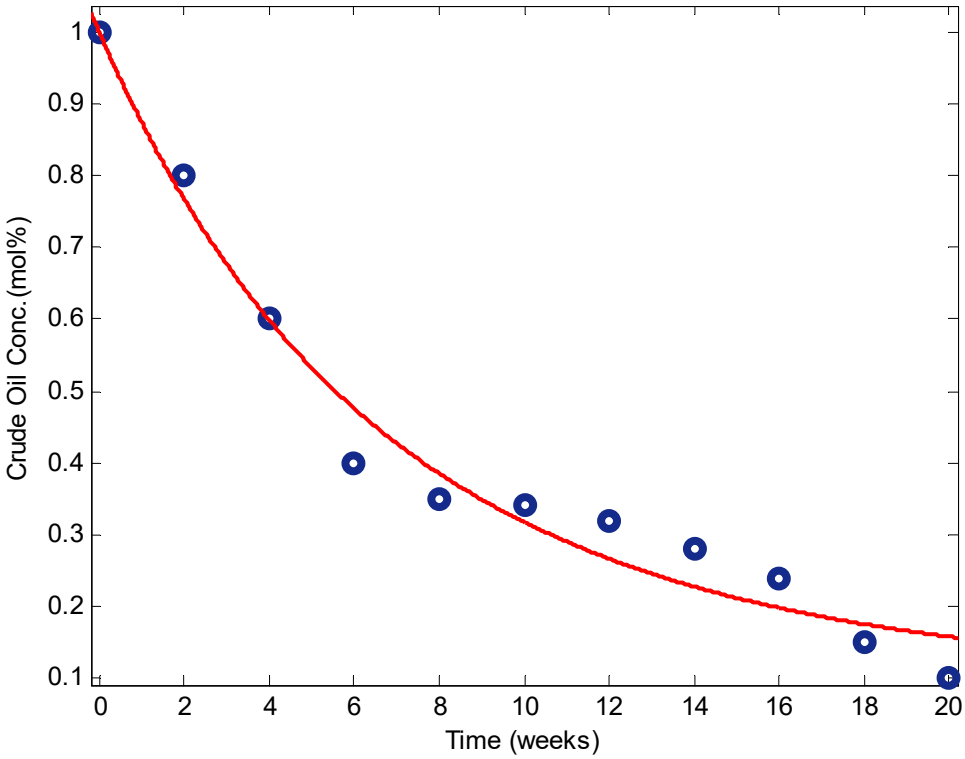

Fig. 4.22: Gaussian model with varying yield Fit for Phytoremediation (Locoweed)

Table 4.10: Numerical Fit Results for the Locoweed assisted Phytoremediation based on the nature of Biomass growth

Rate Model equation	$\frac{X_o}{Y_G}$ or Y_{mG}	μ	γX_o or k	R^2	Adj-R^2
$S = S_0 + \frac{X_0}{Y_G}\left[1 - e^{\mu t}\right]$	660	7.973e-5	-	0.6872	0.6524
$S = S_o \exp\,(\mu t)^{\frac{-1}{Y_G}}$	7.319	0.8162	-	0.9585	0.9538
$S = S_0 + \frac{X_0}{Y_G}\left[1 - \frac{e^{\mu t}}{1 - \gamma X_0\left(1 - e^{\mu t}\right)}\right]$	36.02	0.1562	0.9764	0.9725	0.9656
$S = S_o\left(\frac{\exp\,(\mu t)}{1 - \gamma x_o(1 - \exp\,(\mu t))}\right)^{\frac{-1}{Y_{mG}}}$	0.00681	0.04897	0.9800	0.9750	0.9687
$S = S_o + \frac{X_o}{Y_G}\,(1 - \exp\,(\mu t\,(k - t/2)))$	446.2	2.133e-5	13.09	0.7848	0.7309
$S = S_o \exp\,(\mu t\,(k - t/2))^{\frac{-1}{Y_{mG}}}$	26.68	0.1195	30.58	0.9737	0.9672

It can be observed from Fig.4.17 to Fig. 4.22 that the model fit to experimental data is relatively good for Fig. 4.18, Fig. 4.19, Fig. 4.20 and Fig. 4.22 but poor for Fig. 4.17 and Fig.4.21. The numerical fit results, based on the Adjusted-R^2 show that the phytoremediation augmented bioremediation process using Locoweed fit most to the model for Logistic growth with varying yield, though the fit does not significantly differ from that of logistic growth with constant yield. This suggests that the biomass growth during phytoremediation augmented bioremediation

process can also be seen as growing according to the logistic model, with inhibition as the amount of substrate is depleted and the rate of production of biomass per unit substrate consumed is constant, which is in agreement with the constant yield idea, in consistency with the previous ones.

4.1.7 GRAPHICAL FIT RESULTS FOR BIO-REMEDIATION BASED ON KINETIC EQUATION MODELS FOR VARIOUS REACTION ORDERS

The graphical fit results for bioremediation based on kinetic equation models for various reaction orders are shown in the following figures.

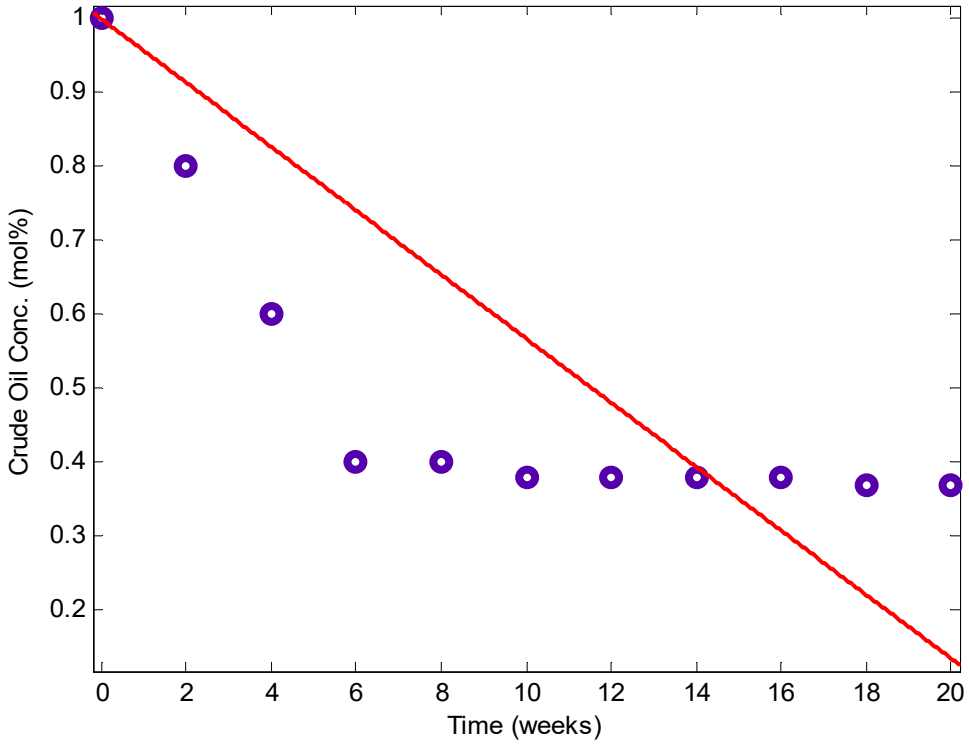

Fig. 4.23: Zero Order Model fit to Bio-remediation data

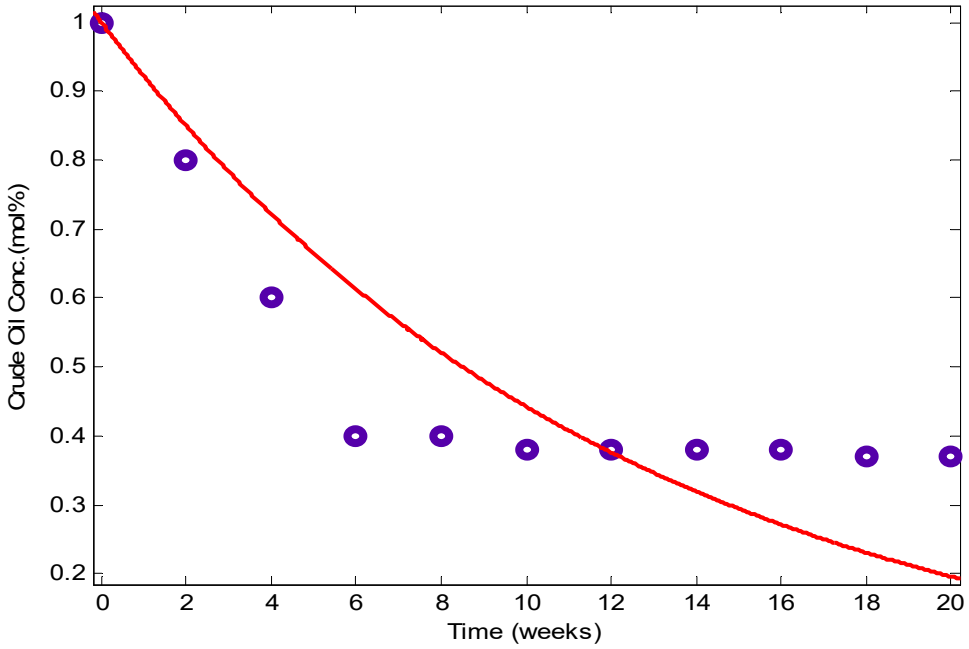

Fig. 4.24: First Order Model fit to Bio-remediation data ($S_\infty = 0$)

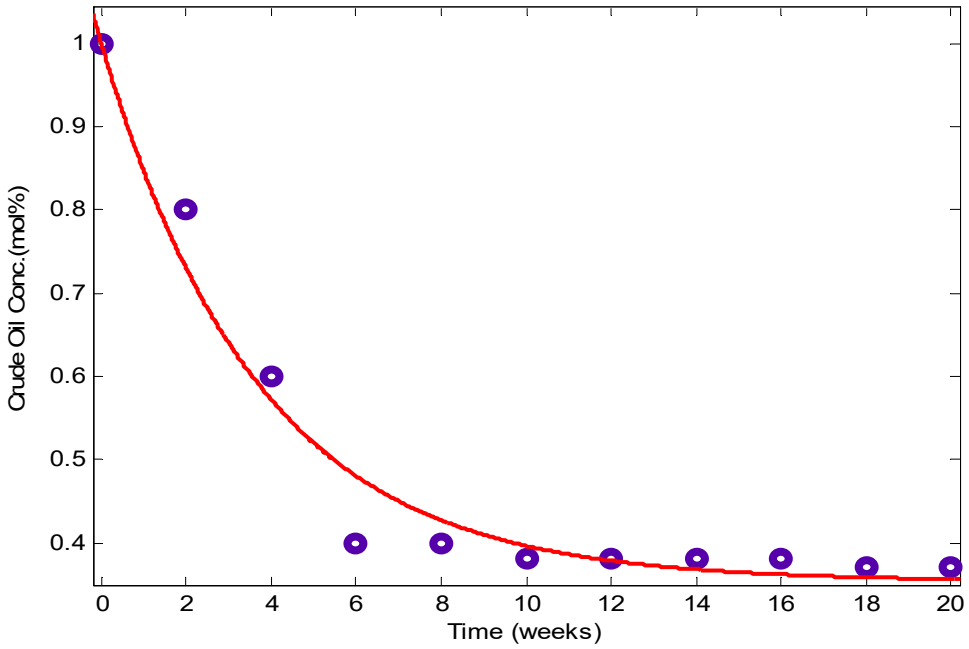

Fig. 4.25: First Order Model fit to Bio-remediation data ($S_\infty > 0$)

