Biosurfactant Enhanced Remediation of a Mixed Contaminated Soil

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A Thesis

In

The Department of

Building, Civil and Environmental Engineering

Presented in Partial Fulfilment of the Requirements

For the Degree of Master of Applied Science at

Concordia University

Montreal, Quebec, Canada.

December 2006

Your file Votre référence
ISBN: 978-0-494-28902-0
Our file Notre référence
ISBN: 978-0-494-28902-0

Abstract

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The most ubiquitous soil contamination problems in the world today are related to hydrocarbons and heavy metals. It is a common phenomenon to find a mixture of heavy metals and hydrocarbons in most contaminated sites in the US and also in Canada. The presence of these contaminants can destroy the balance in the natural habitat. Therefore there is a need for remediation to mitigate these effects on humans and the environment at large. Heavy metals, such as copper (Cu), zinc (Zn), and nickel (Ni), have received considerable attention with regard to their accumulation in soils, uptake by plants and contamination of groundwater by leaching. Since they cannot be degraded, they pose a serious problem to the environment. Hydrocarbons, e.g. diesel oil, also pose a similar risk when present in large quantities in soil. This research focuses on using biosurfactants; rhamnolipids, saponin and mannosyl-erythritol lipids to remediate a natural soil contaminated with a mixture of heavy metals and hydrocarbons (diesel fuel). The soil contained 894 mg/kg of zinc, 216 mg/kg of copper, 167 mg/kg of nickel and 228 mg/kg of the total petroleum hydrocarbons (TPH) content.

Experiments carried out showed that after a series of five washings of the soil using biosurfactants; the highest removal of zinc (88% and 79%) was achieved using saponin (30 g/L), pH 3 and pH 5 respectively.

The maximum copper removal (46%) was obtained with 2% rhamnolipids at pH 6.5. Highest nickel removal (76%) was obtained with saponin (30 g/L) pH 5.

The TPH level in the soil after multiple washings dropped drastically from an initial concentration of 228 mg/kg to concentrations in the range of 14 - 67 mg/kg with biosurfactants and the control.

Sequential extraction performed on the untreated soil showed that copper exists more in the organic fraction (50%), zinc in the oxide fraction (36%) and nickel exists more in the exchangeable and carbonate fractions (50%). After a series of five washings with biosurfactants it was evident that the oxide fraction of zinc, organic fraction of copper, exchangeable and carbonate fractions of nickel were substantially reduced, compared to the control and the untreated soil.

The results of the study clearly indicated the feasibility of reducing zinc, copper, nickel and the total petroleum hydrocarbon content of a mixed contaminated soil with the anionic biosurfactants tested.

Acknowledgments

I like to sincerely thank my supervisor Dr C.N Mulligan for her immense support and encouragements during the course of this research.

My appreciation also goes to my elder brother, Viano for his assistance. I remain grateful to my dear husband Emmanuel and my little girl Tochi, for their understanding during the course of this work.

Finally I would like to thank Mahmood AliMahmoodi for his advice in the lab work and Luciana Oghenekevwe for her help in proof reading the thesis.

Dedication

This work is dedicated to my dear Mum for all she did for me.

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List of Abbreviations

AAS	Atomic Absorption Spectrophotometer
ASTM	American Society of Testing Materials
ASTDR	Agency for Toxic Substances and Disease Registry
CEC	Cation Exchange Capacity
DCE	Dichloroethene
EPA	Environmental Protection Agency
GC	Gas Chromatography
MEL	Mannosyl erythritol lipids
NPRI	National Pollutant Release Inventory
PAH	Polycyclic Aromatic Hydrocarbon
PCP	Pentachlorophenol
ROD	Record of Decision
SDW	Soil Dry Weight
SOC	Soil Organic Content
STDEV	Standard Deviation
TCE	Trichloroethene
TPH	Total Petroleum Hydrocarbon
VOC	Volatile Organic Compound

Chapter One: Introduction

1.1 Problem Statement

In the world today, there is a growing concern about changes in the environment. Scientists and engineers have become more concerned about the maintenance of a healthy environment. This concern stems directly from world population growth, an increase in the number of industries and the constant advancement in technology (Ontario Ministry of Environment and Energy, 1994).

The pollution of the environment as it is known today is a worldwide problem, which can lead to the uptake and accumulation of toxic chemicals in food chains causing harm to the flora and fauna of the affected habitat. These toxic chemicals released into the environment are to a greater measure from anthropogenic sources owing to the diversity of human activities (McKeague and Wolynetz 1980). Contaminants are released through several industrialized processes, for instance, during the manufacture and use of products and during disposal of the generated waste.

Although substantial progress has been made in reducing industrial releases over recent years, major releases still occur. In addition, a considerable number of polluted sites have been identified and new ones are continually being discovered (Banat 1995). Many of these sites as noted by Mulligan et al (2001b) are contaminated with hydrocarbons and heavy metals.

Hydrocarbons are used as fuels and they also form the major raw material for the manufacture of pharmaceuticals, plastics, pesticides, herbicides, and detergents; while the heavy metals are used in plating, and in battery and car manufacturing industries.

At Superfund sites with signed Records of Decision (ROD) metals are the only contaminants at 16% of the sites whereas metals and organic compounds are found at 49% of the sites (Mulligan et al., 1999a). Therefore, metal and organic contamination in soil is a major concern as it contributes to the contamination of drinking water supplies and thereby constitutes a substantial health hazard for current and future generations.

To rectify the situation, numerous remediation technologies have been developed. Primarily due to cost and time, land filling of contaminated soil is currently the most widely used remediation method (Mulligan et al., 2001a). However biological remediation in isolation or in combination with other methods has gained an established place as a soil restoration technology (Makkar and Rockne 2003).

Biosurfactants are gaining more ground in soil remediation in recent times due to the fact that they are environmentally friendly, easily biodegradable and non-toxic compared to their synthetic counter-parts (Mulligan et al., 2001b). Although a lot of investigation has been done on the use of biosurfactants in heavy metal or hydrocarbon remediation, little work has been done on how to treat a mixture of heavy metal and hydrocarbon contamination.

1.2 Research Objectives

In this research the objectives involve the remediation of soil contaminated with a mixture of heavy metals and hydrocarbons using biosurfactants. The objectives are as follows:

- Determine the efficiency of the biosurfactants in remediating the heavy metals and hydrocarbon contaminants in a natural soil sample under various conditions.
- Establish the optimum conditions for the remediation of the soil
- To determine which fractions of metals are removed by the biosurfactants.

Chapter Two: Literature Review

2.1 Soil Environment

The environment beneath the earth's surface is composed of porous material containing water, air and organic matter. The porous material consists of both consolidated (rock) and unconsolidated (e.g., sand, gravel or clay) formations. Generally the uppermost mantle of the earth's surface is referred to as soil, (Lagrega et al., 2001).

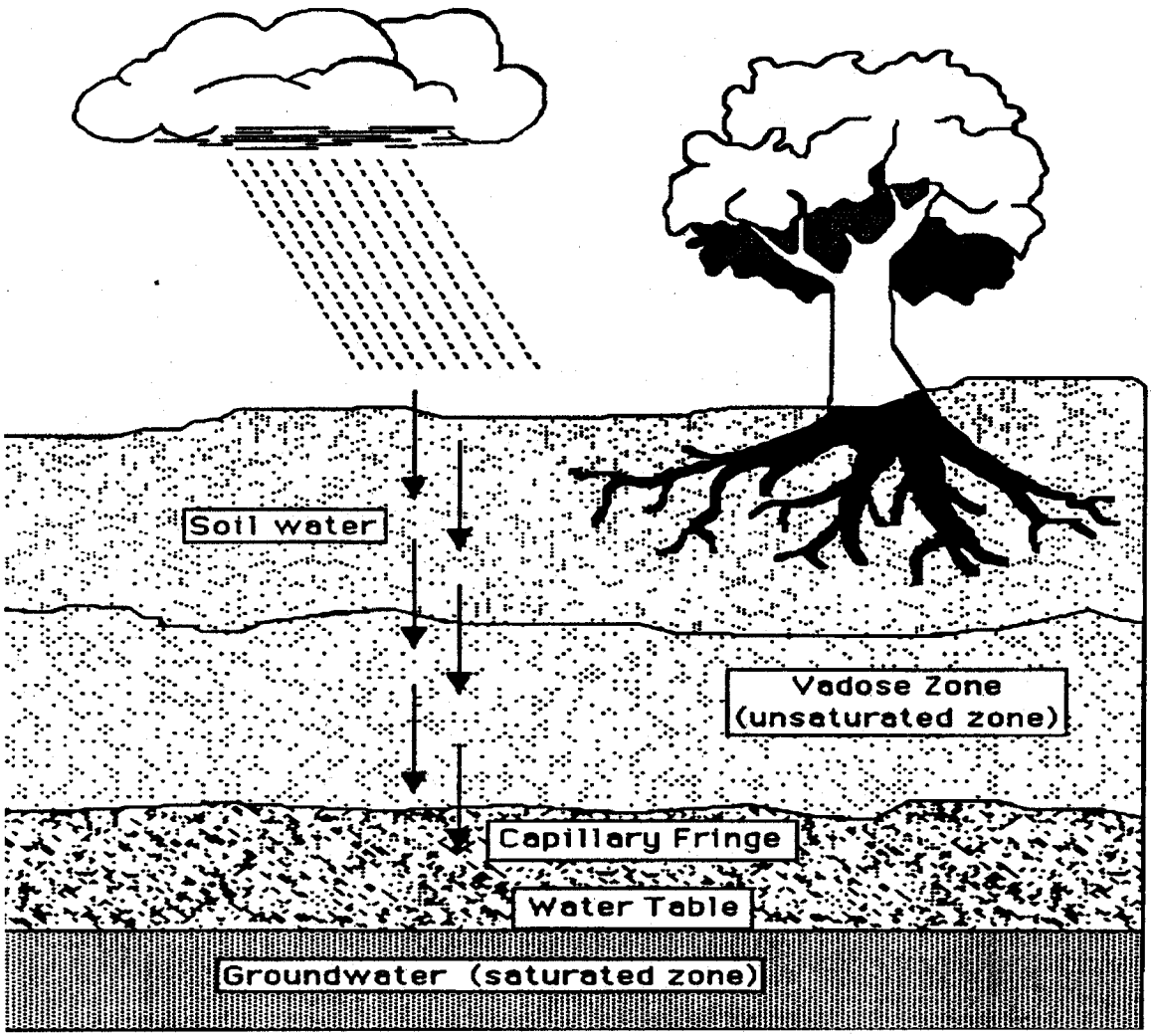


Fig. 2.1 Representative soil environment (Pierzynski et al., 1994)

Soil is a porous material through which solutions and suspensions can move. It is a highly absorbent material that preferentially adsorbs molecules and particles from solution or suspension. The porous nature of soils makes it a highly suitable habitat for living things. Microorganisms and plant roots find the water, oxygen and nutrients required to support life as shown above in Fig 2.1 (Pierzynski et al., 1994).

The formation of soil begins when weathering of the parent rock or unconsolidated sediments, causes these particles to be transported to other sites and deposited. The physical and chemical characteristics of the soil vary greatly with location, depth and time. These characteristics are dependent primarily on the parent material, climatic conditions (wind, water and temperature) and topography (slope and relief) (Pierzynski et al., 1994). Soil as it is known is a mixture of different inorganic and organic materials. The inorganic fraction consists mostly of fine mineral grains that are further subdivided based on grain size distribution (Table 2.1).

Table 2.1 Classification of mineral grain size in soil (adapted from Lagrega et al., 2001)

Classification	Description	Size
Clay	Microscopic mineral particles of colloidal nature, laminated as layers of plates	< 0.002 mm diameter (effective diameter)
Silt	Fine particles composed of minerals from the parent materials	>0.002 mm diameter <0.075 mm diameter
Sand	Granular particles composed of minerals from the parent formation	> 0.75 mm diameter < 2 mm diameter
Gravel	Coarse particles composed of minerals from the parent formation	>2 mm diameter <75 mm diameter

The percentage by weight of gravel, sand, silt, and clay provides a basis for classifying the soil by texture (Fig 2.2).

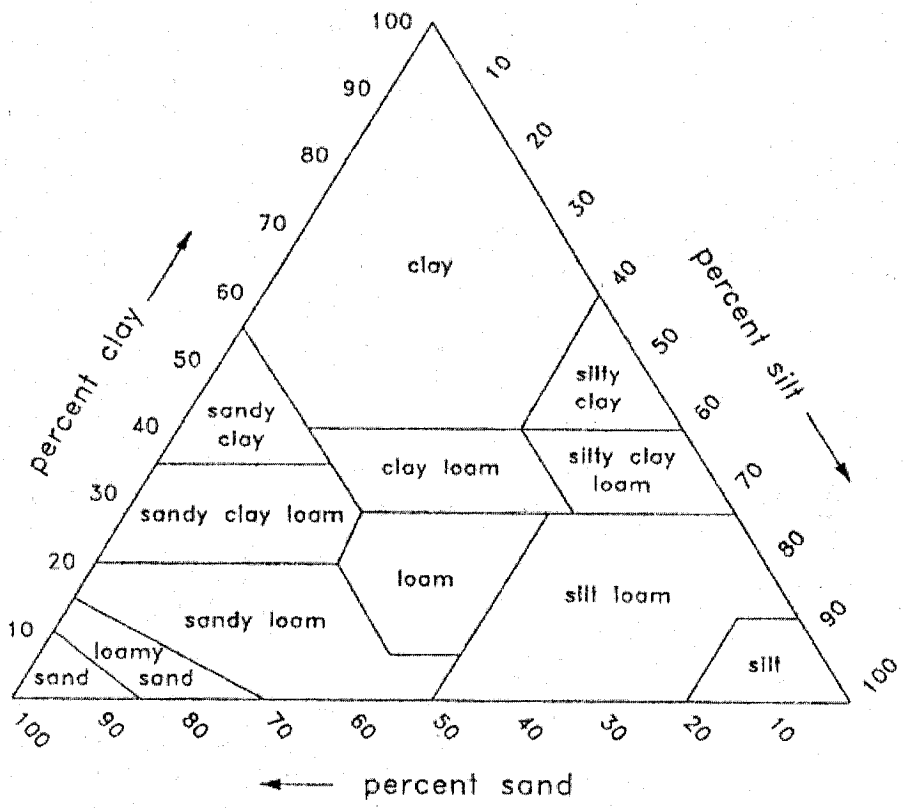


Fig. 2.2 Soil classification by grain size (adopted by US Department of Agriculture 2002)

The mineralogy of clays varies but most are formed from silicates. Common clays include kaolinite, illite and montmorillonite (smectite). The individual colloidal particles agglomerate forming aggregates in soil. Predominant soil inorganic elements include silicon, aluminium and iron with a great number of micro and trace elements. Soil also contains an appreciable amount of organic matter made up of decaying plants or humus. However the organic content decreases with depth in the vertical dimension converse to that in the horizontal dimension (Pierzynski et al., 1994).

The organic fraction in soil acts as a stabilizer that binds the inorganic particles together as aggregates in various sizes and structures (Fig 2.3).

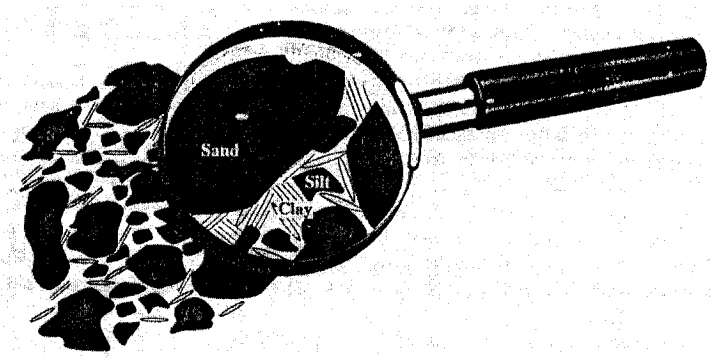


Fig. 2.3 Soil aggregates (Lagrega et al., 2001)

In soil a combination of the organic and inorganic fractions play an important role in contaminant interaction and in attenuation processes.

2.2 Contaminants in Soil

Naturally occurring soil contains some elements that are hazardous substances. Average background concentrations in soil for some of these elements can be seen in Table 2.2.

Table 2.2 Hazardous Substances in Natural Soil (adapted from Ministère du Développement durable, de l'Environnement et des Parcs "A" Soil Criteria 2006)

Metal	Background Concentration in Natural Soil (mg/kg)[a]
Zinc (Zn)	110
Lead (Pb)	50
Chromium (Cr)	85
Cadmium (Cd)	1.5
Nickel (Ni)	50
Copper (Cu)	40

a - Background levels for St Lawrence Lowlands Geological Province

The naturally occurring elements differ from the ones that are introduced by man in that they are in the insoluble form and are slowly released into the soil by weathering. Anthropogenic sources, from human activities; are released through the different industrialised processes and from the treatment and disposal of generated waste. Upon release the contaminants respond to a number of factors either natural or synthetic. They move slowly or quickly to living receptors subject to whether they are in their original or altered form.

The class of contaminants varies depending on the types of activities predominant in the area or the surrounding environment. Typical examples of contaminants that are released into soil are shown in Table 2.3 below.

Table 2.3 Contaminant Release into Soil (adapted from Lagrega et al., 2001)

Source	Volume of release	Contaminant concentration
Transport spills	Partial to entire volume of vessel	High (e.g. often pure products)
Storage spills	Partial to entire contents of storage vessels	High (e.g. often pure products)
Leaks	Minimal rate; yet could continue indefinitely, particularly if underground	High (e.g. often pure products)
Treatment effluent	Varies often high	Low (required by regulatory permits)
Surface seeps	Minimal rate; yet could continue indefinitely	Medium to high

The contaminants can be dissolved into the pore space liquid or adsorbed onto the solid phase of the soil. Common contaminants include hydrocarbons and/or heavy metals like lead, chromium, cadmium, nickel, zinc and copper. Heavy metals form a group of contaminants commonly found in several kinds of wastes including sludge, municipal and industrial wastes, landfill leachate etc. that are highly toxic to humans, animals and aquatic life. Their concentrations can range from a few parts per million (ppm) to tens of thousands parts per million (ppm) (Mulligan et al., 2005). Upon release the metals are retained in soil in the form of oxides, hydroxides, exchangeable cations and/or bound to the organic fractions in the soil. The amount retained depends on the type of soil, species of metal ions present and other prevailing factors (Yong et al., 1992). The presence of heavy metals such as lead, chromium, nickel, zinc, cadmium, and copper in soil can cause significant damage to the environment and human health due to their solubility and high mobility.