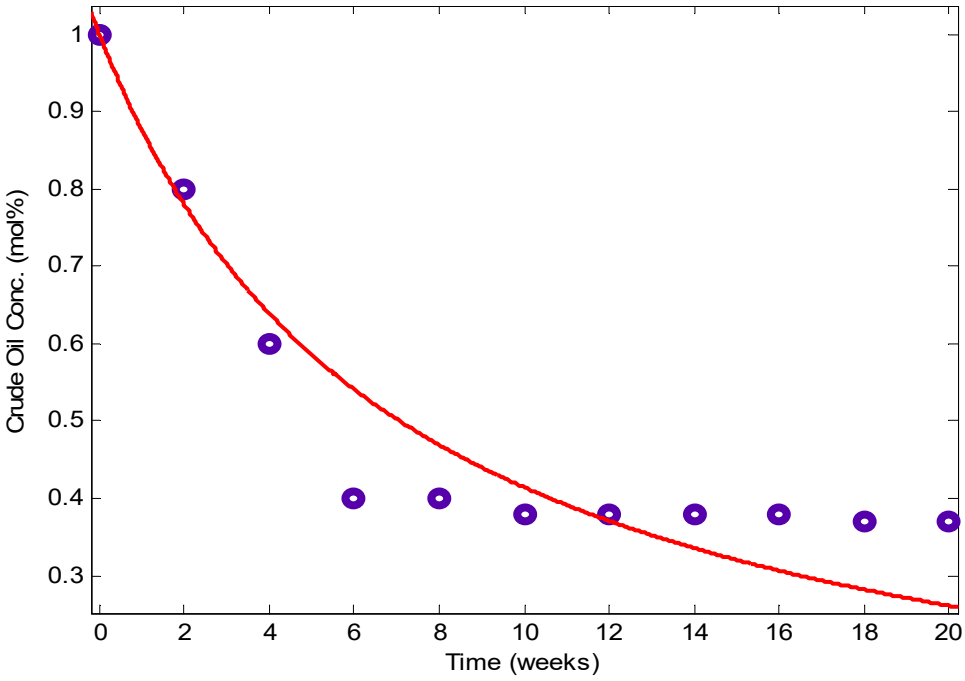

Fig. 4.26: Second Order Model fit to Bio-remediation data ($S_\infty = 0$)

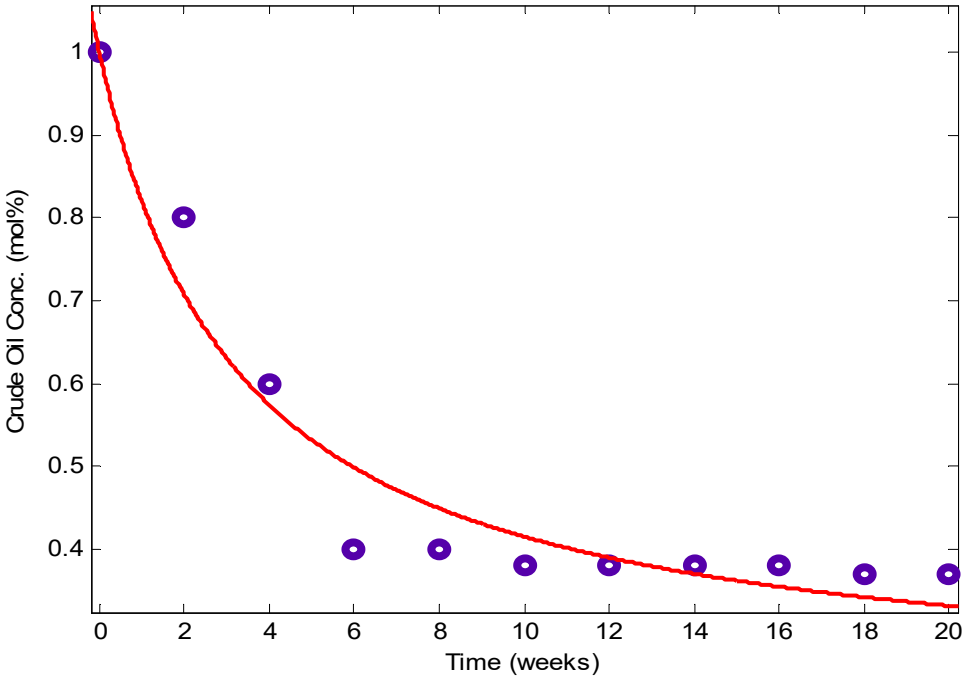

Fig. 4.27: Second Order Model fit to Bio-remediation data ($S_\infty > 0$)

4.1.8 NUMERICAL FIT RESULTS FOR BIO-REMEDIATION

The summary of the numerical fit result for the bioremediation based on kinetic equations for different reaction orders is given in table 4.51 below.

Table 4.11: Numerical fit result summary of the different reaction orders for bioremediation

Kinetic equation	k	S∞	R^2	Adj-R^2	RMSE	SSE
$S = S_o - k\,t$ (Zero Order)	0.04323	-	0.1916	0.1916	0.1931	0.3729
$S = S_o \exp(-k\,t)$ (First Order)	0.08137	-	0.6823	0.6823	0.1210	0.1465
$S = S_\infty + (S_o - S_\infty)\exp(-k\,t)$ First Order	0.2712	0.3535	0.9695	0.9661	0.03954	0.01407
$S = \frac{S_0}{1+S_0\,k\,t}$ (Second Order)	0.1412	-	0.8812	0.8812	0.07401	0.05478
$S = \frac{S_o + S_\infty(S_o - S_\infty)k\,t}{1 + (S_o - S_\infty)\,k\,t}$ Second Order	0.3856	0.2208	0.9443	0.9381	0.05344	0.02571

It can be observed from Fig. 4.23 to Fig. 4.27 that Fig. 4.25 and Fig. 4.27 which represent First Order and Second order reactions with an ultimate substrate concentration greater than zero (S∞> 0) respectively fit well to the experimental data, while other models had poor fits. The numerical fit results reveal that the First Order reaction with an ultimate substrate concentration

greater than zero (S∞> 0) gave the best fit. Thus suggesting that bioremediation is a first order reaction.

4.1.9 GRAPHICAL FIT RESULTS FOR PHYTOREMEDIATION USING LOCOWEED BASED ON THE KINETIC EQUATION FOR DIFFERENT REACTION ORDERS

The graphical results for the locoweed assisted phyto-remediation based on the kinetic equation for different reaction orders are given in the following graphs.

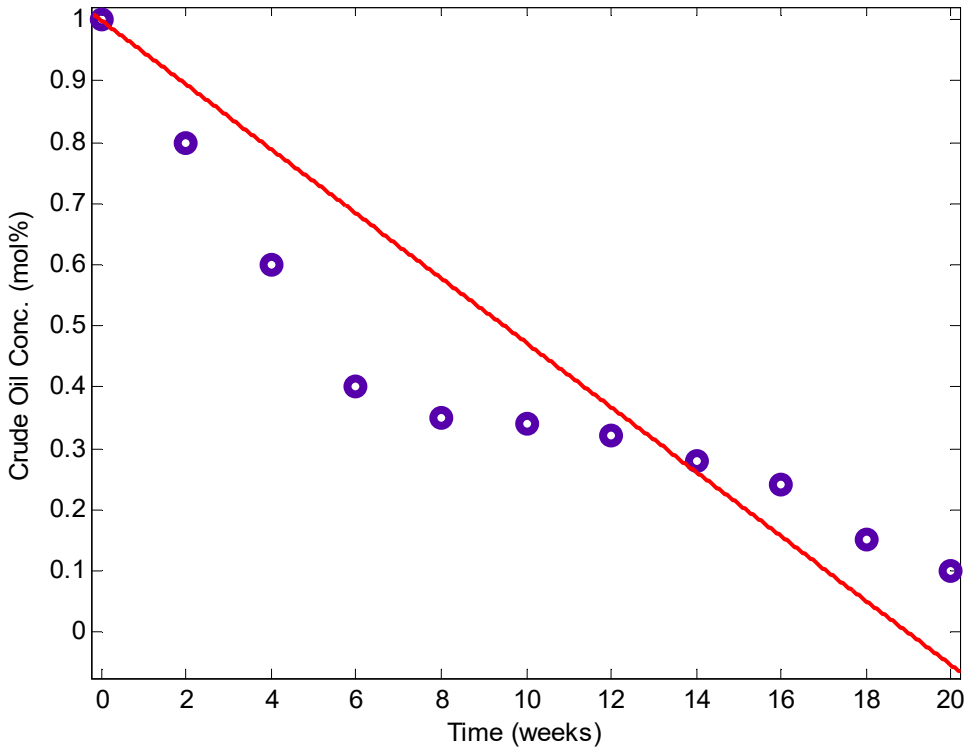

Fig. 4.28: Zero Order Model fit to phyto-remediation using Locoweed

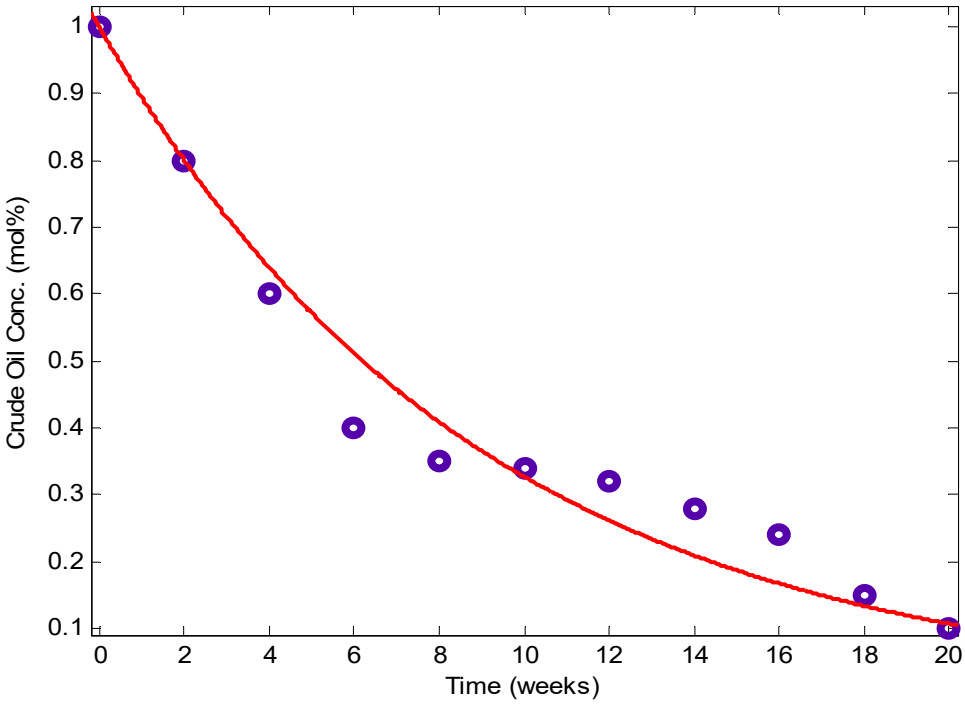

Fig. 4.29: First Order Model fit to phyto-remediation using Locoweed (S∞=0)

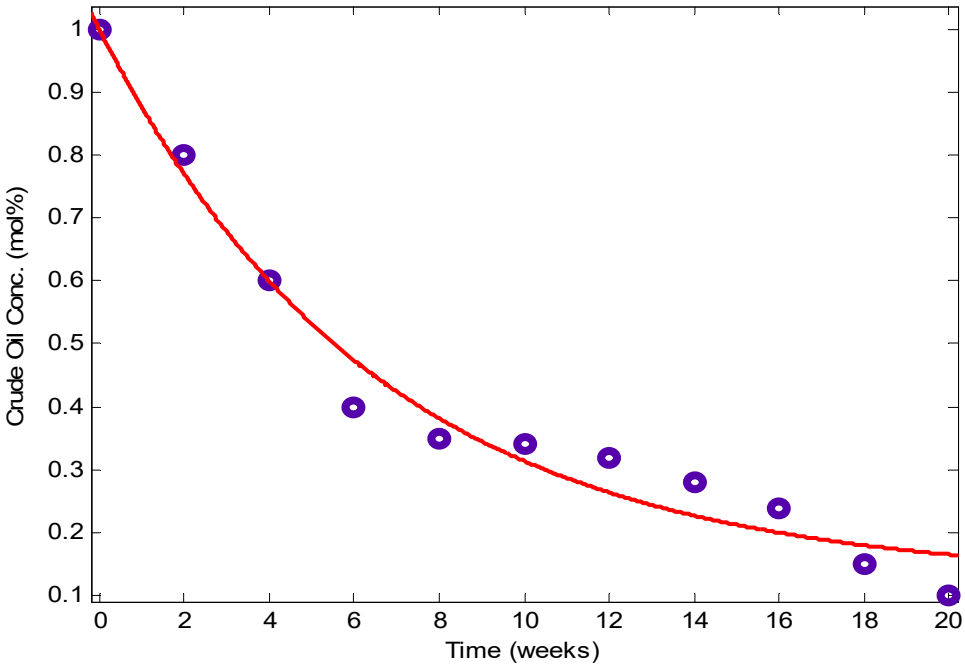

Fig. 4.30: First Order Model fit to phyto-remediation using Locoweed (S∞>0)

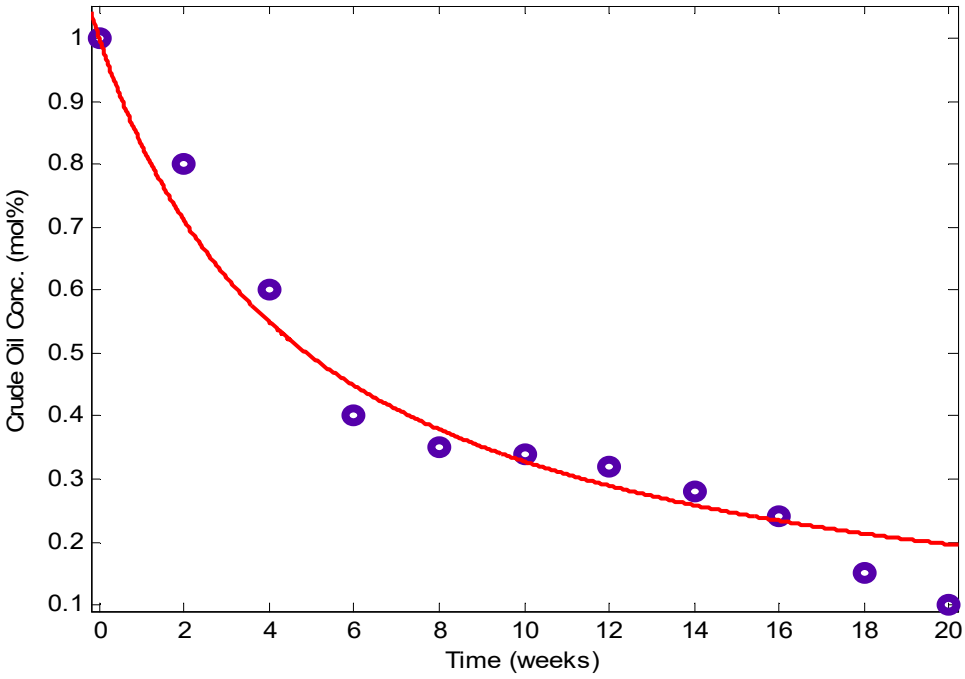

Fig. 4.31: Second Order Model fit to phyto-remediation using Locoweed (S∞=0)

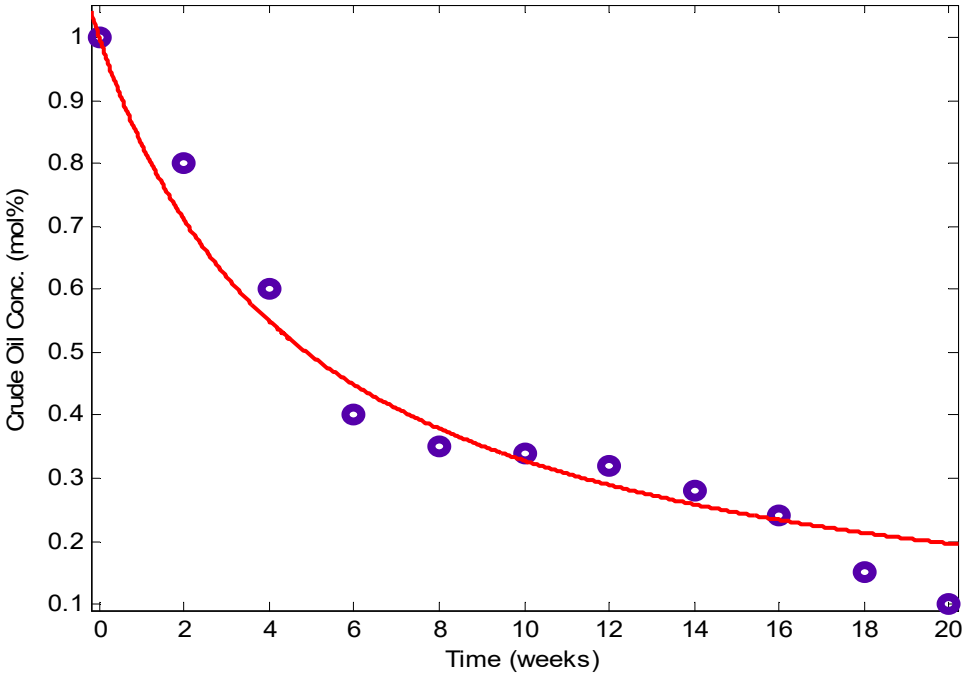

Fig. 4.32: Second Order Model fit to phyto-remediation using Locoweed (S∞>0)

Table 4.12: Numerical Fit Results for Locoweed Enhanced Phytoremediation Based on Kinetic Models for Different reaction orders.

Kinetic equation	k	S∞	R^2	Adj-R^2	RMSE	SSE
$S = S_o - k\,t$ (Zero Order)	0.05266	-	0.6876	0.6876	0.1543	0.2381
$S = S_o \exp(-k\,t)$ (First Order)	0.1115	-	0.9585	0.9585	0.05626	0.03166
$S = S_\infty + (S_o - S_\infty)\exp(-k\,t)$ First Order	0.1527	0.1241	0.9727	0.9696	0.04811	0.02083
$S = \frac{S_o}{1+S_o\,k\,t}$ (Second Order)	0.2045	-	0.9622	0.9622	0.05367	0.02881
$S = \frac{S_o + S_\infty(S_o - S_\infty)k\,t}{1 + (S_o - S_\infty)\,k\,t}$ Second Order	0.2045	2.121e-11	0.9622	0.9622	0.05367	0.02881

It can be observed from Fig. 4.28 to Fig. 4.32 that Fig. 4.29, Fig. 4.30, Fig. 4.31 and Fig. 4.32 which represent First Order and Second order reactions respectively fit well to the experimental data, while the zero model had a poor fit. The numerical fit results reveal that the First Order reaction with an ultimate substrate concentration greater than zero (S∞> 0) gave the best fit. Thus suggesting that bioremediation is a first order reaction and the ultimate substrate concentration will never go to zero.

4.1.10 GRAPHICAL FIT RESULTS FOR SUNFLOWER ENHANCED PHYTO-REMEDIATION BASED ON KINETIC MODEL FOR DIFFERENT REACTION ORDERS

The graphical results for the sunflower enhanced phyto-remediation based on the kinetic equation for different reaction orders are given in the following graphs.

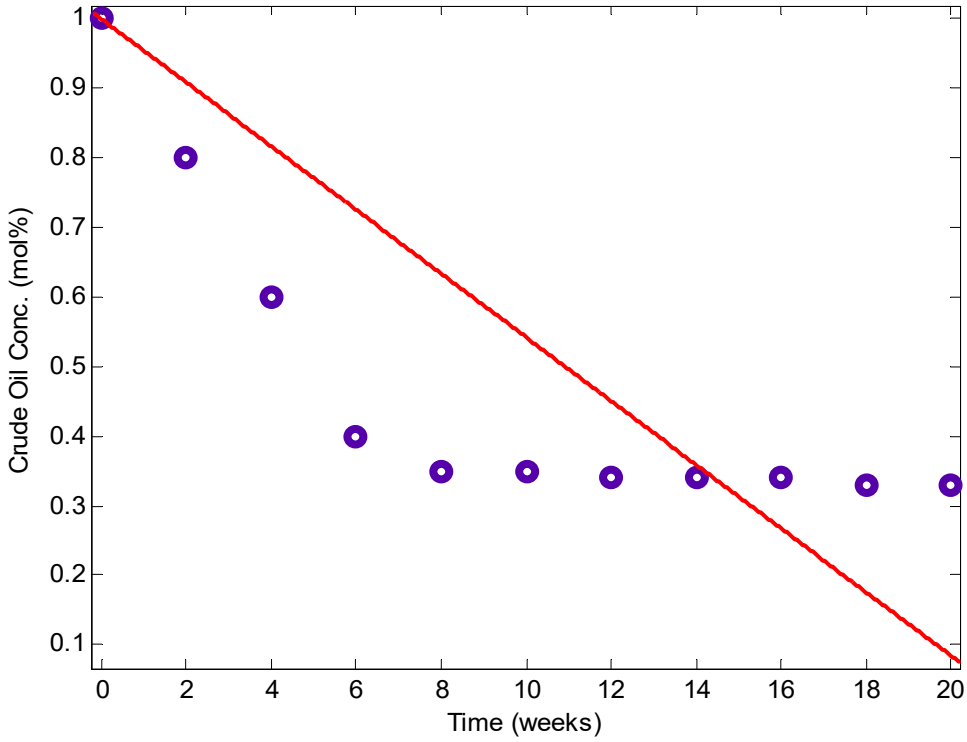

Fig. 4.33: Zero Order Model fit to phyto-remediation using Sunflower

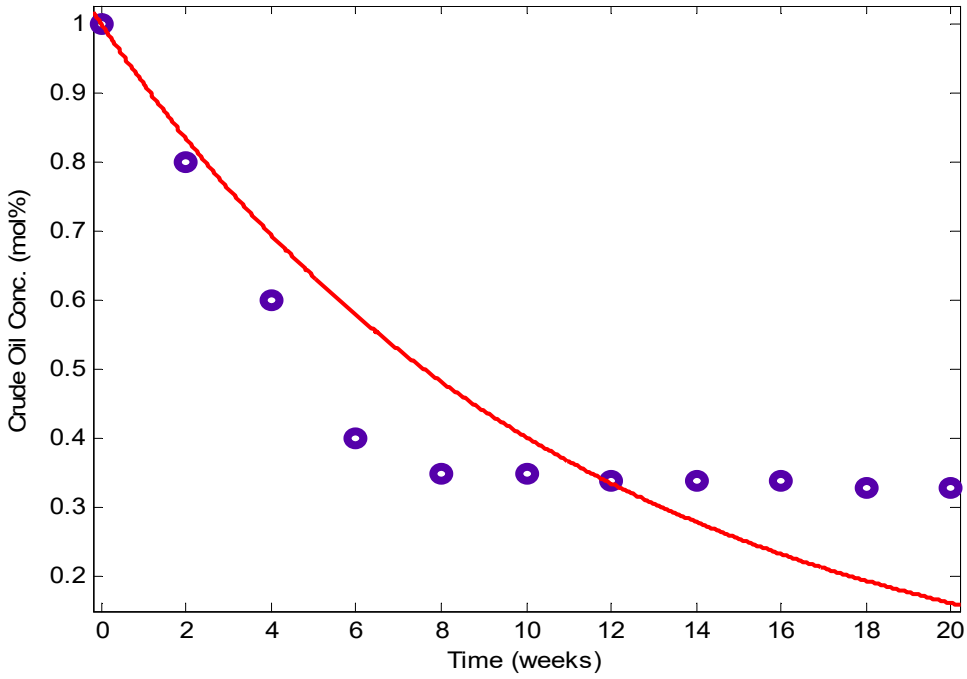

Fig. 4.34: First Order Model fit to phyto-remediation using Sunflower ($S\infty = 0$)