2.2.1 Nickel

Nickel is a naturally occurring element. Pure nickel is a hard, silvery-white metal used to make stainless steel and other metal alloys. Skin irritations are the most common effects in people who are sensitive to nickel. Nickel is found in all soil and is emitted from volcanoes. Nickel has been found in at least 862 of the 1636 National Priority List sites identified by the Environmental Protection Agency (EPA, 1998). Nickel compounds are used in nickel plating, colouring ceramics, batteries, and as catalysts to increase the rate of chemical reactions.

2.2.2 Zinc

Zinc is the 23rd most abundant element in the earth's crust; it is the second most common trace metal, after iron, naturally found in the human body. It is a bluish-white, lustrous metal that is used to coat iron or steel in a process called galvanization to prevent rust. It is often found in association with cadmium but is not as toxic as cadmium (Columbia Electronic Encyclopaedia, 2006). In acidic medium, zinc is in the divalent form, which is quite mobile. Natural levels of zinc in soils are 30-150 ppm while levels of 400 ppm are toxic (Mulligan et al., 2001a). Galvanized parts, bronze alloys, glass paints, televisions, tires and zinc-based alloys form sources of zinc contamination in soil.

2.2.3 Copper

Copper is one of the most important metals; it is reddish with a bright metallic lustre. It is malleable, ductile, and a good conductor of heat and electricity (second only to silver in electrical conductivity). Its alloys, brass and bronze, have very important uses. The most important compounds are the oxides and the sulphates (blue vitriol) (EPA, 1998).

Copper binds strongly to organic matter and clay minerals in soil thereby increasing its accumulation in the environment. The average content of copper in soil is 2 to 100 ppm; plants can accumulate copper to the levels of up to 5-30 ppm with toxic levels reaching 20-100 ppm (Mulligan et al 2001a).

The increased level of copper can be attributed to the use of fertilizers, pesticides, sprays, rayon, building materials and agricultural and industrial wastes. All copper compounds, unless otherwise known, are being treated as if they were toxic (ASTDR, 2006).

2.2.4 Petroleum Hydrocarbons

A hydrocarbon is any organic compound composed solely of the elements hydrogen and carbon. The hydrocarbons differ both in the total number of carbon and hydrogen atoms in their molecules and in the proportion of hydrogen to carbon. The hydrocarbons can be divided into various homologous series. Each member of such a series shows a definite relationship in its structural formula to the members preceding and following it, and there is generally some regularity in changes in physical properties of successive members of a series e.g. alkanes, alkenes and alkynes (Columbia Electronic Encyclopaedia, 2006).

Many common natural substances, e.g., natural gas, petroleum, and asphalt, are complex mixtures of hydrocarbons. These complex mixtures can be refined by fractional distillation into simpler mixtures or pure substances e.g. diesel fuel, gasoline etc. The hydrocarbons differ in chemical activity. The alkanes are unaffected by many common reagents, while the alkenes and alkynes are much more reactive, as a result of the presence of unsaturation (i.e., a carbon-carbon double or triple bond) in their molecules, (Columbia Electronic Encyclopaedia, 2006).

Many important compounds are derived from hydrocarbons. This occurs either by substitution or replacement by some other chemical group or element of one or more of the hydrogen atoms on the hydrocarbon molecule, or by the addition of some element or group to a double or triple bond (in an unsaturated hydrocarbon). Such derivatives include alcohols, aldehydes, ethers, carboxylic acids, and halocarbons.

In soil the hydrocarbon adsorbs to the organic fractions and has only a weak interaction with clay particle surfaces. The presence of these hydrocarbons poses a great risk to the existence of the fauna and flora of the affected habitat (Yong et al., 1992).

2.3 Soil Properties and Contaminant Transport

In the subsurface environment (soil) contaminant transport is strongly dependent on a number of factors e.g. groundwater flow, soil properties etc. It is important, therefore, to understand the nature and characteristics of the soil as it relates to contaminant transport.

Contaminants found in soil exist in various forms (solid, liquid and gaseous phases) and their transport is greatly affected by groundwater (pore water) flow. Some contaminants from the soil matrix dissolve as the water flows and are transported with the ground water. The water and the dissolved solutes flow around the solid soil particles through interconnected pore spaces (Fig 2. 4). In this way the contamination spreads throughout the soil.

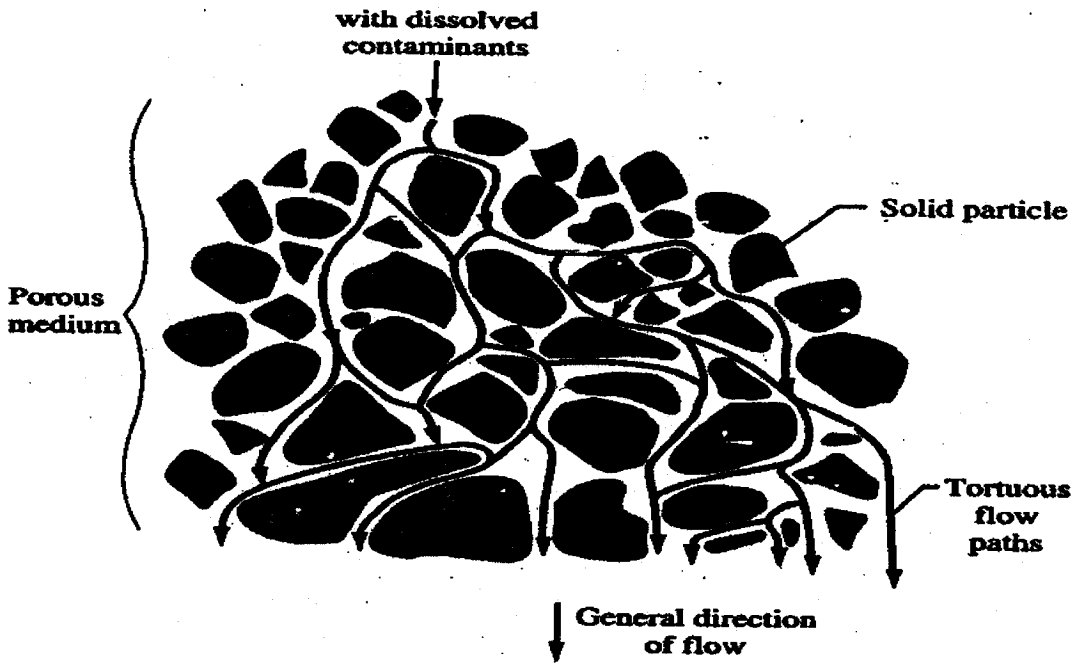


Fig. 2.4 Flow of pore water with dissolved contaminants in soil (Lagrega et al., 2001)

The properties of soil as it relates to contaminant transport can be subdivided into physical and chemical properties.

2.3.1 Physical Properties

2.3.1.1 Permeability

This is the property of soil that relates to the rate of flow of fluid through a representative volume of soil. Permeability defines the ease with which a fluid passes through a porous medium, thus if V represents the velocity of flow of the permeating fluid, and I the hydraulic gradient, the following relationship is obtained: (Hermond et al., 2001)

$V = KI = K \Delta h / \Delta x$

Where K is a constant defined as the hydraulic conductivity or the coefficient of permeability.

Δ = Change.

h = Hydraulic head

x = Spatial distance

I = Hydraulic gradient.

This relationship is referred to as Darcy's law and K is Darcy's coefficient.

2.3.1.2 Porosity

This is the ratio of the volume of voids to the total volume of the soil. Most times it is given in fractions or percentages.

2.3.1.3 Bulk Density

Bulk density is a measure of the mass per volume of soil. Its measurement is useful for estimating the type of soil minerals present and the degree of compaction. Bulk density is calculated on an oven dried weight basis. Soils with higher bulk densities have slow water infiltration and permeability (Yong et al., 1992).

2.3.1.4 Water Saturation

The degree of saturation of a soil sample is the fraction of pore spaces in the soil that is filled with water i.e. the water content of the soil. It is related to the soil pore pressure; such that when the soil pore pressure is zero the medium is at its saturation content. The relationship between the pore pressure and water content is determined empirically by the pore pressure-water content curves (Lagrega et al., 2001).

2.3.1.5 Soil Structure

Soil is composed of solid materials ranging in size from stones to fine clays. The larger materials are chemically or physically weathered over a long period of time to smaller soil

particles of sand, silt and clay. The soil particles are held together forming aggregates or peds that define the soil structure (Lagrega et al., 2001). Soil structure influences the amount of water that enters the soils (infiltration) and the amount of gas that diffuses in at the soil surface. Structure also plays an important role in the movement of liquid and gaseous substances through soil; porosity is a function of soil structure.

2.3.1.6 Specific Surface Area

This refers to the total surface area per unit weight of dry soil; generally expressed as m^2/g of soil. Thus individual particles in the soil contribute their surface area to the total

surface area of the soil. The higher the percentage of fines in the soil mass the greater the specific surface area. This property of soil plays a major role in the accumulation of toxic chemicals in soil.

2.3.2 Chemical Properties

The major chemical properties of soil include: cation exchange capacity, surface functional groups, soil buffering effects, organic matter content and chemical reactions (pH).

2.3.2.1 Organic matter content

Organic matter is comprised of decomposed plant and animal remains. It is a complex mixture of carbon compounds containing nitrogen, sulphur and phosphorus. Organic matter is made up of humic substances and other biochemical compounds (Pierzynski et al., 1994). Its content in the soil varies from less than 1% in coarse textured soils and soils of arid regions to 100% in peat soils. Typical topsoil contains 2-10% of organic matter and this often influences the physical and chemical properties of soil. (Yong, 2001)

2.3.2.2 Surface Functional Groups

The soil fractions with reactive surfaces include the layer silicates (clay minerals), soil organics, oxides, carbonates and sulphates. Layered silicates have structures that are in the form of 1:1, 2:1, and 2:1:1 tetrahedral or octahedral sheets. The tetrahedral sheets are composed of silicon and oxygen, while the octahedral sheets have aluminium and magnesium cations present in the layer matrix. Sometimes a disruption of the layers during formation and other like processes causes the bonds present on the surface and edge of each layer to be broken. Cations and anions present in the pore water compensate for these broken bonds through a mechanism of interaction known as sorption.

Surface functional groups are chemically reactive groups associated with the surfaces of layer silicates and with soil organic matter. The functional groups associated with layer silicates include hydroxyl group, hydrous oxides and amorphous silicates. Those associated with soil organics are the carbonyl, methoxyl, carboxyl, and amine groups. They can protonate or deprotonate depending on the pH of the aqueous environment (Yong, 2001).

2.3.2.3 Cation Exchange Capacity

Cation exchange capacity refers to the number of exchange sites, available for the adsorption and release of cations. It is the most significant property of soil as it relates to contaminant accumulation and transport. Cations of lower charge substitute for cations of

higher charge, thereby resulting in a permanent charge being formed on the charge site. Charge sites can also develop as a function of the pH of the soil environment.

2.3.2.4 Soil pH

Soil pH or soil reaction is an important condition of soil that affects the health of plants and animals. It is defined as the negative logarithm of the hydrogen ion concentration in soil. Soil pH is an indication of the acidity or alkalinity of soil and is measured in pH units. The pH scale goes from 0 to 14 with pH 7 as the neutral point. From pH 7 to 0 the soil is increasingly acidic and from pH 7 to 14 the soil is increasingly alkaline or basic. The effect of soil pH on the solubility of minerals, nutrients or contaminants present in the soil is considerable. Most minerals, nutrients and contaminants are more soluble in acid soils than in neutral or slightly alkaline soils (Yong et al., 1992, Pierzynski et al., 1994). Soil pH provides various clues about soil properties and is easily determined. The most accurate method of determining soil pH is using a pH meter.

2.4 Fate and Transport Process of heavy metals

The presence of heavy metals in soil can cause significant damage to the environment and human health due to their solubility and high mobility. Their presence in soil is a potential source of groundwater contamination. They are transported from the source in response to advection, hydrodynamic dispersion and molecular diffusion processes taking place in the subsurface. Apart from these transport processes a myriad of other complex

chemical and microbiological processes affect the fate of contaminants in soil. These processes serve to retard the movement of contaminants if not attenuate their concentration. Table 2.4 summarizes some of the processes that affect the fate of contaminants in soil (subsurface).

2.4.1 Retardation Processes

Retardation processes refer to those processes that impede further spreading of contaminants by removing or immobilizing them from their free state. For metals they include; precipitation, sorption ion exchange, filtration, redox reactions, biological uptake, dissolution and complexation (Yong et al., 1992).

2.4.2 Attenuation Processes

Attenuation processes are those which result in irreversible removal or transformation of the contaminants. Metal attenuation processes include biological uptake and oxidation-reduction reactions. Some processes tend to increase the mobility of the metals rather than immobilise or remove them; examples of such processes are dissolution and complexation.

Table 2.4 Summary of Natural Processes affecting the Fate of Hazardous Constituents in Soil (adapted from Lagrega et al., 2001)

Process	Class of chemical	Effect
Sorption	Organic/ Inorganic	Retardation
Precipitation	Inorganic	Retardation
Ion exchange	Inorganic	Retardation
Filtration	Organic/ Inorganic	Retardation
Chemical Oxidation-reduction	Organic/ Inorganic	Transformation/ Retardation
Biological uptake	Organic/ Inorganic	Retardation
Biodegradation	Organic	Transformation
Hydrolysis	Organic	Transformation
Volatilization	Organic	Elimination by inter-medium transfer
Dissolution	Organic/ Inorganic	Mobility enhancement
Cosolvation	Organic	Mobility enhancement
Ionization	Organic	Mobility enhancement
Complexation	Inorganic	Retardation mobility enhancement
Immiscible phase	Organic	Various partitioning

2.5 Fate and Transport Processes of Hydrocarbons

Over the years there has been an increase in the production and use of organic chemicals (Pierzynski et al., 1994). Their wide-spread use has also resulted in an increase of incidents where organic compounds have accidentally spilled into surface water, soil, or the atmosphere. The introduction of organic chemicals into the environment can occur by design, accident or neglect. This is often the case in some industrial sites which have the

potential of contaminating surface water, groundwater and soil if appropriate measures are not taken. These organic compounds can have adverse effects on certain microorganisms in the soil, plants and human health (Urum et al., 2003). It becomes necessary then to understand the processes that serve to reduce or eliminate these compounds. Figure 2.5 shows some of the processes and the fate of organic chemicals in the environment (Pierzynski et al., 1994).

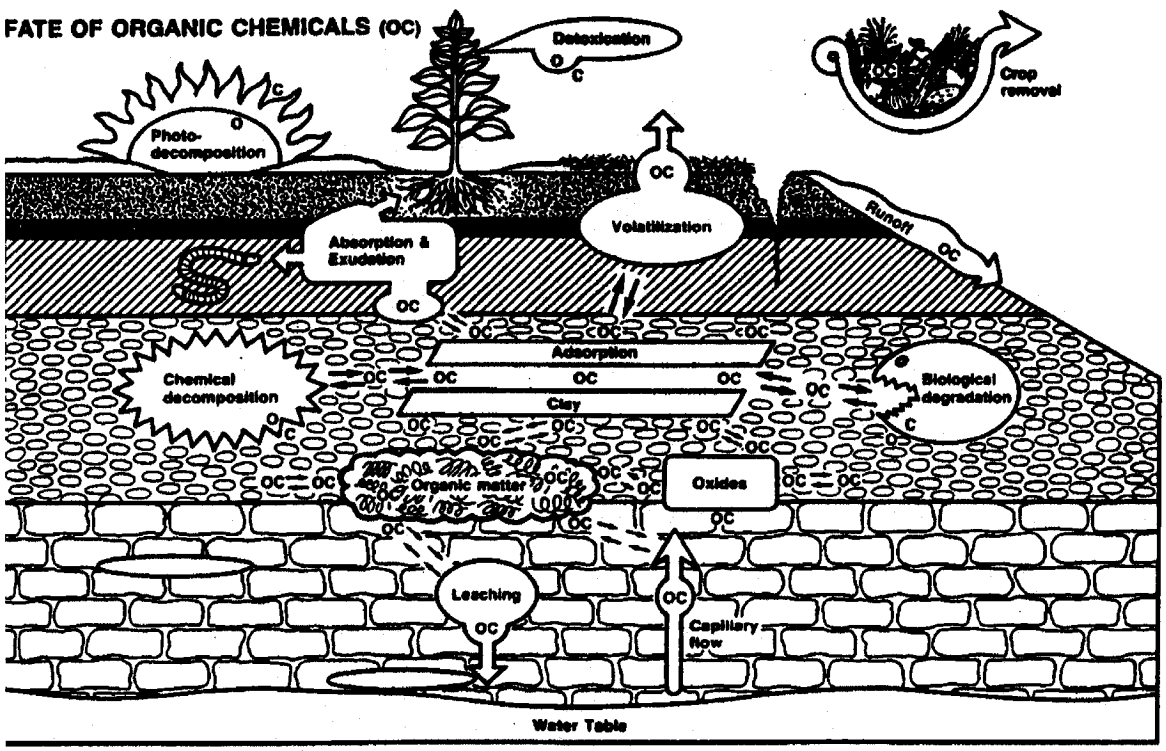


Fig. 2.5 Fate of organic chemicals in the environment ([Pierzynski et al., 1994)

2.5.1 Plant Uptake

Organic chemicals can be absorbed through roots or above ground foliage. Uptake is dependent on the plant species and the organic chemical. Above ground absorption could happen through stems, buds, and leaves (Pierzynski et al., 1994). Once organic chemicals are absorbed several reactions can occur to transform the chemicals into inorganic end products or other intermediary organic products (Lagrega et al., 2001). Examples of the reactions include oxidation-reduction, hydrolysis, dehalogenation and dealkylation.

2.5.2 Sorption

Sorption is an important process that helps to retard the movement of organic chemical contamination in soil. Sorption of organic chemicals by clay and organic matter materials occurs through one or more of the following interactions; van der Waals forces, hydrogen bonding, dipole-dipole interactions, ion exchange, covalent bonding, protonation, ligand exchange, cation bridging, water bridging and or hydrophobic partitioning (Yong et al., 1992). These processes are effective in reducing the mobility of the organic compounds.

Modelling the sorption of organic compounds in soil is frequently done using adsorption isotherms. The data are most commonly represented by either the Freundlich or Langmur equation.

The Freundlich adsorption equation is given as:

$X/m = KC^{1/n}$

Where;

X/m= mass of organic chemical adsorbed per unit weight of soil.

K, n = empirical constants

C = equilibrium concentration of the chemical.

The value of K is a measure of the extent of sorption. A plot of log (x/m) vs log C should produce a straight line, with 1/n as slope and intercept as log K.