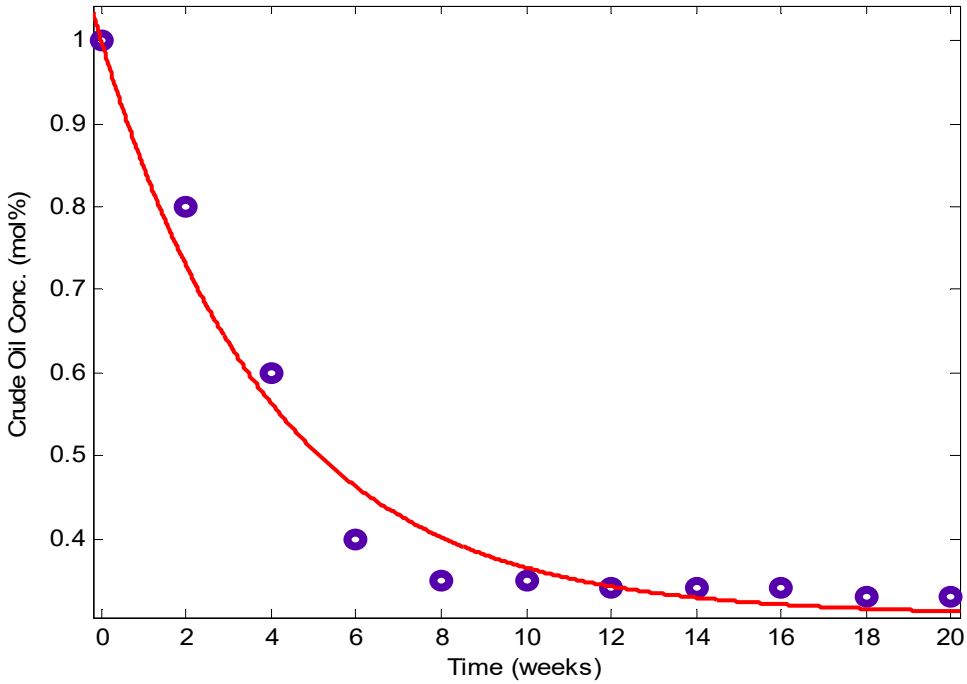

Fig. 4.35: First Order Model fit to phyto-remediation using Sunflower ($S\infty > 0$)

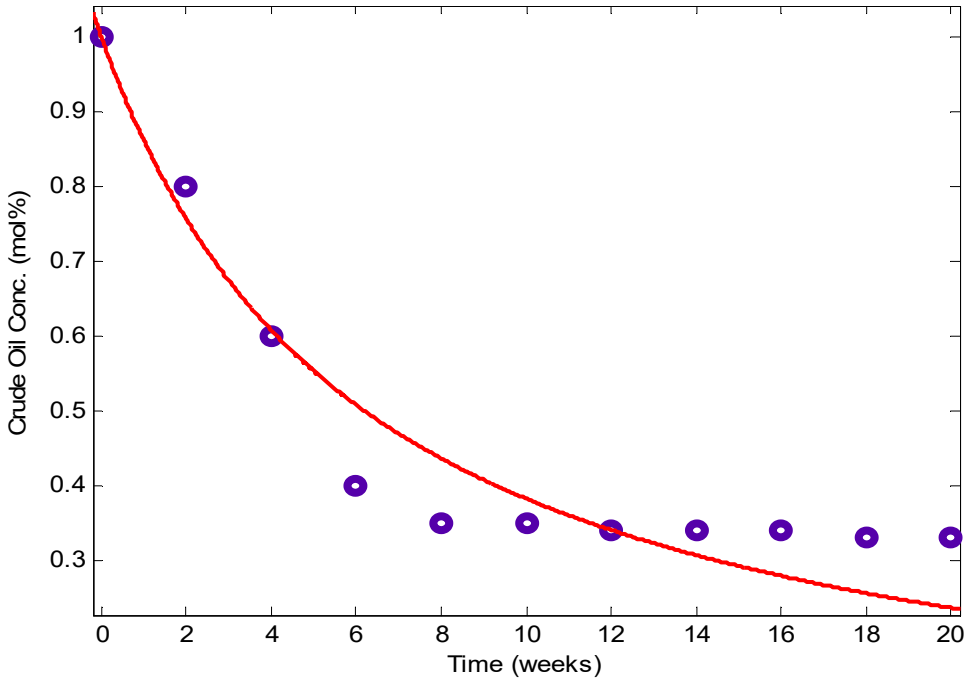

Fig. 4.36: Second Order Model fit to phyto-remediation using Sunflower ($S\infty = 0$)

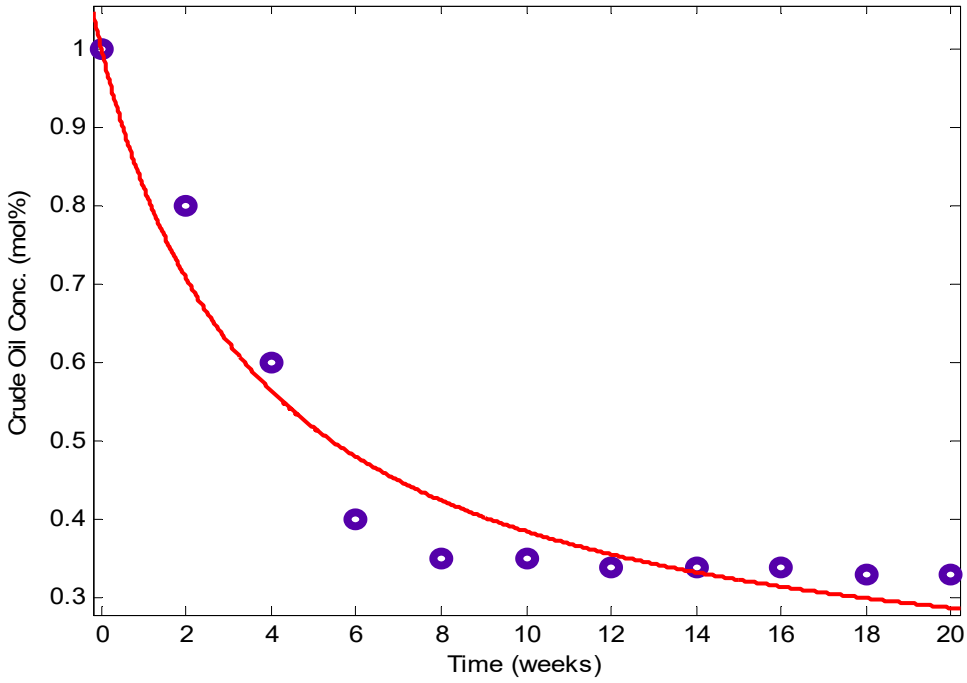

Fig. 4.37: Second Order Model fit to phyto-remediation using Sunflower ($S\infty > 0$)

Table 4.13: Numerical Fit Results for Sunflower enhanced Phytoremediation based on the Kinetic Models for different reaction orders.

Kinetic equation	k	S∞	R^2	Adj-R^2	RMSE	SSE
$S = S_o - k\,t$ (Zero Order)	0.04577	-	0.2759	0.2759	0.1960	0.3840
$S = S_o \exp(-k\,t)$ (First Order)	0.09095	-	0.7655	0.7655	0.1115	0.1244
$S = S_\infty + (S_o - S_\infty)\exp(-k\,t)$ First Order	0.2493	0.3082	0.9728	0.9698	0.04002	0.01441
$S = \frac{S_o}{1+S_o\,k\,t}$ (Second Order)	0.1612	-	0.9223	0.9223	0.0642	0.04121
$S = \frac{S_o + S_\infty(S_o - S_\infty)k\,t}{1+(S_o - S_\infty)\,k\,t}$ Second Order	0.3127	0.1530	0.9494	0.9437	0.05462	0.02685

It can be observed from Fig. 4.33 to Fig. 4.37 that Fig. 4.35 and Fig. 4.37 which represent First Order and Second order reactions with the ultimate substrate concentration being greater than zero (S∞> 0) respectively fit well to the experimental data, while the other models have a poor fit. The numerical fit results reveal that the First Order reaction with an ultimate substrate concentration greater than zero (S∞> 0) gave the best fit. Thus suggesting that bioremediation is a first order reaction and the ultimate substrate concentration will never go to zero.

It can be observed that the First order model fit well to both Bioremediation and Phytoremediation processes with the ultimate substrate concentration never going to zero. The summary of the fit is given in table 4.13 below.

Table 4.14: First Order Model Fit Results for all Three Processes

Remediation Process	k	S∞	R^2	Adj-R^2	RMSE	SSE
Bioremediation	0.2712	0.3535	0.9695	0.9661	0.03954	0.01407
Phytoremediation (Locoweed)	0.1527	0.1241	0.9727	0.9696	0.04811	0.02083
Phytoremediation (Sunflower)	0.2493	0.3082	0.9728	0.9698	0.04002	0.01441

The first order model obtained from this study thus agrees with the first order assumption based on which equations (2.36) to (2.38) were obtained.

We have from our study:

$$S = S_\infty + (S_o - S_\infty)\exp(-k\,t) \qquad 2.39$$

$$\left(\frac{S-S_\infty}{S_o-S_\infty}\right) = \exp(-k\,t) \qquad 2.40$$

$$kt = -\ln\left(\frac{S-S_\infty}{S_o-S_\infty}\right) \qquad 2.41$$

$$t = -\ln\left(\frac{S-S_\infty}{S_o-S_\infty}\right) / k \qquad 2.42$$

Equation (2.42) gives the time to achieve a specified reduction in contaminant concentration using Bioremediation or phytoremediation.

Comparison of equation (2.38) and equation (2.42) gives:

$$\left(\frac{M}{M_o}\right) = \left(\frac{S - S_\infty}{S_o - S_\infty}\right) \qquad 2.43$$

Since the kinetic model obtained in this work can be used to obtain k and S∞ (the first order rate constant and ultimate substrate concentration respectively), the value of k can be substituted into equation (2.36) to obtain U (uptake rate of contaminant) as:

$$U = kM_o = k\,(S_o - S_\infty) \qquad 2.44$$

The value of U can be substituted into equation (2.35) to obtain TSCF (Transpiration Stream Concentration Factor) as:

$$TSCF = \frac{U}{(T)(C)} \qquad 2.45$$

Where, T and C are Transpiration rate of vegetation (l/day) and ground water (mg/l) respectively to be obtained experimentally.

Thus, the kinetic model obtained in this work can be used to determine the relevant phytoremediation parameters and constants.

4.1.11 STATISTICAL ANALYSIS (OBITE SOIL SAMPLE) (Data: Table 4.4)

The Analysis of Variance (ANOVA) for effect of Time and Dilution Rate on Bioremediation and other statistical analysis are given in the following tables and graphs.

Table 4.15: Analysis of variance for effect of time and dilution rate on bioremediation.

Source	Sum Sq.	d. f.	Mean Sq.	F	Prob> F
X_1	3.08964	3	1.02988	15.85	0.0029
X_2	1.21588	2	0.60794	9.36	0.0143
Error	0.38987	6	0.06498		
Total	4.69539	11			

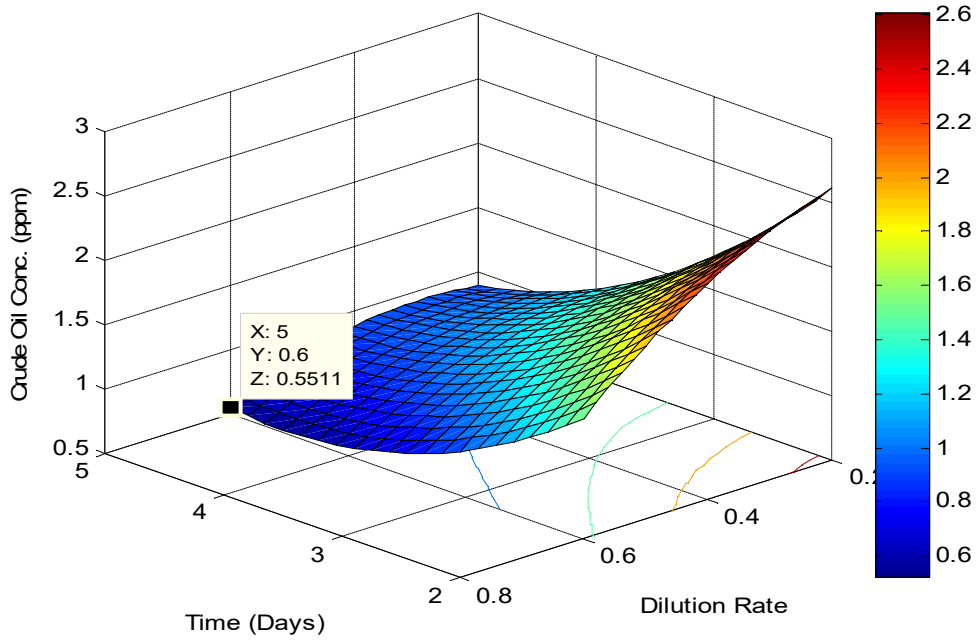

Fig 4.38: Interaction of Remediation time and dilution rate with Contaminant concentration.

Table 4.16: Response Surface Model Statistics

Variables	Coefficients	Std. Error	t-stat	P-val	F-stat
Constant	5.5316	0.88249	6.2682	0.00766	Sse = 0.22738
Time	-1.6826	0.41525	-4.0520	0.0067109	Dfe = 6
Dilution Rtae	-1.1455	2.63890	-0.43409	0.6794	Dfr = 5
Time*Dil. Rate	0.7005	0.30780	2.2758	0.063163	Ssr = 4.468
Time^2	0.1383	0.056197	2.4616	0.049015	F = 23.579
Dilution Rate^2	-4.000	2.9803	1.3421	0.2281	p-val = 0.00070511
	R-sq. = 0.9516	Adj.R-Sq. =0.9112			

$Y = a_1+a_2*x_1+a_3*x_2+a_4*x_1*x_2+a_5*x_1^2+a_6*x_2^2$

The Analysis of variance table above represents Time as x_1 and Dilution rate as x_2. A variable is significant if the Prob>f value is less than or equal to 0.05 (using 95% confidence interval). Dilution rate and time are significant in bioremediation. The surface plot reveals that there is a slightly quadratic relationship between these two variables and substrate concentration and the contours lines are non-parallel lines showing that these two factors interact. It also reveals that increase in time and dilution rate reduce substrate concentration. The Response surface model statistics reveal that a quadratic model fit well to the relation between time, dilution rate and substrate concentration. The t-statistics value reveal that the constant term, time, Time*Dilution

rate and time^2 are the significant variables in the model since the absolute values of their t-stat are equal to or greater than 2, these variables are also significant at 95% confidence based on the p-val except for time*dilution rate which is significant at 90% confidence. The F-statistics shows that the model is adequate with a p-val of 0.0007 and the R^2 shows that the model explains 95% of the observed variability in the experimental data.

Table 4.17: Values used for Response Surface Model (Obite Sample)

Time (Days)	Dilution Rate	Substrate Conc. (Bonny Light)
2	0.2	2.834
3	0.2	1.467
4	0.2	1.169
5	0.2	0.975
2	0.4	2.138
3	0.4	1.425
4	0.4	1.128
5	0.4	0.879
2	0.6	1.325
3	0.6	0.988
4	0.6	0.708
5	0.6	0.394

4.1.12 STATISTICAL ANALYSIS OF OTHER PARAMETERS THAT CHANGE DURING BIOREMEDIATION.

The statistical analyses from the fit of other parameters and how they vary during the bioremediation are given below.

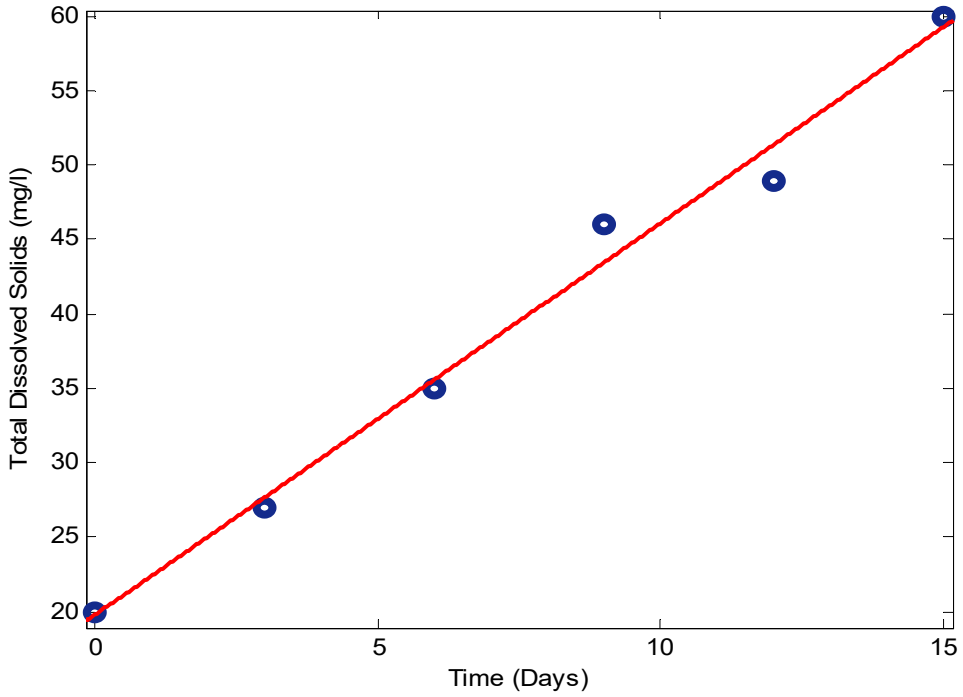

Fig. 4.39: Total Dissolved Solid variation with time during Bioremediation

Linear model Poly1:

$f(x) = p_1*x + p_2$

Coefficients (with 95% confidence bounds):

$p_1 =$ 2.638 $p_2 =$ 19.71

Goodness of fit:

SSE: 13.37 RMSE: 1.828

R-square: 0.9879 Adjusted R-square: 0.9849

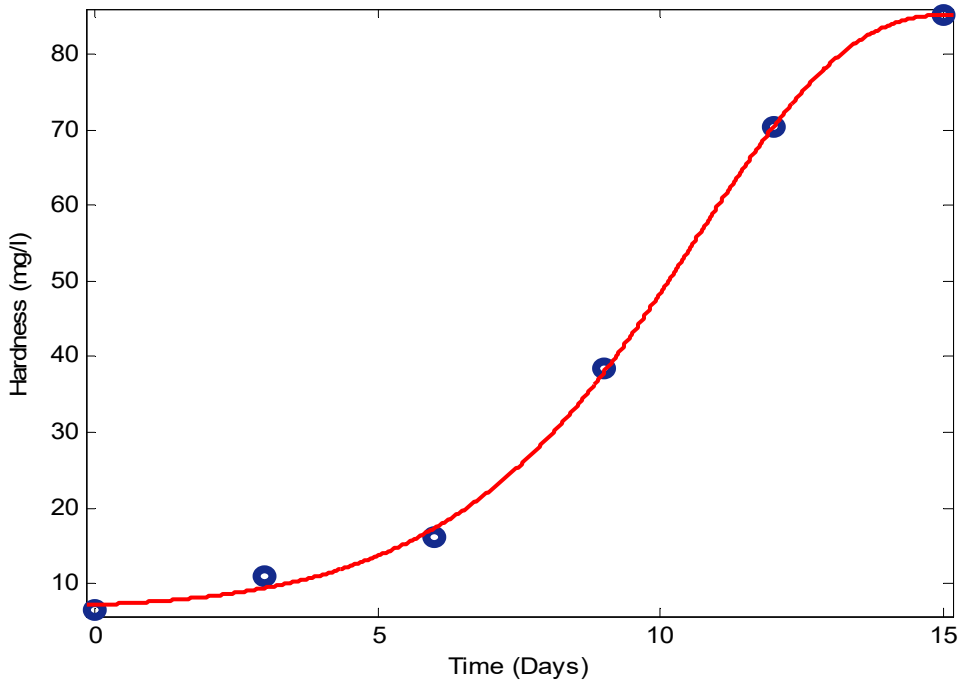

Fig. 4.40: Hardness variation with time during Bioremediation

General model Rat22:

$f(x) = (p_1*x^2 + p_2*x + p_3) / (x^2 + q_1*x + q_2)$

Coefficients (with 95% confidence bounds):

p_1 = 20.23

p_2 = -123.3 p_3 = 1340

q_1 = -24.29 q_2 = 186.8

Goodness of fit:

SSE: 4.737

R-square: 0.9991

Adjusted R-square: 0.9957

RMSE: 2.176

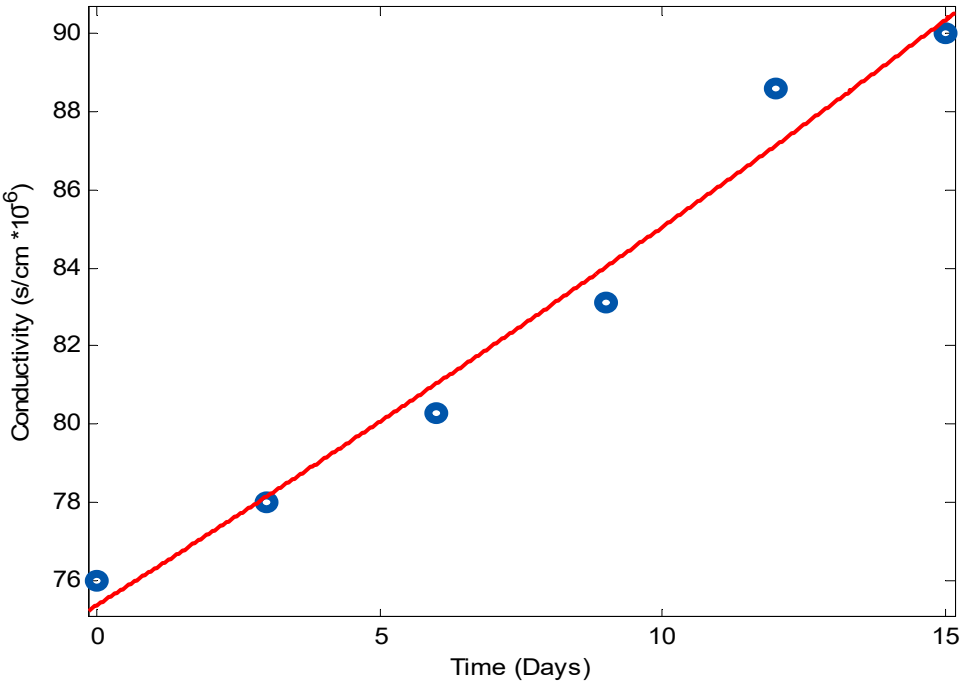

Fig. 4.41: Conductivity variation with time during Bioremediation

General model Exp1:

f(x) = a*exp(b*x)

Coefficients (with 95% confidence bounds):

a = 75.35 b = 0.0121

Goodness of fit:

SSE: 4.105

R-square: 0.9745

Adjusted R-square: 0.9681

RMSE: 1.013

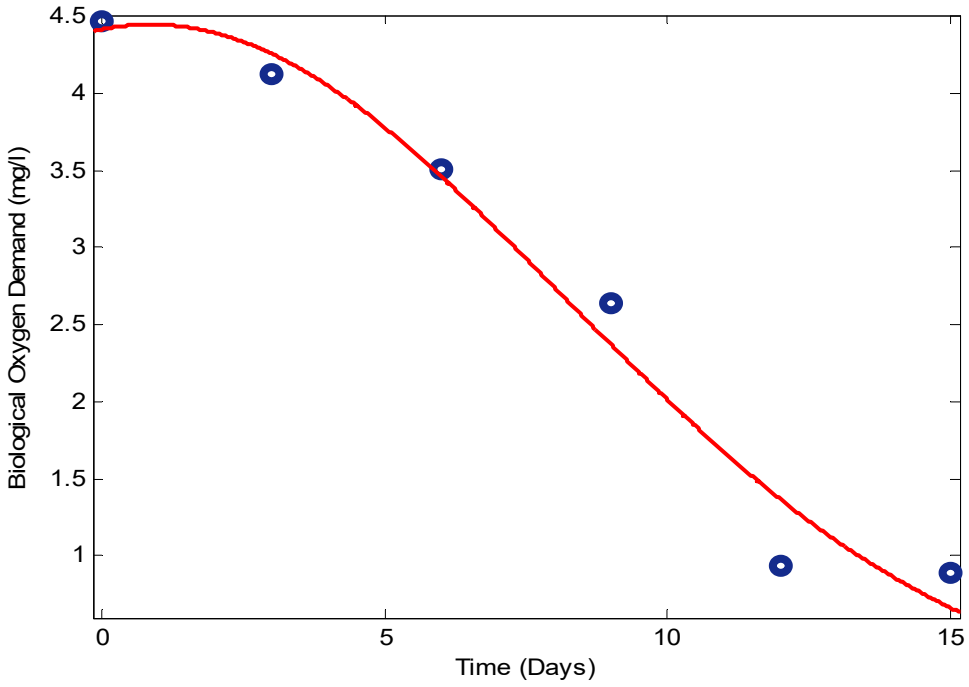

Fig. 4.42: Biological Oxygen Demand variation with time during Bioremediation

General model Gauss1:

f(x) = a1*exp(-((x-b1)/c1)^2)