The Langmur adsorption equation is given as:

$X/m = kbc (1+kc)$

Where,

X/m = mass of organic chemical adsorbed per unit weight of soil.

c = equilibrium concentration of the organic chemical

k = adsorption constant related to binding strength.

b = maximum amount of organic chemical that can be sorbed by the soil.

The linear form $c/ (x/m) = 1/ (kb + c/b)$

When a plot of c (x/m) vs c gives a straight line, then the adsorption data conforms to the Langmur equation.

2.5.3 Abiotic and Biotic Transformation

In soil, both biotic and abiotic reactions result in the transformation of organic chemicals in soil. The degradation process may occur through biotic reactions, however, the abiotic reactions may occur simultaneously. The principal abiotic processes that occur include

hydrolysis and oxidation- reduction reactions. They take place mainly in the liquid phase, i.e., the soil solution. Clays, organic matter and metal oxides (soil solids) are capable of catalyzing these abiotic reactions in the soil environment (Hermond and Fechner-Ley 2001).

Organic chemicals can also be lost through volatilization from soil. The rate of volatilization depends on the chemical and physical properties of the organic compounds (i.e., solubility, vapour pressure, half-life), soil properties (soil moisture, porosity, organic matter and clay content and density) and environmental factors (temperature, humidity, and wind speed). The initial step of volatilization is the ability of the compound to evaporate (change from solid or liquid to vapour). The vapour then moves through the soil and disperses into the atmosphere (Pierzynski et al., 1994).

The biotic process involves microorganisms in soil which are capable of transforming organic chemicals to inorganic products (CO_2, H_2O and mineral salts) in a process know as biodegradation. These micro-organisms in soil include bacteria, fungi, algae, yeast and specific protozoa. Biodegradation can occur under aerobic (presence of oxygen) or anaerobic conditions (absence of oxygen).

2.6 Soil Remediation Technology Review

Past industrial and waste management activities have contaminated soil and ground water at several sites both in Canada and the US (Yong et al., 1992, Urum et al., 2003). In the

US 60000 sites have been identified that are potential threats to public health and or the environment. In Canada, according to the National Pollution Release Inventory, industrial operations such as metal processing releases in approximate amounts 13300 metric tons of copper, 9500 metric tons of zinc, 1300 metric tons of lead and 33 metric tons of cadmium into air, water and soil (NPRI 1995). Professionals are faced with a great challenge in the remediation of these sites.

The selection of the most appropriate soil remediation technology depends to a large extent on the site characteristics, concentration and type of contaminants and the end use of the soil (Pierzynski et al., 1994). A number of remedial technologies for heavy metal contaminated soils have been in existence. Examples include isolation, immobilization, physical separation and extractions etc. However, very few technologies have been developed to deal with the problem of soil with a mixture of heavy metals and hydrocarbons. The remediation technologies available can be subdivided into physical, chemical and biological processes.

2.6.1 Physical Processes

2.6.1.1 Thermal Treatment

This method relies on the use of heat to remove the contaminants either by evaporation or by destroying the contaminants in a process known as incineration. For soil with organic contamination, the volatile organic compounds (VOC) are eliminated by evaporation and

the rest converted to carbon dioxide, water and other products of combustion (Lagrega et al., 2001).

By destroying the organic fractions and converting them to carbon dioxide and water vapour, thermal treatment reduces the organics. However, the inorganic content is not affected and can be even more toxic to the environment along with the products of combustion of organic contaminants.

2.6.1.2 Solidification/Stabilization

Solidification/Stabilization can be accomplished physically or chemically where the soil is bound into a solid mass. The contaminated soil is solidified to form a barrier thus preventing further migration of the contaminant. Solidification is a physical encapsulation of the contaminants while stabilization includes some form of chemical reaction to reduce the mobility of the contaminant. These processes often include the use of cement and plastic binding materials (Lagrega et al., 2001).

Some metals such as arsenic, chromium (VI) and mercury are not suitable for this type of treatment since they do not form hydroxides (Mulligan et al., 2001a). Another form of the solidification process in use also is vitrification; it requires thermal energy supplied through electrodes which are inserted into the soil. The soil is heated to high temperatures and cooled. This process has been used to treat arsenic, lead and chromium contaminated soil. High clay and moisture content can affect the efficiency of this method (Mulligan et al., 2001a).

2.6.1.3 Mechanical Separation

This method includes treatment that physically separates the contaminants from soil. The process of size selection is used such that larger cleaner particles are separated from smaller more contaminated ones. Hydrocyclones can separate larger particles from smaller ones by centrifugal force. Fluidised beds also cause a similar separation by gravimetric settling and flotation. Magnetic separation uses magnetic properties of the contaminants to remove them from soil. Mechanical separation techniques are more commonly used to remove metal contamination in a particular form or in combination with other processes.

2.6.1.4 Air /Steam Stripping

Air or steam stripping can remove volatile organic chemicals from soil. This method is particularly suitable for removing water-soluble hydrocarbons (e.g. methanol, ethanol, phenol etc), water immiscible hydrocarbons (e.g. benzene toluene, xylene) and halogenated hydrocarbons (trichloroethene TCE, dichloroethene DCE).

2.6.2 Chemical Processes

Chemical decontamination technologies are continuously being developed. Contaminants establish bonds with soil constituents, which when chemically treated detach or release the sorbed contaminants. Chemical treatments by reductive or oxidative mechanisms are

in use to detoxify or decrease the mobility of contaminants in soil (Evanko and Dzomback, 1997).

2.6.2.1 Treatment Walls

Treatment walls contain a reactive substance, physical, chemical or biological or in combination to reduce contaminants in ground water at contaminated sites.

However the treatment walls do not play an active role in the remediation of contaminated soil as a whole. They are only effective in treating contaminated ground water (Yong, 2001).

2.6.2.2 Electrokinetics

The electrokinetic process involves passing a low intensity electric current between a cathode and an anode embedded in the contaminated soil. Ions and charged particles are transported between the electrodes (Elektorowicz, 2004), as shown in Fig 2.6. Other processes like electroplating, precipitation and ion exchange can then remove the metals collected between the electrodes. Electrokinetics can be used in-situ in saturated soil with a low ground water flow rate, or excavated soil with metals such as zinc, copper, lead, arsenic, cadmium, chromium and nickel (Elektorowicz, 2004).

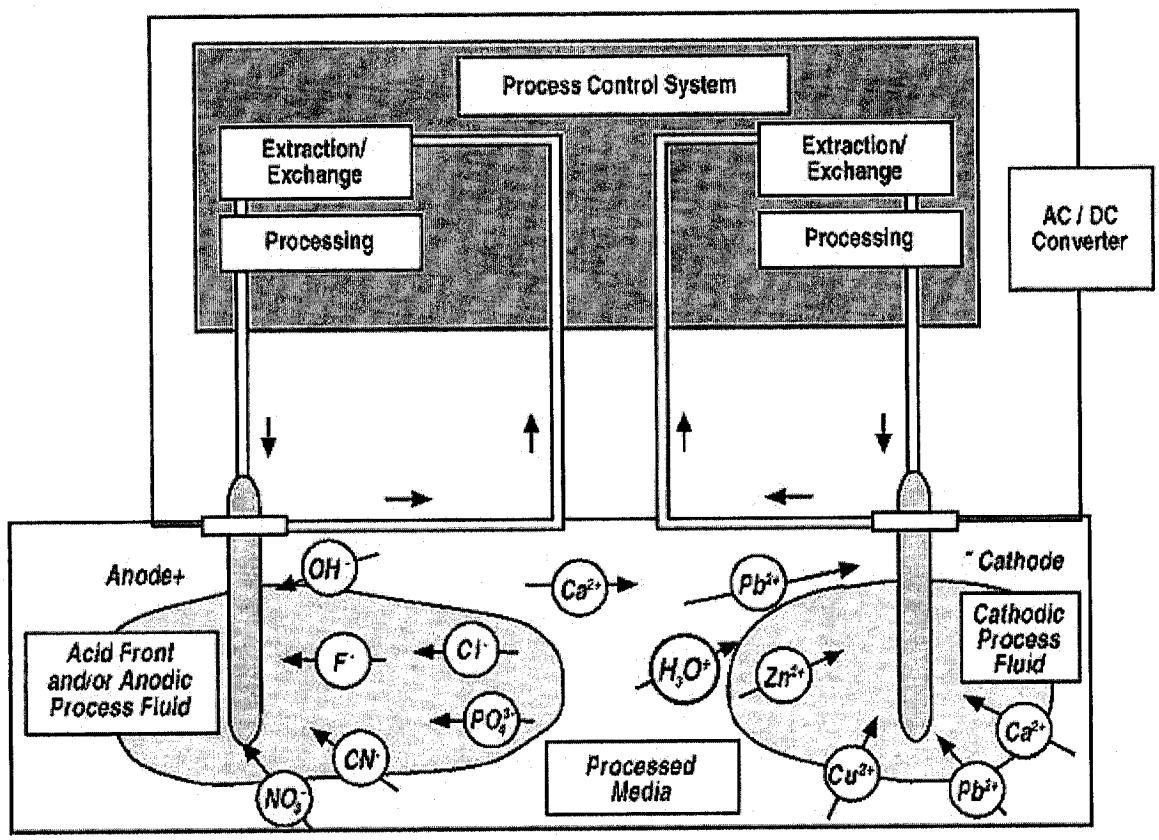


Figure 2.6 Electrokinetic process for soil remediation (adapted from HSRC, Michigan State University, 2005)

2.6.2.3 Ion Exchange

The ion exchange process involves the sorption of ions in solution onto oppositely charged discrete sites on the surface of a soil particle. It is driven by the attractive force of maintaining neutrality where electric charges are balanced by free ions of opposite charge. As such a previously held ion of weaker affinity is exchanged by the soil for stronger ions in solution. In the process ions compete for exchange sites on the soil solid particles, displacing previously held ions. Exchange affinity depends on pH, hydrated radius, electric charge and molecular configuration (Yong, 2001).

2.6.3 Biological Processes

2.6.3.1 Land Treatment

In land treatment the contaminated material is mixed into or dispersed over the surface of the soil. This process is acquiescent to the organic contaminants in the soil. Microbial degradation of the organic chemical is enhanced over-time as the microorganisms become acclimatised to the contaminants in the soil. Other land treatment methods include bio-pile, composting and land farming (Lagrega et al., 2001).

2.6.3.2 Biodegradation

Biodegradation of contaminated soil is relatively new and still under development (Riser-Roberts, 1998). It has gained considerable attention for insitu or on site remediation of soil contaminated with organic compounds. Considering the wide range of organic chemicals in use today, there exist different strains of bacteria, fungi, yeast and other microorganism in soils to carry out the degradation process.

Many other methods or technologies that use microorganisms to biodegrade the contaminants include; bioventing, biosparging, biofiltration, biotransformation, biotraps, biorestoration and bio-stripping (Yong, 2001). However the biodegradation process is not amenable to inorganic contaminants which most often are present with the organic

compounds. The toxicity, persistence and mobility of intermediary organic metabolites which can accumulate, are also concerns that serve as setbacks in this method.

2.6.3.3 Phytoremediation

Phytoremediation is an in-situ treatment of soil that relies on the ability of plants to accumulate or detoxify organic and inorganic chemicals. Although this method of soil remediation has not been fully studied, the fact remains that plants have the ability to absorb and metabolize contaminants in soil (Elektorowicz, 2004). Phytoremediation methods include processes such as rhizofiltration, phytoextraction and phytostabilization. Moreso, it is most often applicable to soils with low levels of contamination. This method however has the draw-back of a longer time of treatment compared to other methods available.

2.7 Biosurfactants

Biosurfactants are a heterogeneous group of surface-active molecules produced by microorganisms from various substrates like sugars, alkanes, oils and wastes. They are produced extra-cellularly or as part of the cell membrane by bacteria, fungi and yeast. (Mulligan et al., 2001b)

Biosurfactants are amphiphilic in nature. They contain a hydrophobic portion, which has little affinity for the bulk medium, and a hydrophilic group that is attracted to the bulk

medium. The hydrophilic moiety is composed of amino acids, peptides, anions or cations and mono, di or polysaccharides. The hydrophobic portion is often made up of saturated or unsaturated hydroxylated fatty acids (Banat, 1995). The surfactant molecules reduce the surface and interfacial tension in both the aqueous solutions and hydrocarbon mixtures (Banat, 1995).

Biosurfactants are grouped as glycolipids, lipopetides, phospholipids, fatty acids, neutral lipids, polymeric and particulate compounds as shown in Table 2.5 (Mulligan et al., 2002). Most of these compounds are either anionic or neutral with only a few being cationic in nature (Mulligan et al., 1999a). The critical micelle concentrations of biosurfactants range from 1 to 200 mg/l and molecular weights range from 500 to 1500 Daltons (Lang and Wagner 1987). Biosurfactants have become promising natural agents used in soil remediation due to their biodegradability, ecological acceptability, non-toxic and environmentally friendly nature. In addition, their uses have also increased in recent times due to their ability to meet most synthetic surfactant requirements. (Hong et al., 2002).

Table 2.5 Type and microbial origin of biosurfactants (adapted from Mulligan & Gibbs, 2002)

Surfactant Class	Microorganism
Trehalose Lipids	*Arthrobacter parafinues, Cornyebacterium spp Mycobacteriumspp., Rhodococus erthropolis*
Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas sp.*
Sophorose lipids	*Candidia apicola, Candida bombicola Candida lipolytica, Candida bogoriensis*
Glucose- fructose-, saccharose lipids	*Arthrobacter sp., Corynebacterium sp., R. erythropolis*
Cellobiose lipids	*Ustilago maydis*
Polyol lipids	*Rhodotorula glutinus, Rhodotorula graminus*
Diglycosyl diglycerides	*Lactobacillus fermentii*
Lipopolysaccharides	*Acinetobacter calcoaceticus(RAGI), Psuedomonas sp*
Lipopeptides	*Arthrobacter sp., Bacillus pumilis, Bacillus licheniformis*
Surfactin	*Bacillus subtilis*
Viscosin	*Psuedomonas fluorescens*
Ornithine, lysine peptides	*Thiobacillus thiooxidans, Streptomyces sioyeansis Gluconobacter cerinus*
Phospholipids	*Acinetobacter sp.*
Sulfonylipids	*T. thiooxidans, Corynebacterium alkanolyticum*
Fatty acids (corynomycolic acids, spiculisporic acids etc.	*Capnocytophaga sp., Penicillium spiculisporum Corynebacterium lepus, Arthrobacter paraffineus Talaramyces trachyspermus, Nocardia erthropolis*

2.8 Biosurfactants application in soil remediation

Biosurfactants are gaining prominence owing to the advantage they have over synthetic surfactants. They are taking over in a number of important industrial uses like de-emulsifier, crude oil recovery, penetrants, and foaming agents. Also the possibility of them being produced from renewable resources and their functionality at extreme conditions make them amenable to a variety of applications.

Biosurfactants have shown great success in soil remediation operations. Mulligan et al., (1999) showed that surfactin, rhamnolipids and sophorolipids could be used to enhance metal removal. They demonstrated the feasibility of using these biosurfactants to remove heavy metals like copper and zinc from oil-contaminated soil in a batch washing process. The soil contained contaminants to the levels of 890 mg/kg zinc and 420 mg/kg copper and 12.6% of oil and grease. The results showed the highest level of zinc and copper removal using 12% rhamnolipids. With a series of five washings the removal efficiency greatly increased such that 70% of copper was removed by 0.1% surfactin/1% NaOH while 4% sophorolipids/0.7% HCl removed 100% of the zinc.

Sequential extraction showed the fractions from which the contaminants were removed. The carbonate and oxide fraction accounted for over 90% of the zinc present in the soil whereas the organic fraction constituted over 70% of the copper. Surfactin removed the organically bound copper while sophorolipids removed the carbonate and oxide bound zinc (Mulligan et al 1999a). This experiment shows the possibility of using the anionic

biosurfactants: rhamnolipids, surfactin, and sophorolipids in removing heavy metals from soil, even when the exchangeable metal fraction is very low.

Hong et al., (2002) with his team of researchers used a plant derived biosurfactant (saponin) to recover cadmium and zinc from soil. They carried out a batch washing process, where the biosurfactant extracted 90-100% of the cadmium and 85-98% of the zinc extraction. The saponin was effective in removing the exchangeable and carbonates fractions of the metals.

Biosurfactants have been demonstrated to successfully solubilize and remove hydrocarbon contaminants from soil (Banat, 1995). In research done by Mulligan et al. (2003) on the removal of pentachlorophenol (PCP) from soil, rhamnolipids in its foam form was used to remove PCP from soil. In a soil contaminated with 1000 mg/kg PCP, rhamnolipids was able to solubilize 61% of the PCP concentration in the soil sample.

Biosurfactants have also been used to enhance biodegradation of hydrocarbon contaminants in soil. Banat, (1995) showed that sophorolipids could enhance the biodegradation of polycyclic aromatic hydrocarbons (PAHs) present in a bilge waste. The result was concurrent with the fact that biosurfactants could help solubilise the hydrophobic contaminants and make them available to the indigenous microorganism thereby reducing the concentration in the soil.

The presence of hydrocarbons and heavy metals in soil can cause significant damage to the environment and human health. The most appropriate remediation technique depends on the soil characteristics, concentration and type of pollutant to be removed and the end use of the soil.

The studies above indicate the possibilities of using biosurfactants to remediate organic or metal contamination. However, in the case of mixed contamination very few technologies are capable of dealing with both types of contaminants (hydrocarbons and heavy metals). This poses a challenge in the mitigation process of soils with both types of contaminants. The need to devise ways of handling the problem of mixed contamination necessitated this research into finding out how biosurfactant use can enhance the remediation process.

2.9 Biosurfactant Enhanced Remediation of Mixed Contaminated Soil

Biosurfactant applications in soil remediation have shown positive results in areas of heavy metals and/or hydrocarbon remediation. Results from studies conducted by Mulligan et al. (1999), 2003), Banat (1995) and Hong (2002) have shown a substantial decrease in the concentration of the contaminant after treatment with biosurfactants. The contaminants were treated by desorption from the soil matrix. The biosurfactants seem to have enhanced the remediation process by influencing the bioavailability of the contaminant (Mulligan et al., 2004).

The biodegradability and low toxicity of the biosurfactants make them a promising choice for use in soil remediation technology. In order to ascertain the success of biosurfactant use in the remediation of a mixed contaminated soil, three biosurfactants (saponin, rhamnolipids, and mannosyl-erythritol lipids) are employed in a soil washing process. The research thus focuses on investigating the feasibility of enhanced remediation of the heavy metals and hydrocarbon contaminated soil by the biosurfactants.