Coefficients (with 95% confidence bounds):

a_1 = 4.447 b_1 = 0.8857 c_1 = 10.23

Goodness of fit:

SSE: 0.3396

R-square: 0.9722

Adjusted R-square: 0.9536

RMSE: 0.3364

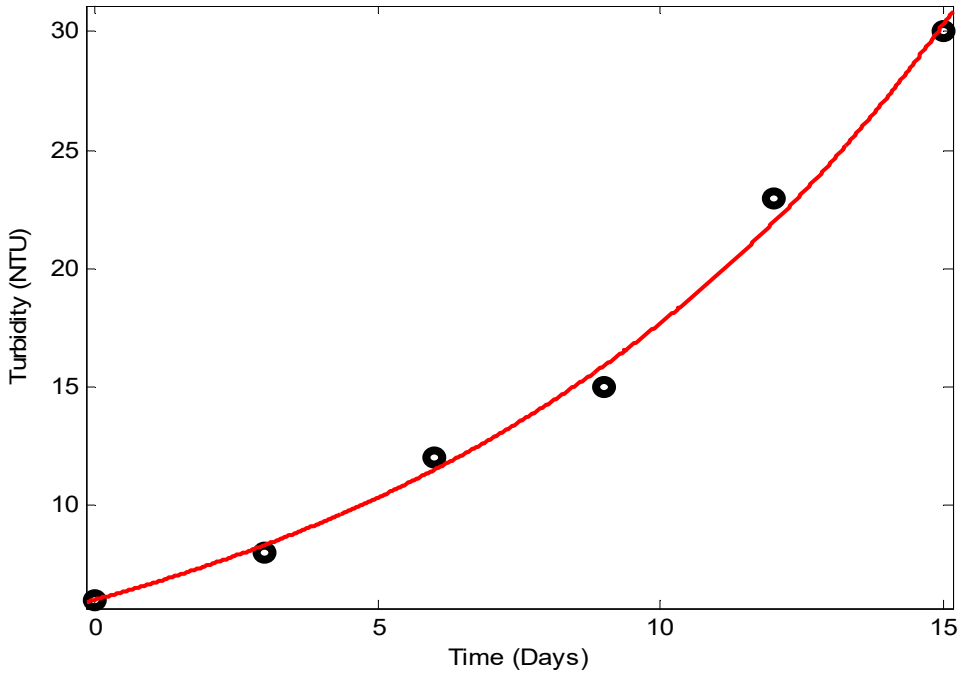

Fig. 4.43: Turbidity variation with time during Bioremediation

General model Exp1:

f(x) = a*exp(b*x)

Coefficients (with 95% confidence bounds):

a = 6.02

b = 0.1079

Goodness of fit:

SSE: 2.349

R-square: 0.9945

Adjusted R-square: 0.9931

RMSE: 0.7664

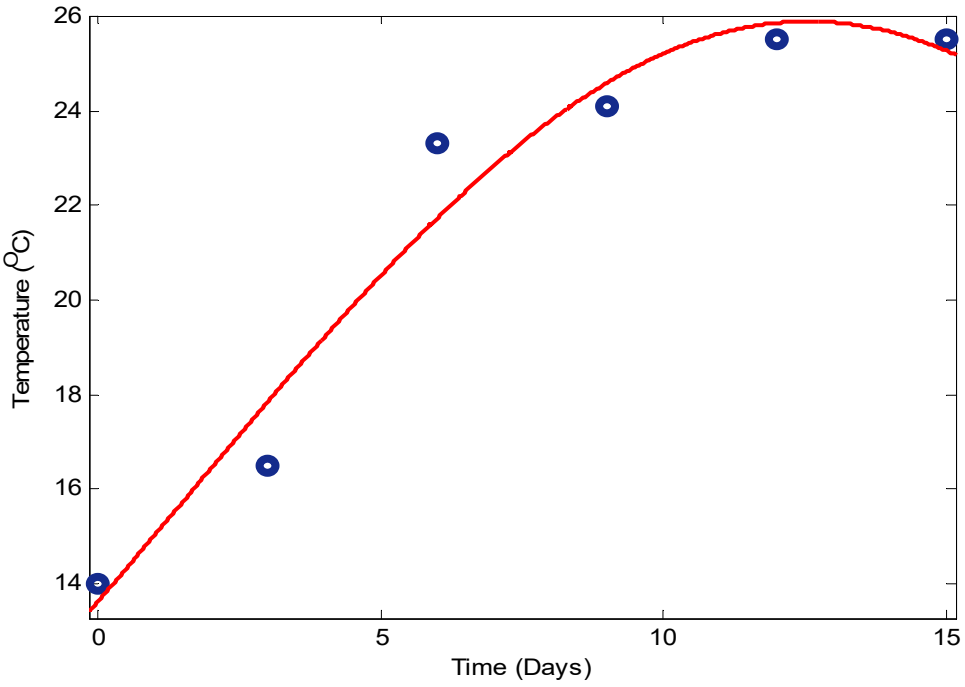

Fig. 4.44: Temperature variation with time during Bioremediation

General model Gauss1:

$f(x) = a_1*\exp(-((x-b_1)/c_1)^2)$

Coefficients (with 95% confidence bounds):

$a_1 =$ 25.89 $b_1 =$ 12.56 $c_1 =$ 15.66

Goodness of fit:

SSE: 4.841

R-square: 0.9607

Adjusted R-square: 0.9345

RMSE: 1.27

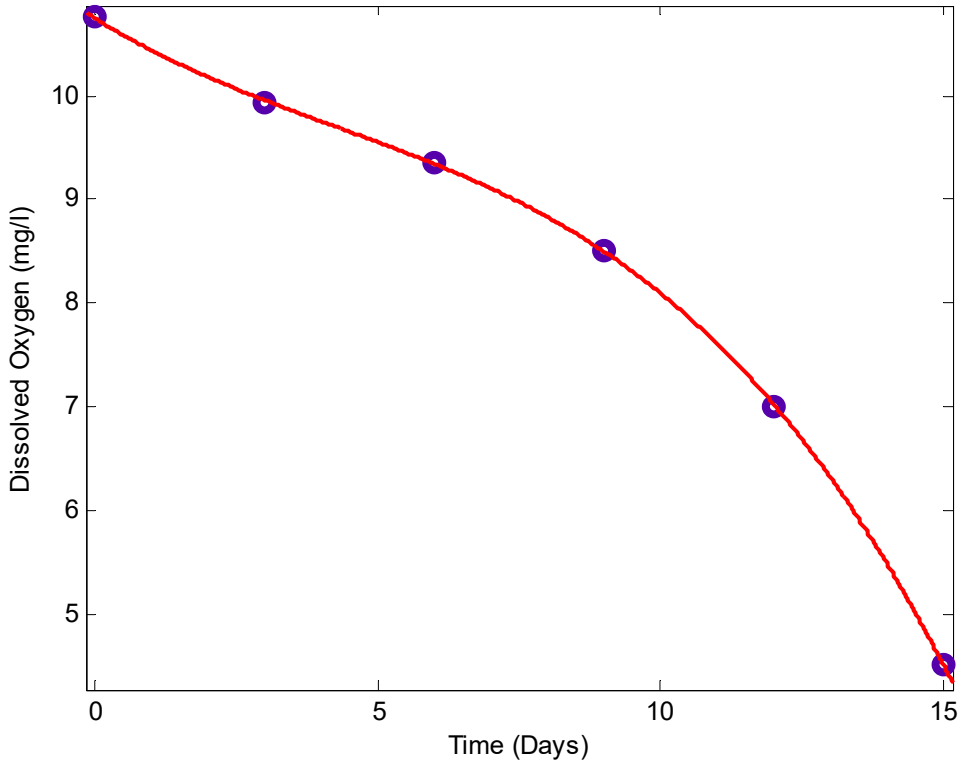

Fig. 4.45: Dissolve Oxygen variation with time during Bioremediation

Linear model Poly3:

$f(x) = p_1*x^3 + p_2*x^2 + p_3*x + p_4$

Coefficients (with 95% confidence bounds):

$p_1 =$ -0.00251 $p_2 =$ 0.03243

$p_3 =$ -0.339 $p_4 =$ 10.75

Goodness of fit:

SSE: 0.001171

R-square: 1

Adjusted R-square: 0.9999

RMSE: 0.0242

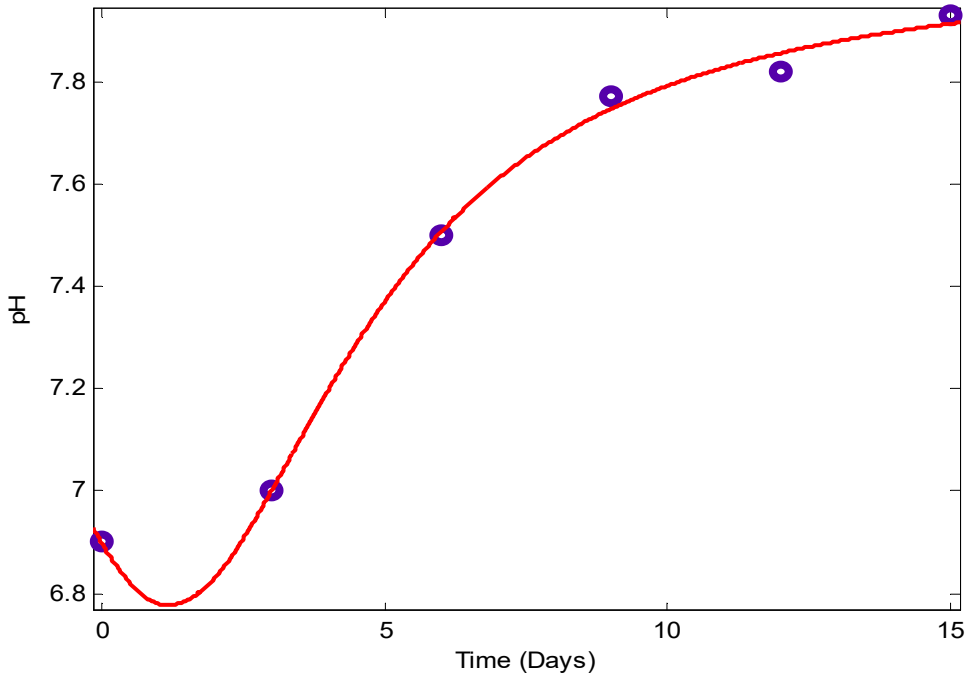

Fig. 4.46: pH variation with time during Bioremediation

General model Rat22:

$f(x) = (p_1*x^2 + p_2*x + p_3) / (x^2 + q_1*x + q_2)$

Coefficients (with 95% confidence bounds):

$p_1 =$ 8.048 $p_2 =$ -15.2 $p_3 =$ 102.9

$q_1 =$ -1.793 $q_2 =$ 14.92

Goodness of fit:

SSE: 0.002175

R-square: 0.9978

Adjusted R-square: 0.9888

RMSE: 0.04664

Fig. 4.39 shows that the Total dissolved solid increases linearly with time during bioremediation.

Fig. 4.40 shows that the hardness of the medium increases with time following a quadratic-quadratic rational model. The increase is slow at the beginning and also slow and asymptotic at the end.

Fig. 4.41 shows that the Conductivity increases exponentially with time during the bioremediation process.

Fig. 4.42 shows that the Biological oxygen demand reduces with time following the Gaussian model

Fig. 4.43 shows that the turbidity increases exponentially

Fig. 4.44 shows that the Temperature increases following the Gaussian model

Fig. 4.45 shows that the Dissolved oxygen reduces following a cubic model

Fig. 4.46 shows that the pH increases following a quadratic-quadratic rational model

4.1.13 COST ESTIMATION OF BIOREMEDIATION AND PHYTOREMEDIATION PROCESS

Kamath et al (2004) presented data for cost of Phytoremediation and pump and Treat process for a period of five years (260 weeks). In their work Phytoremediation cost $250,000 which is equivalent to $961.54 per week, while Pump and treat cost $660,000 which is equivalent to $2538.46 per week. Agunwamba and Mbogu (2013) compared cost of Bioremediation and Phytoremediation and presented minimum costs of N185,000 and N120,000 for Bioremediation and Phytoremediation respectively. This gives a ratio cost of Bioremediation to Phytoremediation of 1.5417. Thus Bioremediation will cost an estimated $1482.37 per week.

The cost of achieving 60% contaminant removal will be computed for comparison of costs of the two methods. Sixty percent contaminant removal is a reduction from 100mol% (1.0) to 40mol% (0.4). This range was chosen so as not to be lower than the maximum ultimate substrate concentration which was obtained for bioremediation as 0.3535.

Using values from Table 4.1 and equation (2.12), we have:

Bioremediation:

$$t = -\ln\left(\frac{0.4 - 0.3535}{1 - 0.3535}\right)/0.2712$$

$$t = 9.7055\ weeks$$

Phytoremediation (Locoweed):

$$t = -\ln\left(\frac{0.4 - 0.1241}{1 - 0.1241}\right)/0.1527$$

$$t = 7.5652\ weeks$$

Phytoremediation (Sunflower):

$$t = -\ln\left(\frac{0.4 - 0.3082}{1 - 0.3082}\right)/0.2493$$

$$t = 8.1014\ weeks$$

Cost of Bioremediation:

9.7055 weeks x $1482.37/week = $14,387.14

Cost of Phytoremediation (Locoweed):

7.5652 weeks x $961.54/week = $7,274.24

Cost of Phytoremediation (Locoweed):

8.1014 weeks x $961.54/week = $7,789.82

Phytoremediation has advantage of being cheaper than Bioremediation, less disruptive for the environment, less need for soil disposal sites, better public acceptance, avoids excavation and transportation, allows a much larger scale clean up and potentially versatile to treat a diverse range of harzardous materials, with Phytoextraction and Rhizofiltration nearing commercialization (Singh and Ward, 2004).

This study has also revealed that:

1. Phytoremediation will produce remediated soil with a lower ultimate substrate concentration that Bioremediation alone.

2. Phytoremediation can achieve same level of contaminant removal in a shorter time than Bioremediation.

4.2 DISCUSSION

4.2.1 MODELS BASED ON SUBSTRATE CONCENTRATION

It can be observed from figure 4.5 that the model for exponential growth with constant yield does not give a good graphical fit to the bioremediation experimental data. The model for exponential growth with varying yield (figure 4.6) does not also give good graphical fit to the bioremediation experimental data. The experimental data for bioremediation gave good graphical fit to the models for logistic growth with constant yield (figure 4.7) and logistic growth with varying yield (figure 4.8). It can be observed from figure 4.9 and figure 4.10 that the experimental data for bioremediation does not give good graphical fit to the model of Gaussian growth with constant yield and Gaussian growth with varying yield respectively.

The numerical fit results of Table 4.7 reveal that the model that best fit the bioremediation data based on the R^2 is that of logistic growth with constant yield, which explains 99.5% of the

observed variability in the experimental data, closely followed by the model for logistic growth with varying yield.

This suggests that during bioremediation the biomass grows according to the logistic model (with inhibition as the amount of substrate is depleted) and the rate of production of biomass per unit substrate consumed is constant (constant yield).

It can be observed from figure 4.11 and figure 4.12 respectively, that the model for exponential growth with constant yield and exponential growth with varying yield does give good graphical fit to the experimental data for phytoremediation augmented bioremediation using sunflower. Similarly, figure 4.15 and figure 4.16 respectively reveal that the model for Gaussian growth with constant yield and Gaussian growth with varying yield does not also give good graphical fit to the experimental data for phytoremediation augmented bioremediation using sunflower. On the other hand figure 4.13 and figure 4.14 reveal that models for logistic growth with constant yield and logistic growth with varying yield give good graphical fit to the experimental data for phytoremediation augmented bioremediation using sunflower.

The numerical fit results of Table 4.8 reveal that the model that best fit the experimental data for phytoremediation augmented bioremediation using sunflower based on the R^2 is that of logistic growth with constant yield, which explains 99.7% of the observed variability in the experimental data, closely followed by the model for logistic growth with varying yield.

This suggests that during phytoremediation augmented bioremediation using sunflower the biomass grows according to the logistic model (with inhibition as the amount of substrate is depleted) and the rate of production of biomass per unit substrate consumed is constant (constant yield).

It can be observed from Fig.4.17 to Fig. 4.22 that the model fit to experimental data from phytoremediation augmented bioremediation using locoweed is relatively good for the models of exponential growth with varying yield (Fig. 4.18), logistic growth with constant yield (Fig. 4.19), logistic growth with varying yield (Fig. 4.20) and Gaussian growth with varying yield (Fig. 4.22) but poor for the models of exponential growth with constant yield (Fig. 4.17) and Gaussian growth with constant yield (Fig.4.21).

The numerical fit results of Table 4.9, based on the R^2, shows that the phytoremediation augmented bioremediation process using Locoweed fit most to the model for Logistic growth with varying yield (0.9750), though the fit does not significantly differ from that of logistic growth with constant yield (0.9750). This suggests that the biomass growth during phytoremediation augmented bioremediation process can also be seen as growing according to the logistic model, with inhibition as the amount of substrate is depleted and the rate of production of biomass per unit substrate consumed is constant, which is in agreement with the constant yield idea, in consistency with bioremediation or phytoremediation augmented bioremediation using sunflower.

4.2.2 MODELS BASED ON ORDER OF REACTION

It can be observed from Fig. 4.23 to Fig. 4.27 that the model for first order reaction with ultimate substrate concentration being greater than zero ($S_\infty > 0$) (Fig. 4.25) and the model for second order reaction with ultimate substrate concentration being greater than zero ($S_\infty > 0$) (Fig. 4.27) gave relatively good fit to the data for bioremediation, while the other models for zero order (Fig. 4.23), first order with ultimate substrate concentration of zero (Fig. 4.24) and Second order with ultimate substrate concentration of zero (Fig. 4.26) had poor fits.

The numerical fit results of Table 4.10 reveal that the First Order reaction with an ultimate substrate concentration greater than zero ($S_\infty > 0$) gave the best fit and explains 96.95% of the experimental data. Thus suggesting that bioremediation is a first order reaction, which is in agreement with the observation of several authors as presented by Kamath et al., 2004 and with a non-zero ultimate substrate concentration.

It can be observed from Fig. 4.28 to Fig. 4.32 that models for first order reaction with zero ultimate substrate concentration (Fig. 4.29), first order reaction with non-zero ultimate substrate concentration (Fig. 4.30), second order reaction with zero ultimate substrate concentration (Fig. 4.31) and second order reaction with non-zero ultimate substrate concentration (Fig. 4.32) fit well to the experimental data for phytoremediation augmented bioremediation using locoweed, while the zero order reaction model (Fig. 4.28) had a poor fit.

The numerical fit results of Table 4.11 reveal that the First Order reaction with an ultimate substrate concentration greater than zero ($S_\infty > 0$) gave the best fit, explaining 97.27% of the observed experimental data. Thus suggesting that bioremediation is a first order reaction and the ultimate substrate concentration will never go to zero.

It can be observed from Fig. 4.33 to Fig. 4.37 that the model for first order reaction with ultimate substrate concentration being greater than zero ($S_\infty > 0$) (Fig. 4.35) and the model for second order reaction with ultimate substrate concentration being greater than zero ($S_\infty > 0$) (Fig. 4.37) gave relatively good fit to the data for phytoremediation augmented bioremediation using sunflower, while the other models for zero order (Fig. 4.33), first order with ultimate substrate concentration of zero (Fig. 4.34) and Second order with ultimate substrate concentration of zero (Fig. 4.36) had poor fits.

The numerical fit results of Table 4.12 reveal that the First Order reaction with an ultimate substrate concentration greater than zero ($S_\infty > 0$) gave the best fit and explains 97.28% of the experimental data. Thus suggesting that bioremediation is a first order reaction, which is in agreement with the observation of several authors as presented by Kamath et al., 2004 and with a non-zero ultimate substrate concentration.

Phytoremediation has advantage of being cheaper than Bioremediation, less disruptive for the environment, less need for soil disposal sites, better public acceptance, avoids excavation and transportation, allows a much larger scale clean up and potentially versatile to treat a diverse range of harzardous materials, with Phytoextraction and Rhizofiltration nearing commercialization (Singh and Ward, 2004).

4.2.3 RESPONSE SURFACE ANALYSIS

The Analysis of variance of Table 4.13 represents time as x_1 and dilution rate as x_2 and reveals that dilution rate and time are significant in bioremediation at 95% confidence (since both p-values of 0.0029 and 0.0143 are less than 0.05) though time of remediation plays a more significant role than dilution rate, since it has a lower. The surface plot of fig 4.38reveals that there is a slightly quadratic relationship between these two variables and substrate concentration and the contours lines are non-parallel lines showing that these two factors interact. It also reveals that increase in time and dilution rate reduce substrate concentration. The Response surface model statistics of table 4.14 reveal that a quadratic model ($Y = a_1 + a_2x_1 + a_3x_2 + a_4x_1x_2 + a_5x_1^2 + a_6x_2^2$) fit well to the relation between time, dilution rate and substrate concentration. The t-statistics value reveal that the constant term, time, Time*dilution rate and time^2 are the significant variables in the model since the absolute values

of their t-stat are equal to or greater than 2, these variables are also significant at 95% confidence based on the p-val except for time*dilution rate which is significant at 90% confidence. The F-statistics shows that the model is adequate with a p-val of 0.0007 and the R^2 shows that the model explains 95% of the observed variability in the experimental data.

4.2.4 PHYSICO-CHEMICAL PROPERTIES.

The Total Dissolved Solid content increases linearly with time during bioremediation (Fig. 4.39), hardness increases exponentially with time based on a rational relationship (Fig. 4.40), conductivity increases exponentially with time also, though the rate is low and approximately linear (Fig. 4.41), Biological oxygen demand reduces exponentially with remediation time following the guassian profile (Fig. 4.42). Turbidity increases exponentially with remediation time at a rate about ten times that of conductivity (Fig. 4.43). Temperature also increases exponentially with remediation time following the Gaussian profile (Fig. 4.44). Dissolved oxygen reduces with remediation time following a third order polynomial profile (Fig. 4.45). pH increases exponentially with remediation time according to a rational function (Fig. 4.46).

Discussion on estimated cost of processes

Phytoremediation has advantage of being cheaper than Bioremediation and based on the estimate from this study would cost about 50% of the cost of using bioremediation alone. It is also known to be less disruptive for the environment, has less need for soil disposal sites, better public acceptance, avoids excavation and transportation, allows a much larger scale clean up and potentially versatile to treat a diverse range of hazardous materials, with Phytoextraction and Rhizofiltration nearing commercialization (Singh and Ward, 2004).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

Bioremediation and phytoremediation augmented bioremediation are based on the same mechanism, based on the result of this study. During both processes the biomass grows according to the logistic model (with inhibition as the amount of substrate is depleted) and the rate of production of biomass per unit substrate consumed can be considered constant. Bioremediation and phytoremediation are first order processes whose rates are directly proportional to the substrate concentration driving force. Phytoremediation enhances the rate of the bioremediation process by reducing the ultimate substrate concentration achievable through bioremediation alone, though the first order rate constant is reduced in the process. Locoweed shows higher effectiveness in enhancing the remediation process in comparison to Sunflower.

Bioremediation reduces the contaminant concentration by about 65%, while when augmented with Phytoremediation, the contaminant concentration is reduced by 69% and 88% for Sunflower and Locoweed respectively.

5.2 RECOMMENDATIONS

In view of the findings and conclusions, the following recommendations are made:

i. Since phyto remediation has been shown to be a viable means for remediation of oil contaminated sites, it is recommended that further work be carried out to determine the applicability of more plant materials for this process. Legislative framework should be provided for the application of phytoremediation as part of a

monitoring function of the regulatory body in all locations where petroleum is handled.

ii. The monitoring body charged with responsibility of monitoring adherence to environmental regulations should be properly funded to discharge its functions and the regulations be regularly revised to keep abreast with advances in technology and international standards and best practice.

iii. Government should encourage further research in the application of genetically engineered microbes in remediation contaminated sites and the use coagulant chemicals to further optimize clean-up process.

iv. Government should implement of extant laws for corporate social responsibilities in developing host communities with benefit to all stakeholders. Corrosion monitoring of pipelines and consistent safety monitoring of Right of Way (ROW) and pipeline and its end facilities would reduce instances of spillage or pollution of the environment.