Chapter Three: Experimental Materials and Methods

3.1 Introduction

This chapter presents the experimental materials and methods used in the research. The materials include; soil samples from a contaminated site, JBR 210, saponin, mannosyl-erythritol lipids, 6N HCl used for the digestion of soil prior to Atomic Absorption Spectrophotometer (AAS) analysis, HNO_3 used for biosurfactant digestion and distilled water used for dilution of samples and in control experiments. 1% NaOH and 1% HCl were used to adjust the pH of solutions. All reagents used were obtained from Fisher Scientific and Sigma-Aldrich.

3.2 Experimental Materials

3.2.1 Soil Sample

Soil samples were collected from a potential residential downtown Montreal site, contaminated with diesel fuel and heavy metals. The soil was air dried; large particles were crushed using a pestle and a series of sieves were then used to establish the particle size distribution of the soil. Particle sizes retained on the 2 mm sieve were discarded while the ones less than 2 mm were homogenised and stored for subsequent remediation experiments. Soil samples weighing between 1- 5 g were used, depending on the experiment. The selected characteristics of the soil are listed in Table 3.1.

Table 3.1 Characteristics of the soil

Parameter	Value
Grain Size Distribution (%)[a]	6 <0.05 mm 65 <2 mm 29 > 2 mm
pH of aqueous soil suspension	7.45
Cation Exchange Capacity (CEC) (cmol/kg)[b]	6
Organic Content (%)[c]	6
Water Content (%)[d]	11.2
Total Petroleum Hydrocarbon (TPH) Content (mg/kg dry soil)	228
Heavy Metal Content (mg/kg dry soil)	449, 894, 261, 167, 33, 16 For Pb, Zn, Cu, Ni, Cr and Cd respectively.

a ASTM method D422-63 (2002) b Appendix A

c Appendix B, d ASTM method D 2216

The heavy metal content in the soil was determined by cold digestion using 6N HCl with shaking at 60 rpm for 24 h. The samples then were centrifuged by an IEC HN-SII centrifuge manufactured by International Equipment Company USA at 3000g for 10 min. The digested solutions were filtered with Whatman No.2 filter paper and analysed for metal content by Perkin Elmer Atomic Absorption Spectrophotometer 100 (AAS). The values of the various heavy metals are presented in Table 3.1.

3.2.2 Contaminants in soil

From the Quebec "B" soil criteria (Ministère du Développement durable, de l'Environnement et des Parcs, 2006), for residential soil use, the contaminants of interest in the soil are shown in Fig 3.1. They are copper, zinc and nickel. Since the total

petroleum hydrocarbon content of the soil was low (below the allowable limit), its final concentration was measured only at the end of the washing process. The results are presented in the next chapter.

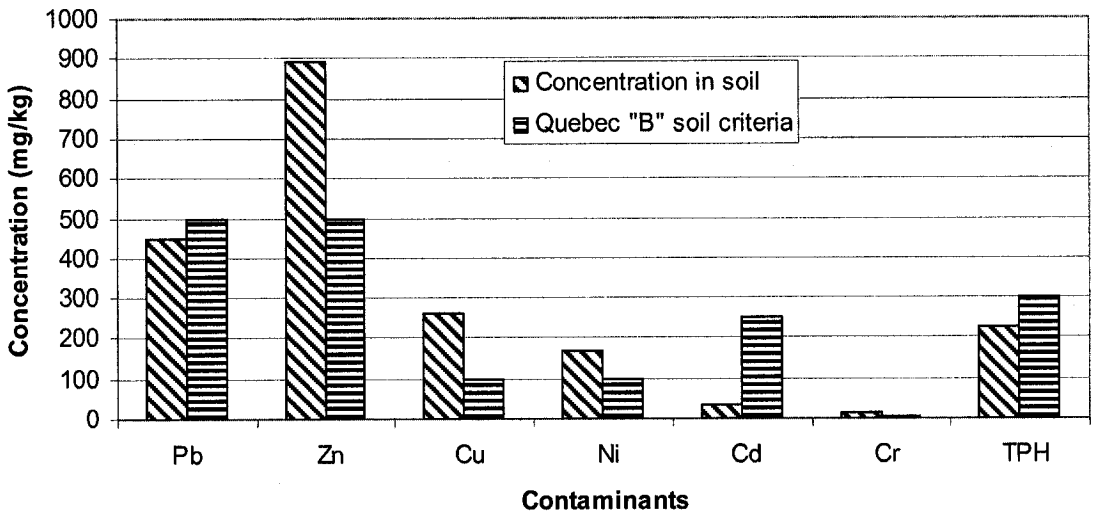


Figure 3.1 Contaminants in soil

Pb = Lead, Zn = Zinc, Cu = Copper, Ni = Nickel, Cd = Cadmium, Cr = Chromium, TPH = Total Petroleum Hydrocarbon.

3.2.3 Saponin

The saponin obtained from Sigma-Aldrich Canada is a plant derived biosurfactant from quillaja bark. It contains a B-D-glucuronic acid with a carboxyl group of sugar moiety in the hydrophilic fraction (Hong et al., 2002) (Fig 3.2).

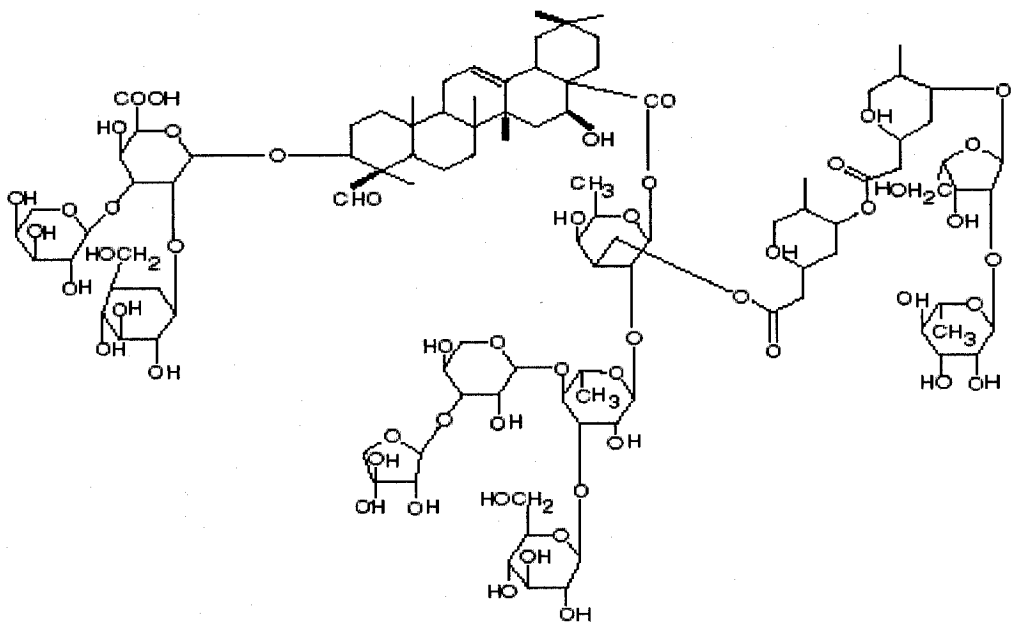


Fig 3.2 Chemical structure of saponin (Urum et al., 2003)

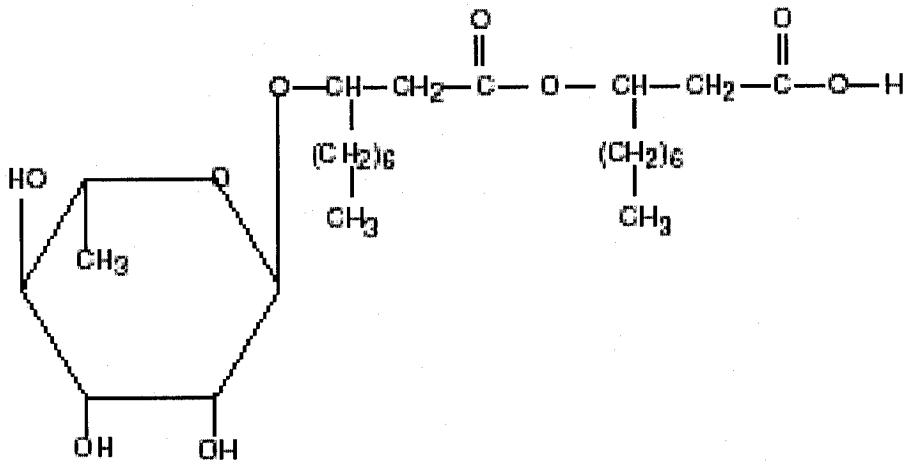
Each concentration of 1, 5, 10, 30, 60, and 100 grams per litre of saponin was prepared by weighing out the required amount in 1 litre of distilled water. The properties of saponin are presented in Table 3.2.

Table 3.2 Properties of Saponin (Adapted from Sigma-Aldrich 2006)

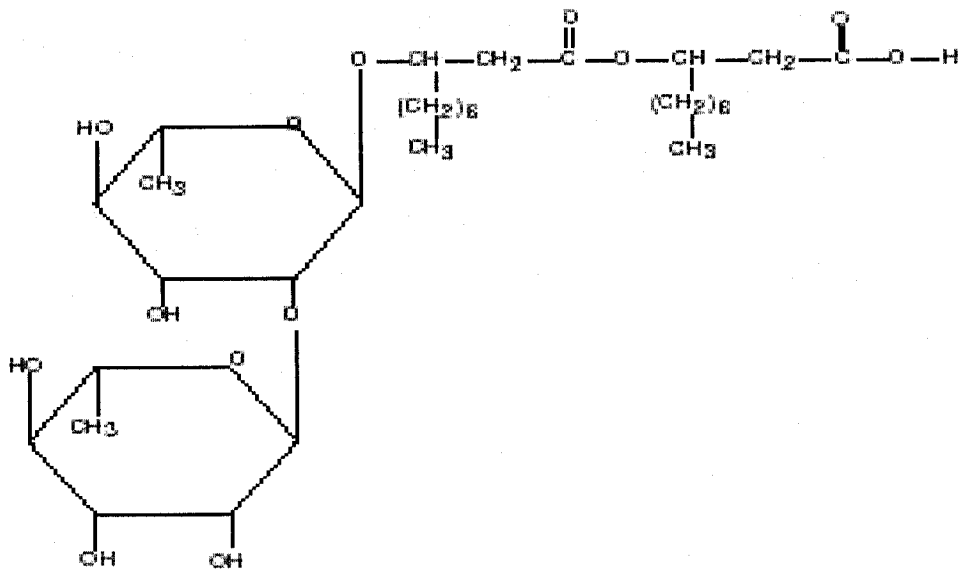
Property	Description
Surface Tension (mN/m)	40
pH of aqueous solution	5.0 -5.5
Critical micelle concentration (g/l)	1
Appearance	Light coloured Powder
Solubility	Soluble
Volatility	Not Volatile
Elemental Analysis	44% carbon, 6% hydrogen 51 % oxygen.

3.2.4 Rhamnolipids JBR 210 (Jeneil Surfactant Company 2006)

Rhamnolipid biosurfactant is produced by *Pseudomonas aeruginosa* as a metabolic by-product (Mulligan et al 2005). It contains two types of rhamnolipids, RL (R1) and RRLL (R2) (Fig 3.3).



R1 (RL)



R2 (RRLL)

Fig 3.3 Structure of rhamnolipids (Mulligan et al 2004)

JBR 210 is an aqueous solution containing 10% rhamnolipids, obtained from Jeneil Biosurfactant Co. USA. A summary of its properties are shown in Table 3.3.

Table 3.3 Properties of Rhamnolipids (adapted from Jeneil Biosurfactant Company 2006)

Property	Description
Surface Tension (mN/m)	30
pH of aqueous solution	6.5-7.5
Critical micelle concentration (mg/l)	25
Appearance	Dark reddish brown solution
Solubility	Soluble at neutral pH
Volatility	Not Volatile
Specific Gravity at 25^0 C	1.05-1.15
Molecular weight	504(R1), 650(R2)
Formula	$C_{26}H_{48}O_9$ (R1) ,$C_{32}H_{58}O_{13}$ (R2)

The 10% rhamnolipid solution was diluted with varying amounts of distilled water to obtain the different concentrations of 0.1%, 0.5%, 2%, and 4% used in this research. These concentrations were chosen based on the results of previous studies conducted by Mulligan et al. (1999a, 2001a) on rhamnolipid use in soil washing. These rhamnolipid concentrations were used in the first phase of the experiments in order to establish the optimum conditions for the soil washing. The actual remediation was conducted with a 2% rhamnolipid concentration.

3.2.5 Mannosyl-Erythritol Lipids (MEL)

MEL is a biosurfactant with surface active properties. It is produced extracellularly during the cultivation of yeast *Kurtzmanomyces* sp I-II with soybean oil as the sole carbon source

(Kakugawa et al 2002a and 2002b) (Fig. 3.4). MEL is a representative of glycolipids and has gained attention in recent years (Kitamoto et al., 1995).

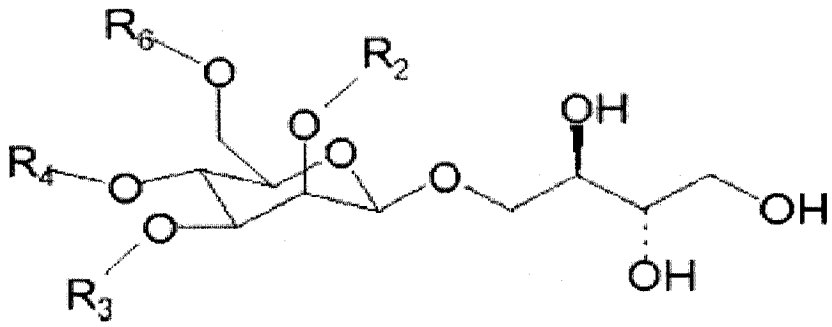


Fig 3.4 Molecular structure of mannosyl-erythritol lipids.
The length and saturation of the fatty acid residues (R2, R3) depend on the substrate and microorganism used. For example, if *Pseudozyma aphidis* DSM 14930 is grown on soybean oil: R2 = R3 = C7-C14 fatty acids, saturated and unsaturated. R4, R6 = acetyl or H (Kitamoto et al. 1995).

It has a surface tension of 29 mN/m and a critical micelle concentration of 2 mg/L (Kim et al., 2002). A summary of the characteristics are presented in Table 3.4.

Table 3.4 Selected Characteristics of MEL (Kakugawa et al., 2002a and 2002b)

Property	Description
Surface tension (mN/m)	29
pH of aqueous solution	5-5.5
Critical micelle concentration (mg/L)	2
Appearance	Light yellow solution
Solubility	Soluble at pH above 4
Volatility	Not Volatile
Molecular weight	634 Daltons

The MEL solution contained 42% of active ingredient. The solution was diluted with the required amounts of distilled water to obtain concentrations of 0.002%, 2%, 4% and 10% that were used in the experiments.

3.3 Experimental Methods and Set-up

In this study, experiments were designed in two phases. The first phase of experiments included batch tests, to establish the optimum conditions with respect to concentration and pH. These conditions were then used in the second phase, which is the actual remediation of the soil. The procedures followed in the course of this research and the specifications of the equipments used are presented in this section.

3.3.1 Soil Characterization

The soil characterization was done using the EPA (APHA 1995) or ASTM standard methods. The organic content of the soil was determined by the method of ashing. Details of the method are presented in Appendix B. The method outlined by Chapman (1965) was used in estimating the cation exchange capacity of the soil (Appendix A). A series of five standard ASTM sieves were used to separate the soil into different particle sizes according to ASTM method D 422-63 (2003). The result is shown in Table 3.1.

For the purpose of this study, particle sizes greater than 2 mm were discarded while sizes of less than 2 mm were homogenised and stored for use in subsequent experiments. The heavy metal content of the soil was determined by the following procedure:

- 3 g of soil were weighed in 3 separate sterile centrifuge tubes
- 20 ml of 6N HCl was then added to each centrifuge tube to digest the metals in the soil.
- The vials were placed on an INNOVA shaker at 60 rpm for 24 h.
- The vials were centrifuged at 3000g for 10 min and filtered to remove the supernatant.
- The supernatant was diluted to 50 ml with distilled water and analysed for metal content with the Atomic Absorption Spectrophotometer (AAS).
- The metal concentration in the supernatant in mg/kg dry soil was then calculated, using equation 3.1.

Metal concentration $_{supernatant}$ (mg/kg dry soil) = (A X B)/ (G) x 1000 g / 1kg .....**Equation 3.1**

Where A= Concentration of metal in digested solution (mg/L)

B= Final volume of digested solution (mL)

G = weight of soil sample in grams.

The diesel in the soil was extracted with hexane according to EPA method 1664/8015C (EPA 2000). Hexane 15 mL was added to 5g of air dried soil and shaken vigorously for 5 min. The vial was allowed to stand for a few minutes then the extract was pour out leaving the soil in the centrifuge tube. Analysis of the extract was done by gas chromatography using a Varian Model 3800 with auto sampler 8400 equipped with a flame ionization detector and capillary column (30 m long, I.D 0.25 Supelco 20). The injector and detector were maintained at 250^0C and the oven temperature was programmed to rise from 70 ^{0}C to 300 ^{0}C at 10 ^{0}C/min increments and hold for 10 min at the final temperature of 300 ^{0}C. The initial holding time at 70 ^{0}C was 5 min. The gas flows used were hydrogen at 30 ml/min, air 300 ml/min with a make up flow of 25 ml/min.

Quantification of the total petroleum hydrocarbon was done by comparing peak areas of the extract from soil samples with the peak areas of known concentrations of diesel fuel in hexane. A sample of the calibration curve is shown in Appendix C.

The TPH content in mg/ kg dry soil was calculated using the following equation;

$$\text{mg TPH}_{\text{supernatant}} = \left(\frac{\text{mg TPH}_{\text{supernatant}}}{\text{kg hexane}}\right) \times \left(\frac{0.68\text{kg hexane}}{\text{L}}\right) \times \left(\frac{1\text{L}}{1000\text{ml}}\right) \times (\text{ml of extract}) \quad \textbf{Equation 3.2}$$

$$\text{mg TPH/ kg dry soil} = \left(\frac{\text{mg TPH}_{\text{supernantant}}}{\text{g soil}}\right) \times \left(\frac{1000\text{g}}{1\text{kg}}\right) \quad \ldots\ldots \textbf{Equation 3.3}$$

3.3.2 Critical Micelle Concentration (CMC) Determination

The critical micelle concentration of the biosurfactants was determined using the Fisher Tensiomat model 21, by measuring the surface tension of the biosurfactant solution at different concentrations. The duNouy ring method was applied here, where the ring was pulled through the biosurfactant solution until it broke through the surface. The value of the surface tension at that point was then recorded. The CMC values were obtained as the value at intersection of the tangents of the curve in a plot of surface tension versus concentration.

3.3.3 Batch Experiments

Batch soil washing experiments were conducted by varying the biosurfactant concentration and pH in centrifuge tubes with a soil: solution ratio of 1:20 w/w. Distilled water alone was used as a control. The vials were placed on an INNOVA 2000 shaker at 60 rpm for 24 h at room temperature and then centrifuged (3000g,10 min).The supernatant was filtered and analysed for metal content (Cu, Zn, Ni) by AAS according to standard methods. The metal content of the soil was then calculated using Equation 3.1. The metal removal from the soil is reported as a percentage of the initial concentration. Below is a schematic diagram of the process (Fig 3.5).

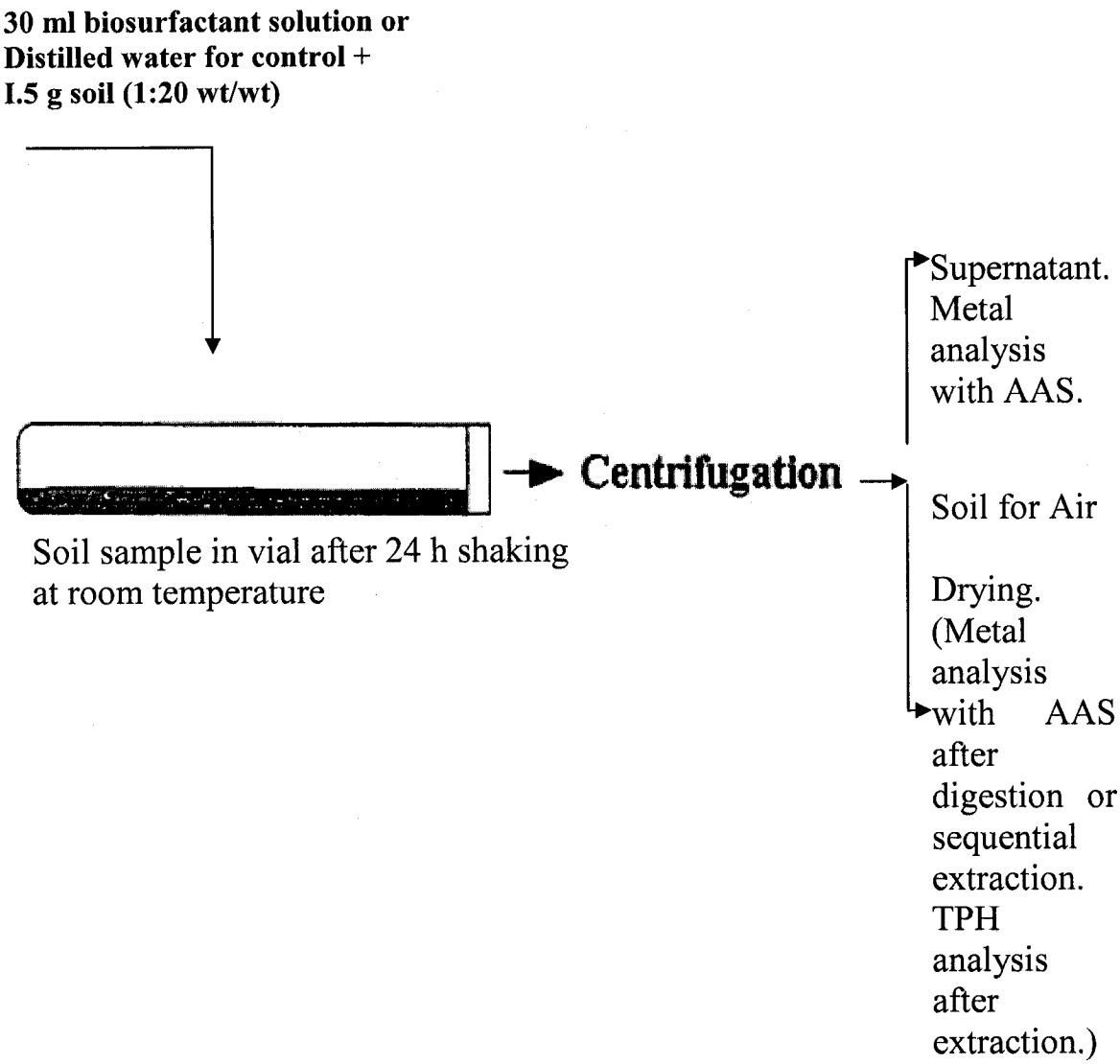


Fig 3.5 Batch washing process for the contaminated soil

The total petroleum hydrocarbon (TPH) levels were determined after the first and fifth wash of the soil so as to have an understanding of how much had been removed. The soil in the vials (about 1.5 g) was dried after the washing process was completed and extracted with 4.5 ml of hexane according to the method detailed in section 3.3.1 above. Analysis of the extract was done with gas chromatography using the conditions stated in section 3.3.1. The amount removed was then calculated using equations 3.2 and 3.3.

Surface tension measurements were used to estimate the amount of surfactant sorbed to the soil, since this has a direct effect on the amount of surfactant available for metal desorption. All experiments were done in triplicate, and the results presented as the average ± standard deviation.

3.3.3.1 Rhamnolipid Soil washing.

A series of washings was carried out using 1.5 g of soil to establish the optimum conditions: concentration and pH for the remediation of the soil using rhamnolipid solution. To do this, 30 ml solutions of four different concentrations (0.1%, 0.5%, 2%, and 4%) of rhamnolipid, pH 6.5 were added to 1.5 g soil in the batch washing process outlined in section 3.3.3. The concentration of rhamnolipid solution with the highest removal of metals after one wash was chosen. Concentrations of 0.5% and 2% were chosen for the study of effect of pH on removal of the metals. pH values of 6.5, 8 and 10 were used. The solution pH was adjusted with 1%
NaOH. The pH value with the highest percentage removal was chosen to be used for the next phase of experiments. The results are presented in the next chapter.

3.3.3.2 Saponin Soil washing

Batch washing of the soil was performed also with saponin as a function of concentration and pH. To determine the optimum concentration of saponin needed for the soil remediation experiments, 1.5 g of soil were weighed out into a series of centrifuge tubes

and 30 ml of solution containing varying amounts of saponin was added. The pH range of the mixture was 5.0-5.5. The vials were sacrificed for TPH and heavy metal analysis after 24 h (single wash) with shaking as detailed in section 3.3.3. Distilled water alone at the same pH range was used as the control.

The effect of pH on removal of the heavy metals was also studied using the concentration with the highest percentage removal of metals, at pH 3, 4, 5, 7 and 10. The TPH content of the soil after washing were determined for the optimum conditions only. The pH of the saponin solution was adjusted with 1 % NaOH or 1 % HCl solutions.

3.3.3.3 Mannosyl-Erythritol Lipids (MEL) Soil Washing

The washing process was also conducted using concentrations of 0.002%, 2%, 4% and 10% crude mannosyl-erythritol lipids solution. The concentration with the highest removal of metals was chosen for the study of the effect of pH on removal rates.

The optimum conditions (concentration and pH) established in the batch tests were then used for the remediation of the soil. Each test was conducted in duplicate and the results presented as an average ± standard deviation.

3.3.4 Multiple Washing of Soil

The optimum conditions of concentration and pH established in the batch experiments with rhamnolipid, saponin and mannosyl-erythritol lipids were used in the multiple washing of the soil. The soil was washed for five consecutive times with the biosurfactants. After each wash (24 h) the supernatant was removed by centrifugation (3000g, 10 min) and a fresh biosurfactant solution was added to the soil.

Since the soil has more heavy metals (above the allowable limits for residential soil use) than TPH, the conditions chosen were that for optimum metal removal. The method applied here was similar to that used in the batch experiments. All the experiments were performed in triplicate and the results presented as an average ± standard deviation.

3.3.5 Sequential Extraction

Sequential extractions were performed to determine the speciation of the metals in the contaminated soil. The method outlined by Mulligan (1998) was used. It entails extracting the metals with solutions of varying strength as shown in Table 3.5. The different metal fractions include; soluble, exchangeable, carbonate oxides and hydroxides, organic and residual (Yong et al., 1999).

Sequential extraction was carried out before and after soil washing with biosurfactant to establish the fraction from which the biosurfactant removed the metals.

To do this 1.5 g of soil were weighed into three different vials and the solution for the extraction of each fraction was added. The concentration of metals in each fraction was measured with the AAS, and the amount of metals (Cu, Zn, Ni) extracted from the soil in each fraction was then calculated using equation 3.1 above

Table 3.5 Sequential Extraction Process (adapted from Mulligan, 1998)

Order of Sequence	Chemical Reagents	Soil Fraction
1	Extraction of metals by biosurfactant and distilled water overnight with 15 ml of solution	Soluble
2	Extraction of metals with 8 ml of 1 M $MgCl_2$ (pH 7) for 1 hour	Exchangeable
3	Extraction of metals with 8 ml of 1 M Sodium Acetate (NaOAc) adjusted to pH 5 with acetic acid for 5 hours	Carbonates
4	Extraction of metals with 20 ml of 0.04 M $NH_2OH.HCl$ in 25% (v/v) acetic acid (pH 2.5) at 96^0C for 6 hours	Oxides and hydroxides
5	Extraction with 3 ml of 0.02 M HNO_3 and 5 ml of 30% H_2O_2 (pH 2) for 2 hours at 85^0C, followed by 3 ml of H_2O_2 (pH 2) for 3 hours at 85^0C and 5 ml of 3.2 M ammonium acetate (NH_4OAc) in 20% (v/v) HNO_3 diluted to 20 ml at room temperature for 30 minutes	Organic
6	Digestion at 90^0C with 25 ml of dilute aqua regia (50 ml HCl 200 ml HNO_3 and 750 ml distilled water) for 3 hours	Residual

Ac denotes Acetate.

Chapter Four: Results and Discussion

4.1 Introduction

In this chapter the results of the research are presented and discussed. The natural soil sample contaminated with heavy metals (Cu, Zn, and Ni) and diesel fuel was remediated using three different biosurfactants (rhamnolipids, saponin and mannosyl-erythritol lipids). Prior to multiple washing of soil, batch washing tests were conducted to establish the optimum conditions with respect to concentration and pH of the washing solution. These conditions were used to determine the maximum percentage removal of the contaminants in the remediation process. The range of parameters studied was chosen based on the related literature review. All the experiments were performed in triplicate and the results presented as an average ± the standard deviation.

4.2 Critical Micelle Concentration (CMC) of Biosurfactants

The structures and properties of the biosurfactants used are given in chapter 5. In this section the results of the CMC determined for the biosurfactants are presented. The CMC values are indicated in Figures 4.1 and 4.2.

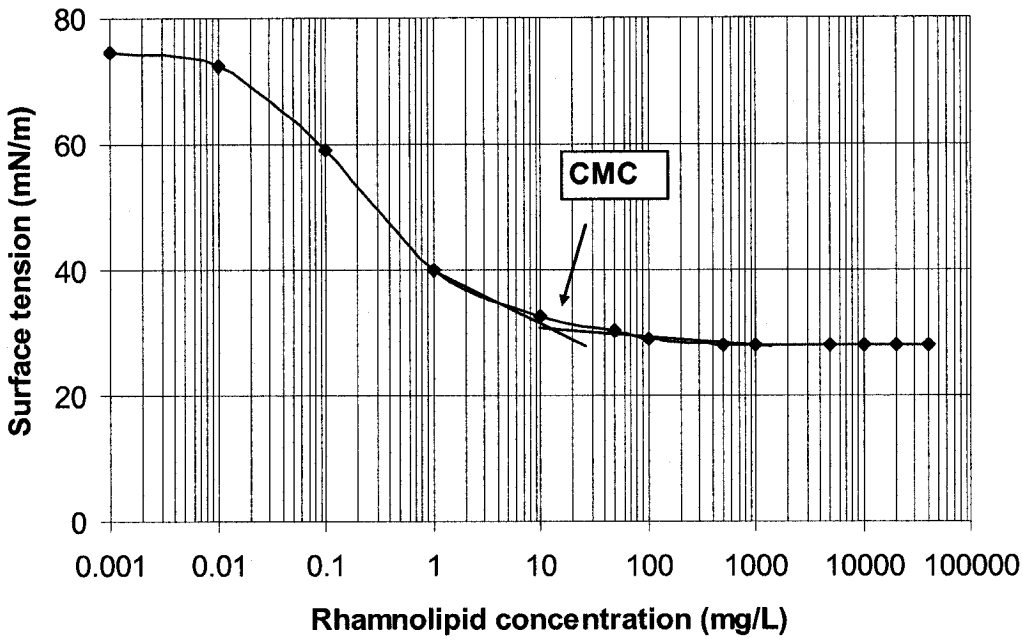


Fig 4.1 CMC of rhamnolipids (JBR 210)

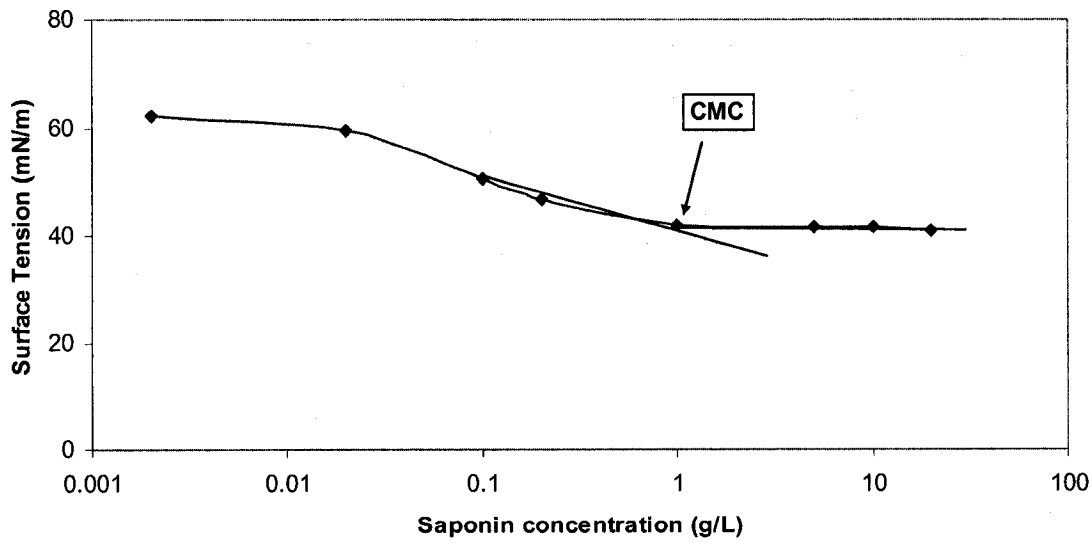


Fig 4.2 CMC of saponin

As indicated in the above figures the value of the CMC was about 12.5 mg/L for rhamnolipids while from literature the value ranges from about 25 -60 mg/L. The CMC for saponin was about 1g/L, this value corresponds to the value obtained by Hong et al., (2002). The CMC of mannosyl-erythritol Lipids is 2 mg/L (Kim et al., 2002). The experiments conducted thereafter were carried out at concentrations above the CMC. This was done to ensure the formation of micelles during the washing process.

4.3 Results and Discussion

4.3.1 Batch Tests

4.3.1.1 Rhamnolipid Soil Washing

To establish the optimum concentration of rhamnolipid solution to be used for the multiple washing of the soil, four different concentrations (0.1%, 0.5%, 2% and 4%) were used in a single washing of the soil. The amount of metals in solution was calculated and the percentage removal determined. Figure 4.3, presents the results of the test for removal of zinc, copper and nickel from soil. Each point on the plot is an average of three replicates.

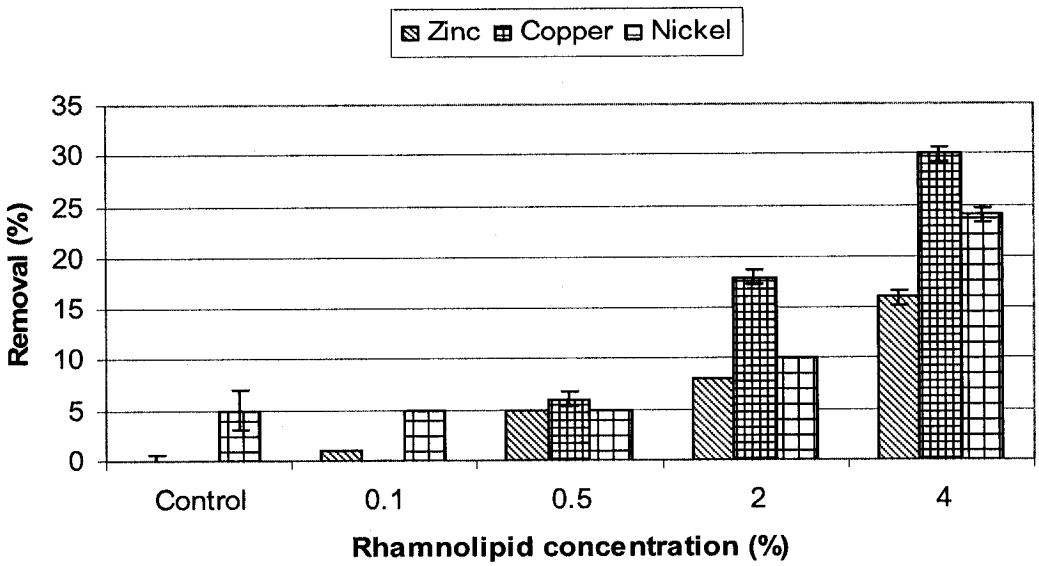


Fig 4.3 Effect of rhamnolipid concentration on removal of zinc, copper and nickel from soil

The results show that as the rhamnolipid concentration increased the percentage removal of zinc, copper and nickel also increased. This phenomenon was also observed by Mulligan et al., (1999a), Darh Azma (2002), and Miller et al. (1995), in their investigations of the removal of heavy metals from soil using rhamnolipids.

As the removal rate increased with each increase in concentration, so did the viscosity of the rhamnolipid solution. Therefore to avoid using very viscous solutions of the biosurfactant in the remediation process and also to avoid introducing large amounts of the surfactant into the soil, 2% concentration was chosen for the study of the effect of pH on the removal of the metals.

Since the rhamnolipids precipitates below pH 5.5 (Dahr Azma, 2005), only pH values above 5.5 were considered (i.e. pH 6.5, 8 and 10). The pH of the solution was adjusted with 1% NaOH. Fig. 4.4 shows the removal of the metals at the different pH values studied.