5.3 CONTRIBUTION TO KNOWLEDGE

i. Suitable model equations for Bioremediation and phytoremediation based on biomass growth and order of reaction have been determined.

ii. A new kinetic model suitable for remediation processes where death of biomass may be encountered was presented in this study.

ii. The kinetic parameters in this study have been related to parameters in phytoremediation (uptake rate of contaminant and Transpiration Stream

Concentration Factor) and computation of remediation time, and thus can be used to obtain these parameters for a selected plant.

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APPENDIX 1

PUBLICATIONS ARISING FROM THE RESEARCH

1. Musa, A. S., Oyoh, K. B., Osoka, E. C., Onyelucheya, O. E. (2015): Modelling Phytoremediation Augmented Bioremediation Based on Biomass and Yield Kinetics. *International Journal of Engineering and Management Research (IJEMR)*, 5(4), 241 – 248.

2. Musa, A. S., Oyoh, K. B., Osoka, E. C., Onyelucheya, O. E. (2016): Modelling Phytoremediation Augmented Bioremediation Based on Order of Reaction. *International Journal of Innovative Science, Engineering & Technology (IJISET)*, 3(4), 512 - 522

APPENDIX 2

ADDITIONAL INFORMATION

STUDY SITE

The study site was Ogba-Egbema-Andoni LGA, located between latitude 4° 55' 55" N and 6° 32' 48" E at altitude of 774ft above sea level (Using 2009 Europa Statellite Imagery GPS 3111 (Accuracy: ±1 Meter). Mean annual rainfall ranged from 2450 – 2500mm, mean annual temperature range of 26.5 – 27.5° C, mean relative humidity of 65 – 75%, evapotranspiration of 1445 – 1450 mm/yr. (B. U. Uzoho, T. V. Okechukwu).

SOIL TYPE AT SITE

Soils of the area have been classified as Eutric Tropofluvent (FDALR, 1985).

Typically consisting of sand, silt and clay.

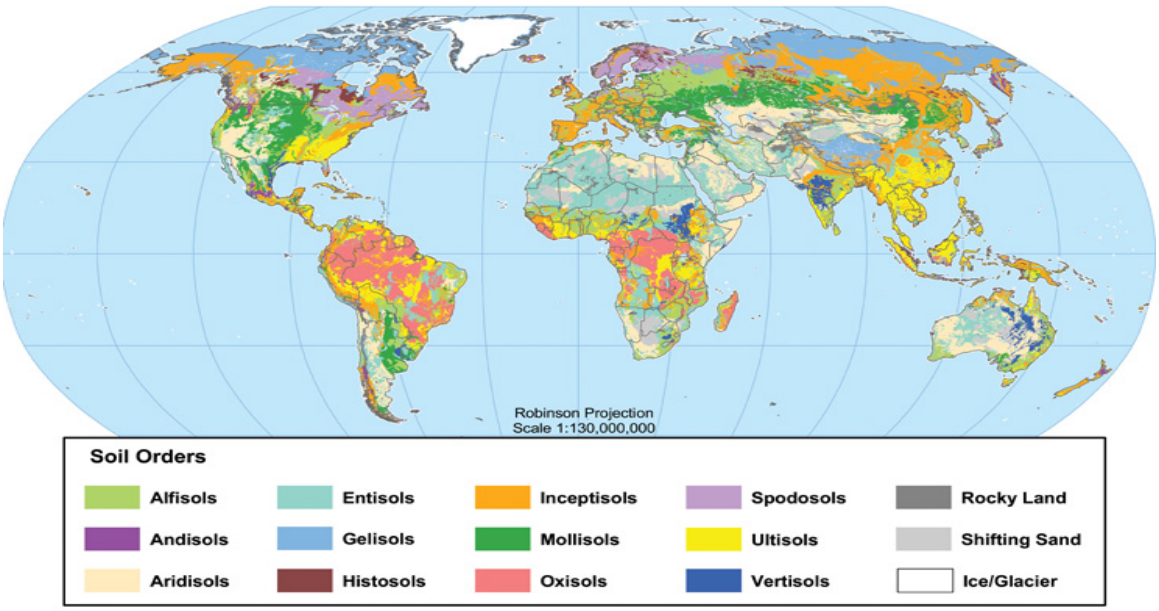
USDA have developed an order in soil taxonomy and they vary around the world. Typical soil orders include, gelisols, alfisols, histosols, inceptisols, mollisols, spodosols, entisols, oxisols, ultisols, vertisols, aridisols, andisols, etc. For instance, oxisols are soil order typical of the tropical types, with average coverage area of about 9.8 million Km2 (7.5% of global soil) and found primarily in the humid climates of Africa and South America. These tropical soils are deep, lack developed horizons and are generally yellow or red. (USDA, 2014).

APPLICABILITY OF THIS KNOWLEDGE IN OTHER SOIL TYPES

Bioremediation augmented phytotechnique applied in this research work is not applicable to all soil types in the world. For example, the artic and antartica are the polar regions that receive less sunlight because of earth tilt and plant & animal life face harsh sub-zero temperatures. The main type of soil in the polar region is ahumic soil.

Soil orders of a general nature such as histosols, inceptisols, molisols and oxisols may require amendments, pH adjustments, addition of organic matter, nutrients, moisture content etc under suitable conditions to support plant growth.

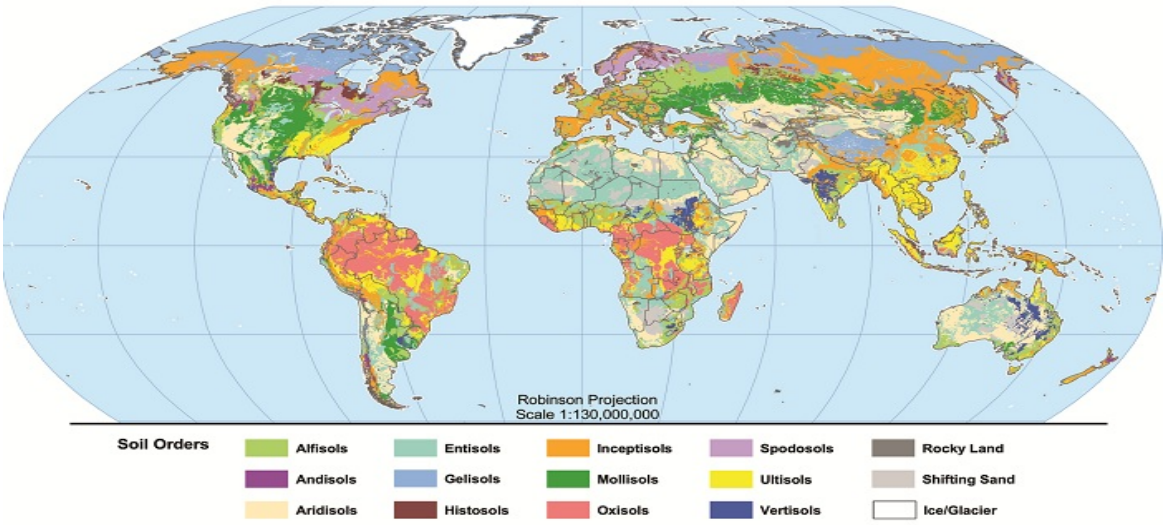
Global Soil Regions

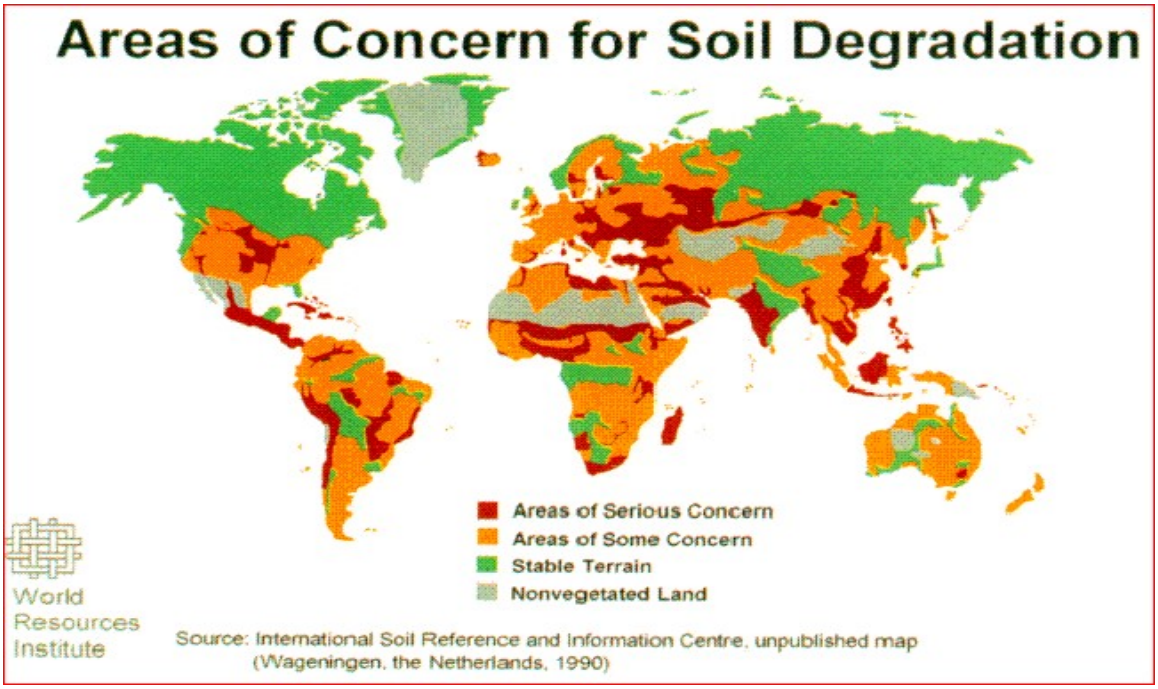


USDA NRCS US Department of Agriculture
Natural Resources
Conservation Service

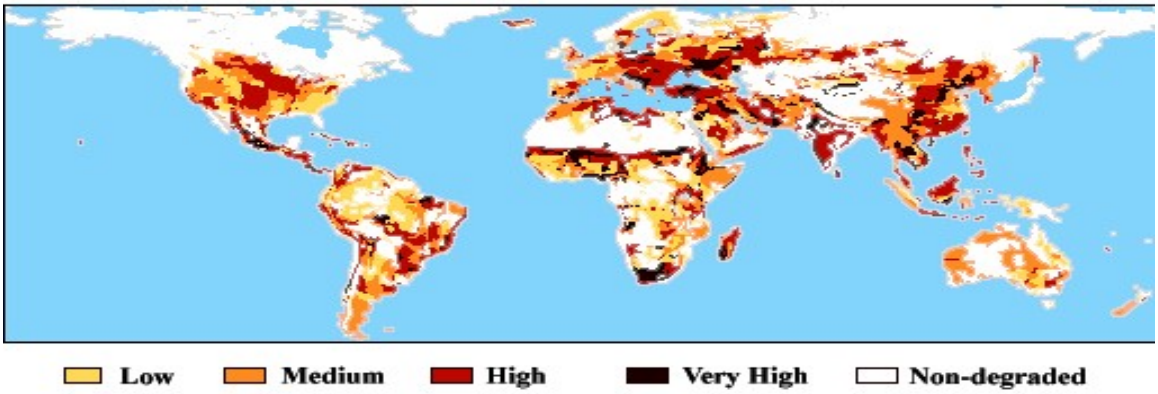
Soil Survey Division
World Soil Resources
soils.usda.gov/use/worldsoils

November 2005





Soil Degradation Severity



Summary:

- Degradation of land includes petroleum pollution, soil erosion, salinization, nutrient depletion, and desertification. The rate of degradation has increased dramatically with growth in human populations and technology.
- Severe land damage accompanies large scale agriculture. Restoration is very problematical.
- Continued loss of arable land will jeopardize our ability to feed the world population, may increase competition and conflicts for land use or ownership.
- Land degradation is worldwide - both developed and developing countries.
- From global view, the time to save our fragile planet earth from environmental degradation (soil, water and air pollutions) is NOW! See above slide – recommendations.