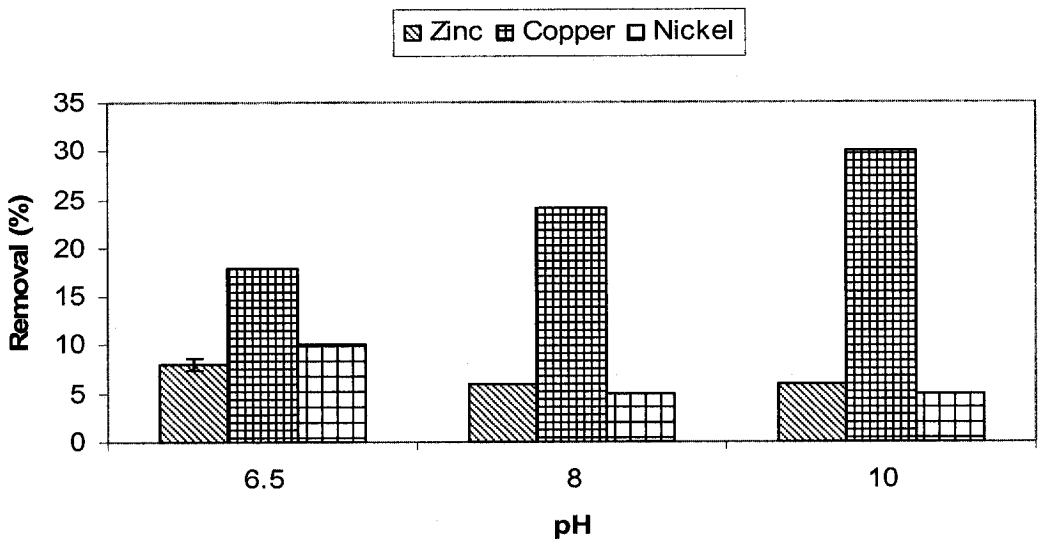


Fig 4.4 Effect of pH on the removal of metals using 2 % rhamnolipid solution

The removal of zinc and nickel appeared to decrease with an increase in pH beyond 6.5, while the removal of copper seemed to increase with pH. This increase might be a result of the fact that copper exists more in the organic fraction of the soil which is very soluble in alkaline or basic pH ranges (Yong et al. 1992, Mulligan et al. 1999a). Moreso the rhamnolipid may be removing more of the organic fraction of the copper, which was later confirmed when sequential extraction was conducted.

The highest percentage removal for zinc (8%) and nickel (10%) occurred at pH 6.5 while that of copper (30%) occurred at pH 10. The optimum pH of the rhamnolipid solution was chosen to be pH 6.5, which happens to be its natural pH. This pH value was chosen since it gives the highest removal of the metal with the highest concentration in the soil (i.e. zinc). The results compared closely with the study conducted by Dahrazma (2005) on heavy metal removal from sediments with rhamnolipids which showed that highest removal of metals occurred at pH ranges of 5.5 - 6.5.

4.3.1.2 Saponin Soil washing

In determining the ideal concentration to use in the washing of the soil, the concentration of saponin was varied between 1 g/L and 100 g/L to see how the percentage removal of the metals was affected. The effect of saponin concentration was expressed as a percentage of the initial metal content of the soil. The results of the investigation are presented in Fig 4.5.

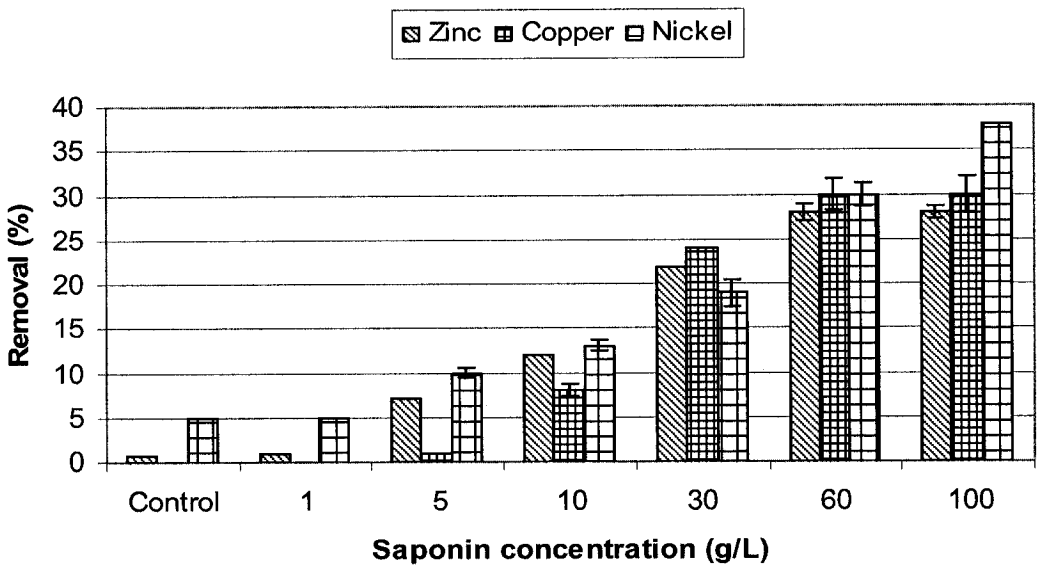


Fig 4.5 Effect of saponin concentration on removal of zinc, copper and nickel from soil

It can be seen that as the concentration of the saponin increased from 1 g/L to 100 g/L there seemed to be a significant increase in the percentage removal of the metals up to 30 g/L. As the concentration was further raised from 30 g/L to 100 g/L, the removal of nickel increased while the removal of zinc and copper reached a plateau. The results of zinc and copper removal were very close to that presented by Hong et al. (2002) on a similar type of soil. The control (distilled water) removed 5% of the nickel, 0.7% zinc and negligible amounts of copper.

From Fig. 4.5 it can also be seen that the increase in the removal rates was minimal beyond a concentration of 30g/L for zinc and copper with the exception of nickel which increased with an increase in concentration. In order to avoid the introduction of excess saponin into the soil, the optimum concentration of saponin was chosen to be 30 g/L for subsequent experiments.

The effect of pH on the removal of metals from the soil after a single wash was studied using the optimum concentration of 30 g/L saponin solution obtained from the previous experiment. The pH ranges used in the study were chosen based on the literature review. They included pH 3, 4, 5, 7 and 10. The pH was adjusted with 1% HCl and 1% NaOH solutions. The results are shown in Fig 4.6.

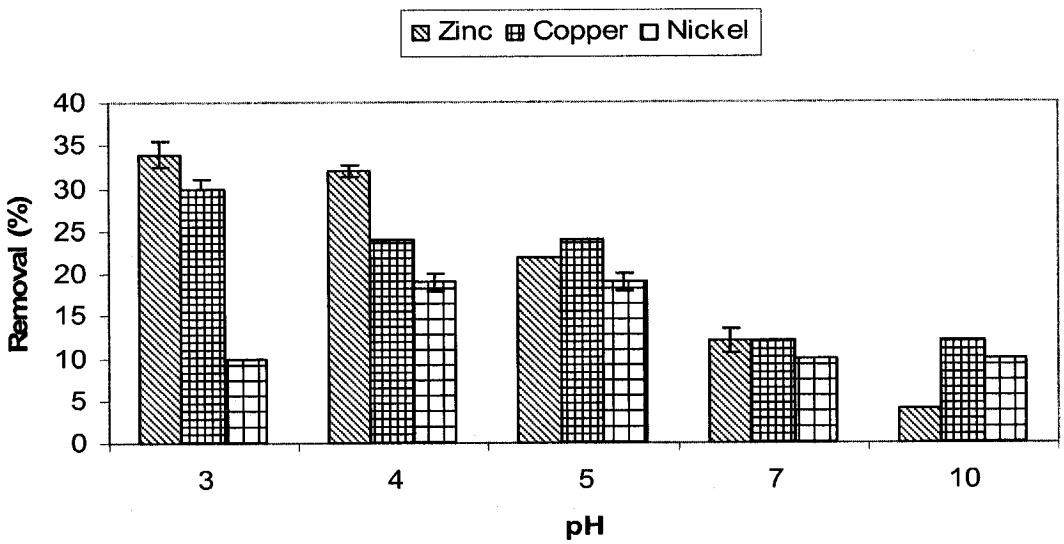


Fig 4.6 Effect of pH on the removal of metals using 30 g/L saponin solution

The removal of the metals using saponin was dependent on the pH of the washing solution as shown in Figure 4.6 above. The removal of zinc and copper seemed to decrease with an increase in pH while that of nickel showed unique behaviour around pH 4.0 and pH 5.5. The highest removal of nickel appeared to be between pH 4.0 and pH 5.5.

The highest removal of zinc (34%) and copper (30%) was around pH 3 and that of nickel (19%) around pH 5. Hong et al., (2002) noted that it was best to treat soil in weakly acidic pH ranges which resulted in less damage to the physical and chemical properties of the soil. Thus the soil treatment with saponin was considered suitable in the pH ranges of 5.0-5.5 (Hong et al., 2002).

Based on this, the optimum pH to be used in the remediation experiment was chosen to be pH 5, which happens to be the natural pH of saponin. However since the highest removal of zinc and copper occurred around pH 3, experiments were also carried out at this pH to see what level of removal could be attained.

4.3.1.3 Mannosyl-erythritol lipids (MEL) Soil Washing

Batch washing tests were carried out using mannosyl-erythritol lipids at concentrations of 0.002%, 2%, 4% and 10%, at pH 5.6 with a soil to solution ratio of 1:20. To establish the optimum concentration to use in subsequent experiments, MEL was used in a single wash of the soil; distilled water alone was used as the control. The amount of metals removed in the supernatant was then determined, the results are shown in Fig 4.7.

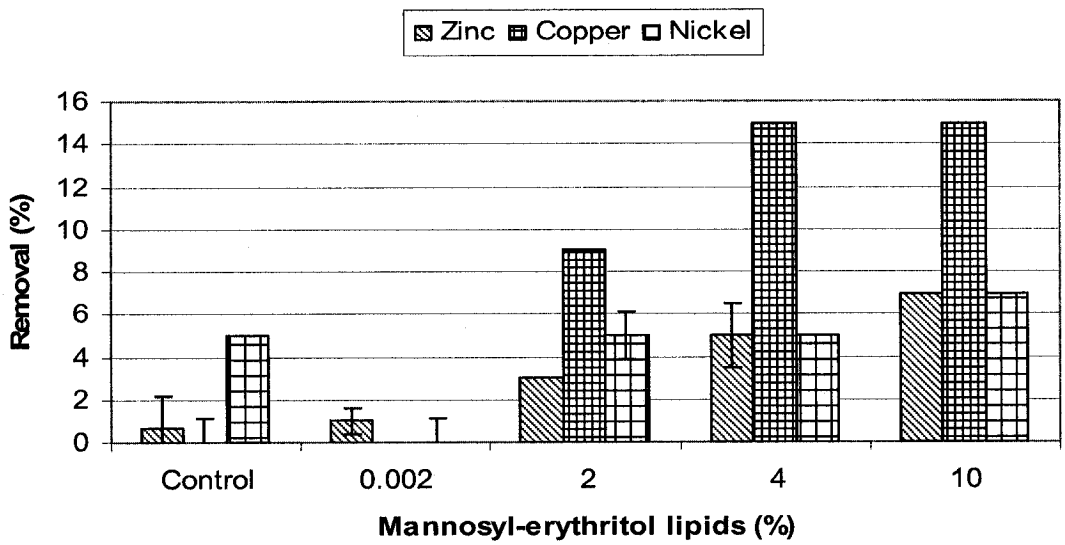


Fig 4.7 Effect of MEL concentration on removal of zinc, copper and nickel from soil

It is apparent that the removal of all three metals increased as the concentration of MEL increased from 0.002% to 4%. Beyond 4% concentration there was no a significant increase in the percentage removal of the metals. The concentration of MEL to be used in the subsequent experiment was chosen to be 4%.

Using the concentration obtained above (4%), the effect of pH on the removal of metals was investigated. From the literature it was noted that MEL is stable between pH values of 4 and 10 (Kim et al., 2002). Thus the pH effect was studied at pH values of 4, 5.6, 8 and 10. The adjustment of pH was done with 1% NaOH and 1% HCl. The results are shown in Fig. 4.8 below.

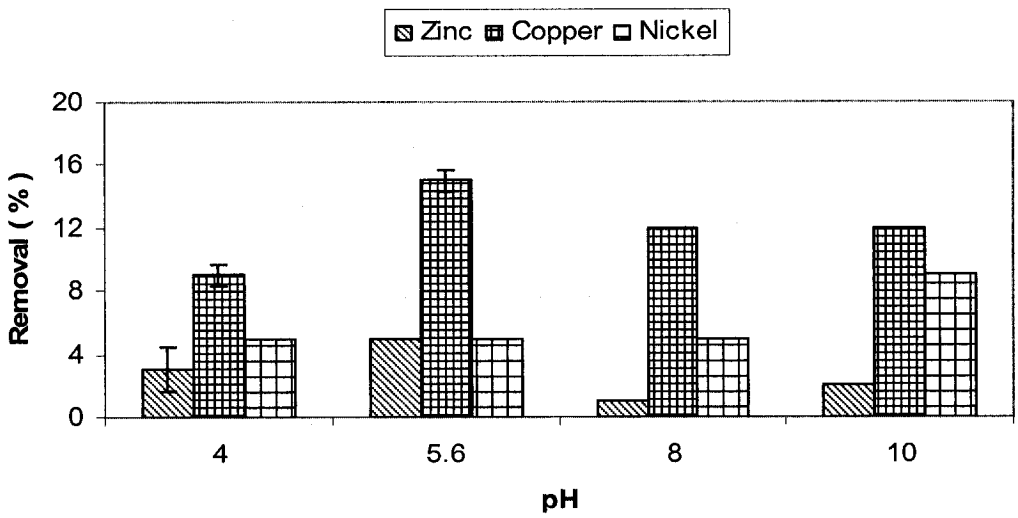


Fig 4.8 Effect of pH on the removal of metals using 4% MEL solution

The removal of the metals seems to be the highest at a pH of 5.6 (5% Zn, 15% Cu, 5% Ni) with the exception of nickel which shows a 9% removal at pH 10 against 5% at pH 5.6. The MEL appears to have a higher affinity for copper than for zinc and nickel as in the case of rhamnolipids (Fig 4.4). A pH of 5.6 was taken as the optimum pH for use in the remaining experiments.

4.3.2 Multiple washing experiment

The optimum conditions (concentration and pH) obtained from the batch washing tests were used for multiple washings of the contaminated soil. Since the contaminants of interest in the soil are heavy metals, the optimum conditions chosen were those that gave the highest removal of metals.

Rhamnolipid at a 2% concentration (pH 6.5), saponin of 30 g/L (pH 3 and 5) and MEL of a concentration of 4% (pH 5.6) were used. Water alone (pH 3, 5, 5.6 and 6.5) was used as the control in the experiments.

In the remediation of the soil, 30 ml of distilled water (control) or each biosurfactant at the concentration and pH stated above was added to vials containing 1.5 g of soil for a series of washings. The soil was washed five consecutive times with the supernatant being removed after each wash and fresh biosurfactant solution added.

In order to monitor the total petroleum hydrocarbon (TPH) content of the soil during the washing process, its concentration was analysed at the first and last wash of the soil. This was done to see what amount of TPH was removed alongside the metals. The results for the control (distilled water at pH 3, 5, 5.6 and 6.5) and each of the biosurfactants used to remove heavy metals of interest (zinc, copper and nickel) are shown in Figs 4.9, 4.10, 4.11 and 4.12.

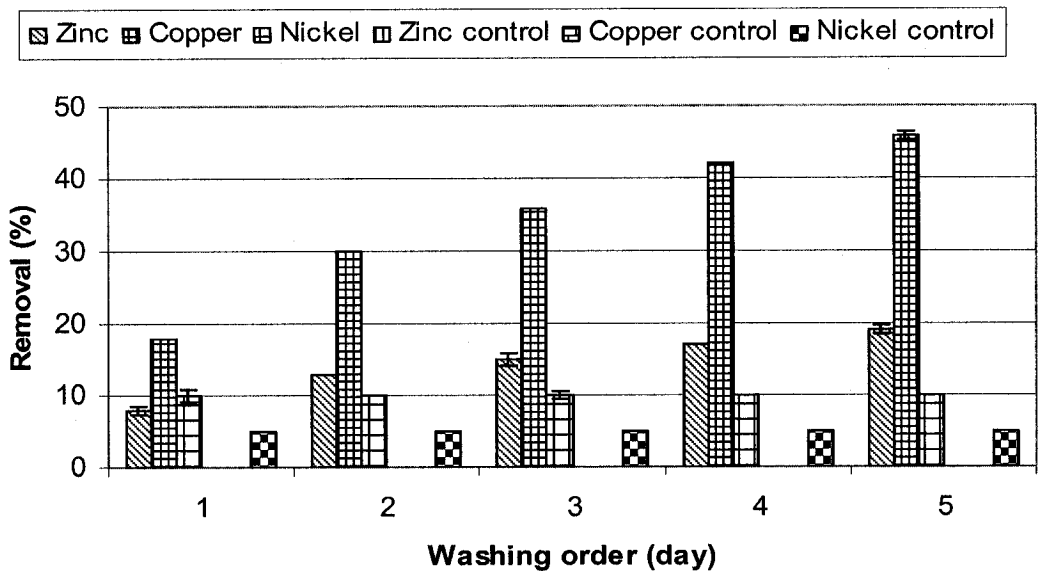


Fig 4.9 Removal of metals with 2% rhamnolipid at pH 6.5

In Fig 4.9, it can be see that after five washings of the soil, rhamnolipids removed 46% of copper, 19% of zinc and 10% of nickel while the control removed 5% nickel and no percentage of zinc and copper. The rhamnolipids seems to have more affinity for copper than for zinc and nickel as shown by the high removal rate. This phenomenon was observed by Darhazma, (2005) where rhamnolipids removed more of copper than zinc and nickel from sediment samples in a batch washing test. Multiple washings appeared to improve the removal of the metals significantly, especially the removal of copper.

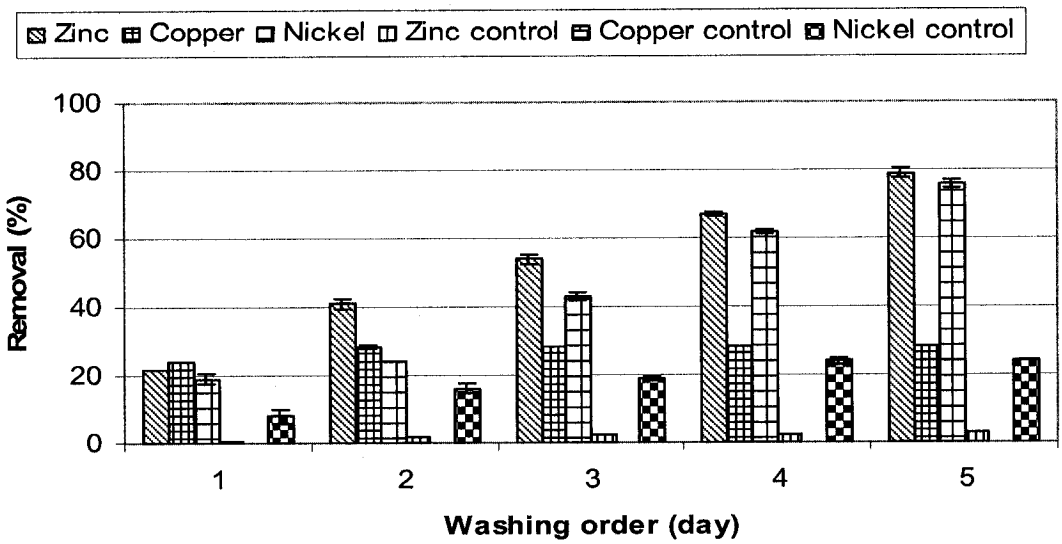


Fig 4.10 Metal removal with saponin at pH 5

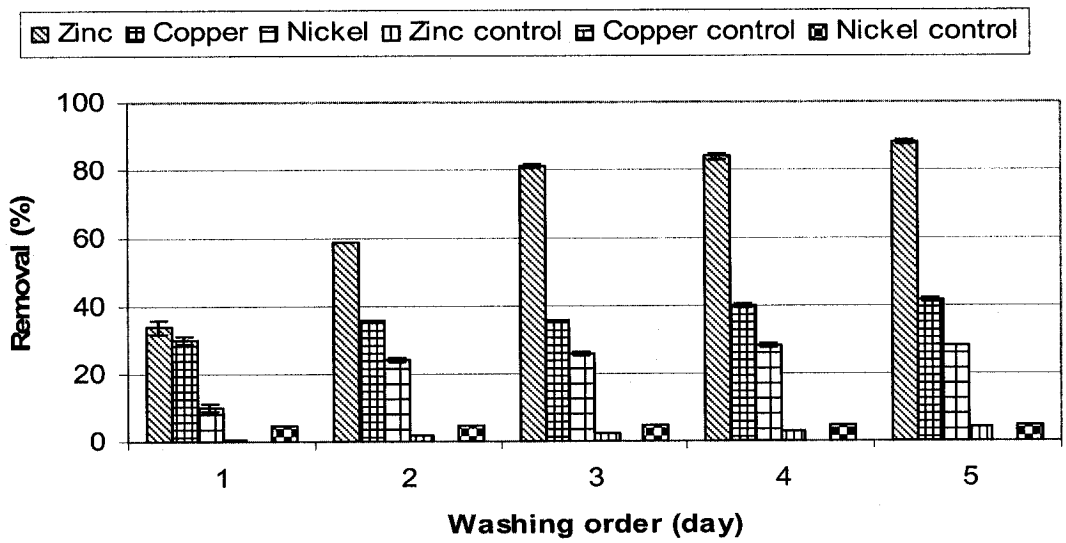


Fig 4.11 Metal removal with saponin at pH 3

Saponin at a concentration of 30 g/L (pH 3 and 5) was used in a series of washings as shown in Figs 4.10 and 4.11. The saponin at pH 5 was able to remove 79% of zinc and 76% of nickel; however the case was different for copper where the removal was 28% after 5 washings. At pH 3 about 88% of zinc, 42% of copper and 28% of nickel were removed by saponin. The control (water); removed minimal amounts of all three metals (4.3% of zinc, <0.1% of copper and 5% of nickel) at pH 3 while at pH 5 a significant amount of nickel was removed (24%). The removal of zinc was 3.2% and negligible amounts of copper were removed. It is also evident from Figures 4.10 and 4.11 that more than one washing of the soil improved the removal efficiency of the metals. Another deduction that can be drawn from Figs 4.10 and 4.11 is that saponin seems to have a stronger affinity for zinc at both pH levels than for copper and nickel.

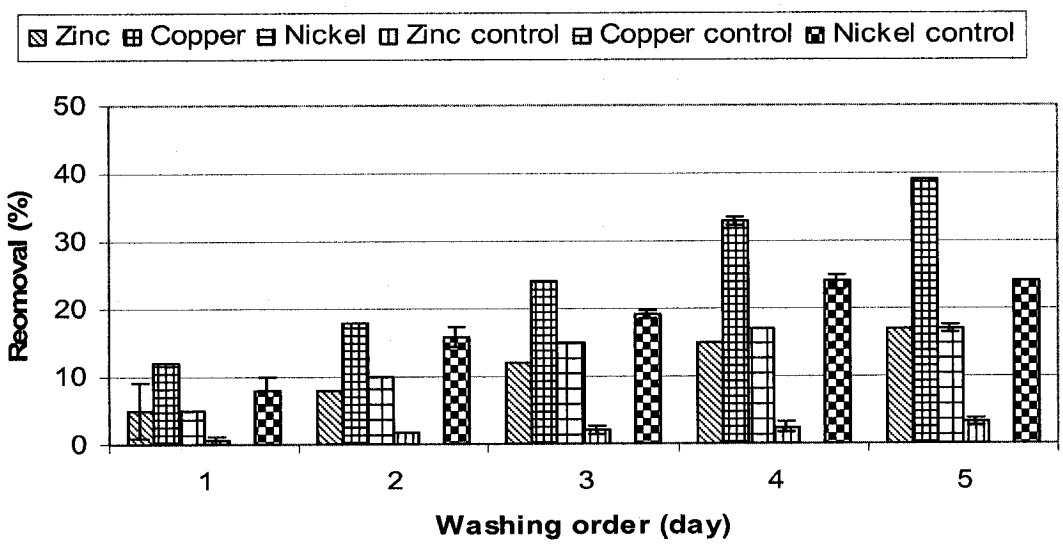


Fig 4.12 Removal of metals using 4% MEL at pH 5.6

The crude form of MEL was used for the remediation process at a concentration of 4% and pH of 5.6 which were previously determined as optimum conditions. A series of five washings proved to be significantly superior to a single washing of the soil using MEL. A single washing yielded 5% removal of zinc and nickel and about 12 % of copper, whereas after five washings the removal was greatly increased to 17 % of zinc and nickel while 36 % of copper was removed. This finding is concurrent with the results of research carried out by Mulligan et al. (1999a) on metal removal from soils and sediments by surfactin, where multiple washing of the soil/sediment greatly improved the removal efficiency of the metals. The control removed 3.2% of zinc, 0% of the copper and about 24 % of nickel. MEL was also seen to have a significant effect on copper removal from soil, much more than zinc and nickel.

4.3.2.1 K factor for multiple washing of soil

There seems to be an exponential removal of the metals for all biosurfactants tested. From the equation;

$\% R = e^{kt}$

Where % R = % removal of metals, t = number of washings.

A plot of ln (% R) vs. t yields a slope equal to k. i.e. k = ln (% R)/ (t)

The k factors for the multiple washing of the soil with the biosurfactants (rhamnolipids, saponin and mannosyl-erythritol lipids) are shown in Figures 4.13, 4.14 and 4.15. A high K value indicates higher affinity of the biosurfactants in the removal of the metals.

As indicated in the Figure 4.14, saponin has more affinity for zinc and nickel than for copper. However rhamnolipids have more affinity for copper than to zinc and nickel (Figure 4.15).

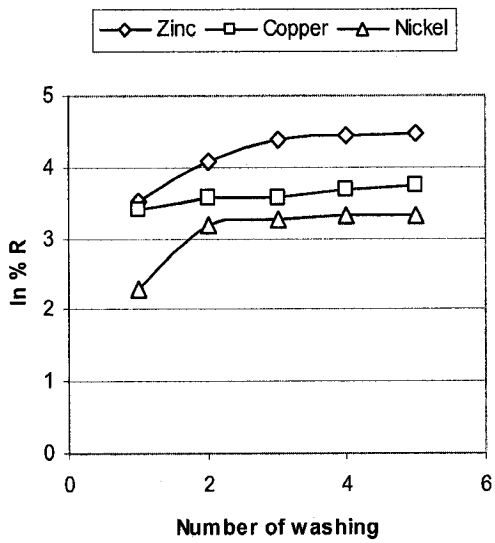


Figure 4.13 K factor for soil washing with with 30 g/L saponin, pH 3

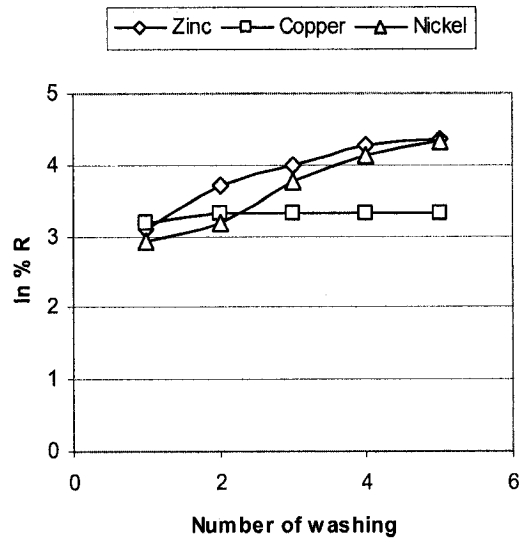


Figure 4.14 K factor for soil washing 30g/L saponin, pH 5.

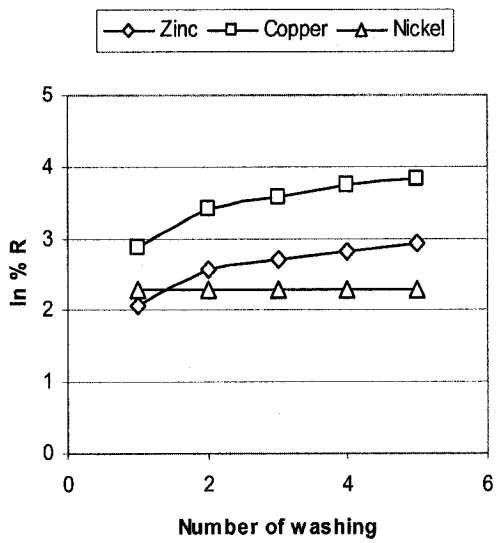


Figure 4.15 K factor for soil washing with with 2 % rhamnolipid, pH 6.5

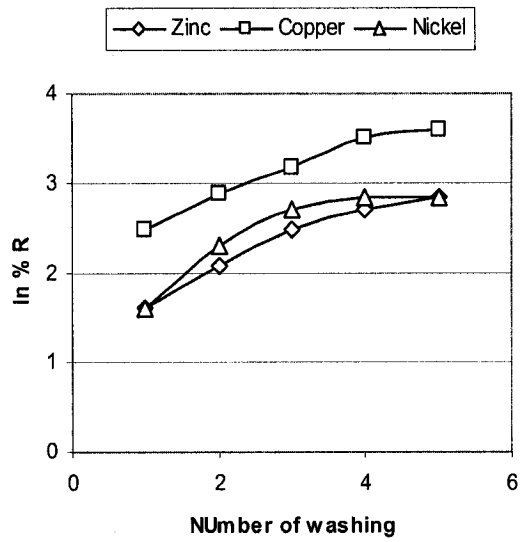


Figure 4.16 K factor for soil washing 4% mannosyl-erythritol lipids, pH 5.6

4.3.3 Sequential Extraction

Sequential extractions were carried out to determine the speciation of the heavy metals (zinc, copper and nickel) contaminants in the soil. To do this, solutions of increasing strength were used to extract the metals from 1.5 g of soil in vials (Table 3.5). The fractions are namely; soluble, exchangeable, carbonates oxides, organic and residual. The exchangeable fractions are the least strongly bound while the residual are the most strongly bound (Mulligan et al., 1999a).

The extractions were carried out before and after treatment of the soil to establish the fraction that was being removed by the biosurfactants. Sequential extraction before treatment indicated that the oxide fraction accounted for 36% of the zinc while the

organic fraction constituted 50% of copper. Nickel was found mainly in the exchangeable and carbonate fractions (about 50% of nickel). The residual fraction seemed to hold about 30% of nickel, 35% of zinc and 35% of copper.

The soil residues obtained from the biosurfactant treatment were dried, weighed and then fractionated by sequential extraction procedures (Table 3.5) (Mulligan et al., 1998), and then compared with soil untreated with biosurfactant. The metal fraction removed by the biosurfactant was designated as the soluble fraction. A decrease in the size of a particular fraction compared to the untreated soil sample indicated that the surfactant dissolved the metal from that fraction into its soluble form in the supernatant in contact with the soil. The sequential extraction results for zinc, copper and nickel are shown in Figs 4.17, 4.18 and 4.19.

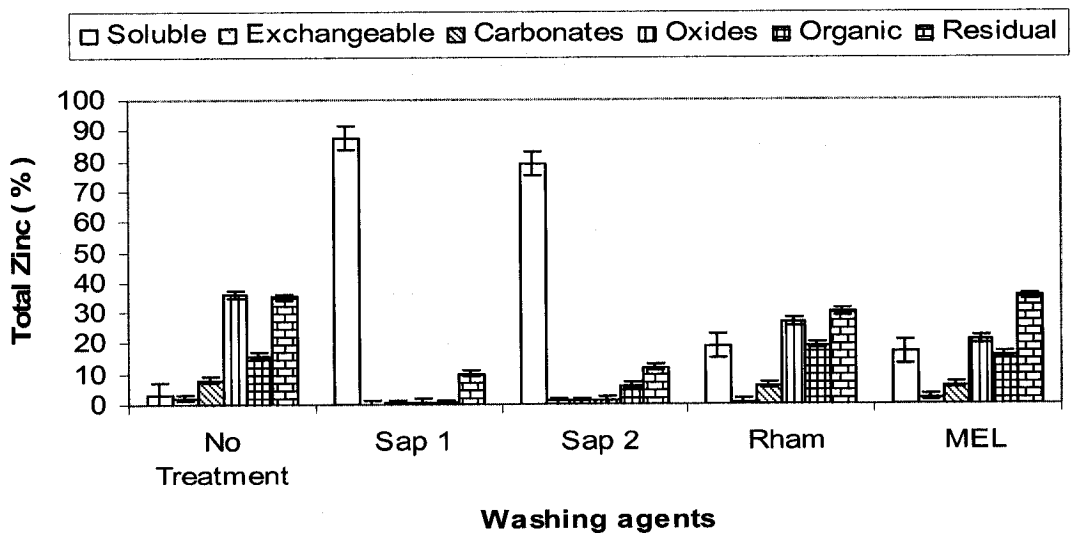


Fig 4.17 Sequential extraction of zinc
Sap 1 = 30g/L saponin at pH 3, Sap 2 = 30g/L saponin at pH 5, Rham = 2% rhanmnolipids at pH 6.5, MEL = 4% mannosyl-erythritol lipids at pH 5.6

Figure 4.17 shows the difference when soil samples were washed with 30 g/L saponin (pH 3 and pH 5), 2% rhamnolipids (pH6.5) and 4% MEL (pH 5.6) in comparison with the untreated or unwashed soil. There is a significant decrease in the oxide, organic, carbonate and exchangeable fractions of zinc in the soil washed with saponin. 2% rhamnolipids (pH 6.5) and 4% MEL (pH 5.6) removed zinc mainly from the oxide and carbonate fractions. The control experiment removed very minimal amounts of zinc from the oxide fraction of the soil (result not shown).

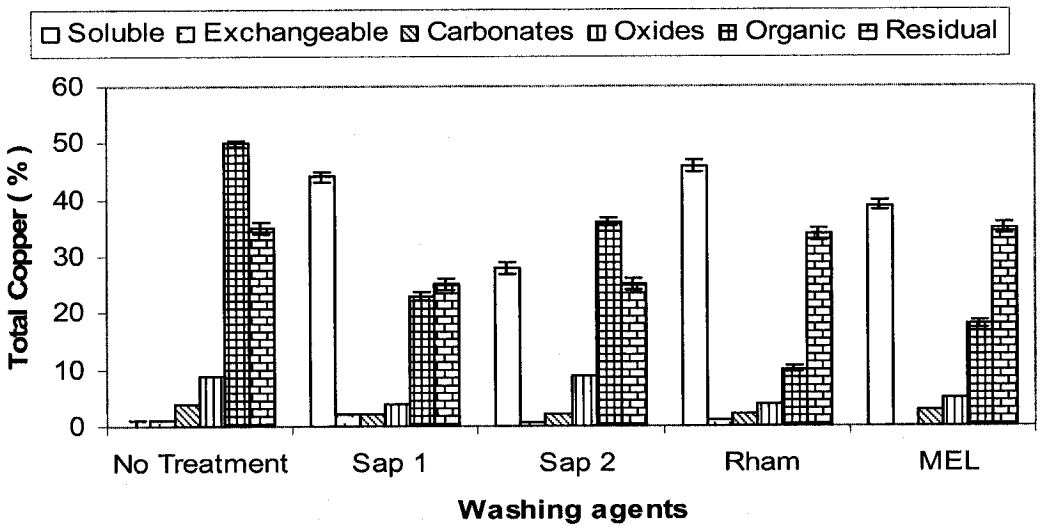


Fig 4.18 Sequential extraction of copper
Sap 1 = 30g/L saponin at pH 3, Sap 2 = 30g/L saponin at pH 5, Rham = 2% rhanmnolipids at pH 6.5, MEL = 4% mannosyl-erythritol lipids at pH 5.6

As can be seen in Fig 4.18, copper in the soil samples was removed more from the organic and oxide fractions, by 2% rhamnolipids (pH6.5), 30 g/L saponin (pH 3 and 5) and 4% MEL (pH 5.6). The effect of the surfactant on the soil was much higher than the

control which did not remove any copper after a series of five washings. This indicates that these biosurfactants can remove copper in the organic fraction as well as the oxide fraction, when added to the washing solution in a soil washing process.

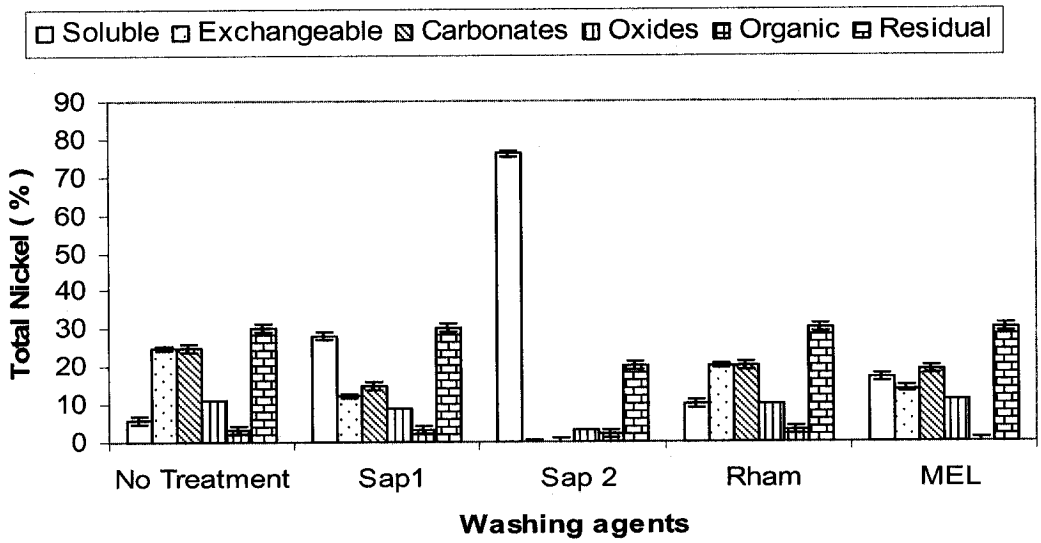


Fig 4.19 Sequential extraction of nickel
Sap 1 = 30g/L saponin at pH 3, Sap 2 = 30g/L saponin at pH 5, Rham = 2% rhanmnolipids at pH 6.5, MEL = 4% mannosyl- erythritol lipids at pH 5.6

For nickel, a substantial decrease was observed in the exchangeable and carbonates fractions after washing the soil with 30 g/L saponin at (pH 5 and pH 3). 2% rhamnolipids (pH 6.5) and 4% MEL (pH 5.6) removed appreciable amounts also from the exchangeable and the carbonate fractions. The control (water at pH 3 and pH 5) removed a significant amount also from the exchangeable and carbonates fractions; however the effect was much less compared to that of 30 g/L saponin (pH 5). The results above show

that when nickel is present in the exchangeable and carbonate fractions, these biosurfactants can be used in a soil washing process to remediate the soil.

4.3.4 Total Petroleum Hydrocarbon (TPH) Analysis after treatment with biosurfatants

The total petroleum hydrocarbon content of the soil before treatment (228 mg/kg) was below the limit allowed by the Quebec "B" soil criteria (300 mg/kg) however the soil can be said to be slightly contaminated with hydrocarbons. As part of the objectives of the research, to investigate the capability of the biosurfactants to remove the hydrocarbons from soil, the TPH levels were monitored during the course of the remediation experiment. The concentrations of the TPH in the soil were analysed after the first and fifth wash of the soil using gas chromatography as stated in the methodology detailed in chapter 3. This was done to have an understanding of the amount that was being removed along with the metals using the biosurfactants; saponin, rhamnolipids or water as control. The results are presented in Table 4.2.

Table 4.2 Total Petroleum Hydrocarbons in soil after treatment with biosurfactants and control

Biosurfactants	Initial TPH concentration (mg/kg)	Concentration after lst wash (mg/kg)	Concentration after 5th Wash (mg/kg)	% Removed
Saponin 30 g/L (pH 3)	228 ± 1.5	46 ± 3	14 ± 3	93
Saponin 30 g/L (pH 5)	228 ± 1.5	39 ± 3	14 ± 3	93
Rhamnolipids 2% (pH 6.5)	228 ± 1.5	65 ± 4	45 ± 4	80
Water (pH 3)	228 ± 1.5	80 ± 8	67 ± 6	71
Water (pH 5)	228 ± 1.5	56 ± 9	46 ± 8	80
Water (pH 6.5)	228 ± 1.5	35 ± 4	33 ± 5	84

4.3.5 Mass Balance Check for Soil Samples

Soil residues from the soil washing process using biosurfactants (saponin and rhamnolipids) were dried and digested with 6N HCl, the supernatant was removed by centrifugation and analysed by Atomic Absorption Spectrophotometer (AAS). The average results from three replicates are shown in Table 4.3.

Table 4.3 Mass Balance check of metals

Biosurfactant	Metal	Removed (%)	Residual (%)	Total (%)	Missing (%)
Saponin 30 g/L (pH 3)	Zinc	87.7	10.5	98.2	1.8
	Copper	42.1	57.5	99.6	0.4
	Nickel	27.6	70.8	98.4	1.6
Saponin 30 g/L (pH 5)	Zinc	78.8	19.9	98.7	1.3
	Copper	28.0	71.4	99.4	0.6
	Nickel	76.4	22.7	99.1	0.9
Rhamnolipids 2% (pH 6.5)	Zinc	19.2	80.2	99.4	0.6
	Copper	45.7	53.1	98.8	1.2
	Nickel	9.6	89.3	98.9	1.1

From the mass balance checks it can be seen that small fractions of the metal are missing. This may be a result of analytical/ experimental error or some may have been lost during the process of filtration after centrifuging soil samples.

4.3.6 Removal of metals by the different biosurfactants

In the study conducted using rhamnolipids, saponin and mannosyl-erythritol lipids in batch washing process of soil contaminated with heavy metals and hydrocarbons, the metal removal from soil with the different biosurfactants tested varied. Rhamnolipids

and mannosyl-erythritol lipids appear to remove more copper than zinc and nickel from the contaminated matrix. Saponin however has more effect on the removal of zinc and nickel than on the removal of copper.

Miller et al. (1995) noted that rhamnolipids may be more effective in soil contaminated with metals that are less sensitive to ion-exchange processes. This may explain why rhamnolipids are more effective in removing copper with strong chemisorption on clays, oxides and humus of soil (Hong et al. 2002).

Hong et al. (2002) in their study of removal of metals from different soils using saponin noted that saponin was more effective in the removal of exchangeable, carbonates and oxides fractions of metals from soil. This explains why saponin was more effective in the removal of zinc and nickel from the soil since these fractions contained a substantial amount of both metals (zinc and nickel).

Chapter Five: Conclusions and Recommendations

5.1 Conclusion of this study

This research was performed to evaluate the possibility of enhanced remediation of a natural soil sample contaminated with heavy metals and hydrocarbons, using three biosurfactants namely saponin, rhamnolipds and mannosyl erythritol lipids (MEL). The contaminants in the soil include, zinc (894 mg/kg), copper (261 mg/kg), nickel (167 mg/kg) and the total petroleum hydrocarbons content (228 mg/kg) (Table 5.1). The presence of these contaminants could pose a threat to humans and the environment and hence the need to clean up the soil in order to correct the problem.

The study was conducted in two phases, the optimum conditions were established in the first phase of experiments and then these conditions were used in the second phase which was the multiple washing of the soil. Then sequential extractions were used to determine the fractions affected by the soil treatment. The conclusions that can be drawn from the experiments conducted so far are presented below.

5.1.1 Optimum conditions

The optimum conditions for the washing of the soil were determined to be 30 g/L saponin at pH 5 (22% Zn, 24% Cu, 19% Ni) (Figure 4.10) and pH 3 (34% Zn, 30% Cu, 10 % Ni) (Figure 4.11), 2% rhamnolipids at pH 6.5 (8% Zn, 18% Cu, 10% Ni) (Figure 4.9) and 4% MEL at pH 5.6 (5% Zn, 15 Cu, 5% Ni) (Figure 4.12).

5.1.2 Remediation of soil

For a single washing of the soil, highest levels of zinc and copper removal were achieved with saponin at pH 3 while nickel's highest removal was attained with saponin at pH 5. However this removal rate was greatly increased with multiple washings of the soil. From the study it appears that zinc and nickel removal was further enhanced in the presence of saponin rather than rhamnolipids. However with copper, rhamnolipids seemed to offer the best results.

The concentrations of zinc and nickel were reduced below the allowable limit with saponin 30 g/L at pH 3 and 5, respectively. The concentration of copper was reduced to levels slightly above the limit. (Table 5.2). After multiple washings with the controls (water at pH 3, 5 and 6.5), none of the metal concentrations were close to the limits except for nickel control (water pH 5) that was slightly close to the allowable limit. In monitoring the total petroleum hydrocarbon content during the experiments, the study showed that hydrocarbons can be removed along with the metals in a soil washing

process. The results clearly indicated the feasibility of removing zinc, copper and nickel from a contaminated soil with the anionic biosurfactants tested

The results obtained by other researchers for the removal of heavy metals from soil/sediment are shown in the Table 5.1.

Table 5.1 Heavy metal removal from soil/sediment

Researchers	Biosurfactant	Medium/No. of washing	Metal	Removal (%)
Mulligan et al (1999b)	Rhamnolipids (0.1% ,1% NaOH)	Soil/ 5 washings	Zinc Copper	17 38
Mulligan et al (2001a)	Rhamnolipids (0.5%, 1% NaOH)	Sediment/single wash	Zinc Copper	18 60
Hong et al (2002)	Saponin (30g/L) (pH 5.0 -5.5)	Soil/single wash	Zinc Copper	60 50
Darhazma (2002)	Rhamnolipids 2% (pH6.5)	Mining residue/5 washings	Copper	29
Darhrazma (2005)	Rhamnolipids 2% (pH6.5)	Sediment/5 washings	Zinc Copper Nickel	7 5 22

Table 5.2 Removal of metals from soil after treatment with biosurfactants and control

Bio surfactant	Metal	Quebec "B" soil criteria concentration (mg/kg)	Initial in soil concentration (mg/kg)	Final Concentration in soil after treatment (mg/kg)	Removal after treatment (%)
Saponin 30 g/L (pH 3)	Zinc	500	894	107	88
	Copper	100	261	151	42
	Nickel	100	167	120	28
Saponin 30 g/L (pH 5)	Zinc	500	894	188	79
	Copper	100	261	188	28
	Nickel	100	167	40	76
Rhamnolipids 2% (pH 6.5)	Zinc	500	894	724	19
	Copper	100	261	141	46
	Nickel	100	167	150	10
MEL 4% pH (5.6)	Zinc	500	894	742	17
	Copper	100	261	159	39
	Nickel	100	167	139	17
Water (pH 3)	Zinc	500	894	858	4
	Copper	100	261	261	0
	Nickel	100	167	159	5
Water (pH 5)	Zinc	500	894	867	3
	Copper	100	261	261	0
	Nickel	100	167	127	24
Water (pH6.5)	Zinc	500	894	894	0
	Copper	100	261	261	0
	Nickel	100	167	159	5

5.1.3 Sequential Extraction

In the sequential extraction of untreated soil, zinc was found to exist mostly in the oxide fraction; copper was found more in the organic fraction, while nickel existed in both the exchangeable and the carbonate fractions. The residual fraction seemed to hold a substantial amount of all three metals.

Soil fractionation after treatment with the biosurfactants showed that the zinc was removed more from the oxide fraction with some appreciable amounts being removed also from the other fractions. Copper was removed mostly from the organic fraction while nickel removal was from the exchangeable and the carbonate fractions.

In summary, removal of the contaminants (heavy metals and hydrocarbons) from the natural soil using rhamnolipids, saponin and mannosyl erythritol lipids was feasible for zinc, copper, nickel and the total petroleum hydrocarbons. However, more research is required to employ this process on a large scale operation.

5.2 Recommendations for Future Research

- In this research the biosurfactants were used in isolation. A mixture of the biosurfactants could be used to evaluate the effect on the overall removal rate of the metals and the hydrocarbon.

- Evaluation of the biosurfactants in the treatment of soil with a high content of heavy metals and hydrocarbons.

- Investigation of the possibility of biosurfactants enhanced biodegradation of hydrocarbons followed by washing of the soil to ascertain the extent of remediation possible with both contaminants (heavy metals and hydrocarbons).

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Appendices

Appendix A

Determination of Soil Cation Exchange Capacity (CEC)

Materials

1. 5 g soil
2. Sterile Centrifuge tubes
3. 1M Potassium Acetate ($KC_2H_3O_2$)
4. Methanol (CH_3OH)
5. 1M Ammonium Acetate (NH4 $C_2H_3O_2$)
6. 100 ml beakers
7. Standard Potassium solutions (5, 10, 20 ppm)
8. Atomic Absorption spectrophotometer (AAS)
9. Weighing balance
10. Table top centrifuge

Procedure

1. Weight out 5 gram of dry soil sample into three different centrifuge tubes
2. Add 20 ml of 1M Potassium acetate to each tube. Cap tubes and shake intermittently for 5 minutes.
3. After shaking remove cap temporarily and wash down soil on the sides or caps of the tubes with distilled water from wash bottle.

4. Recap tubes and place them in a centrifuge and centrifuge for 15 minutes at 3000g. Discard the clear supernatant.
5. Add another 20 ml 1 M Potassium acetate and centrifuge again for 15 minutes, discard the supernatant.
6. Add 20 ml methanol to the soil pellets and shake until soil is re-suspended.
7. Wash down soil on the caps and sides of tubes back into the liquid with distilled water.
8. Centrifuge at high setting for 15minutes discard the supernatant.
9. Repeat steps 6-8.
10. Add 25 ml of 1 M Ammonium acetate to the soil pellets, cap tubes and shake until soil is re-suspended.
11. Rinse caps and sides of tubes to wash down solid materials back into the liquid. Cap tubes and centrifuge at 3000g for 15 minutes.
12. Pour clear supernatant into 100 ml beaker.
13. Repeat steps 10-12, once again pouring the clear supernatant into same beaker.
14. Measure the K concentration in the supernatant using the Atomic Absorption spectrophotometer (AAS). Using the value of the K concentration to determine the CEC of each soil sample in $cmoles^{+}/kg$ of dry soil

Results

The result of the experiment and calculations are summarised in Table A.1 below

Table A.1 Experimental Results and Calculation of CEC

Sample	$[K^+]$ by AAS (ppm)	$[K^+]$ in supernatant (ppm)	$[K^+]$ Mass (mg)	CEC ($cmoles^+/kg$)
1	14.0	224.0	11.2	5.74
2	14.0	224.0	11.2	5.74
3	13.0	208.0	10.4	5.33

The CEC of the soil is the average of the three values.

$$CEC = \frac{5.74 + 5.74 + 5.33}{3} = 5.60 \text{ (cmoles}^+\text{/kg)}$$

Standard deviation

$$STDEV = \sqrt{\frac{(5.74-5.60)^2 + (5.74-5.60)^2 + (5.33-5.60)^2}{2}} = 0.24$$

The CEC of the soil is given as 5.60± 0.24 $cmoles^+/kg$

Appendix B

Measurement of Soil Organic Content

Materials

1. Soil samples
2. Crucibles
3. Balance
4. Desiccators
5. Muffle Oven
6. Furnace

Procedure

1. Weigh crucibles
2. Weigh soil samples about 1g into each of three crucibles. Dry soil in oven at 105 ^{0}C for 48 hours.
3. Cool down soil in desiccators for 30 minutes, Weigh crucible + dried soil.
4. Ash dry soil in 550 ^{0}C muffle furnace for 2 hours, leave samples in the furnace overnight. Weight sample as described in 3 above.
5. Calculate the soil organic content (SOC) according to the following equation:

% SOC = (SDW before ashing – SDW after ashing)/ (SDW before ashing) X 100 %

Results

The results of the experiments are summarised in the Table B.1 below.

Table B.1 Experimental Results and Calculation of SOC

Sample	Crucible weight (g)	Total weight before ashing (g)	Total weight after ashing (g)	Soil weight (g)	Organic content (%)
1	15.7334	16.7238	16.6651	0.9904	5.93
2	18.4502	19.4390	19.3811	0.9888	5.86
3	18.1100	19.0990	19.0365	0.9890	6.32

The organic content is the average of the three values.

$$\text{Organic Content} = \frac{5.93 + 5.86 + 6.32}{3} = 6.04\ \%$$

Standard deviation;

$$\text{STDEV} = \sqrt{\frac{(5.93-6.04)^2 + (5.86-6.04)^2 + (6.32-6.04)^2}{2}} = 0.25$$

The organic content of the soil is given as 6.04± 0.35 %

Appendix C

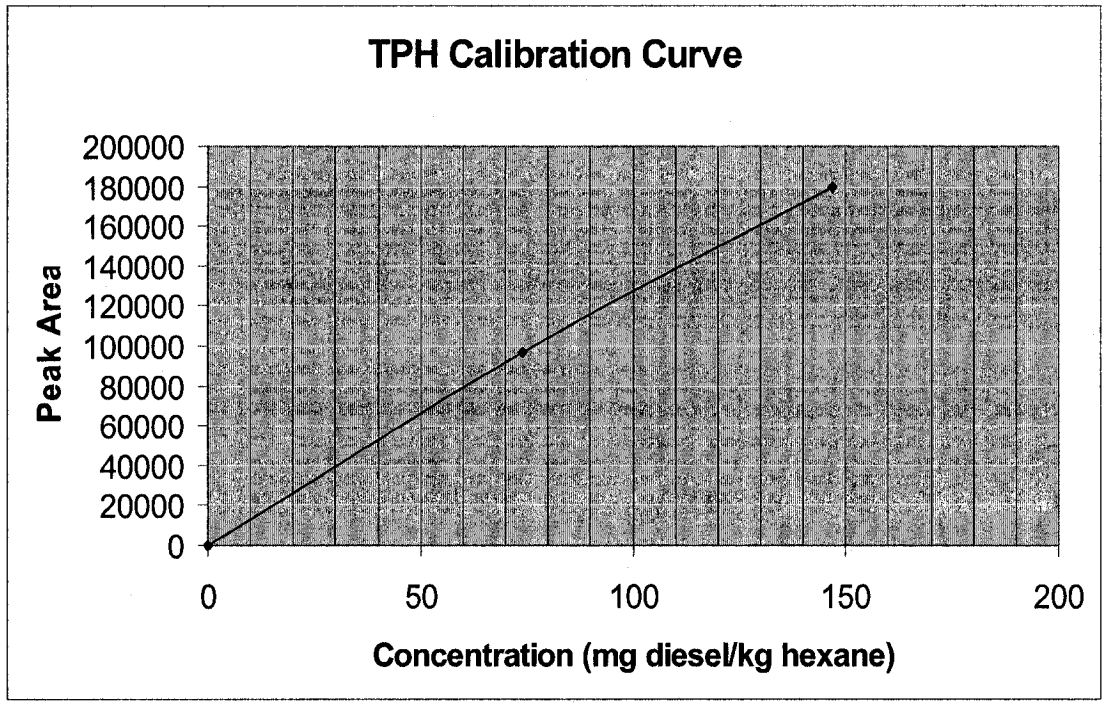

Figure C.1 Total Petroleum Hydrocarbon (TPH) Calibration Curve